

## 平成 28 年度 委託研究開発成果報告書

### I. 基本情報

事業名：(日本語) 医療分野国際科学技術共同研究開発推進事業 地球規模課題対応国際科学技術協力プログラム (SATREPS)

(英 語) International Collaborative Research Program Science and Technology Research Development for Sustainable Development (SATREPS)

研究開発課題名：(日本語) モンゴルにおける家畜原虫病の疫学調査と社会実装可能な診断法の開発

(英 語) Epidemiological Studies on Animal Protozoan Diseases in Mongolia and Development of Effective Diagnostics Measures

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実 施 期 間：平成 28 年 4 月 1 日 ~ 平成 29 年 3 月 31 日

### II. 成果の概要（総括研究報告）

#### ・ 研究開発代表者による報告の場合

こう疫トリパノソーマの野生株 (*Trypanosoma equiperdum*) を 2015 年 4 月に引き続き、2016 年 4 月に 1 種類分離培養することに成功し、本プロジェクトでモンゴル国立獣医学研究所 (IVM) に設置したセルバンクに凍結保存した。2016 年 5 月には 4 種類のピロプラズマ病病原体 (*Babesia*) の野生株の分離培養に成功し、トリパノソーマと同様セルバンクで保管している。いずれの原虫サンプルも必要に応じて実験材料として供給可能な体制を整えた。

トリパノソーマ野生株に特有の抗原をコードする遺伝子を特定するために、原虫株の全ゲノム解析とトランスクリプトーム解析を実施している。また、タイレリア (*Theileria*) 及びバベシア (*Babesia*) から診断用抗原をコードする EMA-2t 及び RAP-1 遺伝子をモンゴル全土の疫学調査で収集した感染血液の DNA サンプルからクローニングした。

組換え GM6-4r 抗原 (rGM6-4r : 動物のトリパノソーマの診断用抗原) を利用した ELISA 法と ICT 法を開発し、両診断法の成績をモンゴル全国から集められた血液の血清サンプルを用いて評価した。その結果、組換え GM6-4r 抗原ベースの試験法は、標準 ELISA 法と良好な相関を示した。この事実から、組換

え GM6-4r 抗原ベースの ELISA と ICT は、モンゴルにおける家畜のトリパノソーマの新しい診断法として用い得る可能性が高いこと明らかにした。

ウマのトリパノソーマ病の感染率が高い農家をプロジェクトの関係者が見つけ出し、3軒の飼育農家と、原虫病予防・対策のモデル地区としての研究活動の受入れを打診した。その結果、2軒の飼育農家の協力を得ることとなった。(2軒のウマ飼育頭数は合計で 340 頭程度)。

トリパノソーマ病に罹患したウマの病理解剖を実施し、全身の組織・臓器サンプル採取して病理組織学的検索のための標本を作製した。また、ウマ病理解剖マニュアルを英語とモンゴル語で作成して 1,000 部印刷し、同大学の獣医学教育に活用するため 300 部をモンゴル国立生命科学大学図書館に寄贈した。

Since April 2015, we have succeeded in isolating and cultivating a wild type *Trypanosoma equiperdum*, a causative agent of dourine (horse trypanosomosis), and successfully isolated another wild type trypanosome from an infected horse. The trypanosomes are cryopreserved in the Cell Bank established at the Institute of Veterinary Medicine (IVM). In May 2016, we had isolated four kinds of wild type Babesia, and they are kept in the Cell Bank. We have established a system that can supply those protozoan parasites as experimental material as needed.

In order to identify genes encoding antigens specific to *Trypanosoma equiperdum*, we recently perform whole genome analysis and transcriptome analysis of the parasite. In addition, EMA-2t and RAP-1 genes encoding diagnostic antigens from *Theileria* and *Babesia* were cloned from DNA samples of infected blood samples collected through epidemiological studies across Mongolia.

We developed ELISA and ICT using recombinant GM6-4r antigen (rGM6-4r: the diagnostic antigen for trypanosomosis), and these methods were evaluated by using the serum samples through epidemiological studies across Mongolia. As a result, the recombinant GM6-4r antigen-based tests showed a good correlation with the standard ELISA method. This fact revealed that the recombinant GM6-4r antigen based ELISA and ICT are likely to be used as a new diagnostic method for trypanosomosis in livestock in Mongolia.

We found 3 farmers with a high infection rate of horse trypanosomosis and requested them to be model farms for trial of prevention and control measures of trypanosomosis. As a result, it was decided to cooperate with two farmers. (The total number of horses raised in 2 farms is about 340 heads).

A pathological autopsy of a horse suffering from trypanosomosis was performed, and samples of tissues and organs of the whole body were sampled to prepare a specimen for histopathological analyses. In addition, 1,000 copies of equine pathology dissection manual were prepared in English and Mongolian, and 300 copies were donated to the Library of the Mongolian National University of Life Sciences for use in improvement of their veterinary education.

### III. 成果の外部への発表

#### (1) 学会誌・雑誌等における論文一覧

(国内誌(和文)2件、モンゴル国内誌(英語)5件、国際誌24件)

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(2) 学会・シンポジウム等における口頭・ポスター発表

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(3) 「国民との科学・技術対話社会」に対する取り組み

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(4) 特許出願

該当なし