5th International Symposium on

Strategies for the Control of Ticks and Tick-borne Diseases

(Supported by JSPS Asia-Africa Science Platform Project)

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CONTENTS

17:00~17:10 **Junya Yamagishi (Hokkaido University, Japan)**
Closing remarks

18:00~20:00 **Reception**

Brief introduction of JSPS Asia-Africa Science Platform Project

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The Japan Society for the Promotion of Science (JSPS) is Japan's leading funding agency and is largely funded through annual subsidies from the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT). Established in 1932, JSPS promotes the advancement of academic research in all disciplines from social sciences and humanities to natural sciences and engineering. Additionally, JSPS administers a number of bilateral and multilateral programs for scientific cooperation and exchange under memorandums of understanding concluded with its various counterpart foreign academic institutions around the world.

Since FY 2012, the JSPS has implemented Core-to-Core Program, comprising two components: (1) Advanced Research Networks and (2) Asia-Africa Science Platforms. This program is designed to create top world-class research centers that partner over the long term with other core research institutions around the world in advancing research in leading-edge fields, on issues of high international priority, and in areas that contribute to the solution of prevailing problems in the Asia-African regions. While advancing research in these fields and building core research and education hubs in the Asia and Africa, the Core-to-Core Program also concentrates on fostering the next generations of trailblazing young researchers.

The title of our project selected by JSPS is "Establishment of International Collaborating Center for Controlling Tick-borne Protozoan Diseases in Africa". We are approaching to the goal through following steps:

<Step 1> Collect about 500~1000 blood samples from livestock (cattle/horses/sheep) in each African country, and perform the molecular survey on TBD.

<Step 2> Identify the regional dominant strains of *Babesia*/*Theileria* based on above molecular screening, and carry out the whole genome/transcriptome sequencing by next generation sequencer.

<Step 3> Develop effective control (diagnostic/preventive/therapeutic) measures against TBD based on above genomic/transcriptomic databases.

Current trends on prevalence and control of ticks and tick-borne diseases in Kenya

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Tick-borne pathogens (TBPs) are mainly viruses, bacteria and protrozoans, which are transmitted by ticks thereby causing diseases in animals and humans in sub-Saharan Africa including Kenya. Some tick-borne viruses are of veterinary and medical significance causing viral-encephalitis and Crimean-Congo haemorrhagic fever in humans. Other tick-borne viruses such as African swine fever virus and Nairobi sheep disease virus cause diseases in livestock. The other major tick-borne protozoans including theileriosis and babesiosis as well as tick-borne bacteria such as anaplasmosis, and heartwater also infect livestock and human. The ticks belonging to the Genera, *Rhipicephalus, Hyalomma, Amblyomma,* and *Haemaphysalis*, are *kn*own to harbor and transmit the TBPs to livestock and human resulting in tick-borne diseases (TBDs). These diseases cause major constraint to livestock production and food security in Kenya. Some of the zoonotic-infections are of public health concern. Although, the prevalence and control measures for most of these TBDs have been reported previously, an understanding of current trends on prevalence and control measures employed for emerging ticks and TBPs in Kenya is still critical. Therefore, this presentation focusses on the current status of prevalent ticks and TBDs in Kenya. Up-to-date research on ticks and TBDs in Kenya is also highlighted. The major emerging problems related to tick infestation and TBDs of veterinary and medical importance are also presented. Additionally, conventional technologies applied in the control of ticks and TBDs as well as recent research done on tick-control technologies in Kenya is presented. Finally, future research areas on ticks-control and TBDs in sub-Saharan Africa including Kenya are proposed in this presentation.

