Supported by
Japan Society for Promotion of Science (JSPS)
Asia-Africa Science Platform Program

The 1st International Meeting for Protozoan Diseases

Date:
September 6 – 7, 2007

Place:
National Research Center for Protozoan Diseases, Obihiro, Hokkaido, Japan

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Time Table

September 6, 2007

13:00 – 14:00	Orientation
14:00 – 17:00	On Demand Discussion

September 7, 2007

Chairman (Dr. Sugimoto)

09:30 – 09:45	Opening remarks (Dr. Inoue)
09:45 – 10:15	P1 (Dr. Thekisoe)
10:15 – 10:30	P2 (Dr. Inoue)
10:30 – 10:45	Tea break

Chairman (Dr. Ouma)

10:45 – 11:00	P3 (Dr. Mbwambo)
11:00 – 11:15	P4 (Dr. Domingo)
11:15 – 11:30	P5 (Dr. Zhang)
11:30 – 11:45	Tea break (Take a group photograph)

Chairman (Dr. Inoue)

11:45 – 12:00	P6 (Dr. Sakurai)
12:00 – 12:15	P7 (Dr. Zhou)
12:15 – 12:30	P8 (Dr. Lun)
12:30 – 14:00	Lunch break

Chairman (Dr. Mbwambo)

14:00 – 14:15	P9 (Dr. Ouma)
14:15 – 14:30	P10 (Dr. Lin)
14:30 – 14:45	P11 (Dr. Lin)
14:45 – 15:00	P12 (Dr. Yin)
15:00 – 15:15	Tea break

Chairman (Dr. Lun)

15:15 – 15:30	P13 (Dr. Kirunda)
15:30 – 15:45	P14 (Dr. Guan)
15:45 – 16:00	P15 (Dr. Kumar)
16:00 – 16:15	P16 (Dr. Li)
16:15 – 16:30	Closing remarks (Dr. Inoue)

17:30 – 19:30	Closing Party

P-1 (09:45-10:15)

Progress on development of loop-mediated isothermal amplification (LAMP) technique for diagnosis of trypanosomosis and bovine theileriosis

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Loop-mediated isothermal amplification (LAMP) method can amplify a few copies of DNA to 10^9 in less than an hour under isothermal conditions with high specificity and sensitivity. Positive LAMP reaction can be visualized by naked eye from a white turbidity which is magnesium pyrophosphate a by-product of the positive reaction or by addition of fluorescent dyes or by real-time turbidimetry. Original LAMP method employs 4 primers but addition of 2 more loop primers accelerates the reaction thereby reducing the reaction time by less than half. Furthermore, LAMP requires only a simple heating device such as laboratory heatblock or waterbath. We hereby report on the progress made so far on development of LAMP for diagnosis of trypanosomosis and bovine *Theileria* infections. Since the advent of LAMP technique, we have developed LAMP assay for detection of *Trypanosoma brucei* sub-species as well as *T. evansi* targeting the PFRA gene. We have further developed species specific LAMP assays for detection of *T. b. gambiense* (5.8-ITS2 and TgsGP genes), *T. b. rhodesiense* (SRA gene), *T. congolense* (P0 and 18S rRNA genes), *T. evansi* (VSG RoTat 1.2), *T. brucei* and *T. equiperdum* (maxicircle kDNA), and for *T. evansi* and *T. equiperdum* (minicircle kDNA). Furthermore, we have developed LAMP assay based on HSP70 for detection of major bovine *Theileria* species, as well as *T. parva* specific LAMP based on the p104 and PIM genes. Although there is still a need for improvement on some of the primers and simple and faster DNA template preparation protocol, LAMP assays developed so far for diagnosis of trypanosomosis and bovine theileriosis can be useful research tools, as alternative detection tools for confirmative diagnosis and for application in epidemiological studies especially in resource poor laboratories.

P-2 (10:15-10:30)

Development of LAMP for human African trypanosomosis (HAT)

