Title of dissertation			
Epidemiological study on tick-borne parasitic diseases in livestock from Tanzania and Kenya （タンザニアとケニアの家畜におけるマダニ媒介性寄生虫症の疫学的研究）			
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SUMMARY

Tick-borne diseases (TBDs) pose a major challenge to the livestock industry in many tropical and sub-tropical countries. In East Africa, Tanzania and Kenya are among the countries located just south of the horn of Africa. This region is popularly known for a large population of livestock. However, TBDs in the region present a serious threat to the livestock sector particularly cattle and small ruminants. The most important TBDs in the region are theileriosis, anaplasmosis, babesiosis and ehrlichiosis. Despite the damage incurred by these diseases in Tanzania and Kenya, limited epidemiological data on the occurrence and distribution of TBDs in the two countries is available. Therefore, several molecular studies were carried out in different locations of the two countries to address the problem.

In chapter 1, a total of 245 blood samples from different breeds of cattle were randomly collected on Pemba Island, in Tanzania. Polymerase chain reaction (PCR) and sequencing was used to detect and identify the tick-borne pathogens (TBPs). The assays were performed using primers based on *Theileria* spp. (18S rRNA), *Babesia bovis* (SBP-2), *B. bigemina* (RAP-1a), *Anaplasma marginale* (MSP-5 and groEL), *Ehrlichia ruminantium* (pCS20), *T. parva* (p104), *T. mutans* (18S rRNA), and *T. taurotragi* (18S rRNA). PCR screening of cattle samples collected on Pemba Island revealed overall infection rates for *Theileria* spp. (62.4%), *B. bigemina* (17.6%), *A. marginale* (15.9%), *E. ruminantium* (7.4%) and *B. bovis* (4.5%). Further analysis using sequences of *Theileria* spp. (18S rRNA) revealed infection of cattle with *T. mutans* (68.6%), *T. taurotragi* (48.4%), *T. parva* (41.2%), and *T. ovis* (1.9%). Co-infections of cattle, with up to six TBPs, were revealed in 46.9% of the samples. Sequence analysis indicated that *T. parva* (p104), *E. ruminantium* (pCS20) and *A. marginale* (MSP-5) genes are conserved among cattle blood samples in Pemba, with 99.3% - 100%, 99.6% - 100% and 100% sequence identity values, respectively. In contrast, the *B. bigemina* (RAP-1a) and *B. bovis* (SBP-2) gene sequences were relatively diverse with 99.5% - 99.9% and 66.4% - 98.7% sequence identity values, respectively. The phylogenetic analyses revealed that *T. parva* (p104), *E. ruminantium* (pCS20) and *A. marginale* (MSP-5) gene sequences clustered in the same clade with other isolates from other countries. In contrast, the *B. bigemina* (RAP-1a) and *B. bovis* (SBP-2) gene sequences showed significant differences in the genotypes, as they appeared in separate clades. The data provided should improve the understanding of the epidemiology of tick-borne diseases, and is expected to improve the approach for diagnosis and control of tick-borne diseases in Tanzania.

In chapter 2, blood samples were collected randomly in 236 cattle of different breeds from Zanzibar Island, Tanzania. The PCR and sequencing were used to screen the samples for detection of TBPs. The assays were performed using primers described in chapter 1. The PCR screening revealed that 64.5% of animals were infected by TBPs, including *T. mutans* (38.1%), *T. parva* (34.3%), *T. taurotragi* (30.9%), *A. marginale* (10.2%), *B. bigemina* (5.1%), *T. velifera* (3.4%) and *B. bovis* (2.1%). Overall, a total of 86 animals (36.4%) were co-infected with up to five pathogens concomitantly. The pathogens mostly involved in the co-infection were *T. parva*, *T. taurotragi* and *T. mutans*. Sequence analysis indicated that *T. parva* (p104) and *B. bigemina* (RAP-1a) genes are diverse among the sampled animals on Zanzibar Island, with 99.64%–100% and 99.51%–100% nucleotide sequence identity value, respectively. In contrast, the *A. marginale* (MSP-5) and *B.*