Seroprevalence and risk factors associated with infection of calves with *Babesia bigemina* and *Anaplasma marginale* in Narok County, Kenya

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Babesia bigemina (B. bigemina) and *Anaplasma marginale (A. marginale)* cause Babesiosis and Anaplasmosis respectively in cattle resulting in economic losses to dairy and beefindustries in many developing countries including Kenya. Despite their economic impact, there is limited knowledge on their risk factors and seroprevalence especially for calves in many regions in Kenya. Therefore, this study estimated current seroprevalence associated with infection of by *B. bigemina* and *A marginale.* The risk factors associated with the infections among calves in Narok County, Kenya was also determined. A cross-sectional study was conducted in Narok County between February and May 2023. A total of 402 calves from 76 farms were randomly selected from 8 villages in Naroosura Majimoto and Ololulunga sub-counties. Closed-ended questionnaires administered to cattle-owners or caretakers. Whole blood and serum were collected from the calves in the selected farms. Giemsa-stained blood smears were examined by microscopy and sera screened by commercial enzyme-linked immunosorbent assay (ELISA) kits for antibodies to *B. bigemina*. *A. marginale*. Mixed effect logistic regression analysis was used to determine the relationship between seropositivity and calf and farm level factors using the farm as a random effect. *B. bigemina* and *A. marginale* antibodies were detected in calves with overall seropositivity of 60 % for both parasites. On microscopic examination, *B. bigemina* was observed in 22.9% of the samples a proportion less than that of *A. marginale* (32.6 %). Factors significantly associated with the seropositivity included: age (for *B. bigemina* (p= 0.007)*, A. marginale* (p=0.000) and *B. bigemina* and *A. marginale* coinfections (p=0.019), acaricide application (for *A. marginale* (p=0.001) and *A. marginale* coinfections (p=0.007)), purchasing of feed (for *B. bigemina* (p=0.009) and *B. bigemina* and *A. marginale* coinfections (p=0.001)). The other factors included education level (for *B.b* (p=0.012)), infection history (for *B. bigemina* and *B. bigemina* coinfections (p=0.001)), fever (for *B. bigemina* and *A. marginale* coinfections (p=0.028)) and vaccination status (for *A. marginale* (p=0.034)). The seroprevalence of *B. bigemina* and *A. marginale* infections in this area is relatively high in apparently healthy cattle. Endemic stability exploitation and strategic acaricide application should be explored further to reduce risk of clinical diseases of both infections.

A review of the epidemiology of crimean-congo hemorrhagic fever in Africa

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Crimean-Congo haemorrhagic fever (CCHF) is a viral haemorrhagic fever usually transmitted by ticks (mainly of the *Hyalomma* genus). It is caused by the CCHF virus, which belongs to the genus *Orthonairovirus* (family *Nairoviridae*, order *Bunyavirales*). CCHF outbreaks constitute a threat to public health services as the virus can lead to epidemics, has a high case-fatality ratio (10–40%), potentially results in hospital and health facility outbreaks, and is difficult to prevent and treat. CCHF is endemic in Africa, but the epidemiology remains unclear. Using a broad database search, we reviewed the literature to understand the prevalence, outbreaks, and distribution of CCHF in Africa. This review aims to sensitize the researchers on the significance of the disease, its potential public health threat, and the need to improve surveillance.

Crimean- Congo Hemorrhagic Fever (CCHF) in Non-human primates in Kenya

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CCHF, an emerging tickborne viral zoonosis, of the family Nairoviridae, is largely endemic in Africa, with a poorly defined epidemiology. Many mammals, reptiles, and birds, although asymptomatically infected, have active virus amplification, putting livestock keepers, slaughterhouses, and healthcare workers in endemic areas at a higher risk of infection. In Kenya, CCHF has been reported in humans, particularly in livestock-keeping communities and livestock-associated workers, and in different animal species, with variable infection patterns where there are sympatric species. However, there is a paucity of data on its occurrence in NHP and associated sequelae. Here, the objective was to characterize exposure to CCHFV in free-ranging vervet monkeys, baboons, and semi-captive chimpanzee populations in Kenya. Seroprevalence was estimated using ID-VET multi-species, double-antigen IgG ELISA from archived sera from Vervet monkeys (Busia, N=41), Baboons (Aberdares Ranges – 67; Nairobi National Park – 6; Maasai Mara National Reserve – 19; Ol Pejeta Conservancy – 14), and semi-captive chimpanzees (2014: N=23; 2021: N=32) from Ol Pejeta Conservancy. The prevalence in baboon populations was 39% (41/106 individuals), and 10% (4/41 individuals) in Vervet monkeys (non-sympatric to chimpanzees and baboons). CCHF prevalence in chimpanzees in 2014 and 2021 was 26% (6/23) and 25% (8/32 individuals), respectively, with all individuals who seroconverted in 2014, being seropositive in 2021, except one. This is the first report of CCHF seropositivity in NHPs in Kenya. In addition, we demonstrate IgG persistence in individual chimpanzees for over seven years, an important consideration for its ecology and epidemiology