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The low levels of parasitemia usually hamper parasitological diagnosis of trypanosomes in humans or animals. Although antibody detection tests are useful for screening purposes, they do not distinguish between past and present infections, and the current reliability of antigen detection tests is limited. Polymerase Chain Reaction (PCR) has developed as one of the most specific and sensitive molecular methods for diagnosis of infectious diseases and has been widely applied for detection of pathogenic microorganisms. However, in spite of the excellent specificity and sensitivity, these molecular biology techniques are not commonly used in the diagnosis of trypanosomosis in countries lacking resources where the disease is endemic. This is due to lack of skilled personnel and expensive automated thermal cyclers for PCR that are not easily available in these countries. Loop-mediated isothermal amplification (LAMP) is a new DNA amplification method that is performed under isothermal conditions. This unique characteristic of LAMP allows us to use simple and inexpensive heating device for incubating LAMP reaction mixture. In addition, LAMP is a rapid and simple technique since it can be carried out within 30 min. Therefore, LAMP has great potential of being used for diagnosis of trypanosomosis in the laboratory and the field, especially in countries that lack sufficient resources needed for application of molecular diagnostic techniques. In collaboration with FIND, we have developed LAMP for the specific detection of *Trypanozoon*, *Trypanosoma brucei gambiense*, and *T. b. rhodesiense*. These LAMP primer sets are highly sensitive, and are capable of detecting as little as 100 fg to 1 pg of trypanosomal DNA, which is equivalent to 1 to 10 trypanosomes. Although our LAMP system should be further improved and standardized for field application, we will discuss possibility and rationale of LAMP as a field molecular diagnostic technique.

P-3 (10:45-11:00)

The application of a combination of diagnostic tests is useful in the control of bovine trypanosomosis

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Four hundred and thirty two cattle in 9 farms in the Coast and Dar Es Salaam regions of Tanzania were bled at different periods from March 2006 to January 2007and specimens prepared for parasitological (buffy coat technique - BCT, blood slide examination) and serological (Antigen, Antibody, PCR ELISAs and LAMP Test) screening for presence of trypanosomes. Alongside this, 1.0 ml of EDTA blood of test animals with Packed Cell Volume (PCV) values less than 25% was inoculated into 20 g mouse for possible detection of trypanosomes in low parasitaemic infections; were followed-up for 60 days.

Four cattle were detected positive for pathogenic trypanosomes, notably *Trypanosoma vivax* in three cattle by blood slide examination and BCT, and *T. congolense* (one case) by mouse inoculation. Serological screening is not yet completed.

90.3% of animals sampled had PCV values above 25%. Forty two cattle (9.7%) had PCV values below 25%. Low PCV values in adult cattle in trypanosomosis-endemic areas suggest presence of trypanosome infections. Subsequent trypanocidal drug treatment of five parasitologically negative cattle with PCV of 15%, 16%, 18%, 20% and 22% resulted into improved PCV values from 25% to 27% by day 30 after treatment with diminazene aceturate 7.0 mg per kg body weight.

Mouse inoculation was discriminately done on blood of parasitologically negative cattle with PCV values below 23%. One pair of mice became positive for *T. congolense* on day 43 and 47 of inoculation. Hence, in the absence of more sensitive diagnostic tests, it is recommended to complement the BCT and mouse inoculation techniques to blood slide examination for improved diagnosis of trypanosomosis.

P-4 (11:00-11:15)

Surra in the Philippines from 2001 to 2005

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Trypanosoma evansi (Surra) is an arthropod borne hemoflagellate parasite which belongs to the subgenus *Trypanozoon*. The disease known as "Bayawak" in the vernacular has been causing havoc among horses and ruminants in the Philippines since 1900's. It is classified (per D. A. AO 12, Series of 1992) under the diseases where prevention and control are of primary concern of the farm. Economic losses amounting to P44.8 M losses ($ 1,149,230.70) due to death in 1990-1998 excluding decreased meat and milk yield, poor reproductive performance, cost of labor and medication has been estimated (Manuel, 1998).

Surra affects domesticated mammals in the Philippines (e.g. dogs, horses, sheep, goats, pigs, cattle, and buffaloes) with horses being very susceptible to the infection leading to their death within weeks or months. Cattle (*Bos indicus*) and buffaloes (*Bubalus bubalis*) are the main reservoirs wherein the infection is sub-clinical.

Clinical manifestations in swamp buffaloes, cattle and horses are: intermittent fever, severe anemia, progressive loss of condition despite fairly good appetite, incoordination, prostration, circling movement, running aimlessly, sudden collapse and death if not treated. Scrotal swelling, anestrus and abortion were reported in cattle.

Transmission is by blood sucking insects that serve as mechanical vectors such as horseflies (*Tabanus* spp.), stable flies (*Stomoxys calcitrans*), buffalo flies (*Lyperosia exigua*) and mosquitoes. Other means are by non-blood sucking flies (*Musca* spp) which feed from blood oozing from wound and mucous membrane of infected animals; ingestion of raw infected meat or blood by carnivores (de Jesus, 1951) and by congenital transmission in calves from *T. evansi* positive dams (Cresencio et al, 1994).

