The trypanosomes of pigs in Arusha, Northern Tanzania

The 1st International Young Researcher Seminar in Zoonosis Control 2009

Programme & Abstract

Duration August 19 – August 21, 2009
Venue Hotel Niseko Ikoinomura

Organized by
Global COE Program, Hokkaido University
"Establishment of International Collaboration Centers for Zoonosis Control"

Organizing Committee

Satoru Konnai
(Lab. of Infectious Diseases, Dept. of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University)

Yuji Sunden
(Lab. of Comparative Pathology, Dept. of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University)

Kentaro Yoshii
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Masatoshi Okamatsu
(Lab. of Microbiology, Dept. of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University)

Tatsuya Sakurai
(Lab. of Parasitology, Dept. of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University)

Organized by

Global COE Program, Hokkaido University
"Establishment of International Collaboration Centers for Zoonosis Control"
GCOE Office
Graduate School of Veterinary Medicine
Hokkaido University
Kita-18 Nishi-9 Kitaku, Sapporo, Japan 060-0818

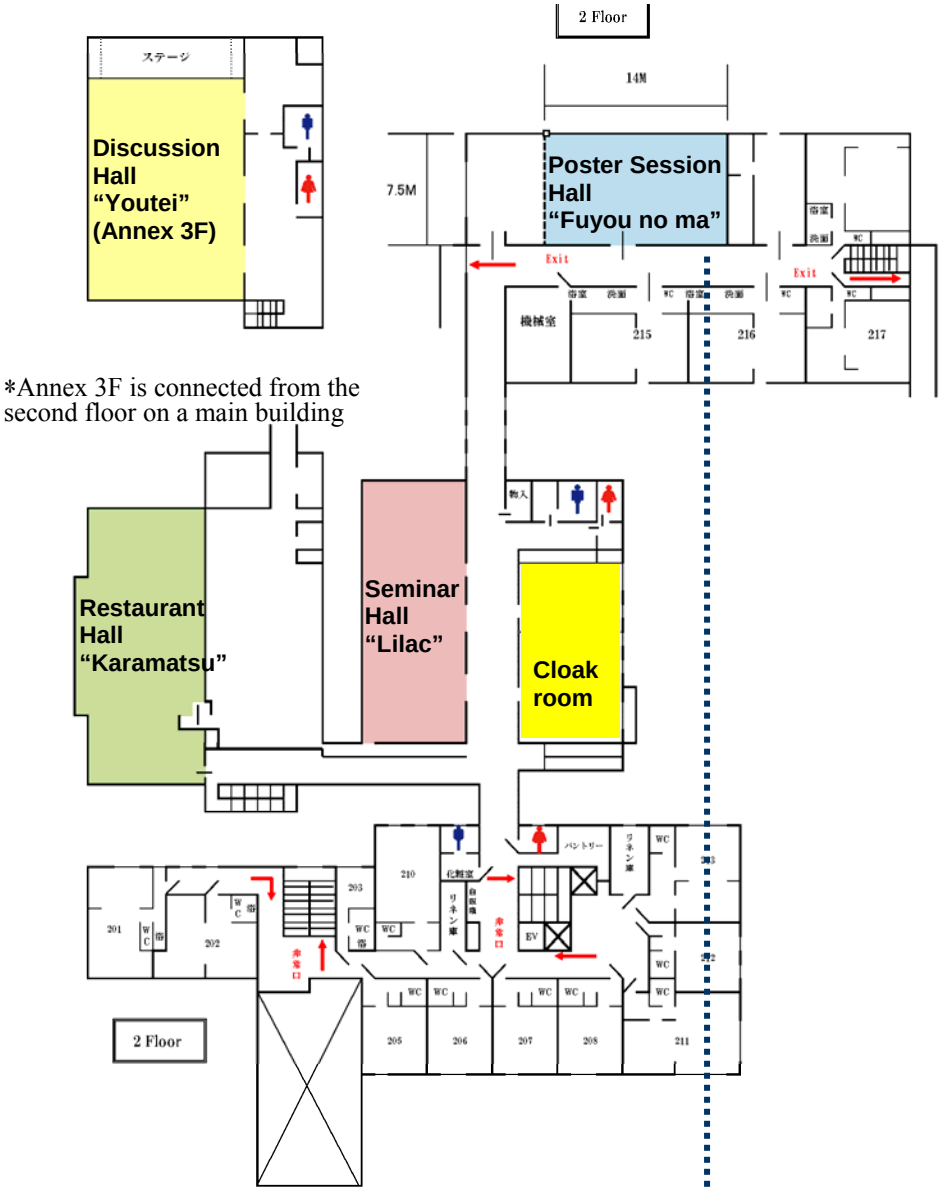
gcoe@vetmed.hokudai.ac.jp
http://www.vetmed.hokudai.ac.jp/gcoe/

FYI
Niseko Village
http://www.town.niseko.hokkaido.jp/welcome/english/
Weather in Niseko
http://www.niseko-village.com/english/winter/index.html
Sapporo city
http://www.welcome.city.sapporo.jp/english/index.html
Narita International Airport (Tokyo)
http://www.narita-airport.jp/en/index.html
New Chitose Airport (Sapporo)
http://new-chitose-airport.jp/en/
JR Sapporo Station map
http://www2.jrhokkaido.co.jp/global/english/access/sapporo.html
Hokkaido University Campus
http://www.hokudai.ac.jp/en/pickup/accesstocampus.html

CONTENTS

Venue:　Hotel Niseko Ikoinomura

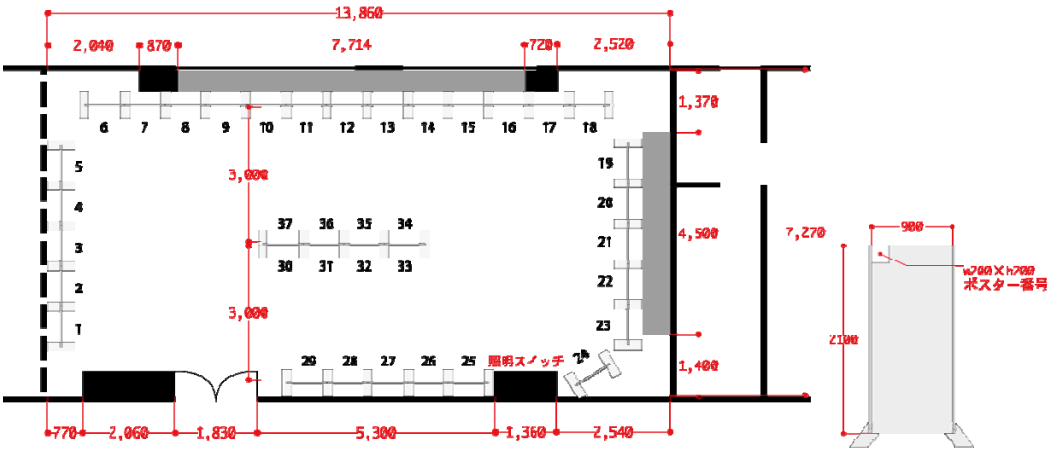
Address/ Niseko473, Niseko, Hokkaido
Phone/ +81 136-58-3111, Fax +81 136-58-2351
http://www.chuo-bus.co.jp/ikoinomura/　(in Japanese)

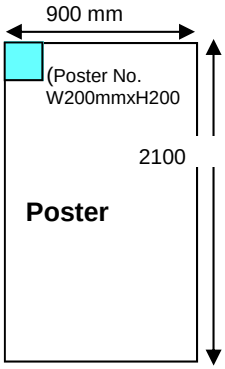


*Annex 3F is connected from the second floor on a main building

BBQ House "Mokumoku tei"

Poster Session Hall "Fuyou no ma"





Time Schedule

Day 1 August 19 (wed)

9:00
Registration
Time appointed for assembling
in front of Veterinary Medicine

9:30
Leaving for Niseko by bus
(Please be punctual without any delay for the bus departure.)