Ticks and tick-borne diseases in Egypt: epidemiology, research and control

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Ticks are small arachnids of the order Ixodida along with mites, they are hematophagous ectoparasites. Toxins of various ticks according to their saliva protein may cause a disease known as tick paralysis, which affects humans, domestic and wild animals; it was nearly fatal, particularly in dogs. Ticks play a major role in transmitting infectious diseases to their hosts. Ticks' feeding habits and other disease-causing aspects have been reported in early historical times from Egypt. *Ornithodoros savignyi* was described in a report on African Ixodoidae as an Egyptian tick specimen. The genera *Hyalomma*, *Rhipicephalus* and *Amblyomma* comprise the most important ixodid ticks infesting animals. *H. dromedarii, H. impeltatum, H. excavatum, H. anatolicum, H. truncatum, H. marginatum, H. rufipes, H. turanicum, R. annulatus, R. sanguineus, R. turanicus, R. guilhoni, R. camicasi, A. lepidum, A. marmoreum,* and *A. variegatum*, were collected from cows, sheep, goats, cattle, buffaloes, and camels from different localities in Egypt. Therefore, many tick-borne diseases (viral, bacterial, and protozoan) have been reported in Egypt. Viral diseases such as Alkhurma Hemorrhagic Fever Virus, Crimean–Congo hemorrhagic fever virus, bacterial diseases such as Anaplasmosis, Ehrlichiosis, Spotted Fever Rickettsioses, Tick-Borne Lyme Borreliosis, Tick-Borne Relapsing Fever and Tularemia, protozoal Diseases such as Babesiosis and Theileriosis were previously recorded from Egypt. The previously mentioned data are important, present a threat to many health and economic problems, and call for the intensive implementation of control measures for such diseases in Egypt. Mostly, in Egypt, farmers prefer to use acaricides, a key component of tick control strategies. But it is not enough and could develop detrimental effects on the environment. The multi-disciplinary approach should be used to tackle the ticks and their diseases by considering all components including environmental and ecological/wildlife as well as a domestic animal and human factors. However, the information regarding the prevalence of these pests and their diseases in Egypt has not been updated in recent years, and still little recent data is available on the prevalence, distribution, and most fundamentally, the genetic diversity of the pathogens causing them. Our project will provide additional information on tick-borne diseases in Egypt and will assist in developing strategies for controlling the diseases.

Current situation of tick control in Tanzania mainland

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An investigation was carried out to understand the ticks and tick-borne diseases affecting cattle in Tanzania. Two sites were chosen for the study, Chamakweza in the Coast region and Madibila in Mbeya region. The ticks found on cattle included: *Rhipicephalus appendiculatus, R. evertsi evertsi, R. decoloratus, R. microplus, R. pulchelus, Hyalomma rufipes, Amblyomma variegatum* and *A. gemma*. It was further observed that cattle were infected with *Anaplasma marginale, A. centrale, Babesia bigemina* and *B. bovis*.