bovis (SBP-2) genes are conserved, with 100% and 99.66%-100% nucleotide sequence identity values respectively. The phylogenetic analyses revealed that *T. parva* (p104) and *B. bigemina* (RAP-1a) gene sequences showed significant differences of genotypes, as they appear in different clades. Meanwhile, *A. marginale* (MSP-5) and *B. bovis* (SBP-2) gene sequences appear in the same clade with other sequences extracted from the NCBI GenBank. The epidemiological findings revealed in this study will provide important information on tick-borne diseases in Tanzania and will be used as scientific basis for planning future control strategies.

In chapter 3, a total of 250 blood samples were randomly collected from indigenous cattle of Tanga region, Tanzania. The assays performed were based on primers described in chapter 1. The results show an overall infection rate for *T. mutans* (48%), *A. marginale* (32.4%), *T. parva* (25.6%), *T. taurotragi* (20.8%) and *B. bigemina* (13.2%). Co-infections of up to four pathogens were revealed in 44.8% of the cattle samples. Sequence analysis indicated that *T. parva* (p104) and *A. marginale* (groEL) genes were conserved among the sampled animals with sequence identity values of 98.92 – 100% and 99.88% – 100%, respectively. On the other hand, *the B. bigemina* (RAP-1a) gene and the (V4 region of the 18S rRNA of *T. mutans* genes were diverse among the sampled cattle, indicating the sequence identity values of 99.27% - 100% and 22.45% - 60.77%, respectively. The phylogenetic analyses revealed that *T. parva* (p104) and *A. marginale* (groEL) gene sequences of this study were clustered in a clade. In contrast, the *B. bigemina* (RAP-1a) and the *T. mutans* (V4 region of the 18S rRNA) gene sequences appeared in the different clades. The findings revealed in this work shows that indigenous breed of cattle reared in free range system in Tanzania can be the source of tick-borne infections in other naïve breeds of cattle in the country. Therefore, this information is useful in the control of tick-borne diseases.

In chapter 4, blood samples were randomly collected from 76 apparently healthy sheep in Machakos and Homa-bay counties in Kenya. The assays were performed using primers based on *Theileria* spp. (18S rRNA), *Anaplasma* spp. (16S rRNA), *Babesia ovis* (18S rRNA), *A. ovis* (AoMSP-4) and *E. ruminantium* (pCS20). The overall infection rates of sheep samples collected in Machakos and Homabay counties in Kenya for *Theileria* spp. (51.3%), *Anaplasma* spp. (40.8%), *A. ovis* (34.2%) and *E. ruminantium* (7.9%). The overall co-infection was (61.8%). All *Theileria* spp. positive samples were confirmed to be *T. ovis* on sequencing. A phylogenetic analysis of (18S rRNA) gene sequences revealed that isolates of this study clustered with *T. ovis* sequences from other regions suggesting this gene is highly conserved. The *E. ruminantium* (pCS20) sequences were in the same clade on the phylogenetic tree. However, three (AoMSP-4) sequences appeared in the same clade while one sequence formed a separate branch revealing genetic divergence to the other sequences. The 16S rRNA sequencing revealed uncultured *Anaplasma* spp. and *A. ovis*. The phylogenetic analyses of uncultured *Anaplasma* spp. revealed that the two sequences formed a divergent clade signifying genetic differences to other isolates. This study provides important information regarding tick-borne pathogens occurrence and their degree of genetic diversity among sheep in Kenya.

In conclusion, the studies reveal that TBDs are well distributed in Tanzania and Kenya. *Theileria* spp. were the most prevalent TBPs in the region led by *Theileria mutans, T. parva* and *T. taurotragi*. Moreover, mixed infection in cattle and sheep were the prominent findings in the study areas. These data provide basement epidemiological background which will contribute to the future control strategies of tick-borne diseases in Tanzania and Kenya.

Photos

Dr. Ringo with certificate of PhD

Certificate of PhD degree