12:00
Arriving at Niseko

12:00-13:30
Submitting presentation data &
Setting up poster , Lunch time
(Discussion Hall "Youtei")

13:30-13:45
Orientation: Satoru Konnai
(Seminar Hall "Lilac")

Opening speech: Hiroshi Kida

13:45-16:00
Oral presentation
(Seminar Hall "Lilac")
O-1. Louise Hamill
O-2. Liam Morrison
O-3. Amy F. Savage
O-4. Brett Eyford
O-5. Kyoko Hayashida

16:00-16:15
Coffee break

16:15-18:15
Oral presentation
(Seminar Hall "Lilac")
O-6. Tomohiro Ishikawa
O-7. Ryo Murata
O-8. Kentaro Yoshii
Special lecture 1. Aaron C. Brault

18:30-20:00
Welcome reception
(Restaurant Hall "Karamatsu")

20:00-21:00
One minute presentation (flash talk)
of 37 posters (Seminar Hall "Lilac")

Day 2 August 20 (thu)

7:00-8:30 Breakfast
(Restaurant Hall "Karamatsu")

8:30-10:00
Oral presentation
(Seminar Hall "Lilac")
O-9. Andrea Marzi
O-10. Keita Matsuno
O-11. David Safronetz
O-12. Shumpei P. Yasuda

10:00-10:15 Coffee break

10:15-11:45
Oral presentation
(Seminar Hall "Lilac")
O-13. Anne M Gaynor
O-14. Tadaki Suzuki
O-15. Gerald S. Baron
O-16. Chang-Hyun Song

11:45-13:00 Lunch
(Restaurant Hall "Karamatsu")

13:00-14:00
Special lecture 2. Wataru Kamitani
(Seminar Hall "Lilac")

14:00-14:15 Coffee break

14:15-15:30
Oral presentation
(Seminar Hall "Lilac")
O-17. Glenn A. Marsh
O-18. Yuji Sunden
O-19. Koichi Araki

15:30-15:45 Coffee break

15:45-16:15
Poster Core time Odd numbers
(Poster session Hall "Fuyou no ma")

16:15-16:45
Poster Core time Even numbers
(Poster session Hall "Fuyou no ma")

16:45-17:45
Poster session Discussion
(Poster session Hall "Fuyou no ma")

18:30-20:30 BBQ
(BBQ House "Mokumoku tei")

20:30- Free Discussion
(Poster session Hall "Fuyou no ma")

Day 3 August 21 (fri)

7:00-8:30 Breakfast
(Restaurant Hall "Karamatsu")

7:00-8:30
*Removing Poster
*Removing things from your room to the cloakroom by 8:30

8:30-9:45
Oral presentation
(Seminar Hall "Lilac")
O-20. Masato Hatta
O-21. Tomohisa Tanaka
O-22. Rashid Manzoor
O-23. Chie Nakajima

9:45-10:00 Coffee break

10:00-10:45
Oral presentation
(Seminar Hall "Lilac")
O-24. Nobuhiko Tokuriki
O-25. Manabu Igarashi

10:45-11:00 Review awards & Break

11:00- Awards & Closing speech

11:15- Preparation for leaving

11:45- Leaving for Sapporo

(Please be punctual without any delay for the bus departure.)

13:30-14:45 Lunch at Otaru

15:45 Arriving at Sapporo

General Information & Guideline

General Information

Registration
*You do not need submit any registration form, just tell your name at the desk of the lobby of Veterinary Medicine at 9:00 am, August 19 (wed). You will have a programme & abstract, name card and information.

Registrationは8月19日（水）朝9時に北大獣医学研究科正面玄関ロビーの受付でおこないます。受付でお名前を申し出てください。特に事前にRegistration formを提出する必要はありません。
8月19日（水） 9時半にバスがでますので時間厳守でお願いします。

(Please be punctual without any delay for the bus departure. Your bus and room no. are indicated on your name card)

[15 Invited speakers]
Staff will pick you up at the lobby of Sapporo Aspen Hotel at 8:30 am, August 19 (wed).

Accommodation in Sapporo for the invited speakers
Sapporo Aspen Hotel
Address/ 5, Kita8-jyo Nishi4-chome, Kita-ku, Sapporo, Hokkaido 060-0808 Japan
Phone / Fax Phone +81 11-700-2111 / FAX +81 11-700-2002
http://www.aspen-hotel.co.jp/english/frame.htm
Single room with breakfast

Accommodation in Niseko for all of the participants
From the night of August 19 to the morning of August 21, 2009, the accommodation will be arranged at the same place where the Seminar will be held.
Accommodation including all meals and transportation between Sapporo and Niseko during the seminar will be provided by the program.

Hotel Niseko Ikoinomura
Address/ Niseko473, Niseko, Hokkaido
Phone/ +81 136-58-3111, Fax +81 136-58-2351
http://www.chuo-bus.co.jp/ikoinomura/ (in Japanese)

[Notice]
All guest rooms are typical Japanese style shared by 2-6 persons or twin rooms without private bath. Public spa (hot spring) is available (Open from 12:00 am to 9:00 am in the next morning)
Valuables should be kept in the reception desk or a safe in the room.
Room Equipment: Bath towel, Face towel, Slippers, Hair dryer, Yukata (Robe)
http://www.japaneseguesthouses.com/about/ryokan/yukata.htm
Internet: Wireless access is available (bring your computer's LAN card)
Amenities: Toothbrush, Razor, Soap, Shampoo, Rinse

General Guideline

Oral presentation
- The laptops running Windows XP Professional operating system, with MS Office 2007 and Macintosh OS X operating system, with MS Office 2004 will be equipped.
- You can have your presentation in your own personal laptop if you use "moving images" or special programs included in your Power Point.
- Please keep the time for the presentation to ensure smooth proceedings.

【2 invited speakers (Special lecture)】
Your oral presentation is allocated 40-45 min with 15-20 min discussion.
【13 invited speakers (Oral presentation)】
Your oral presentation is allocated 20 min with 10 min discussion.
【The above 15 Invited speakers 】
Please bring your presentation loaded in USB thumb drive (flash disk) or CD-ROM at the arrival of the Venue on August 19 (wed) to the staff.

【12 speakers from Hokkaido University】
Your oral presentation is allocated 10 min with 5 min discussion.
* Please bring your presentation loaded in USB thumb drive (flash disk) or CD-ROM by August 17 (mon) to GCOE office or at the arrival of the Venue on August 19 (wed) to the staff.

*The above 27 speakers do not need to prepare poster.

General Guideline for Poster Presentation
- All young researchers and students from Hokkaido University except oral speakers should present poster.
- Each board size is 900 mm W x 2100 mm H (See page 4)
- A0 size: 841 mm x 1189 mm may be appropriate if you prepare your poster as a single sheet.
- Boards with Poster No. card and pushpins for poster setup will be provided.
- Each poster will be provided a poster number as indicated in the Programme & Abstract booklet.
- Poster is to be mounted at lunch time on August 19 in the hall “Poster session Hall (Fuyou no ma) .
- Poster is to be removed by 8:30 am on August 21.

One minute presentation (flash talk) of 37 posters
One minute presentation will be held on August 19 from 20:00 to 21:00 at Seminar Hall “Lilac”.
- The presentation should be one minute and prepare one ppt slide by August 10 to GCOE Office [more details on GCOE web site]

【One minute presentation (Flash talk) の進め方】
1) 一人あたりの持ち時間は70秒とする（約60秒発表）。
2) 全ての発表者は次演者待機場所に発表順に一列に並んでください。
3) 座長は名前と所属だけを紹介し、呼ばれたら1名ずつ壇上に上がり、スライド1枚、約60秒間で発表を行う。
（残りの10秒は発表者の移動と座長による発表者紹介の時間とする。また、この場で討論の時間はない。）

Program Day 1

August 19 (wed), 2009

	9:00	**Registration *Time appointed for assembling (in front of Veterinary Medicine)**
	9:30	**Leaving for Niseko by bus**
	12:00	**Arriving at Niseko**
12:00～	13:30	**Submitting presentation data & Setting up poster , Lunch time (Discussion Hall "Youtei")**
13:30～	13:45	**Orientation : Satoru Konnai (Hokkaido University, Japan) (Seminar Hall "Lilac")** **Opening speech: Hiroshi Kida** **(Leader of Global COE program, Professor, Hokkaido University, Japan)**
		Chair persons: Tatsuya Sakurai & Hirohisa Mekata
13:45～	14:15	**O-1.** Domestic pigs as potential reservoirs of human and animal trypanosomiasis **Louise Hamill (University of Edinburgh, UK)**
14:15～	14:45	**O-2.** The genetics of Human African Trypanosomiasis outbreaks – implications for spread and control of the disease **Liam Morrison (University of Glasgow, UK)**
14:45～	15:15	**O-3.** Characterization of Unknown GPI Anchored Proteins in *Trypanosoma brucei brucei* **Amy F. Savage (Yale University, USA)**
15:15～	15:45	**O-4.** Differential protein expression throughout the life cycle of *Trypanosoma congolense,* a major parasite of cattle in Africa **Brett Eyford (University of Victoria, Canada)**
15:45～	16:00	**O-5.** Aberrant expression of MDM2 and dysfunction of p53 pathway in *Theileria parva*-infected cells **Kyoko Hayashida (Hokkaido University, Japan)**
16:00～	16:15	**Coffee break**
		Chair persons: Kentaro Yoshii & Tadaki Suzuki
16:15～	16:45	**O-6.** A novel chimeric flavivirus vaccine; prM/E to prM/E/NS1 strategy **Tomohiro Ishikawa (Kobe University, Japan)**
16:45～	17:00	**O-7.** N-linked glycosylation in the envelope protein of West Nile virus influences viral growth in vitro and in vivo **Ryo Murata (Hokkaido University, Japan)**
17:00～	17:15	**O-8.** Construction of a full length infectious cDNA clone for Omsk hemorrhagic fever virus **Kentaro Yoshii (Hokkaido University, Japan)**
17:15～	18:15	**Special lecture 1** A murder of crows: Molecular identification of the emergence of avian virulence determinants of West Nile viruses **Aaron C. Brault (University of California, Davis & Centers for Disease control and Prevention, USA)**
18:30～	20:00	**Welcome reception (Restaurant Hall "Karamatsu")**
20:00～	21:00	**One minute presentation (flash talk) of 37 posters (Seminar Hall "Lilac")**

