4th International Symposium on

Strategies for the Control of Ticks and Tick-borne Diseases

(Supported by JSPS Asia-Africa Science Platform Project)

October 5, 2022

National Research Center for Protozoan Diseases,
Obihiro University of Agriculture and Veterinary Medicine,
Obihiro, Japan

Contents

13:40~13:55	**Athanase Badolo (University Joseph KI-ZERBO, Burkina Faso)** Bionomics and populations genetics of *Aedes aegypti*, dengue vector in Burkina Faso
13:55~14:10	**Ferda Sevinc (Selcuk University, Turkey)** Ovine babesiosis: the main tick-borne disease of sheep in Turkey
14:10~14:25	**Aaron Ringo (Zanzibar Livestock Research Institute, Tanzania)** Molecular investigation and genetic diversity of tick-borne haemoparasites isolated from East African Zebu cattle in Tanga region, Tanzania
14:25~14:40	**Kumar Uday (Sher-e-Bangla Agricultural University, Bangladesh)** Molecular detection of ticks and piroplasma of livestock in Bangladesh
14:40~14:55	**Eloiza Galon (Obihiro University, Japan)** Utility of targeted amplicon deep sequencing for the characterization of bovine piroplasma populations in the Philippines
14:55~15:10	**Shengwei Ji (Obihiro University, Japan)** Efficacy of the antimalarial MMV390048 against *Babesia* infection reveals phosphatidylinositol 4-kinase as a druggable target for babesiosis
15:10~15:40	**Break**
15:40~15:55	**Iqra Zafar (Obihiro University, Japan)** *Babesia microti* and *Plasmodium berghei* co-infection in mice
15:55~16:10	**Hang Li (Obihiro University, Japan)** Identification of three members of the multidomain adhesion CCp family in *Babesia gibsoni*
16:10~16:25	**Boniface Chikufenji (Obihiro University, Japan)** Molecular detection and characterization of cattle tick-borne piroplasms in southern Malawi
16:25~16:35	**Naoaki Yokoyama (Obihiro University, Japan)** Closing remarks
18:00~20:00	**Reception**

Brief introduction of JSPS Asia-Africa Science Platform Project

Xuenan Xuan
National Research Center for Protozoan Diseases,
Obihiro University of Agriculture and Veterinary Medicine, Japan

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.

Equine piroplasmosis -diagnosis and chemotherapy-

Ikuo Igarashi
National Research Center for Protozoan Diseases,
Obihiro University of Agriculture and Veterinary Medicine, Japan

Equine piroplasmosis (EP) is a tick-borne disease of equids caused by two hemoprotozoan parasites, *Theileria equi* and *Babesia caballi*. EP is responsible for significant sanitary and economical losses in the horse industry due to impaired performance and to the constraint to the international movement. EP is endemic in tropical and temperate areas and its distribution is correlated to that of vectors such as *Dermacentor*, *Hyalomma*, and *Rhipicephalus.* Fever, inappetence, malaise, hemolytic anemia and hemoglobinuria are frequently observed as major clinical signs.

Diagnoses of EP include parasite detection, serological methods and molecular detection. Microscopy Parasite finding with microscopic examination of thin blood smears is most useful during the acute phase of EP infection. *In vitro* culture has been employed successfully for the identification of *B. caballi* and *T. equi* in blood samples in carrier equids. Several serological assays have been developed to increase diagnostic sensitivity in equids chronically infected with *B. caballi* and *T. equi*. Some of these diagnostic assays include the compliment fixation test (CFT), enzyme-linked immunosorbent assay (ELISA), the immunochromatographic test (ICT), Western blot, and immunofluorescence antibody test (IFAT). Each of these serological techniques has some advantages or disadvantages. The polymerase chain reaction (PCR) has been developed as molecular detection of EP and is more sensitive than any other methods and is best for diagnosis of animals in chronic infection with EP. Loop-mediated isothermal amplification (LAMP), using four or six primers, is rapid, highly sensitive, and cost effective.

The use of imidocard dipropionate has shown considerable efficiency in eliminating both *T. equi* and *B. caballi* parasites during the chronic stage of infection. Other drugs such as clotrimazole, ketoconazole, artesunate and clofazimine, have been reported to be effective in inhibiting the growth of EP parasites *in vitro*. Further study is necessary to treat infected horses with EP in the future.

