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Molecular detection and characterization of tick-borne pathogens of domestic animals in Malawi

（マラウィの家畜におけるマダニ媒介性病原体の分子学的解析）

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SUMMARY

Tick-borne diseases (TBDs) remain one of the major challenges to the improvement of livestock production in the tropics where the vector ticks are widespread. The commonly diagnosed TBDs of domestic animals are anaplasmosis, babesiosis, ehrlichiosis, hepatozoonosis and theileriosis. The molecular epidemiological data on the occurrence of TBPs are important as they provide science-based information for the formulation of disease control measures and monitoring of the effectiveness of the measures in place. In Malawi, although ticks that infect domestic animals have been documented for over a century, there has been no molecular study conducted to characterize the tick-borne pathogens (TBPs) of domestic animals. This thesis aimed at detecting and characterizing the TBPs in cattle, sheep, goats and dogs in Malawi using molecular techniques.

In chapter I, 191 cattle DNA samples collected from the central and southern regions of Malawi were screened for tick-borne piroplasms and *Anaplasmataceae*. The screening of *Anaplasmataceae* was done using the heat shock protein gene (*groEL*) nested PCR assays. The screening of piroplasms was done using 18S ribosomal RNA gene (rDNA) nested PCR assays. A new 18S rDNA multiplex PCR assay was designed for the identification of *Babesia* and *Theileria* species (*Babesia bigemina*, *Babesia bovis*, *Theileria mutans*, *Theileria parva*, *Theileria taurotragi* and *Theileria velifera*). The positive rate for *Anaplasmataceae* was 57.6% (n = 110) while that of piroplasms was 80.1% (n = 153). Overall, 177 (92.7%) of the samples were positive for at least one TBP species. Co-infection of different species was observed in 152 (79.6%) of the samples analyzed. *Anaplasma bovis*, *Anaplasma marginale*, *Anaplasma platys*-like, *Anaplasma* sp., *Ehrlichia* sp., *B. bigemina*, *T. mutans*, *T. parva*, *T. taurotragi* and *T. velifera* were detected in cattle for the first time using molecular techniques in Malawi.

In chapter II, the study focused on *T. parva* because it was the most pathogenic and economically important for cattle in Malawi among the TBPs detected in the chapter I. A total of 446 cattle blood collected at three sites in the central region of Malawi were tested by the *T. parva*-specific nested PCR which showed a *T. parva* positive detection rate of 54.5% (n = 243). The PCR-positive samples were further analyzed for two *T. parva* genes (*Tp1* and *Tp2*) recognized by bovine CD8$^+$ T cells in order to determine the genetic diversity of *T. parva* in cattle in Malawi and the sequence polymorphisms among vaccinated and unvaccinated cattle in Malawi. Single nucleotide polymorphisms were observed at 14 positions (3.7%) in *Tp1* and 156 positions (33.1%) in *Tp2*,

plus short deletions in *Tp1*, resulting in 6 and 10 amino acid variants in the *Tp1* and *Tp2* genes, respectively. Most sequences were either identical or similar to *T. parva* Muguga, Kiambu 5 and Serengeti transformed strains, the components of Muguga cocktail (MC) vaccine. This may suggest the possible expansion of vaccine components into unvaccinated cattle, or that a very similar genotype to vaccine components existed in Malawi. This study provides information that supports the use of *T. parva* MC vaccine to control East Coast fever (ECF) in Malawi.

In chapter III, the study focused on the genotyping and population structure of *T. parva* in Malawi using 100 *T. parva*–positive samples from six study sites in the central and southern regions of Malawi. A total of 9 satellite markers were employed in this study. Linkage equilibrium in 4 of the 6 populations was observed when the populations were treated separately but, when treated as a single population there was linkage disequilibrium. There was sub-structuring among some samples from Katete and Likasi farms as shown by the principal coordinate analysis (PCoA). The analysis of molecular variance (AMOVA) showed that the differences were mainly within (99%) than between (1%) populations. Majority of the isolates clustered with the *T. parva* Muguga reference strain, suggesting that the isolates in Malawi are closely related to the MC vaccine component. The data support the current use of MC vaccine to control ECF.

The study in chapter II showed that there were three groups of *T. parva* circulating in cattle in Malawi (those related to MC vaccine strains, those related to buffalo strains and those related to Zambian strains). The study in chapter III has shown that there are two populations (those related to MC strains and those related to Zambian strains). Those samples that clustered with buffalo strains from Nkhotakota in chapter II clustered with MC strains in chapter III. This observation may be due to lower resolution power of *Tp1* and *Tp2* gene sequencing analysis conducted in chapter II than satellite analysis conducted in chapter III. These findings have shown that satellite markers provide a comprehensive genetic profile as compared to *Tp1* and *Tp2* genes which cover a limited region in the *T. parva* genome. These findings show that *T. parva* in Malawi has two types of strains in which majority are closely related to MC strains and other to Zambian strains. More studies are required to ascertain the impact of the other strains on the efficacy of MC vaccine.

In chapter IV, molecular detection and characterization of TBPs of sheep and goats in Malawi was conducted. A total of 107 blood samples from sheep (n = 8) and goats (n = 99) were collected from animals that were apparently healthy from two farms in the central and the southern regions of

Malawi. The V4 hypervariable region of the 18S rDNA and the V1 hypervariable region of the 16S rDNA PCR assays were used for detection of tick-borne piroplasms and *Anaplasmataceae*, respectively. Almost the full-length 18S rDNA and *groEL* gene sequences were used for genetic characterization of the piroplasms and *Anaplasmataceae*, respectively. The results showed that 76.6 % of the examined animals (n = 107) were positive for at least one TBP. The overall co-infection with at least two TBPs was observed in forty-eight animals (45.9%). The detected TBPs were *Anaplasma ovis* (65.4%), *Ehrlichia ruminantium* (3.8%), *Ehrlichia canis* (1.8%), *Babesia* sp. closely related to *Babesia gibsoni*-like (0.6%), *Theileria ovis* (52.4%), *T. mutans* (2.6%), *Theileria separata* (1.8%), *Anaplasma* sp. (0.6%) and *Theileria* sp. strain MSD-like (16.8%). These results provide a significant milestone in the knowledge of occurrence of TBPs in sheep and goats in Malawi, which is prerequisite to proper diagnosis and control.

In chapter V, molecular detection and characterization of TBPs of dogs in Malawi was conducted. The occurrence of TBPs of the genera *Babesia*, *Hepatozoon*, *Anaplasma* and *Ehrlichia* were investigated in 209 owned and stray dogs in three major cities in Malawi through molecular techniques. Among the examined dogs, 93 (44.5%) were infected with at least one TBP. The detection rates were 23.1% for *Babesia rossi*, 2.9% for *Babesia vogeli*, 19.1% for *Hepatozoon canis*, 2.4% for *A. platys* and 3.8% for *E. canis*. This is the first molecular study that has provided evidence that dogs in Malawi are infected with TBPs. Sensitization is required for veterinary practitioners, dog handlers, and pet owners as the detected pathogens affect the animals' wellbeing. Further studies focusing on rural areas with limited or no access to veterinary care are required to ascertain the extent of the TBP infection in dogs.

The studies in this thesis have shown that domestic animals in Malawi are infected with a wide range of TBPs. These studies have also shown that domestic animals do not always present clinical symptoms even when infected with certain TBPs and serve as reservoirs of TBPs, which could be the source of TBP infections to other susceptible domestic animals. The molecular epidemiological data generated in these studies are important in monitoring the control measures currently in place and conceiving novel methods to control ticks and TBDs in Malawi.