Program Day 2

August 20 (thu), 2009

7:30～8:30		**Breakfast (Restaurant Hall "Karamatsu")**
		Chair persons: Kenta Shimizu & Eri Nakayama (Seminar Hall "Lilac")
8:30～	9:00	**O-9.** Cross-protective efficacy of Vesicular-Stomatitis-Virus-based Ebola vaccines **Andrea Marzi (National Institutes of Health, NIAID, USA)**
9:00～	9:15	**O-10.** Different efficiency of C-type lectin-mediated entry between Marburg virus strains **Keita Matsuno (Hokkaido University, Japan)**
9:15～	9:45	**O-11.** Replication deficient Adenovirus vectors expressing Andes virus nucleoprotein or individual glyco-proteins protect hamsters from lethal hantavirus infection **David Safronetz (National Institutes of Health, NIAID, USA)**
9:45～	10:00	**O-12.** *Hantavirus* genome quantification in experimentally infected laboratory rats and naturally infected wild rats (Rattus norvegicus) **Shumpei P. Yasuda (Hokkaido University, Japan)**
10:00～	10:15	**Coffee break**
		Chair persons: Chang-Hyun Song & Yasuko Orba
10:15～	10:45	**O-13.** Identification and characterization of WU polyomavirus, a novel virus isolated from a patient with acute respiratory tract infection **Anne M Gaynor (Washington University, USA)**
10:45～	11:00	**O-14.** Viroporin activity of JCV agnoprotein **Tadaki Suzuki (Hokkaido University, Japan)**
11:00～	11:30	**O-15.** Towards imaging prion replication and spread **Gerald S. Baron (National Institutes of Health, NIAID, USA)**
11:30～	11:45	**O-16.** Effect of transplantation of bone marrow-derived mesenchymal stem cells on mice infected with prions **Chang-Hyun Song (Hokkaido University, Japan)**
11:45～	13:00	**Lunch (Restaurant Hall "Karamatsu")**
		Chair persons: Satoru Konnai & Naoki Yamamoto
13:00～	14:00	**Special lecture 2** A novel strategy to suppress host gene expression by SARS coronavirus nsp1 protein **Wataru Kamitani (Osaka University, Japan)**
14:00～	14:15	**Coffee break**
		Chair persons: Satoru Konnai & Yosuke Nakayama
14:15～	14:45	**O-17.** Progress towards an understanding of the broad host-range and pathogenesis of Henipaviruses and pathogenesis of Henipaviruses **Glenn A. Marsh (CSIRO Livestock Industries, Australia)**
14:45～	15:00	**O-18.** Suppression of rabies virus propagation in mice brain by intracerebral immunization of inactivated virus **Yuji Sunden (Hokkaido University, Japan)**
15:00～	15:30	**O-19.** Virus specific T cells generated in the presence of calcineurin-inhibitor FK506 cause lethal disease: Implications for transplantation **Koichi Araki (Emory University, USA)**
15:30～	15:45	**Coffee break**
15:45～	16:15	**Poster Core time (Odd numbers) (Poster session Hall "Fuyou no ma")**
16:15～	16:45	**Poster Core time (Even numbers)**
16:45～	17:45	**Poster session (Discussion)**
18:30～	20:30	**BBQ (BBQ House "Mokumoku tei")**
20:30～		**Free Discussion (Poster session Hall "Fuyou no ma")**

Program Day 3

August 21 (fri), 2009

7:30～8:30	**Breakfast (Restaurant Hall "Karamatsu")**
7:30～8:30	**Removing Poster** **Removing things from your room to the cloakroom by 8:30**
	Chair persons: Masatoshi Okamatsu & Yuji Sunden (Seminar Hall "Lilac")
8:30～9:00	**O-20.** The molecular determinants for the pathogenicity of H5N1 influenza A virus **Masato Hatta (University of Wisconsin-Madison, USA)**
9:00～9:15	**O-21.** Experimental mouse model of human influenza-associated encephalopathy in childhood **Tomohisa Tanaka (Hokkaido University, Japan)**
9:15～9:30	**O-22.** PB2 protein of a highly pathogenic avian influenza virus strain A/chicken/Yamaguchi/7/2004 (H5N1) determines its replication potential in pigs **Rashid Manzoor (Hokkaido University, Japan)**
9:30～9:45	**O-23.** Characterization of a new mycobacterial species belonging to Mycobacterium tuberculosis complex isolated from monkeys in Bangladesh **Chie Nakajima (Hokkaido University, Japan)**
9:45～10:00	**Coffee break**
	Chair person: Kimihito Ito
10:00～10:30	**O-24.** The unique strategy of viral proteins for robustness and evolvability **Nobuhiko Tokuriki (University of Cambridge, UK)**
10:30～10:45	**O-25.** Prediction of *N*-glycosylation potential of influenza virus hemagglutinin:a bioinformatics approach **Manabu Igarashi (Hokkaido University, Japan)**
10:45～11:00	**Review awards & Break**
11:00～	**Awards & Closing speech: Chihiro Sugimoto** **(Member of Global COE program, Professor, Hokkaido University, Japan)**
11:15～	**Preparation for leaving**
11:45～	**Leaving for Sapporo**
13:30～14:45	**Lunch at Otaru**
15:45	**Arriving at Sapporo**

Profile of Invited speakers

Special Leture 1

Aaron C. Brault

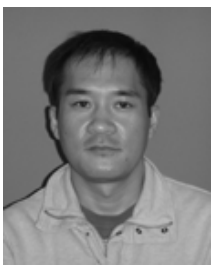
Associate Professor (adjunct)/ Research Microbiologist
Division of Vector-borne Infectious Diseases,
Centers for Disease Control and Prevention
Department of Pathology, Microbiology and Immunology
University of California, Davis

ACADEMIC DEGREES:
B.S. 1995 Texas A&M University (Zoology)
Ph.D. 2001 University of Texas Medical Branch (Virology/Experimental Pathology)

PROFESSIONAL APPOINTMENTS:
2001 - 2003 ASM postdoctoral scholar Centers for Disease Control and Prevention
2003 - 2007 Assistant Professor, University of California, Davis
2007 - Associate Professor, University of California, Davis
2009- Research Microbiologist, Centers for Disease Control and prevention

RESEARCH INTERESTS:
Viral emergence mechanisms
Genetic determinants of Pathogenesis
Viral Evolution

Special Leture 2



Wataru Kamitani

Associate Professor
Global COE Program
Research Institute for Microbial Diseases
Osaka University

ACADEMIC DEGREES:
D.V.M.1999 Rakuno Gakuen University (Veterinary Medicine)
Ph.D. 2003 Osaka University (Molecular Medicine)

PROFESSIONAL APPOINTMENTS:

April, 2003-March, 2004	Postdoctoral Fellow, Department of Virology, Research Institute for Microbial Diseases, Osaka University, JAPAN
May, 2004-March, 2009	Postdoctoral Fellow, Department of Microbiology and Immunology, The University of Texas Medical Branch at Galveston, Galveston, Texas, USA
April, 2009-present	Associate Professor, Global COE Program, Research Institute for Microbial Diseases, Osaka University

RESEARCH INTERESTS:
I am most interested in understanding the molecular basis of pathogenesis of emerging viruses. Severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV) is the etiological agent of the recently emerged human respiratory diseases SARS. Currently, I am studying the effect of SARS-CoV proteins, nsp1, on host cells. My studies have suggested that nsp1 protein inhibits host mRNA accumulation in SARS-CoV-infected cells. I speculate that SARS-CoV uses this property of the protein to shut off the expression of genes that are involved in host innate immune responses. I would like to understand the mechanism of suppression of host gene expression by nsp1 protein.

Oral presentation 1

Louise Hamill

MSc by Research Student
Centre for Infectious Diseases,
College of Medicine and Veterinary Medicine,
Royal Dick School of Veterinary Science,
The University of Edinburgh

ACADEMIC DEGREES:
BSc Biological Sciences with Honors in Microbiology and Infection, 2.1 (University of Edinburgh, 2004-2008)
MSc by Research, The Molecular Epidemiology of Trypanosomiasis in Uganda (University of Edinburgh, 2008-2009, completion Autumn 2009)

PROFESSIONAL APPOINTMENTS:
BSP student member since January 2009
Biological Sciences student representative, September 2006
Immunology 3 class representative, 2006
Biological Sciences Student mentor, 2005-2008

RESEARCH INTERESTS:

My primary research interest is the epidemiology of human and animal trypanosomiasis, with a strong emphasis on zoonotic *Trypanosoma brucei rhodesiense*. As part of my undergraduate degree I completed a laboratory based research project entitled "The trypanosomes of pigs in Arusha, Tanzania", a project I secured independently and in which I was awarded a first. The results of the project are currently going through the departmental editing process, with a view to submitting for publication soon.