Establishment of the Tick Biobank and its application to vector biology research

Rika Umemiya-Shirafuji
National Research Center for Protozoan Diseases,
Obihiro University of Agriculture and Veterinary Medicine, Japan

In the past decades, omics data including genomes, transcriptomes and proteomes of ticks of medical and veterinary importance have become available worldwide as web-based resources. In addition, laboratory colonies and cell lines of these ticks have been established and now become essential tools for the research advancement of ticks and tick-borne diseases. Nevertheless, such research resources of the ticks distributed in Japan are limited to *Haemaphysalis longicornis*, a major hard tick species in Japan and a vector of various microorganisms that are harmful to human and animals. *H. longicornis* has long been used as an "experimental model of hard tick" for biological and physiological studies and for validation of effectiveness of insecticides or acaricides in research institutions. The parthenogenetic tick is a suitable research tool because of its ease of stable supply.

In 2017, the National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine started a project titled “Establishment of the Tick Biobank and its application to vector biology research”, supporting by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan. The goal of this project is the establishment of the ‘Tick Biobank’ which includes the construction, maintenance and supply of colonies of globally important tick species and the compilation of multi-omics data. In addition to the accumulation of omics data, physiological events such as blood feeding and reproduction need to be standardized at cell/tissue/whole-body levels. For example, we recently reported the developmental process of oocytes from the unfed period through the oviposition period and proposed the classification criteria for oocyte development in parthenogenetic *H. longicornis*. Comprehensive understanding of ticks at genome, transcript, protein, cell/tissue and whole-body levels will be an indispensable concept for the progress of tick studies. Offering and sharing the materials (tick colonies) and omics data to the tick-research community will lead to efficient progress of tick research. Here, I introduce the multifaceted activities in our ‘Tick Biobank’ project.

Novel *Babesia bovis* exported proteins that modify properties of infected red blood cells

Hassan Hakimi[1,2], Thomas J. Templeton[1], Miako Sakaguchi[3], Junya Yamagishi[4,5], Shinya Miyazaki[1], Kazuhide Yahata[1], Takayuki Uchihashi[6], Shin-ichiro Kawazu[2], Osamu Kaneko[1], Masahito Asada[1,2]

[1]Department of Protozoology, Institute of Tropical Medicine, Nagasaki University, Japan; [2]National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Japan; [3]Central Laboratory, Institute of Tropical Medicine, Nagasaki University, Japan; [4]Division of Collaboration and Education, Research Center for Zoonosis Control, Hokkaido University, Japan; [5]International Collaboration Unit, Research Center for Zoonosis Control, Hokkaido University, Japan; [6]Department of Physics, Nagoya University, Japan

Babesia bovis causes a pathogenic form of babesiosis in cattle. Following invasion of red blood cells (RBCs) the parasite extensively modifies host cell structural and mechanical properties via the export of numerous proteins. Despite their crucial role in virulence and pathogenesis, such proteins have not been comprehensively characterized in *B. bovis*. Here we describe the surface biotinylation of infected RBCs (iRBCs), followed by proteomic analysis. We describe a multigene family (*mtm*) that encodes predicted multi-transmembrane integral membrane proteins which are exported and expressed on the surface of iRBCs. One *mtm* gene was downregulated in blasticidin-S (BS) resistant parasites, suggesting an association with BS uptake. Induced knockdown of a novel exported protein encoded by BBOV_III004280, named VESA export-associated protein (BbVEAP), resulted in a decreased growth rate, reduced RBC surface ridge numbers, mis-localized VESA1, and abrogated cytoadhesion to endothelial cells, suggesting that BbVEAP is a novel virulence factor for *B. bovis*.

High prevalence of tick-borne protozoan parasites in sika deer and wild boar from western region in Japan

Tatsunori Masatani

Laboratory of Zoonotic Disease, Faculty of Applied Biological Sciences, Gifu University, Japan

We have previously collected ticks from mountain forests in various parts of Kagoshima Prefecture and detected the protozoa harbored by these ticks using our newly developed PCR method, and detected many unidentified piroplasma genes (Masatani et al., Tick Tick Borne Dis 2017). However, basic information such as distribution status, host range, and pathogenicity are lacking to assess the risk of these protozoa to livestock health and public health. Here we aimed to clarify the distribution of tick-borne protozoa in wild animals in the western Japan using universal protozoa detection PCR detection.

A total of 276 Japanese deer blood or liver samples were collected from Yamaguchi, Oita, Kagoshima, Okayama, Ehime, Kochi, and Tokushima during 2013-2019; a total of 219 wild boar liver or blood samples were collected from Yamaguchi, Kagoshima, and Nagasaki/Tsushima prefectures during 2015-2018. DNA extracted from these samples was used to amplify the protozoan genes by piroplasma-specific nested-PCR. Phylogenetic analysis of the obtained gene sequences was performed.