Prevalence of Surra was found to be decreasing. In 2001, there were 236 Surra cases out of 5,849 samples (4.03%); in 2002, there were 323 Surra cases out of 6,990 samples (4.62%); in 2003, there were 154 cases out of 10,854 samples (1.42%); in 2004, there were 135 cases out of 5,753 samples (2.35%); and finally in 2005, there were 27 Surra cases out of 4,980 samples examined (0.54%).

Surra occurs in all 13 regions of the country particularly in Regions 3, 4 and 5 of Luzon, Regions 6 and 8 of Visayas and Regions 11 and 13 of Mindanao. From 2001 to 2005, the Philippine Animal Health Center of the Bureau of Animal Industry reported 875 cases of Surra out of 34,426 samples (2.54%) submitted for blood parasite examination. Proportion of Surra cases (n=875) from 2001-2005 according to origin showed Mindanao with 72% (628/875) followed by Luzon with 21% (183/875) and lastly, Visayas with 7.3% (64/875). The significant number of cases in Mindanao still showed that the disease is still one of the major concern for

animal health in the region. However, according to prevalence odds ratio it was found that animals in the Visayas are 6.7 times at risk and animals in Mindanao are 4 times at risk of contracting Surra when compared to animals in Luzon. In general, animals in the Philippines are 2.5 times at risk of contracting Surra depending on the origin they come from .

It was also observed that areas with Type II and IV climates, regardless of whether they are located in Luzon, Visayas or Mindanao, had the highest prevalence of Surra. Type II has no dry season and with very pronounced maximum rain period from November to January. Whereas, Type IV has a more or less even distribution of rainfall throughout the year.

For instance, out of 875 Surra detected cases using blood parasite examination, 61.37% (537) came from Type II climate and 31% (271) from Type IV climate. Whereas, 3.54% (31) came from Type I and 4.11% (36) from Type III. However, based on prevalence odds ratio, it was found that animals in Type IV climate were 14.5 times at risk; animals in Type II climate are 11 times at risk, and those in Type III are 3.6 times at risk of having Surra as compared to animals in Type I climate. In general, animals in the Philippines are 8 times at risk of contracting Surra based on the type of climate they are exposed to.

Types II and IV climates imply that they can create a conducive ecology that could support the proliferation of all insect vectors of Surra. This ecological condition is characterized by high humidity and warm ambient temperature, high rainfall distribution throughout the year, subsequent formation of stagnant pools and swamps, lush vegetation and plenty of decaying vegetable matter.

Among the different animal types, swamp buffaloes had the highest proportion of cases (58%) followed by cattle (29.5%) then by horses (7.4%).Goats, sheep and pigs were also infected but of low percentage. Surra is highly fatal to horses fluctuating from 12% to 28% for the past several years. Swamp buffaloes, sheep, goats and pigs serve as important reservoir hosts and harbor *T. evansi* without clinical manifestations. However, if they are exposed to severe stress conditions such as long distance travel, lack of feed, overworked, decreased resistance due to secondary or concurrent viral, bacterial or parasitic infection they succumb to Surra. Swamp buffaloes and cattle that come down with Surra become susceptible to opportunistic diseases such as *Pasteurella* and anthrax. Mortality in ruminants had been at an average of 4.5% annually.

In case no positive cases were diagnosed thru microscopy (Blood Parasite Examination), CATT, microhematocrit method and mouse inoculation test were used. Lately, the Philippine Carabao Center had been using polymerase chain reaction to survey Surra among murrah buffaloes, cattle and swamp buffaloes in different regions of Luzon.

The government has created the National Control Program for Surra in the Philippines last January of 2002 in an effort to curb Surra. Also the region-wide Minadanao Unified Surra Control Approach (MUSCA) was created, with the Da-RFU XI on the lead. The concerted campaign against Surra in Mindanao is being conducted with assistance from the Australian Center for International Agricultural Research (ACIAR) and the International Livestock

Research Institute (ILRI) in collaboration with other agencies and institutions like the University of Southern Mindanao (USM), Central Mindanao University (CMU) and PCARRD.

ACKNOWLEDGEMENT

The author wishes to thank with sincerest gratitude the organizing committee of the JSPS AA Science Platform Program and the National Research Center for Protozoa Diseases at Obihiro University of Agriculture and Veterinary Medicine for sponsoring the expenses for travel and accommodations. Special thanks are due to Prof. Shin-ichiro Kawazu and Assoc. Prof. Noboru Inoue of the same laboratory at the NRCPD; and for the National Animal Health Center of the Bureau of Animal, Dept. of Agriculture that provided the National Animal Surra Situation reports from 2001 to 2005.