My masters project is entitled "The molecular epidemiology of trypanosomiasis in Uganda". It aims to assess the impact of the SOS re-treatment intervention on the prevalence of *T. vivax*, *T. b. brucei* and *T. b. rhodesiense*; more information relating to this programme can be found at www.sleepingsickness.org. Twenty villages were randomly selected from within the re-treatment area, of which ten had reported human sleeping sickness cases. Molecular analysis if cattle blood samples from these twenty villages is currently underway, using both PCR and loop-mediated isothermal amplification (LAMP) protocols. During the course of my project so far I have undertaken a large amount of both collaborative and individual work, both here in laboratory in Edinburgh as well as in the collection of field samples in Uganda.

Oral presentation 2

Liam Morrison

Postdoctoral Research Associate
Wellcome Centre for Molecular Parasitology
Faculty of Veterinary Medicine
University of Glasgow

ACADEMIC DEGREES:

B.Sc.	1998	University of Glasgow (Parasitology)
B.V.M.S.	2000	University of Glasgow (Veterinary Medicine)
Ph.D.	2004	University of Glasgow (Molecular Parasitology)

PROFESSIONAL APPOINTMENTS:

2003-2005	Research Assistant, Wellcome Centre for Molecular Parasitology, University of Glasgow
2005-2010	Postdoctoral Research Associate, Wellcome Centre for Molecular Parasitology, University of Glasgow

Profile of Invited speakers

RESEARCH INTERESTS:

My research interests focus on the genetics of parasites, specifically African trypanosomes, and the implications that genetic diversity and genetic exchange have upon the epidemiology of disease, particularly with reference to the spread of traits such as drug resistance and pathogenesis. I work both on the trypanosomes species that infect domestic livestock (*Trypanosoma brucei*, *Trypanosoma congolense* and *Trypanosoma vivax*), as well as the trypanosomes that cause Human African Trypanosomiasis, or sleeping sickness (*Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*). I am interested in the determinants of disease pathogenesis, treatment and spread at the levels of both the host and parasite, and the translation of lab based studies into the clinical setting. I use both population genetics approaches to understand the dynamics of trait transmission in field populations, and also laboratory based studies using a classical genetic approach to identify genes in the parasite responsible for phenotypes. An example of the latter from my current work is the identification of trypanosome genetic loci involved in causing pathology of the liver and spleen in the host.

Oral presentation 3

Amy F. Savage

Doctoral Candidate, Division of Epidemiology of Microbial Disease
Yale School of Epidemiology & Public Health
Yale School of Medicine
Yale University

ACADEMIC DEGREES

A.S.	1996	University of Connecticut (Applied Science)
B.S.	2001	University of Connecticut (Pathobiology, Animal Science)
M.S.	2003	University of Florida (Veterinary Parasitology)
M. Phil.	2007	Yale University (Epidemiology of Microbial Disease)

PROFESSIONAL APPOINTMENTS

Intern and Technician, Biocomplexity of Introduced Avian Disease, US Geological Survey, Pacific Island Ecosystems Research Center

RESEARCH INTERESTS

I am interested in both zoonotic and vector-borne eukaryotic parasites of importance to human and animal health. Specifically, I am interested in the host-parasite interactions which allow the parasites to establish and maintain a presence in their host. Additionally, I am interested in the role parasites play in the decline of host populations.

Oral presentation 4

Brett Alexander Eyford

Doctoral Student of Professor Terry Pearson
Department of Biochemistry and Microbiology
Faculty of Graduate Studies
University of Victoria

ACADEMIC DEGREES:

B.Sc. (honours)	2003 - 2007	University of Victoria (Biochemistry)
Ph.D	2007 –	University of Victoria (Biochemistry)

RESEARCH INTERESTS:
Mass spectrometry based analysis of African trypanosomes; Studies of differential protein expression throughout the *Trypanosoma congolense* life cycle; Development of new diagnostics for human African trypanosomiasis; Antibodies for enrichment of low abundance plasma proteins/peptide; Killing of trypanosome by modified host defense peptides.

Oral presentation 6

Tomohiro Ishikawa

Research resident
Department of International Health
Graduate School of Health Sciences
Kobe University

ACADEMIC DEGREES:

B.A.	2001	Kobe University (Health Sciences)
M.A.	2003	Kobe University (Health Sciences)
Ph.D.	2006	Kobe University (Health Sciences)

PROFESSIONAL APPOINTMENTS:

2006 - 2008	Post-doctoral Fellow, Department of Pathology University of Texas Medical Branch
2008 -	Research resident, Department of International Health, Kobe University Graduate School of Health Sciences

RESEARCH INTERESTS:
Virology, Vaccinology, Infection-immunology

Oral presentation 9

Andrea Marzi

Visiting fellow
Laboratory of Virology
Rocky Mountain Laboratories, NIAID, NIH
903 South 4th Street
Hamilton, MT 59840, USA

ACADEMIC DEGREES:

B.Sc.	10/2000	University Erlangen-Nürnberg, Germany (Biology)
M.Sc.	03/2003	University Erlangen-Nürnberg, Germany (Microbiology & Virology)
Ph.D.	06/2007	University Erlangen-Nürnberg, Germany (Virology & Molecular Biology, "*summa cum laude*")

Profile of Invited speakers

PROFESSIONAL APPOINTMENTS:

07/2007 – 08/2007	postdoctoral fellow in Dr. Stefan Pöhlmann's laboratory Institute for Clinical and Molecular Virology, Friedrich-Alexander-University Erlangen-Nürnberg
09/2007 – 09/2008	NSERC visiting fellow in Dr. Heinz Feldmann's laboratory Public Health Agency of Canada, National Microbiology Laboratory, Special Pathogens Program, Winnipeg, Canada
since 10/2008	visiting fellow in Dr. Heinz Feldmann's laboratory Laboratory of Virology, Rocky Mountain Laboratories, NIAID, NIH, Hamilton, Montana, USA

RESEARCH INTERESTS:

The focus of my research lies on the filovirus glycoprotein essential for productive infection of target cells.
First of all, the glycoprotein mediates the attachment of viral particles to the cell and receptor engagement finally leading to the fusion of viral and cellular membrane and to infection of the cell. I am interested in analyzing the single steps involved in this process like binding to cell surface molecules, uptake of viral particles into the cell via endocytosis and the cleavage of the virus glycoprotein triggering membrane fusion. The process itself is identified but the underlying molecular determinants are not very well understood and need further study.

Second, I am working with the Vesicular-Stomatitis-Virus (VSV)-based vaccine against Ebola and Marburg virus developed in Dr. Feldmann's lab with the focus on cross-protection. There are five different Ebola virus species known so far and it would be great to have just one single vaccine able to induce immune response against them all. The vaccine contains the filoviral glycoprotein as antigen and we are currently investigating using animal models, if we can achieve cross-protective immunity with one single dose of this vaccine.

Oral presentation 11

David Safronetz

Visiting Fellow
Laboratory of Virology
National Institutes of Health
National Institute of Allergy and Infectious Disease

ACADEMIC DEGREES:

B.Sc.	2000	University of Saskatchewan (Medical Microbiology and Immunology)
M.Sc.	2003	University of Manitoba (Medical Microbiology)
Ph.D.	2009	University of Manitoba (Medical Microbiology)

PROFESSIONAL APPOINTMENTS:

2008 -	Visiting Fellow, Laboratory of Virology, NIH, NIAID

RESEARCH INTERESTS:

My primary research interest is hantaviruses, with emphasis on ecology of virus transmission and disease prevention. My past research has included field work looking at seroprevalence and virus transmission, both long term studies as well as those surrounding hantavirus pulmonary syndrome (HPS) case investigations. My current work is focused on pathogenesis of Andes virus in the hamster model of HPS as well as vaccine studies aimed at dissecting the protective immune response.

Oral presentation 13

Anne M Gaynor

PhD Candidate
Division of Biology and Biological Sciences
Program in Molecular Microbiology and Microbial Pathogenesis
Washington University in St. Louis, MO, USA

ACADEMIC DEGREES:
Bachelor of Science in Forensic Biology with Distinction, 2000
Juniata College, Huntingdon PA, USA

RESEARCH INTERESTS:
My interests have been focused on the study of infectious diseases of unknown etiology, specifically respiratory disease. My current thesis work has focused on the characterization of a novel polyomavirus called WU Polyomavirus. This virus was identified in our laboratory using unbiased sequencing techniques from a nasopharyngeal aspirate from a child with acute respiratory tract illness of unknown etiology. Currently I am trying to understand the tissue tropism of this virus by developing an *in-vitro* tissue culture system where we can study the replication cycle of the virus. Upon the completion of my doctoral dissertation I hope to utilize my research background in the global public health field.