The three tick species (*Rhipicephalus microplus, Rhipicephalus appendiculatus* and *Amblyomma variegatum)* of economic importance were selected to be assessed *in vitro* and *in vivo* for sensitivity to commercially available acaricides. The larval packet test (LPT) was used for *in vitro* studies, while cattle and goats were used for *in vivo* experiments. All three tick species showed high levels of resistance to chlorfenvinphos and amitraz. In addition, *Rhipicephalus microplus* had high levels of resistance to alpha-cypermethrin and cypermethrin. For *A. variegatum* and *Rhipicephalus appendiculatus* a medium level of resistance to fluralaner was observed.

Experiments using cattle found that fipronil pour on were effective against *R. microplus* ticks. The other commercially available acaricides tested (Cymiazole and cypermethrin, and ivermectin) were only partially effective against *R. microplus.* Flumethrin was effective in the control of *Amblyomma variegatum.* Weekly application of flumethrin, amitraz or a combination of cymiazole and cypermethrin controlled *Rhipicephalus appendiculatus*. It was further observed that fipronil pour-on product offered residual control for four weeks in the control of *R. appendiculatus*.

Complexity of managing tick acaricide resistance in African setting

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Ticks and Tick-borne diseases (TTBD) are arguably one of Africa's greatest challenge to livestock productivity. The tropical climate in most African countries favor the fecundity of ticks and year-round tick infestation of livestock, leading to infection with tick-borne diseases. Cattle and small ruminants grazed under extensive production system are worst affected due to the high risk of exposure to ticks. Consequently, farmers have to rely mainly on acaricides to control ticks and reduce its economic impact. Unfortunately, multitudes of factors such as inappropriate acaricide application practices, weak regulatory controls on acaricide registration and rotation, poor farm facilities for acaricide application, weak knowledge among farmers on integrated tick control and inadequate access to technical veterinary services by farmers have invariably led to emergence of tick acaricide resistance in many parts of Africa. Among the compounding drivers for excess acaricide application that drives selection pressure in ticks is the progressive shift in breeds from local to exotic through cross-breeding. In this paper, I will share the current trends of tick acaricide resistance in Africa and how acaricide resistance is more complex to mitigate in the African setting. Key recommendations for sustainable approach to management of acaricide resistance based on lessons learnt outside Africa will also be discussed to inform practical actions for acaricide resistance management in Africa.

Analysis in host specificity of *Babesia bovis* and pathogen detection using NGS

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Study in host specificity and evolution in *Babesia* species

Babesia species are apicomplexan parasites which infect to red blood cells (RBCs) and transmitted by ticks. They cause babesiosis in a variety of animals and some of them are known to cause zoonosis. *B. bovis* causes bovine babesiosis and significant economic damage to livestock industry even though it is a non-zoonotic pathogen. Interestingly, a preliminary study demonstrated its in vitro adaptation to human RBCs (hRBCs) 15 years ago (personal communication) then I am attempting to find molecular basis of the adaptation. Firstly, I have demonstrated comparative genomics among parental strain and adapted derivatives. However, there was no possible polymorphism. Secondly, comparative transcriptomics was conducted then several differentially expressed genes were identified. Among them, merozoite *surface antigen-2a2* which can be involved in invasion and *multi transmembrane* (mtm) family genes known as potential nutrition transporter were hypothesized to be involved in the adaptation.

Development of novel diagnostic methods based on mNGS

Establishing better diagnostic methods is essential for control of infectious diseases. Among various principles and strategies, metagenomic next generation sequencing (mNGS) will be the future standard because of their cost effectiveness and comprehensiveness. Then, I am developing a couple of novel derivatives of the approach. Firstly, mNGS targeting bacterial 16S rRNA was improved for multiplex sequencing by nanopore sequencers. Secondary, the 16S system was expanded to 18S rRNA for eukaryotic pathogens. Amplification from host 18S was suppressed by adding a mammalian-specific blocking primer to increase sensitivity for pathogen 18S. Thirdly, pan-XXX-nanopore system targeting the conserved region among genus or family of target viruses is expanding to paramyxoviruses. Lastly, comprehensive RNAome sequencing is optimizing for diagnosis. In particular, I came up with an effective sample preparation by integration of mNGS and group testing algorithm, namely mNGS-screening enhanced by a group testing algorithm (mEGA). Proof of concept study has been successfully completed using small scale. Now, I am scaling the system to 512 samples. One of the current humper is ratio between host and pathogen reads. To increase the latter, target enrichment using the Twist viral panel was applied and increased population of viral reads was observed.