P-5 (11:15-11:30)

Epidemiological evaluation of bovine trypanosomosis in Southeast Uganda

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Animal trypanosomosis is caused by several salivarian trypanosome species such as *Trypanosoma brucei, T. congolense*, *T. equiperdum*, *T. evansi*, *T. simiae*, *T. suis*, and *T. vivax*. Bovine trypanosomosis is a serious constraint to livestock development in large part of Africa. Infections with pathogenic trypanosome species affect various aspects of cattle productivity, often resulting in death. Livestock production in Uganda is undoubtedly hampered by trypanosomosis. In this study, epidemiological survey of bovine trypanosomosis was conducted in Southeast Uganda on July 2006. A total of 203 cattle blood samples were collected from rural area of Tororo district. Blood samples were collected by jugular vein with heparinized syringe, and examined by microhematocrit method at the site of sample collection. Blood samples were also blotted onto the FTA card for DNA extraction. Sera were prepared by centrifugation at NaLIRRI, and stored at -20^{o}C. DNA samples from FTA cards were examined by PCR. Serum samples were examined by 3 different antibody detection ELISA methods. Each ELISA method employs recombinant *T. congolense* ribosomal P0 antigen expressed in *E. coli*, *T. brucei* bloodstream form crude antigen, or *T. congolense* bloodstream form crude antigen, respectively. In the microhematocrit test, we detected trypanosomes from 20 cattle (10%). The seroprevalence of bovine trypanosomosis was 50% based on recombinant P0 ELISA, and 45% based on two crude antigen ELISAs. These results clearly shows that bovine trypanosomosis is highly endemic in Southeast Uganda. Concordance of the results obtained from those ELISA methods was 73%. According to the results from species-specific PCR tests, it was revealed that most prevalent trypanosome species were *T. brucei*, followed by *T. congolense*, and *T. vivax* in Tororo district. The prevalence based on the PCR analyses was 25%, and this result has low concordance with crude antigen (51%) and recombinant P0 ELISA (55%). Although PCR positive result indicates only current infection, positive result of ELISA includes both current and past infection. Such feature of each diagnostics may be the major reason of discrepancy between ELISA and PCR results. Although both microhematocrit method and PCR can prove current infection, our results indicate low sensitivity of microhematocrit method as compared to PCR. This is therefore an urgent need to develop highly sensitive, specific, and easy-to-use molecular diagnostics (such as LAMP) for trypanosomosis.

P-6 (11:45-12:00)

Identification and molecular characterization of a novel adhesion molecule expressed on the surface of *Trypanosoma congolense* epimastigotes

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Trypanosoma congolense expresses life-cycle stage specific surface molecules involved in adaptation to different host and vector environments. Here we report the discovery and molecular characterization of a novel stage-specific GPI-anchored surface glycoprotein that is selectively expressed in the epimastigote life cycle stage of *T. congolense*. Culture supernatants of epimastigotes but not of procyclic culture forms promoted adhesion of parasites in an *in vitro* assay. Biosynthetic labeling experiments showed that these epimastigote culture supernatants contained a 100 kDa trypanosome derived protein that was not present in procyclic supernatants, a molecule that we named Trypanosome Adhesion Molecule" (TAM). The gene encoding TAM was isolated from an epimastigote cDNA library after immunoscreening. The multicopy gene had a 2,070 bp open reading frame that encodes a polypeptide of 689 amino acids with a predicted mass of 72.9 kDa. The discrepancy between the predicted (72.9 kDa) and observed (100 kDa) masses can be explained by extensive glycosylation of the molecule which has 6 potential N-glycosylation sites and a predicted GPI-anchor. Indeed metabolic-labeling of TAM with [^{3}H] ethanolamine revealed that TAM was a GPI-anchored protein. TAM induced adhesion of EMF and this was significantly inhibited by incubation with specific antiserum raised against recombinant TAM. Confocal laser scanning microscopy showed that TAM was expressed only on the surface of epimastigote stages of the parasite. Taken together the results suggest that TAM may be a molecule involved in adhesion of *T. congolense* to the proboscis of tsetse and that it is required for successful transmission of the parasites.