Oral presentation 15

Gerald S. Baron

Rocky Mountain Laboratories
National Institutes of Health
Laboratory of Persistent Viral Diseases
903 S. 4th St.
Hamilton, MT 59840

DEGREES
1993 BSc (Biochemistry) University of Victoria, Victoria, B.C.
1998 PhD (Biochemistry) "Isolation and characterization of two genetic loci from the intracellular pathogen *Francisella novicida.*" Supervisor: Francis E. Nano, University of Victoria, Victoria, B.C.

PROFESSIONAL TRAINING AND POSITIONS

1991-1993	Undergraduate Research (laboratory of Francis E. Nano), Dept. of Biochemistry and Microbiology, University of Victoria, Victoria, B.C. "Characterization of a serum-sensitive mutant of *Francisella novicida* defective for growth in macrophages." "Production of antiserum against GseA, a KDO-transferase of *Chlamydia trachomatis*."
1993-1998	Graduate Research (laboratory of Francis E. Nano), Dept. of Biochemistry and Microbiology, University of Victoria, Victoria, B.C. "Identification of factors of *Francisella* required for growth in macrophages."
1995	Graduate Directed Studies Research (laboratory of Terry Pearson), Dept. of Biochemistry and Microbiology, University of Victoria, Victoria, B.C. "Amino acid microsequencing of the kinetoplastid membrane protein-11 (KMP-11) from African trypanosomes."
1998-2003	Visiting Postdoctoral Fellow (laboratory of Byron Caughey), Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, Hamilton, MT "Effect of membrane association on prion protein interactions." "Development of high-throughput assays for identification of modulators of prion protein interactions."
2003-2005	Research Fellow (laboratory of Byron Caughey), Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, Hamilton, MT
2005-date	Tenure-track investigator, Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, Hamilton, MT

Profile of Invited speakers

Oral presentation 17

Glenn Marsh

Postdoctoral Fellow
Australian Animal Health Laboratory
Livestock Industries
Commonwealth Scientific and Industrial Research Organisation
Geelong, Australia

ACADEMIC DEGREES:

BAppSci	1998	RMIT University, Melbourne, Australia
Ph.D.	2004	RMIT University (Virology), Melbourne, Australia

PROFESSIONAL APPOINTMENTS:

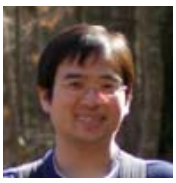
1998 - 2004	Lecturer and tutor, Department of Biotechnology and Environmental Biology, RMIT University, Melbourne, Australia
2004 - 2007	Postdoctoral Fellow, Department of Microbiology, Mount Sinai School of Medicine, New York, NY USA
2007 -	Postdoctoral Fellow, Australian Animal Health Laboratory, CSIRO, Geelong Australia

RESEARCH INTERESTS:

Development of reverse genetics systems for Henipaviruses, SARS and SARS-like Coronaviruses, with the overall goal of this work being the characterization of the molecular determinants of virulence in these highly pathogenic viruses.

Examination of bats as the natural reservoir of these viruses, with the goal of understanding how bats are infected with no signs of illness.

Oral presentation 19



Koichi Araki

Postdoctoral fellow
Emory Vaccine Center
Department of Microbiology and Immunology
Emory University School of Medicine

ACADEMIC DEGREES:

D.V.M 2000	Hokkaido University (Veterinary Medicine)
Ph.D 2004	Hokkaido University (Veterinary Medicine)

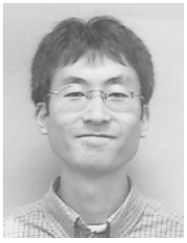
PROFESSIONAL APPOINTMENTS:

2004 ~ Postdoctoral fellow Emory University School of Medicine

RESEARCH INTERESTS:

Viral immunology, Immunological memory, CD8 T cells, Viral pathogenesis

Oral presentation 20



Masato Hatta

Research Assistant Professor
Influenza Research Institute,
Department of Pathobiological Sciences,
School of Veterinary Medicine,
University of Wisconsin-Madison

ACADEMIC DEGREES:

B.S.	1996	Hokkaido University (Veterinary Medicine)
DVM	1998	Ministry of Agriculture and Fishery, Japan
Ph.D.	2000	Graduate School of Veterinary Medicine, Hokkaido University (Virology)

PROFESSIONAL APPOINTMENTS:

1999 - 2003	Research Associate, Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison
2003 - 2005	Assistant Scientist, Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison
2005 -	Research Assistant Professor, Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison

RESEARCH INTERESTS:
Molecular basis of virulence of influenza viruses

Oral presentation 24

Nobuhiko Tokuriki

Dr
Department of Chemistry
University of Cambridge

ACADEMIC DEGREES:

B.A.	1998	Osaka University (Biotechnology)
M.A.	2000	Osaka University (Biotechnology)
Ph.D.	2004	Osaka University (Biotechnology)

PROFESSIONAL APPOINTMENTS:

February 2004- May 2009	Weizmann Institute of Science, Israel, Postdoctoral Fellow
June 2009 -	University of Cambridge, UK, Postdoctoral Fellow (Marie Curie fellowship)

RESEARCH INTERESTS:
Biophysics and Biochemistry, in particular understanding of the dynamics of protein evolution.

Abstract Special Leture

Special Leture 1
(Day 1: 17:15-18:15 on Aug. 19)

A murder of crows: Molecular identification of the emergence of avian virulence determinants of West Nile viruses

Aaron C. Brault
Associate Professor (adjunct), Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis; Research Microbiologist, Division of Vector-borne Infectious Diseases, Centers for Disease control and Prevention, Fort Collins, Colorado

The genotype of West Nile virus (WNV) introduced to North America in 1999 has been associated with virulence in a number of avian species, with American crows (AMCRs) and other corvid species being the most susceptible. Selection modeling using 21 complete WNV genomes identified a single WNV genetic loci (NS3-249) to be under the effect of positive selection. Experimental infection of AMCRs with chimeric WNVs from strains with variable avian virulence phenotypes implicated the same amino acid residue within the helicase domain (NS3-P249T) to be instrumental in the viremia and mortality response in this species. We subsequently engineered four WNVs containing point mutations at the NS3-249 site within the NY99 genotype including: NS3-P249H, P249A, P249T and P249D, representing genetic polymorphisms of naturally occurring WNV genotypes for experimental avian infection studies. The NS3-P249T virus was nearly completely attenuated in AMCRs, with 12.5% mortality and peak viremia of 5 log10 PFU/mL while the 249P virus inflicted 100% mortality with a peak viremia of 9.3 log10 PFU/mL. Incorporation of either a His or Asp acid were sufficient to maintain 100% mortality; however, peak viremias were reduced by 100-fold as compared to the NS3-P249 genotype. Incorporation of an Ala residue generated a virus with an intermediate virulence level (75%) and 6 log10 PFU/mL. These results confirm the vital role of this locus for avian virulence potential and indicate the selective advantage of the NS3-249P for increased AMCR replication within the NY99 WNV genetic backbone. Furthermore, differences identified in replication rates among these different mutants in cultured mosquito cells indicate the potential selective role of mosquitoes in the emergence and enzootic persistence of particular NS3-249 WNV genotypes.

Special Leture 2
(Day 2: 13:00-14:00 on Aug. 20)

A novel strategy to suppress host gene expression by SARS coronavirus nsp1 protein

Wataru Kamitani
Associate Professor,
Global COE Program,
Research Institute for Microbial Diseases,
Osaka University

Severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV), an enveloped, single-stranded, and positive-sense RNA virus, is the etiological agent of a newly emerged disease. SARS originated in southern China in 2002 and spread to various areas of the world in the 2003 epidemic. During SARS-CoV replication, viral genomic-sized RNA plus eight subgenomic mRNAs are produced. These subgenomic mRNAs encode the spike glycoprotein (S), M glycoprotein (M), nucleocapside protein (N), and E protein (E). The 5'-end two-thirds of viral genomic RNA, gene 1, encodes 16 mature proteins. Probably most of the gene 1 proteins are important for viral RNA synthesis. Some gene 1 proteins are predicted to have biological functions related to RNA synthesis, while some gene 1 proteins may have functions other than viral RNA synthesis.

In our studies expression of nsp1, the most N-terminal gene 1 protein, prevented Sendai virus-induced endogenous IFN-β mRNA accumulation without inhibiting dimerization of IRF-3, a protein that is essential for activation of the INF-β promoter. Furthermore, nsp1 protein suppressed host gene expression by promoting host mRNA degradation and host translation inhibition both in expressing cells and SARS-CoV-infected cells.