Detection of *Babesia microti* in wild small rodents and ticks captured in the suburbs of Gifu City, Japan

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Background and Purpose

Babesia microti is a piroplasma parasite of small rodents. Since some genotypes infect humans, this protozoan is also known as a zoonotic protozoan. Although some surveys have been conducted in Hokkaido and other areas of Japan, reports of *B. microti* infections in wild animals in other regions of Japan are limited. In this study, we investigated the infection status of *B. microti* in wild small rodents and ticks in the suburbs of Gifu City, central Japan.

Materials and Methods

DNA was extracted from the spleens of all 73 rodents captured near Gifu City in 2021-2023 and from all 97 adult ticks collected in the same area in 2022 and 2023. A partial 18S rRNA gene was amplified and sequenced by Nested-PCR (Masatani et al., Ticks Tick Borne Dis, 2017), which can detect a wide range of piroplasmids. Based on the results obtained, phylogenetic analysis was conducted using the maximum likelihood method.

Results and Discussion

Among a total of 73 rodents, piroplasma genes were detected in two *Apodemus speciosus,* small field mouse species endemic to Japan. These sequences were 100% identical to those of unidentified *Piroplasmida* sp. OtsuMNR (Accession number AB188086), which was previously detected in *A. speciosus* captured in Otsu City, Shiga Prefecture (near Gifu Prefecture), and *B. microti* AsAW/KH002, which was detected in *A. speciosus* captured on Awaji Island, Hyogo Prefecture, respectively.

Of the 97 ticks, two ticks from the same species showed high identity (99.93% and 100%, respectively) to *Piroplasmida* sp. OtsuMNR. This indicates that a lifecycle of the unidentified *Piroplasmida* sp. may be established between the ticks and the *A. speciosus*. We are currently expanding the number of tick and rodent samples and the areas where they are collected in order to elucidate the lifecycle of these protozoan parasites.

Identification and characterization of *Babesia bovis* exported proteins

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Babesia are apicomplexan tick-borne parasites that have a great economic impact on the livestock industry, companion animals and wildlife health, and a growing concern of human health due to accidental infections by zoonotic *Babesia*. Among *Babesia* species, *Babesia bovis* causes the most pathogenic form of babesiosis in cattle. Like other apicomplexan parasites, *B. bovis*-induced modification of host cells is crucial for its survival. However, our knowledge of *Babesia* surface exposed proteins is limited to variant erythrocyte surface antigen1 (VESA1), which is responsible for fatal cerebral babesiosis. To identify the novel exported proteins in *B. bovis*, we performed red blood cell (RBC) surface biotinylation and mass spectrometry. We identified two novel proteins and characterization was performed by using CRISPR/Cas9 genome editing and *glmS* gene knockdown system. One of the proteins was determined to be essential for parasite development and pathogenicity. Induced knockdown of this protein resulted in a decreased growth rate, reduced RBC surface protrusions created by the parasite, mis-localized VESA1, and abrogated cytoadhesion to endothelial cells. VESA1 is a ligand for cytoadhesion of iRBCs to capillary endothelial cells which leads to blockage of capillaries and causes cerebral symptoms. The second identified protein is encoded by a large multigene family. The gene was downregulated in blasticidin-S resistant parasites, suggesting that the protein mediates entry of blasticidin-S and likely other solutes across the iRBC membrane. This is the first description of a putative channel or transporter molecule on the surface of Babesia-iRBC. Our results provide new insights into the host cell modifications by *B. bovis* and their pathogenicity.