P-7 (12:00-12:15)

Comparison of different variable antigenic types (VATs) from a cloned *Trypanosoma evansi*

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Antigenic variation is a typical biological character of *Trypanosoma evansi,* which leads to produce a serial of different surface antigen trypanosomes in infected host. These different surface antigen trypanosomes are named as variable antigenic types (VATs). To understand whether antigenic variation of trypanosome is accompanied with variation of other physiology and biochemistry property. We isolated 4 VATs produced by a cloned *T. evansi* and compared their growth characteristic, sensitivity to human serum and pathogenicity to mice. ShTat1.1, ShTat1.2, ShTat1.3, and ShTat1.5 were isolated and distinguished by cloning in mice and immunological tests from a cloned Anhui Buffaloes *T. evansi* isolate. The VSG genes of four VATs were cloned and their sequences showed 30-40% low homologies. ShTat1.1 showed gathering in mice blood slide, while the other VATs showed dispersing in mice blood slide; ShTat1.1 showed least sensitive to human serum than the other VATs. However, the four VATs have no difference in pathogenicity to mice. These results indicated biological characteristic of different VATs were not all the same, which implied that antigenic variation of *T. evansi* was accompanied with changes of other physiology and biochemistry property.

P-8 (12:15-12:30)

Analysis of gene expression profiles in the liver and spleen of mice infected with *Trypanosoma brucei brucei* by using a cDNA microarray

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Trypanosoma brucei brucei infects many domestic animals and cause the disease Nagana in livestock in Africa, resulting in serious economic loss in the endemic regions. Although many studies have described the histopathology induced in the organs of mice infected with *T. b. brucei*, few studies have been conducted on gene expression in these organs. Here we used cDNA microarray to analyse the gene expression profiles in the liver and spleen of mice infected with *T. b. brucei* (STIB 920) at the peak parasitemia (12 days after infection). A total of 14,000 sequences including full length and partial complementary DNAs (cDNAs) representing novel, known and control genes of mouse were analyzed. Results from GeneOntology annotation showed that 44 genes in the liver and 138 genes in the spleen were up-regulated in the infected mice, while 79 genes in the liver and 251 genes in the spleen of infected mice were down-regulated compared with control (non-infected) mice. Most of these genes are transport, metabolism, protein biosynthesis, transcription factors and nucleic acid binding protein-related genes. The changes of some important genes, such as heat shock protein 70 and Pcna, were confirmed by quantitative RT-PCR and immunohistochemistry. TUNEL analysis results revealed that extensive apoptosis occurred in the liver of infected mice at the peak of parasitemia. Our results have provided a comprehensive profile of changes in gene expression in the liver and spleen of mice infected with *T. b. brucei* and may be useful in understanding the pathogenesis of Nagana at a molecular level.

(Supported by National Science Foundation of China, grants No.30570245 and 30670275)

P-9 (14:00-14:15)

Spatial and temporal genetic structure of *Glossina pallidipes* (Diptera: Glossinidae) in south-western Kenya

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Tsetse flies, *Glossina* spp (Diptera: Glossinidae) are medically and economically important insects confined to sub-Saharan Africa. They are exclusively blood feeding and are the sole vectors of African trypanosomiases. Proper understanding of rates of gene flow and genetic differentiation among tsetse populations is key to the success of area-wide tsetse control using genetic methods. Ecological studies indicate that tsetse flies in the *morsitans* group have great capacity for dispersal and colonization of suitable habitats. In principle, such rapid dispersals would homogenize allele frequencies among populations and thus prevent genetic differentiation by drift. However, genetic data from nuclear and mitochondrial DNA markers seem to indicate otherwise. The apparent inconsistency between ecological and genetic estimates calls for further research.

We investigated the breeding structure of the tsetse fly, *Glossina pallidipes* at micro- and macro-geographic scales by analyzing spatial and temporal variation at eight autosomal microsatellite loci to test hypotheses about endemism and immigration. The research had three broad objectives: (1) to determine the degree of isolation of Lambwe Valley *G. pallidipes* from other conspecific populations by examining genetic distances and rates of gene flow among resident populations; (2) to examine effects of season and immigration in shaping the breeding structure of *G. pallidipes* in the Nguruman; (3) to determine if earlier control operations had an impact on the effective population sizes of *G. pallidipes.* Specifically, the following hypotheses were tested: (1) allele frequencies are homogenous among sites and blocks incorporating Lambwe and Kodera; (2) allele frequencies are homogeneous across a transect incorporating Serengeti, the Rift Valley escarpment bordering Tanzania, Nguruman, and adjacent lowlands; (3) allele frequencies are temporally homogeneous.