Of the total number of deer samples, 259 (93.8%) were PCR-positive, of which 99.6% (258/259) were unidentified *Theileria* sp. (sika1) of the same species. No regional variation in positivity rates was observed. Several samples were mixed infections with another unidentified *Theileria* species, *Theileria* sp. (sika2). These results indicate that the majority of deer in western Japan harbor *Theileria* sp. (sika1).

For wild boar, 140 of the total samples were PCR-positive (63.9%), and since all the sequences were identical, they were determined to be a single, unidentified species of *Babesia*. Positive rates in all regions ranged from 39-84%, with a high trend in Tsushima (84%). Genetic analysis showed that the *Babesia* sp. is the same species as that recently detected by the applicants in Kagoshima, Japan, in the *Amblyomma testudinarium*, indicating that this tick may be the vector. Further phylogenetic analysis showed that this *Babesia* is closely related to *Babesia gibsoni*, a dog *Babesia*, among the known *Babesia* species.

The effect of atovaquone on the mitochondrial membrane potential of *Babesia gibsoni*

Aiko Iguchi

Veterinary Laboratory Medicine, Faculty of Agriculture, Tottori University, Japan

Atovaquone (ATV) has a growth inhibitory effect against *Babesia gibsoni*. The target site is considered mitochondria, as in the case of Plasmodium spp.; ATV would collapse the mitochondrial membrane potential. *B. gibsoni* has also reported that single nucleotide polymorphisms in cytochrome b of mitochondria are involved in ATV susceptibility. However, the details are still unknown. The study aim was to measure the mitochondrial membrane potential of *B. gibsoni* and evaluate the effect of ATV alone and combined with proguanil (PG) on the mitochondrial membrane potential. As a result of exposure of wild-type *B. gibsoni* to ATV alone, the number of cells with decreased mitochondrial membrane potential increased. When wild-type *B. gibsoni* was exposed to the ATV + PG combination, the peak value of mitochondrial membrane potential was larger than that when exposed to ATV alone. It was suggested that ATV alone affects the mitochondrial membrane potential of *B. gibsoni*, and the effect is enhanced by the combination of ATV and PG. The effect of ATV was weakened for *B. gibsoni* having reduced sensitivity to ATV (*B. gibsoni* with M121I), and the effect was not enhanced by the combination of ATV and PG. Although we still need to elucidate the mechanism of ATV and PG for *B. gibsoni*, these results strongly suggests that the target of ATV for *B. gibsoni* is also cytochrome b of mitochondria.

Whole genome sequencing of *Babesia caballi*

Junya Yamagishi[1], Taishi Kidaka[1], Masahito Asada[2], Akihiro Ochi[3]

[1]International Institute for Zoonosis Control, Hokkaido University, Japan; [2]National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Japan; [3]Equine Research Institute, Japan Racing Association, Japan

Babesia caballi, one of the causative agents of equine piroplasmosis, is an intraerythrocytic parasite belonging to the phylum Apicomplexa and infects equids. Main vectors of *B. caballi* are tick species of the family *Dermacentor*, *Haemaphysalis*, *Hyalomma*, and *Rhipicephalus*. Horses infected with *B. caballi* show anemia and associated systemic illness including fever, lethargy, anorexia, and peripheral edema. The parasite has a worldwide distribution, and equine piroplasmosis is endemic in tropical, subtropical, and some temperate areas. In this study, we sequenced *B. caballi* genome DNA using a sub-cloned USDA strain and nanopore long read sequencer expecting acquisition of complete genome including multiple gene families and repetitive sequences which hamper assembly.

The parasite was cultured *in vitro* in RPMI1640 medium and a sub-clone, USDA-D6B2 strain was established by limiting dilution. Genomic DNA was extracted from *B. caballi*-infected RBCs. The library for MinION was constructed and sequencing was performed. De novo genome assembly were performed Canu and a gene model was estimated by AUGUSTUS. The resulting genome sequence were consisted of nine contigs with 12.9M bp. Out of seven contigs except the apicoplast and mitochondrion genome, three contigs had the telomeric sequence at their both terminals and three contigs had at one side, and one was absent. It suggests the three contigs correspond to the complete chromosomes. Besides, 5,910 protein coding genes were estimated in total. We specified 274 orthologs among *B. bovis, B. bigemina, B. ovata, B. microti, B. divergens, Babesia* sp. Xinjiang, *P. falciparum, T. gondii,* and *B. caballi*. Their amino acid sequences were aligned each other, concatenated then a phylogenetic tree was described. Based on the genome wide analysis, it was suggested that *B. caballi* was close to *B. bigemina* and *B. ovata*. Using hmm model, 481 putative ves1-like genes were identified. Mutual similarity among other ves and SmORF genes in *B. bovis, B. bigemina, B. divergens*, and *Babesia sp*.. Xinjiang were visualized and demonstrated that there was a specific ves family in *B. caballi*. Finally, a set of repetitive sequences were found. The repetitive unit consisted of 297 bp coding 99 amino acids and the largest one consisted of the 17.9 repetitive units. They were unique among Babesia spp. therefore, they can be a potential target of species specific NAT test.