To understand the relationship between the nsp1-mediated translational suppression and the host mRNA degradation promotion, biological functions of purified nsp1 protein in rabbit reticulocyte lysate (RRL) were examined. Nsp1 protein efficiently inhibited translation of capped reporter RNA transcripts, whereas it failed to degrade them. Nsp1 bound to the 40S ribosomal subunit and inhibited 80S monomer formation. Furthermore, the nsp1-40S ribosome complex induced the modification at the 5'-region of the capped mRNA template and made the template RNA to be translationally incompetent. We suspect that host mRNAs that underwent the nsp1 -mediated modification are delivered to normal host mRNA degradation machinery and quickly degraded.

Oral Presentation Day 1: Aug. 19, 2009

O-1 Domestic pigs as potential reservoirs of human and animal trypanosomiasis

Louise Hamill
Centre for Infectious Diseases, The University of Edinburgh, Edinburgh, UK

Trypanosomiasis affects a number of mammalian species, including domestic livestock and humans, causing considerable morbidity, mortality and economic loss. Both domestic and wild pigs are capable of becoming infected with various species of trypanosomes, and hence could be acting as reservoirs of infection for other target populations. Except in the case of *T. simiae* infections, pig trypanosome infections caused by *T. congolense*, *T. brucei s.l.* and other trypanosome species are often asymptomatic (Ilemobade and Balogun, 1981). Therefore, the infection is not a problem for the pig population, but becomes significant considering the potential of pigs to act as reservoirs for these species of trypanosome, which, when passed to other livestock species, such as cattle, are extremely pathogenic. Cattle are considered an extremely important livestock animal in Sub-Saharan Africa, bestowing upon their owners elevated social status as well as the many benefits these animals can bring to farming overall. Few studies have been carried out investigating the prevalence of trypanosomiasis in pigs compared to the vast amount of research conducted on other animal species. Pigs are seldom considered when trypanosomiasis control programmes are being designed and implemented. In this study we look at the levels of trypanosome infection in domestic pigs in Tanzania, and suggest the role that these animals may play as potential reservoirs of cattle and human infective trypanosomes.

Blood samples were collected on Whatman FTA cards from 168 domestic pigs from four different districts, Aru Meru, Babati, Mbulu and Dodoma, situated in the Arusha region of Northern Tanzania. PCR analysis identified 28 (16.7%) pigs infected with one or more species of trypanosome, 5 of which were multiple infections. The parasites identified as circulating in this area include *T. vivax* ,*T. simiae*, *T. b. brucei*, *T. b rhodesiense*, and some suspected *T. godfreyi* infections.

These results suggest domestic pigs may play a role as reservoirs for *T. vivax* and *T. brucei* s. l. infection in cattle, and while these parasites cause mild or asymptomatic infection in porcine species, *T. vivax* is known to cause serious disease in cattle and other economically important livestock. More importantly this study suggests that domestic pigs should be seriously considered as a potential reservoir species for *T. b. rhodesiense*. This parasite, which is the causative agent of acute human sleeping sickness, was found in 4.76% of domestic pig samples overall. However, the *T. b. rhodesiense* prevalence was not evenly distributed across the 4 districts from which samples were collected. Notably, prevalence reached 10.8% in Aru Meru district, a heavily populated area where tsetse species with a predilection for feeding on both porcine and human hosts are also found.

Pig keeping in East Africa is on the increase (Phiri *et al.,* 2003), however these animals are rarely considered for treatment against trypanosomiasis, as they are usually assumed to be free of the disease (Katunguka – Rwakishaya, 1996). The results of this study suggest pigs should be seriously considered as a potential reservoir species for trypanosomiasis, and the socio-economic implications of these findings will be discussed. It is also clear that pigs could act as a reservoir for human infective t. b. rhodesiense and as such should be included when designing and implementing treatment and control programmes for this disease.

O-2 The genetics of Human African Trypanosomiasis outbreaks – implications for spread and control of the disease

Liam Morrison
Wellcome Centre for Molecular Parasitology, University of Glasgow, United Kingdom

The African trypanosome, *Trypanosoma brucei*, infects a wide range of domestic livestock and wild mammals. Two subspecies of *T. brucei*, *T. brucei rhodesiense* and *T. brucei gambiense*, are also able to infect humans and cause a lethal disease affecting approximately 500,000 people per year. *T. brucei* has been shown to undergo genetic exchange in the laboratory, but controversy exists as to the role of genetic exchange in natural populations. The degree of mating has implications for the adaptability of parasites to their environment, and the spread of important traits such as drug resistance. We have used multilocus microsatellite genotyping to determine if there is evidence of genetic exchange in different foci of disease, of both *T. b. gambiense* and *T. b. rhodesiense* sleeping sickness. The results from several foci suggest that *T. b. gambiense* undergoes genetic exchange very rarely, and predominantly reproduces clonally. Different foci, however, are very genetically distinct, and this raises the possibility that human serum resistance in *T. b. gambiense* has arisen through several independent events. Examination of *T. b. rhodesiense* foci reveal dramatic differences in parasite population structure in different locations within a single subspecies. These include (1) a clonal population structure, where there is significant linkage disequilibrium and no evidence for mating; (2) an epidemic population structure, in which linkage disequilibrium results from temporal expansion of a few clones in an otherwise sexual population and (3) a panmictic or randomly mating population structure. In summary, our results suggest that *T. b. gambiense* is predominantly a clonal organism, whereas *T. b. rhodesiense* utilizes a variable mating strategy in which the proportion of clonal propagation can vary, probably depending on environmental conditions. These findings are discussed with respect to the epidemiology of the disease and the role of the reservoir hosts, and implications for control strategies.

O-3 Characterization of Unknown GPI Anchored Proteins in *Trypanosoma brucei brucei*

Amy F. Savage
Epidemiology of Microbial Disease,
Yale Graduate School of Arts and Sciences, Yale University

An in-silico screen of the genome of the animal pathogen *Trypanosoma brucei* yielded several unknown hypothetical

proteins containing glycosylphosphatidylinisotol (GPI) anchor structures. GPI anchors typically bind proteins to cell membranes and as such, these hypothetical proteins may be expressed on the parasite cell surface. The trypanosome undergoes several differentiation steps during development in the tsetse fly, each occurring in the lumen of different fly organs. Upon ingestion by a fly, bloodstream trypanosomes differentiate into the procyclic form and inhabit the fly midgut. Procyclic parasites migrate to the proventriculus and differentiate into epimastigote cells. These epimastogotes then continue to the fly salivary glands where they attach and differentiate a third time, to the mammalian infective metacyclic trypomastigotes. Metacyclics develop into the final bloodstream form after inoculation by the fly during a bloodmeal on an appropriate host, completing the cycle. The genes carrying GPI anchor motifs were searched for signal peptides and other conserved domains. Homology with genes in the related pathogens *Leishmania major* and *Trypanosoma cruzi* were noted. Of the twelve genes selected for further evaluation, 9/12 had known conserved domains and 9/12 were shared with *L. major* and *T. cruzi*. Gene specific primers were designed for each gene. To determine if these unknown genes were expressed in particular developmental stages preferentially, RNAs were prepared from parasite infected salivary glands, proventriculus, and midguts, as well as procyclic culture and bloodstream form parasites. cDNAs were prepared and normalized by PCR using trypanosome *alpha-tubulin*. Then, the normalized templates were tested with gene-specific primers for the twelve hypothetical unknown genes. No detectible product was obtained for 3/12 primer pairs. All other primer pairs yielded bands, and showed differential expression in different host tissues. Further examination using quantitative PCR will be used to quantify the degree to which these genes are up- or down-regulated in different parasite life stages. These data will provide further information on the role these genes may play in infection processes in the tsetse fly or vertebrate hosts, and increase our broader understanding of this complex interaction.

O-4 Differential protein expression throughout the life cycle of *Trypanosoma congolense*, a major parasite of cattle in Africa

Brett Eyford
Department of Biochemistry and Microbiology,
Faculty of Graduate Studies, University of Victoria

Trypanosoma congolense is the major protozoan parasite responsible for the economically devastating disease trypanosomiasis, in livestock of sub-Saharan Africa. The World Health Organization considers this disease (as well as the symptomatically similar diseases caused by *T. brucei* and *T. vivax*) to be one of the largest impediments to the economic development of Africa. Animal trypanosomiasis leaves approximately 10 million km^2 of sub-Saharan African unfit for livestock production, an area larger than that of the United States. The economic impact can be measured in billions of dollars per annum.

T. congolense has four major life cycle stages, with one stage in the blood and/or cerebrospinal fluid of a mammalian host and three in the midgut and mouth parts of the tsetse fly vector. The goal of my research is to, 1) Identify which proteins are present in each life cycle stage and 2) monitor the change in protein expression from one life cycle stage to the next. We have used a technique known as isobaric Tag for Relative and Absolute Quantitation (iTRAQ) in conjunction with mass spectrometry analysis to measure differential expression of proteins throughout the *T. congolense* life cycle. Three complete biological replicates of all four life cycle stages were studied using iTRAQ. The data were analyzed by comparison to a recently completed six frame translated *T. congolense* genome sequence. A total of 9214 tryptic peptides were identified and localized to 2237 open reading frames representing about 27% of the genes predicted to be in the *T. congolense* genome. Our data are being used as an aid to annotation of the parasite genome.