Samples were obtained at seasonal intervals from trap sites in Lambwe Valley and Nguruman in western and southwestern Kenya, respectively. There were 15 trapping sites in Lambwe and 27 sites in Nguruman. Sites were separated by 200 m to 14 km and arranged into blocks. *G. pallidipes* populations nearest to Lambwe and Nguruman were also sampled to test hypotheses about origins of populations in Lambwe and Nguruman. Our data suggest that *G. pallidipes* in Lambwe and Nguruman are geographically isolated from known nearby populations leading to strong measures of genetic differentiation. Genetic differentiation by genetic drift was much less among trapping sites within Lambwe and Nguruman ($F_{ST} \leq 0.049$) than between them ($F_{ST} = 0.232$). High genetic differentiation was also recorded between Serengeti and Nguruman ($F_{ST} = 0.16$) and Kodera Forest and Lambwe ($F_{ST} = 0.15$). Kodera and Lambwe are only about 50 km apart. Genetic variance in *G. pallidipes* attributed to season (0.33%) was about 20% of the variance among collection dates (1.6%), thereby indicating reasonable temporal stability of genetic variation. Allele frequencies in Kodera and Serengeti

differed significantly from Lambwe and Nguruman, thereby falsifying the hypothesis that immigrant flies repopulated Lambwe and Nguruman. Harmonic mean effective population sizes were 180 in Lambwe and 551 in Nguruman. These data suggest that *G. pallidipes* in Lambwe and Nguruman have been endemic for long intervals. In addition, the results show that *G. pallidipes* tend to remain close to their neighborhoods even when such neighborhoods are within a kilometer or two of each other. Staying close to neighborhoods may have adaptive significance in terms of conservation of water and energy reserves. Implications of these findings for tsetse and trypanosomosis control using genetic methods are discussed.

P-10 (14:15-14:30)

Identification of the RH50 protein from the tick *Rhipicephalus haemaphysaloides* and evaluation of its vaccine potential against tick feeding

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A cDNA coding a glycine-rich protein was identified from the *Rhipicephalus haemaphysaloides* tick. The cDNA herein named RH50 was 1,823 bp, including a single open reading frame (ORF) of 1,518 nucleotides. The ORF encodes a polypeptide of 506 amino acid residues with a size of 50 kDa, as calculated by computer. The predicted amino acid sequence of RH50 showed a low homology to sequences of some known extracellular matrix-like proteins. The native protein was identified in both the fed tick salivary gland lysates and extracts of cement material using the serum against the recombinant protein. RT-PCR results showed that RH50 mRNA was only transcribed in partially fed tick salivary glands, not in unfed tick salivary glands or partially fed tick midgut, fat body, or ovary. The differential expression of RH50 protein in fed tick salivary glands was confirmed by immunofluorescence. The low attachment rate both in the adult and nymphal tick, and the high mortality of immature ticks (nymph) feeding on rRH50-immunized rabbits were found. These results show that the RH50 protein could be a useful candidate for anti-tick vaccine development.

P-11 (14:30-14:45)

Gender and stage differentially expressed genes and proteins of *Schistosome japonicum*

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Schistosomiasis is a serious public health problem in China. At present, there are around 800,000 persons suffering from this zoonosis. Developing an effective vaccine against Schistosomiasis is urgent for the controlling of the disease. The differentially expressed molecules may involved in the growth, development, pairing, sex maturing, egg producing of the schistosome, and may be the key targets for finding the vaccine or new drug candidates. We screened and identified Gender and stage differentially expressed genes and proteins by suppressed subtractive hybridization and cDNA microarray analysis, two dimensional electrophoresis and mass spectrum. A total of 108 stages differentially expressed gene clones were sequenced, the results showed that these clones represented 44 unigenes. A total of 855 gender differentially expressed gene clones were selected and sequenced. The results showed that these clones represented 297 unigenes. One of differentially expressed protein named SjGCP was further studied for it biological function by RNA interference.

P-12 (14:45-15:00)

Importance of ticks and tick borne diseases in China

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Ticks have a long history in China, in 121 A.D., in a book "Paraphase of Chinese words", tick was recorded as "Pi", parasitized on cattle. In 4th century, in "lexicon of Chinese words", it was clearly indicated that ticks were parasite. Systematic studies of ticks were started in 30s, 20th century, especially after 1950. Up to now, 101 species or subspecies of Ixodides have been recorded, which includes *Ixodes, Haemaphysalis, Dermancentor, Hyalomma, Rhipicephalus, Boophilus* and *Amblyomma.* Six species of Argasidae have been described in genus of *Argas* and *Ornithdoros*.