Strategies for the control of ticks and tick-borne diseases in Africa especially Kenya

Gabriel Oluga Aboge

Department of Public Health Pharmacology and Toxicology, Faculty of Veterinary Medicine,
University of Nairobi, Kenya

Many ticks transmit various tick-borne pathogens (TBPs) thereby causing tick-borne diseases (TBDs) especially in sub-Saharan Africa including Kenya. These diseases are responsible for major constraint to livestock production and cause public health problem particularly when the TBDs are zoonotic. Previously, a number of ticks and TBDs have been reported to be highly prevalent in Kenya. For example, East Coast fever caused by *Theileria parva*, babesiosis caused by *Babesia bigemina*, anaplasmosis caused by *Anaplasma marginale* and heartwater resulting from infection with *Ehrlichia ruminantium* have been reported. The corresponding prevalent ticks such as *Rhipicephalus appendiculatus*, *Rhipicephalus decoloratus* and *Amblyomma variegatum* have been documented. Recently, other highly prevalent emerging begnin TBPs such as *Theileria* spp., Theileria *taurotragi*, *Theileria mutans*, and *Theileria velifera* have also been reported in sheep. The major issues related to tick infestation and TBDs currently facing Kenya include mortalities and morbidities resulting in losses in production of milk, meat, and other livestock by-products. These losses cause severe economic constraints to dairy and beef farmers in Kenya. The other major issue includes public health problems related to infections with tick-borne pathogens. Subsequently, the current prevalent ticks and tick-borne diseases in Kenya is presented. The current research focusing on ticks and TBDs in Kenya is also highlighted. The major issues related to tick infestation and TBDs are also reviewed. Lastly, strategies for the control of ticks and TBDs in Kenya are proposed with the overall goal of improving public health as well as production in Dairy and Beef Industries.

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

Hany M. Ibrahim
Immunology and Physiology Unit, Department of Zoology, Faculty of Science,
Menoufia University, Egypt

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.

Tick Acaricide Resistance Management: Lessons from our negative experience in Uganda

Vudriko Patrick

Research Center for Tropical Diseases & Vector Control Laboratory, College of Veterinary Medicine, Animal Resources & Biosecurity, Makerere University, Uganda

Tick acaricide resistance is increasingly becoming a global challenge. In Africa, Uganda is arguably the worst affected country. The emergence of acaricide resistance in more than one tick species and multiple acaricide molecules is causing the country billions of shillings in losses. In 2021, Uganda launched a 5-year strategic plan (2021-2026) for the management of ticks and tickborne diseases. In this seminar, I will share the highlights of the above strategy and discuss our experiences regarding complexities involved in mitigating tick acaricide resistance whenever it occurs. The experiences that I will share will provide key lessons for other countries in the Africa and tropics so that they are better prepared to tackle acaricide resistance learning from Uganda's negative experience.

A pilot study to investigate the current status of ticks and tick-borne diseases of cattle in Tanzania

Elikira Kimbita, Haji Ishihaka, Jahash Nzalawahe
Department of Veterinary Microbiology and Parasitology,
College of Veterinary Medicine and Biomedical Sciences, Sokoine University, Tanzania

A study was conducted to establish the current status of tick and tick-borne diseases of cattle in Tanzania. Two sites were chosen for sampling, Chamakweza along the coast and Madibira on the southern highlands. Half body counts of ticks were conducted over four seasons of the year. The ticks were identified in the laboratory using standard taxonomical keys. Blood smears were made, fixed and stained using Giemsa's method for microscopic examination. Ticks were found attached to cattle throughout the year, but the highest counts were seen during the rainy season. The following ticks were identified: *Rhipicephalus appendiculatus, R. microplus, R. decoloratus, R. pulchellus*, *R. evertsi, Amblyomma variegatum, Amblyomma gemma* and *Hyalomma rufipes*. The exotic *R. microplus* has replaced the indigenous *R. decoloratus* in the coastal village of Chamakweza. The *R. decoloratus* was found in the highlands village of Madibira where the *R. microplus* was absent. About 71.9% of the cattle were infected with *Anaplasma marginale* and 33.4% with *Babesia* organisms; while about 3.6% had *Anaplasma centrale*. It was noted that about 14% of the cattle had mixed infections of *A. marginale* and *B. bigemina* and another 5% had mixture of *A. marginale* and *B. bovis*. Tick control by acaricide application is recommended but the frequency should be increased during the rainy season.