All protein sequences were used in a BLAST search of the non-redundant database. Many of the sequences drew strong hits with a related African trypanosome (*T. b. brucei*) or other kinetoplastids (*T. cruzi, Leishmania sp.*). There were eleven sequences that were identified with high confidence in all three replicates that drew no strong sequence similarity to any other sequences in the database. There were an additional eleven sequences that were present in two of the three replicates that also had no strong database hits. These sequences represent new proteins that have not been described in any organism and may be unique to *T. congolense.*

O-5 Aberrant expression of MDM2 and dysfunction of p53 pathway in *Theileria parva*-infected cells

Kyoko Hayashida
Dept.of Education and Collaboration,
Research Center for Zoonosis Control, Hokkaido University,

Theileria parva, and *T. annulata* are tick-transmitted protozoan parasites causing fatal lymphoproliferative diseases known as “East Coast fever” and “tropical theileriosis”. African countries have been suffered from huge economic loss due to these diseases since effective and affordable therapeutic agents have not been developed. The schizont stage of the parasite resides in the lymphocytes, which induces unlimited proliferation of the host cell, like tumor cell. However, the mechanisms that underlie host cell transformation caused by *Theileria* parasites have not been fully elucidated.

To identify a signaling pathway involved in unlimited proliferation of *Theileria*-infected lymphocytes, we screened anti-cancer compounds library. Out of 190 compounds screened, an anti-mdm2 compound, Trans-4-Iodo 4’ boranyl chalcone (TIBL) was found to specifically inhibit expansion, and induced apoptosis of *Theileria*-infected lymphocytes.

MDM2, an ubiquitin ligase for p53, play a central role in regulation of the stability of p53, and has oncogenic potential when overexpressed in the cells. When we measured expression level of this protein, overexpression of Mdm2 in *T. parva* or *T. annulata*-infected cells was demonstrated. This overexpression seems to be resulted from enhanced transcription of *mdm2* gene as confirmed by real-time PCR. Moreover, several spliced variants of *mdm2* mRNA which coexisted with full-length transcripts were detected in *T. parva*-

infected lymphocytes.

Consequently, in *T. parva*-infected lymphocyte, functional p53 accumulation after DNA damage was impaired, whereas *TP53* mRNA was normally transcribed. Treatment of TIBL in *T. parva*-infected lymphocyte stabilized p53, suggesting functional p53 response was hindered by MDM2. The findings indicated that the abnormal p53 response in *T. parva* -infected cells is associated with aberrant expression of MDM2, and MDM2 might contribute to uncontrolled proliferation of the host lymphocytes.

O-6 A novel chimeric flavivirus vaccine; prM/E to prM/E/NS1 strategy

Tomohiro Ishikawa[1], Douglas G. Widman[2], Ernesto Infante Jr.[3], Eiji Konishi[1], Nigel Bourne[3], Peter W. Mason[3]

[1]Department of International Health, Kobe University Graduate School of Health Sciences, [2]Department of Microbiology and Immunology, University of Texas Medical Branch

[3]Department of Pathology, University of Texas Medical Branch

Flavivirus infections caused by Japanese encephalitis virus (JEV), West Nile virus (WNV), dengue virus, and tick borne encephalitis virus are public health problems in the world. Although inactivated-vaccines and live-attenuated vaccines are currently available against some of the flaviviruses, those suffer from problems such as multiple-dose requirement or reversion to virulent. Recently, we developed a single-cycle flavivirus vaccine (SCFV) using WNV model. Genetically engineered chimeric flaviviruses encoding prM/E gene of the target flavivirus have been known as an effective vaccine, since the E protein possesses most of the neutralization epitopes. Therefore, we constructed a chimeric SCFV against JEV by replacing prM/E gene (RepliVAX JE). In addition, one of the nonstructural protein, NS1, has been known to induce protective immunity. Thus, we developed a novel chimeric SCFV encoding JEV prM/E/NS1 gene (TripliVAX JE).

To evaluate and compare those vaccines, mice are immunized with a single dose of either RepliVAX JE or TripliVAX JE via intraperitoneal route. At 3-week post immunization, animals were bled and then challenged with a lethal dose of JEV at 4-week post immunization. Collected sera were examined for neutralization test and ELISA against E and NS1 proteins. Animals were monitored for 2 weeks following challenge.

Single immunization of RepliVAX JE and TripliVAX JE as low as $2.5x10^3$ IU could induce detectable neutralizing antibodies in mice (1:80). Animals immunized with TripliVAX JE developed higher immune responses against E protein than animals with RepliVAX JE. Animals immunized with TripliVAX JE did not show antibody responses against WNV NS1 protein. In contrast, animals immunized with RepliVAX JE and TripliVAX JE showed similar immune responses against JEV NS1 protein. Following Challenge, all animals immunized with RepliVAX JE or TripliVAX JE were protected from the challenge, whereas 90% of control animals died.

These results indicated that TripliVAX is a superior vaccine to RepliVAX in terms of induction of immune responses against E protein. Since immunity induced by TripliVAX is not interfered by pre-exsiting anti-NS1 immunity against back-bone flavivirus, the prM/E/NS1 chimerization strategy can contribute to develop more effective and safer vaccine against variety of flaviviruses.

O-7 N-linked glycosylation in the envelope protein of West Nile virus influences viral growth in vitro and in vivo

Ryo Murata

Lab. of Public Health, Dept. of Environmental Veterinary Sciences, Graduate School of Veterinary Medicine, Hokkaido University

Many West Nile (WN) virus isolates associated with significant human outbreaks, including the recent North American epidemic, possess the glycosylation site on the envelope (E) protein. Our previous study has shown that the E protein glycosylated variants of New York (NY) strains of WN virus are more neuroinvasive in mice than non-glycosylation variants.

To determine how the E protein glycosylation affects the interactions between WN virus and avian hosts, we inoculated young chicks with possessing either E protein glycosylated or non-glycosylated variants of WN virus NY strains. The glycosylated variants were more virulent and had higher viremic levels than the non-glycosylated variants. The virus multiplication and dissemination in mosquitoes in vivo did not show any difference between the glycosylated and non-glycosylated variants. The multiplication characteristics of the glycosylated and non-glycosylated variants in vitro were examined in three different tissue culture cells of mosquito (C6/36), mammalian (BHK) and avian (QT6) origin, at different temperature. The glycosylated variants showed more heat stable propagation than the non-glycosylated variants in BHK and QT6 cells, but no difference was observed between the glycosylated and non-glycosylated variants in C6/36 cells. The heat stable propagation of the glycosylated variant of NY strain was confirmed with the viruses generated by the infecrious cDNA methodology. These results suggest that the E protein glycosylation may be a basic requirement for efficient transmission of WN virus from avian hosts to mosquito vectors.

O-8 Construction of a full length infectious cDNA clone For Omsk hemorrhagic fever virus

Kentaro Yoshii

Lab. of Public Health, Dept. of Environmental Veterinary Science, Graduate School of Veterinary Medicine, Hokkaido University

Omsk hemorrhagic fever virus (OHFV) is a member of the tick-borne encephalitis (TBE) serocomplex of flavivirus. Al-

though the TBE complex is largely represented by viruses causing encephalitis, OHFV are known to cause hemorrhagic disease. However, the specific viral and host response mechanisms involved in OHFV pathogenesis are not well understood.

To investigate the molecular mechanisms involved in OHFV pathogenesis, we set out to construct a full length infectious cDNA clone for OHFV. PCR-amplified fragments of OHFV strain Guriev cDNA were readily assembled into a subgenomic cDNA that could be used to produce replicon RNAs containing large deletions in the structural region. OHFV proteins were produced in replicon-transfected cells. Reporter genes were inserted into OHFV replicons and they were successfully expressed. By *trans*-expression of OHFV structural proteins, replicon RNAs were packaged into single-round infectious VLPs. A genome length cDNA (OHF-IC) was constructed by assembling the subgenomic replicon cDNA and cDNA encoding the structural region. Virus recovered infectious clone exibited biological characteristics similar to those of the parental Guriev strain. These infectious cDNA clone and subgenomic replicons canbe useful useful platfoms for investigating molecular mechanism basis of OHFV pathogenicity.

Oral Presentation Day 2: Aug. 20, 2009

O-9 Cross-protective efficacy of Vesicular-Stomatitis-Virus-based Ebola vaccines

Andrea Marzi
Visiting fellow, Laboratory of Virology
Rocky Mountain Laboratories, NIAID, NIH

Members of the family *Filoviridae*, Ebola virus (EBOV) and Marburg virus, cause severe hemorrhagic fever in humans with lethality rates up to 90%. Outbreaks of filovirus hemorrhagic fever mostly occur in Africa, but globalization and worldwide traveling have led to the importation of single cases into Europe and America. In addition, filoviruses are classified as class A bioterrorism agents. Currently there is no approved vaccine or therapeutic available to protect against infection.