Ticks have played an important role in transmitting diseases in China. The viral diseases transmitted by ticks in China include Russian spring summer encephalitis，Crimean-Congo haemorrhagic fever, Japanese E encephalitis, and bacterial diseases covered Tularaemiam, brucellosis, Lyme disease, anaplamosis, dermatophylosis, North Asia tick typhus, Q fever, ehrlichiosis. For the parasitic diseases, the following were important ones, e.g. babesiosis, theileriosis.

Some of ticks are of veterinary importance, such as *Ixodes persulcatus, Haemaphysalis punctata, H. qinghaiensis, H. longicornis, Dermacentor silvarum, D. nuttalli, Hyalomma anatolicum anatolicum, H. detritum, Rhipicephalus haemaphysaloides haemaphysaloide* and *Boophilus microplus*, because these ticks not only cause severe toxic conditions such as toxicosis, irritation and allergy, but also transmit variety of pathogenic microorganisms, protozoa, rickettsiae and virus to animal and human being.

P-13 (15:15-15:30)

Assessment of the impact of recent technologies in the control of selected vector-borne protozoan diseases in Uganda

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A cross-sectional study to assess the impact of the recently introduced technologies to the control of selected livestock diseases was done in Uganda. In the study, livestock farming areas were randomly selected based on agro-ecological zones that are mainly involved in livestock keeping, with more emphasis put on indigenous cattle reared in the pastoral and agro-pastoral farming systems. The study involved the use of a reconnaissance survey by use of rapid appraisal methods such as focus group discussions with farmers, as a starting stage, and use of a structured questionnaire administered to mainly the randomly selected cattle keeping farmers including examination of animal samples in laboratory. The information on the situation of major livestock vector-borne protozoan diseases at the time was obtained and is compared with that obtained from the previous studies, following a review of some articles. The prevalence, morbidity and mortality of these diseases were used as the basis for assessing the impact of the technologies. The diseases considered included those transmitted by ticks; East Coast Fever (ECF), anaplasmosis, babesiosis and heartwater (cowdriosis). *Theileria parva, Anaplasma marginale, Babesia bigemina* and *Cowdria ruminantium* as the major parasites transmitted by the ticks *Rhipicephalus appendiculatus* and *Boophilus decoloratus* were considered. For trypanosomosis, the most predominant trypanosome species, namely, *Trypanosoma vivax, T. congolense* and *T. brucei* and their vectors *Glossina fuscipes fuscipes* and *G. pallidipes*. The use of ECF vaccine for immunization of cattle against the disease, the use of Haemoque as a technique for field easy diagnosis of trypanosomosis, TBDs and the use of Trypanosomosis Agglutination Card Test (ACT), for simple field diagnosis of *T. rhodesiense* in man and the prophylactic treatment with isometamidium chloride combined with live bait technology (deltamethrin application on cattle) for control of trypanosomosis were the technologies used. The study: Assessing of major vectors and diseases of livestock in Uganda, revealed that the prevalence of East Coast Fever was 78.8%; anaplasmosis 60.8%, babesiosis 6.6% and heartwater 7.9%. The prevalence of trypanosomosis was 5.6%. The review of recent works and the consideration of the results of our study (2006) shows that the prevalence of ticks and tick-borne diseases in Uganda has decreased except for ECF, the most important tick-borne disease in Uganda (Otim, 1999). The prevalence of trypanosomosis, which had gone down (5.2%) due to control activities by FITCA-Uganda project is now raising due to the ending of the project. More sensitization and sustainability of the major vector-borne diseases control technologies are highly recommended. And easy to use and cheaper technologies for the control of these diseases be developed and introduced.

P-14 (15:30-15:45)

The preliminary biological character of a large *Babesia* sp. Xinjiang-2005 infective to sheep in China

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The pathogenicity and morphology in the stage of erythrocyte and tick of a large *Babesia* sp. Xinjiang-2005 were observed in present study. The splenectomized sheep infected by the parasite just showed mild fever and low parasitemia and then would recover. The parasite was almost not virulent for the intact sheep. The splenectomized cattle could not be infected by the *Babesia* species. Narrow and long double piriform parasite was the typical form of the *Babesia* species in the erythrocyte and the average size was 2.42 (±0.35) μm×1.06 (±0.22) μm. The merozoites were found in the gut, salivary gland, haemolymph, ovary and egg, respectively, of engorged female ticks *Hyalomma anatolicum anatolicum* on infected sheep. The results of experimental transmissions showed that the larval and adult ticks of *H. a. anatolicum* could transmit the *Babesia* species to sheep. Nevertheless, we could not get the positive results when we transmitted the parasite using the nymphs.