A systematic review and meta-analysis of prevalence of ticks and tick-borne diseases in Southern African Development Community (SADC) region from 1980 until 2021

Oriel Thekisoe, Mpho Tawana, ThankGod Onyiche, Tsepo Ramatla
Unit for Environmental Sciences and Management, North-West University, South Africa

We conducted a systematic review and meta-analysis of articles published on ticks and tick-borne diseases (TBDs) in Southern African Developing Community (SADC) region following PRISMA 2020 guidelines. Articles were initially identified through PubMed, ScienceDirect, Google Scholar, AJOL and Springer Link and after assessment of titles, abstract and eligibility only 61 articles which reported the prevalence of ticks and tick-borne pathogens (TBPs) fulfilled criteria for inclusion. Common livestock tick genera in SADC countries includes *Amblyomma*, *Haemaphysalis*, *Hyalomma* and *Rhipicephalus*. Different ticks and TBPs were detected from genera *Amblyomma*, *Boophilus*, and *Rhipicephalus* reported from 21 studies from South Africa (n=7); Tanzania (n=3); Zambia (n=2), Zimbabwe (n=2); Madagascar (n=2); Angola (n=1); Mozambique (n=1) and Comoros (n=1). Overall pooled prevalence estimate (PPE) of TBPs in animals is 52.2% (95%CI: 43.9 - 60.3%) with highest PPE in cattle [51.2%], followed by sheep [45.4%] and goats [29.9%]. For TBPs, *Anaplasma marginale* had a PPE of 45.9% followed by *A. centrale* [14.7%], *A. phagocytophilum* [2.52%] and *A. bovis* [0.88%] whilst the *Ehrlichia ruminantium* had PPE of 4.2%. *Babesia bigemina* and *B. bovis* had PPE of [20.8%] and [20.3%] respectively. *Theileria velifera* had the highest PPE of 43.0% followed by *T. mutans* [29.1%]; *T. parva* [25.0%] and other *Theileria* spp. [14.06%]. Our analysis revealed highest PPE of tick-borne pathogens in Mozambique [62.9%] followed by Tanzania [57.8%], Angola [54.3%]; South Africa [52.2%], Zambia [41.7%], Zimbabwe [35.11%] and Botswana [19.43%] with most numbers of studies recorded from South Africa (n=18) and the least in Zimbabwe (n=1). Data analyzed in this study has showed that other SADC countries lack published scientific information on ticks and TBDs.

Bionomics and populations genetics of *Aedes aegypti*, dengue vector in Burkina Faso.

Athanase Badolo

Laboratoire d'Entomologie Fondamentale et Appliquée, Université Joseph Ki-Zerbo, Burkina Faso

The western African region is at risk of dengue and several countries in this region have reported increasing number of dengue cases. For outbreak prevention and control, information on bionomics of the dengue vector, *Aedes aegypti* are needed to devise strategies. We collect baseline data on *Aedes* populations including characterization of breeding sites, resting and feeding behaviors, to assess dengue risk in urban, peri-urban and rural localities in or near Ouagadougou the capital city.

Vacuum aspirators were used to collect indoor and outdoor resting adult mosquitoes. Potential breeding sites were identified, their characteristics recorded and immature stages collected and reared in the laboratory to adulthood. Adults from aspiration and larval rearing were morphologically identified and if blood-fed, PCR was used to identify bloodmeal origin. Dengue risk was assessed through traditional Stegomyia indices and modeled as a function of locality, climate conditions.

A total number of 1,166 houses were sampled divided approximately equally amongst urban (1200 Logements), peri-urban (Tabtenga) and rural (Goundry) communities in 2016 and additional 625 houses were sampled in 2017. The breeding sites diversity and numbers as well as Aedes mosquitoes densities decrease from urban to rural locality. Modelling adults densities showed that locality and outdoor location are the main driving factors of *Aedes aegypti densities*. Locality and breeding sites characteristics including water volume, breeding sites types impact larval densities.

These data are reference data for *A. aegypti* surveillance in West Africa, and could support preparedness and response to outbreaks of *A. aegypti* transmitted arboviruses in other countries.

Ovine Babesiosis: The main tick-borne disease of sheep

Ferda Sevinc, Onur Ceylan, Mutlu Sevinc
Department of Parasitology, Faculty of Veterinary Medicine, Selcuk University, Turkey

Babesiosis is one of the most important tick-borne protozoan diseases of livestock. Several epidemic and endemic babesiosis cases have been reported in sheep in the European, African, Asian, and the Far Eastern countries.

Babesia ovis is the main etiological agent of ovine babesiosis. The most prominent clinical symptoms of B. ovis infections are fever, hemolytic anemia, icterus, and hemoglobinuria. In addition to these clinical symptoms, fatigue, loss of appetite, weight losses, and abortions also occur. Untreated cases usually end up with the death of sick animals. Some sick animals may die in spite of specific drug administration in the case of delayed diagnosis. Some recurrences can also occur in animals during the post-treatment period. Therefore, serious economic losses have been experienced due to disease-related factors such as death cases, yield losses, and cost of pharmaceutical drugs. An early and accurate diagnosis is the most important part of the disease control. The diagnosis is based on observation of the clinical signs and demonstration of the parasites by microscopic examination. However, it is extremely hard to diagnose the disease by microscopic and clinical examination methods in cases where the number of parasites is low. The serological diagnostic methods such as IFAT and ELISA basing on antibody detection have been used to provide data on the epidemiology of infections in population screenings rather than acute infection diagnosis. The immunoreactive proteins, which are abundantly found in the parasite structures and predominantly localized in the cytoplasm of infected erythrocytes, are released into the bloodstream during the asexual development of the parasites. In future, the current infections can be diagnosed by detecting these proteins in the blood samples by antigen detection methods.

Molecular investigation and genetic diversity of tick-borne haemoparasites isolated from East African Zebu cattle in Tanga region, Tanzania

Aaron Edmond Ringo

Zanzibar Livestock Research Institute, Tanzania

Tick-borne diseases are a major hindrance in livestock production in pastoral communities in Africa. Although information on tick-borne infections is necessary in setting up control measures, in pastoral communities of Tanzania it is limited. To provide the overview of the tick-borne infections in Tanzania, a total of 250 blood samples were collected from Zebu cattle in Handeni district. We conducted a molecular study using Polymerase Chain Reaction (PCR) and gene sequencing to detect and identify pathogens. PCR assays was conducted using primers based on *Theileria* spp. (18S rRNA), *Theileria parva* (p104), *Theileria mutans* and *T. taurotragi* (V4 region of 18S rRNA), *Babesia bigemina* (RAP1a), *B. bovis* (SBP-2), *Anaplasma marginale* (heat shock protein *groEL*), and *Ehrlichia ruminantium* (PCS20). PCR screening revealed the overall infection rate of 42.8% for T*heileria* spp., 13.2% for *B. bigemina*, and 32.4% for *A. marginale.* However, the *Theileria* spp. positive samples were further analyzed and revealed 48% were *T. mutans,* 20.8% *T. taurotragi* and 25.6% *T. parva.* Co-infections with up to five pathogens were revealed in 37.9% of the sampled cattle. Sequence analysis indicated that *T. parva* (p104) and B. *bigemina* (RAP1a) genes were relatively diverse among the sampled animals with sequence identity values of 98.64% – 99.71% and 97.42% – 99.73%, respectively. On the other hand, the *A. marginale* heat shock protein *groEL* and the V4 region of 18S rRNA of *T. mutans* genes were conserved among the sampled cattle, indicating the sequence identity values of 99.75% to 100% and 99.83% - 100%, respectively. The phylogenetic analyses revealed that *T. parva* (p104) and *B. bigemina* (RAP1a) gene sequences of this study appeared in different clades. In contrast, the *A. marginale* heat shock protein *groEL* and the *T. mutans* V4 region of the 18S rRNA gene sequences clustered in the same clades. This study provides important basement data for understanding the epidemiology of tick-borne diseases, and will improve the diagnosis and control measures of tick-borne diseases in Tanzania.

Molecular detection of ticks and piroplasma of livestock in Bangladesh

Mohanta Uday Kumar
Department of Microbiology and Parasitology, Faculty of Animal Science and Veterinary Medicine,
Sher-e-Bangla Agricultural University, Bangladesh