P-15 (15:45-16:00)

Recent advances in diagnosis of *Babesia equi* and *Trypanosoma evansi* infections in India

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Equine babesiosis, a tick transmitted haemoprotozoan disease caused by *Babesia equi* is widely distributed among equids in India. The disease got attention in 1976 when clinical form of *B. equi* infection was observed in mares which were imported from Poland and since then infection has been found endemic in many areas. Forty to fifty per cent sero-prevalence of *B. equi* antibodies were recorded in North–Western parts of the India. The earlier conventional sero–tests available were not sufficient for sensitive and specific diagnosis of the latently infected equids. In the last decade new improved diagnostic methods were designed for accurate and rapid diagnosis. As per OIE recommendation CFT was standardized and used for diagnosis of *B. equi* infection in the equid population. Due to many inherent drawbacks in CFT, various versions of ELISA were standardized viz. conventional ELISA, Dot–ELISA and single dilution ELISA. These assays were reported to be more specific and sensitive than CFT. Recently we standardized ELISA using the recombinant antigen and the test was validated which indicated its high sensitivity and specificity. We also designed the PCR test for specific diagnosis of *B. equi* target DNA and results will be discussed.

In India infection due to *Trypanosoma evansi* is wide–spread and has been reported from many species viz. horse, camel, cattle, buffalo and recently humans. Many diagnostic tests have been standardized for detection of antigen and specific antibodies in the serum. Circulating *T. evansi* antigen and DNA were successfully detected by Ag-ELISA and PCR, respectively and relatively PCR was found more sensitive. Genetic variation in different India stocks of *T. evansi* was also studied by RAPD. RAPD results showed some degree of microheterogenicity in *T. evansi* stocks isolated form buffaloes, horses and camels. Recently we have cloned and express the RoTat 1.2 VSG gene and analyzing its potential use in ELISA for specific antibodies detection in serum. We have also exploited this gene for diagnosis in PCR and results will be discussed.

P-16 (16:00-16:15)

Induced nitric oxide synthase (iNOS) and arginase play opposite roles in the rat peritoneal macrophages resistance to *Toxoplasma gondii* infections

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Rat macrophages are naturally resistant to *Toxoplasma gondii* infection in contrast to those of mice which are susceptible to this parasite. iNOS produces nitric oxide to restrict *T. gondii* proliferation, whereas arginase which shares the same substrate with iNOS can modulate the NO production and produces polyamine which is necessary for cell and parasite growth. In this present study, we assessed the role of iNOS and arginase in rat resident peritoneal macrophages infected with *T. gondii*. Generally, *T. gondii* can not normally proliferate in the rat macrophages but leads severe infection in mice macrophages. We found that the rat macrophages could express a high iNOS mRNA level, resulting in abundant NO production with a little arginase activity. However, this phenomenon is opposite to the mice macrophages in which iNOS mRNA expression was undetectable, while a high arginase mRNA level and its activity were detected. Interestingly, however, our results indicated that the NO production and iNOS mRNA expression were reduced in the rat macrophages after infection with *T. gondii*, while arginase I, II and arginase activity level were induced significantly. In addition, when iNOS of rat macrophages was induced by LPS, the proliferation of *T. gondii* was inhibited resulting in most of parasites died. By contrast, *T. gondii* increased significantly when iNOS was inhibited by NOS inhibitor, L-NAME.

In conclusion, the high concentration of the rat peritoneal macrophages is one of the key factors to resist *T. gondii* infection. The parasite infects the macrophages through improving arginase activity and inhibiting iNOS acitivity. In this process, NO plays an important role in resistance to *T. gondii* reproduction, whereas, arginase is very beneficial for the parasite proliferation. Therefore, we consider that the host iNOS and arginase represent a pair of opposite markers of resistance/susceptibility to *T. gondii* infection.

(Supported by “985” project)

Acknowledgements

I am thankful to all participants for their contribution to the meeting. I also thank all the members of Research Unit for Advanced Preventive Medicine, National Research Center for Protozoan Diseases for their assistance. This meeting was made possible by Grants from the Japan Society for Promotion of Science (JSPS), Asia-Africa Science Platform Program.

I am pleased to inform you that this abstract will be published in the next issue (Vol. 17, No. 2 – Dec. 2007) of the Journal of Protozoology Research. I will provide the Journal to the all participants.

Dr. Noboru Inoue, D.V.M., Ph.D.

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Issue Date: 2007-09-01