Ticks are economically important ectoparasites of humans and animals around the globe, and account considerable losses to the livestock industry through blood loss, damage to the hides and role as vectors for pathogens. Therefore, the aims of this study were to identify the tick species and piroplasma of livestock in Bangladesh. A total of 1765 ticks and 276 blood samples were collected from 276 animals in 5 different areas. Three types of ticks, *Amblyomma* sp. (4), *Rhipicephalus (Boophilus) microplus* (97) and *Haemaphysalis bispinosa* (1664) were identified. *Haemaphysalis bispinosa* was found to be distributed all over the country whereas *Amblyomma* sp. was restricted to hilly areas. On the other hand, *Rhipicephalus* (*Boophilus*) *microplus* was found to be distributed in hilly areas and Bogura. A primary screening by analyzing 18S rRNA (V4 hypervariable region) showed a higher prevalence of piroplasmosis (216/276, 78.26%) in livestock. Analysis by species specific PCR and gene sequencing showed 49.27% (136/276), 0.72% (2/276) and 1.08% (3/276) infection of livestock by *Babesia bigemina*, *B. bovis* and *B.naoakii* n. sp., respectively. The results of blood analyses suggested that Bangladesh is endemic for *B. bigemina*. This study may contribute to proper area mapping to limit the spread of non-major tick species and pathogen in the country.

Utility of targeted amplicon deep sequencing for the characterization of bovine piroplasma populations in the Philippines

Galon Eloiza May
National Research Center for Protozoan Diseases,
Obihiro University of Agriculture and Veterinary Medicine, Japan

Targeted amplicon deep sequencing (Ampliseq) is a next generation sequencing-based, highly targeted approach that allows efficient analysis of genetic variations in specific genomic regions. In addition, its higher sensitivity enables detection of low-frequent variants, which is useful in low parasitemia animals. In this study, the utility of Ampliseq for the detection of piroplasma was evaluated in cattle samples from the Philippines. Genomic DNA was isolated from 162 cattle blood samples collected in 3 provinces in the Philippines. Initial detection was performed with a PCR assay targeting the V4 hypervariable region of the 18S rRNA gene of piroplasma. Then, a 2-step PCR protocol was performed. The amplicon library was sequenced using Illumina MiSeq and the AMPTk pipeline was leveraged to produce amplicon sequence variants (ASVs). In total, 95 of 162 (58.64%) samples tested positive for piroplasma. After denoising the reads, 2,179 ASVs were generated, of which 175 ASVs had their taxonomy assigned by BLASTn search. Clustering of ASVs by vsearch obtained distinct operational taxonomic units, namely *Babesia bovis*, *B. bigemina*, *Babesia* sp., *Theileria orientalis*, *T. annulata*, *T. equi*, *T. mutans*, *Hepatozoon canis*, and *Sarcocystis cruzi*. The results of this study demonstrated the usefulness of Ampliseq in elucidating the bovine piroplasma populations in the Philippines, and shall be beneficial in the formulation of tick-borne disease control programs in the country.

Efficacy of the antimalarial MMV390048 against *Babesia* infection reveals phosphatidylinositol 4-kinase as a druggable target for babesiosis

JI Shengwei
National Research Center for Protozoan Diseases,
Obihiro University of Agriculture and Veterinary Medicine, Japan

The present study aimed to evaluate the anti-*Babesia* effect of MMV390048, a drug that inhibits *Plasmodium* by targeting the phosphatidylinositol 4-kinase (PI4K). The half inhibitory concentration (IC50) of MMV390048 against the *in vitro* growth of *Babesia gibsoni* was 6.9 ± 0.9 μM. In immunocompetent mice, oral treatment with MMV390048 at a concentration of 20 mg/kg effectively inhibited the growth of *B. microti* (Peabody mjr strain). The peak parasitemia in control group was 30.5%, whereas the peak parasitemia in MMV390048-treated group was 3.4%. Meanwhile, MMV390048 also showed inhibition on *B. rodhaini* (Australia strain) growth, a highly pathogenic rodent *Babesia* species. All MMV390048-treated mice survived, whereas the mice in control group died within 10 days post infection (DPI). The first 7-day administration of MMV390048 in *B. microti*-infected severe combined immunodeficiency (SCID) mice delayed the rise of parasitemia by 26 days. Subsequently, the second 7-day administration was given upon recurrence. At 52 DPI, parasite relapse (n = 1/5) and a mutation in the *B. microti* PI4K L746S, a MMV390048 resistance-related gene, was detected. Although the radical cure of *B. microti* infection in immunocompromised host SCID mice was not achieved, results from this study showed that MMV390048 has excellent inhibitory effects on *Babesia* parasites, opening a new treatment strategy for babesiosis by targeting the *B. microti* PI4K.

Babesia microti and *Plasmodium berghei* co-infection in mice

Zafar Iqra

National Research Center for Protozoan Diseases,

Obihiro University of Agriculture and Veterinary Medicine, Japan

Human cerebral malaria (HCM) is an encephalopathy caused by *Plasmodium falciparum* developed in infected individuals and responsible for most deaths. *P. berghei* ANKA (PbA) infection in C57BL/6, is globally employed to study the mechanisms involved in cerebral malaria as an alternative to study HCM.

Babesia microti causes babesiosis, in animals as well as humans all around the world, it is also rising as zoonosis. Co-infections can be inconsequential, deleterious, or even beneficial and have complex interactions, including modulation of the host response. By the use of mice co-infection models, cases of cross-protection were illustrated between *Babesia* spp. and *Plasmodium* spp. However, whether the same immune dynamics occur during *Plasmodium berghei* ANKA (PbA) and *Babesia microti* Peabody mjr co-infection is not known. Thus, this study was designed for the investigation of host immune response to elucidate the effect of *B. microti* and *P. berghei* co-infection on disease in mice.

Mice were injected (i.p) with *B. microti* (10^8 iRBCs) and then challenge infected with *P. berghei* ANKA (10^3 iRBCs) at Day 7 post-primary infection (Bm/PbA7). The Control group was injected with *P. berghei* ANKA only (PbA). We monitored the course of parasitemia and elucidate its effect on the host and its survival during the acute stage. All mice from the control died within 12 days on the other hand Bm/PbA7 mice survived longer than the control mice. Although, primary infection with *B. microti* Peabody mjr does not confer complete cross-protection against *P. berghei*. Apparently, co-infection of *B. microti* and PbA decreases the severity of the disease during the acute stage of infection as indicated by the decrease in Evans blue leakage in the brain of Bm/PbA7 mice compared with PbA mice.

However, additional research is required to fully comprehend the situation, including the flow cytometric analysis of the brain to observe the trend in the rise and fall of immune cells especially, $CD8^+$ T and $CD4^+$ T cells in the brain.

Identification of three members of the multidomain adhesion CCp family in *Babesia gibsoni*

Hang Li
National Research Center for Protozoan Diseases,
Obihiro University of Agriculture and Veterinary Medicine, Japan

Canine babesiosis is caused mainly by *Babesia gibsoni*, a parasite transmitted by *Haemaphysalis longicornis* tick. Within the tick, the *Babesia* parasite undergoes the sexual conjugation and the sporogony process of its life cycle. In order to control *B. gibsoni* infection, prompt and effective treatment of acute infections and curing chronic carriers are urgently needed. Gene disruptions of *Plasmodium* CCp resulted in blocking the transition of sporozoites from the mosquito midgut to the salivary glands, showing that these proteins are potential targets for the development of a transmission-blocking vaccine. Here, we characterized three members of the CCp family, named CCp1, CCp2, and CCp3 in *B. gibsoni*. The *B. gibsoni* sexual stages was induced *in vitro* by exposing parasites to 50, 100, 150, 200, 500 μM xanthurenic acid (XA) at 27 and 37 °C. Among them, 100 μM XA-exposed at 27 °C *B. gibsoni* presented diverse morphologies, including parasites with long projections, gradually increased free merozoites, and aggregated and round forms indicative of sexual stage induction. Anti-CCp polycolonal antibodies were raised in ICR mice. Then, the expression of CCp proteins were confirmed by immunofluorescence. Future experiments will focus on the functional characterization and analysis of roles of molecules in gamete binding in the tick through knock out the CCp genes.

Molecular detection and characterization of cattle tick-borne piroplasms in southern Malawi

Chikufenji Boniface Onesmo
National Research Center for Protozoan Diseases,
Obihiro University of Agriculture and Veterinary Medicine, Japan

Tick-borne pathogens (TBPs) cause animal diseases and a major constraint to the development of the livestock industry worldwide, regularly resulting in poor animal health and significant economic losses. Control of tick-borne diseases lies in proper diagnosis, treatment, and prevention. Molecular studies done on cattle piroplasms in Malawi have been inadequate. Therefore, this study aimed to detect cattle piroplasms using polymerase chain reaction (PCR) assays. Two hundred and twenty (n=220) blood samples were collected from apparently healthy cattle from six districts of southern Malawi and the blood DNA samples were screened for piroplasms targeting the V4 hypervariable region of 18S rRNA gene. A total of 148 (67.3%) were recorded positive for piroplasma. Further analysis was done by species specific PCR and sequencing analysis. *Babesia bigemina* (34.5%, 76/220), *B. bovis* (7.3%, 16/220), *B. naoakii* (2.7%, 6/220), *Theileria parva* (15.5%, 34/220), and *T. mutans* (4.1%, 9/220) were detected in the samples. Although it was reported that northern and central Malawi are endemic for piroplasma, it is evident that the detection rates of TBPs in this study are relatively high in the southern region. As such, to ensure steady cattle production, control measures need to be taken. The present study reveals new data on the piroplasm species of bovine populations in southern Malawi.