Four different species are known for EBOV: *Zaire*, *Sudan*, *Cote d'Ivoire*, and *Reston ebolavirus* – the recently discovered *Bundibugyo ebolavirus* is proposed as a fifth species. The goal of this study was to determine whether a Vesicular-Stomatitis-Virus (VSV)-based vaccine expressing the respective EBOV glycoprotein (EBOV-GP) of the different EBOV species could protect rodents against a lethal challenge with *Zaire ebolavirus*. In mice, vaccination with the Sudan, Cote d'Ivoire and Reston EBOV-GP expressing VSV vaccines demonstrated cross-protection against lethal *Zaire ebolavirus* infection. In contrast, guinea pigs could only be protected against *Zaire ebolavirus* infection when vaccinated with a VSV vaccine expressing the homologous, but not a heterologous, EBOV-GP.

In additional experiments, guinea pigs infected with wild-type *Zaire*, *Sudan*, *Cote d'Ivoire*, and *Reston ebolavirus* showed no signs of illness and were subsequently challenged after 21 days with a lethal dose of guinea pig-adapted *Zaire ebolavirus*. All animals were fully protected against lethal disease independent on the source of the initial infection.
These data suggest that cross-protection against multiple EBOV species is possible, however the EBOV-GP alone does not seem to be sufficient in inducing protective immunity against lethal *Zaire ebolavirus* challenge. We are currently in the process of defining additional EBOV proteins responsible for cross-protective immunity.

O-10 Different efficiency of C-type lectin-mediated entry between Marburg virus strains

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Filoviruses cause lethal hemorrhagic disease in human and nonhuman primates. The viral surface glycoprotein (GP) is the only spike protein of the virion and responsible for virus entry into cells. It has been shown that GP interacts with cellular C-type lectins for virus attachment to the surface of the cells. Since primary target cells of filoviruses, such as macrophages, dendritic cells, and hepatocytes, express C-type lectins, C-type lectin-mediated entry is thought to be a possible determinant of virus tropism and pathogenesis.

VSV pseudotyped with Angola GP (VSV-Angola) infected more efficiently K562 cells expressing the C-type lectins, hMGL or DC-SIGN, than VSV pseudotyped with Musoke GP (VSV-Musoke). Unexpectedly, there was no strong evidence to indicate that the binding affinity of the C-type lectins to GPs correlated the different efficiency of C-type lectin-mediated entry.

Middle one third of GP molecule include the mucin-like region (MLR) which has a large number of carbohydrate chains and principally interacts with C-type lectins. MLR was essential for C-type lectin-mediated entry but it was not important for the difference between MARV strains. Mutagenesis identified the amino acid at position 547 which converted the efficiency of C-type lectin-mediated entry. A three dimensional model of Angola GP revealed that this amino acid is in close proximity to the putative site of cysteine protease cathepsin processing.

Interestingly, inhibitors of cysteine protease cathepsin B and L reduced infectivity of VSV-Angola less efficiently than that of VSV-Musoke in C-type lectin expressing cells while only a limited difference was found in control K562 cells. We finally found that the amino acid at position 547 was critical for the different effects of the inhibitors in the virus infectivities. These results suggest that the efficiency of C-type lectin-mediated entry of filovirus is controlled not only by binding affinity between the carbohydrates on GP and C-type lectins but also by GP susceptibility to proteolytic processing most like by cathepsins during internalization of the virus accompanied with C-type lectins.

O-11 Replication deficient Adenovirus vectors expressing Andes virus nucleoprotein or individual glycoproteins protect hamsters from lethal hantavirus infection

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Hantaviruses (genus hantavirus, family bunyaviridae) are a closely related group of rodent-borne viruses which are of significant medical importance worldwide. Clinically, hantaviruses are responsible for two syndromes in humans; hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). In North and South America, HPS is a rare but often fatal disease for which no licensed vaccines or therapeutics currently exists. The long-term goal for the prevention of hantavirus disease in humans is the development of effective vaccines capable of inducing complete, sterile immunity in naïve individuals. The purpose of this study was to develop and evaluate recombinant Adenovirus (Ad) vectors as potential vaccine candidates against Andes virus (ANDV), a highly pathogenic South American hantavirus, and to analyze the protective immune response associated with individual structural proteins. We constructed replication deficient Ad vectors which express ANDV nucleocapsid protein (AdN) or glycoproteins (AdG_N and AdG_C). These vectors were tested for the ability to induce a protective immune response against ANDV challenge in the lethal Syrian hamster model of HPS. Vaccination by all three Ad vectors, individually or in combination, elicited a robust immune response that protected hamsters from lethal infections without boosting. Immunized hamsters did not show any obvious clinical signs of disease following ANDV challenge however; only those which were co-immunized with AdG_N and AdG_C were completely protected against ANDV infection, as demonstrated by a lack of detectable ANDV RNA in tissue samples collected at 6 and 9 days post-infection and relatively static antibody titers post-challenge. Based on low titers of neutralizing serum antibodies post-immunization, the protective immune response associated with the Ad vectors was likely due to a potent cellular immune response. These recombinant Ad vectors represent the first vaccines to prevent lethal hantavirus disease in the hamster model of HPS. The Ad vectors are useful tools for dissecting the protective immune response associated with hantavirus disease and warrant further investigation as vaccine candidates for HPS and HFRS.

O-12 *Hantavirus* genome quantification in experimentally infected laboratory rats and naturally infected wild rats (*Rattus norvegicus*)

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Seoul virus (SEOV), a species in the viral genus *Hantavirus*, is a causative agent of hemorrhagic fever with renal syndrome (HFRS). The natural reservoir of SEOV is the Norway rat (*Rattus norvegicus*) and it is transmitted horizontally from persistently infected rats to uninfected ones. However, the details of this in nature are still unclear.

To study the ecology of SEOV in rodent colonies in more detail, we first examined laboratory rats (WKAH strain, female, six weeks) that were experimentally infected with SEOV strain SR-11 ($6.0x10^4$ ffu/rat, intra-peritoneal route). The fluctuations in the IgM and IgG antibody titers to SEOV were measured using enzyme-linked immunosorbent assays (ELISA) and the virus genome in the lungs was examined using a real-time PCR method. The amount of the genome was expressed relative to the amount of GAPDH mRNA. IgM antibody appeared 6 days post infection (dpi) and IgG antibody appeared 9 dpi. The IgM antibody titers began to decrease 13 dpi, whereas the IgG antibody titer increased until 16 dpi. The virus genome peaked at 6 dpi.

Wild rats were captured in Saigon Harbor and Ho Chi Minh City, Vietnam. Forty-five rats that were SEOV antibody positive in a preliminary examination were re-examined using several diagnosis methods. Ultimately, 21 were determined seropositive (10 rats, anti-SEOV IgM and IgG positive; 11 rats, IgG positive). The relative virus genomes in the lungs of some of the rats examined were 100~1000 times higher than in experimentally infected rats. The amount of virus genome tended to be correlated to the IgM antibody titer. These results confirm the persistence of *Hantavirus* in rodents in nature. Further studies examining the relationship between age and infection are needed to understand the mode of transmis-

sion in rodent colonies.

O-13 Identification and characterization of WU polyomavirus, a novel virus isolated from a patient with acute respiratory tract infection

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WU Polyomavirus is a novel human polyomavirus initially detected in a nasopharyngeal aspirate from a three year old child diagnosed with pneumonia. A random library was generated from nucleic acids extracted from the nasopharyngeal aspirate and analyzed by high throughput DNA sequencing. Sequences from this sample had only limited homology 35-50% to known members of *Polyomaviridae*. We subsequently sequenced the entire genome of 5229bp which revealed features characteristic of *Polyomaviridae* but phylogenetic analysis revealed that it is a novel member. Screening respiratory specimens from 4772 patients with acute respiratory tract infections in Brisbane, Australia (location of original case) and St. Louis, USA using WU virus specific primers yielded an overall WU prevalence rate of 2.4%. The presence of multiple instances of this virus in two continents and over a period of several years suggests that the virus is widespread in the human population and raises the possibility that WU virus may be a human pathogen. To begin to address this question a full length genomic clone has been generated to investigate the tissue tropism and replication properties of WU polyomavirus *in vitro.*

O-14 Viroporin activity of JCV agnoprotein

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Most non-enveloped viruses exit their host cells following cell lysis, which involves breakdown of the cell membrane and which presumably results from increased plasma membrane permeability. JC virus (JCV) the causative agent of Progressive Multifocal Leukoencephalopathy belongs to the family of Polyomaviruses, which have non-enveloped virions. It has been suggested that extracellular release of the progeny virions of Polyomaviruses occurs when cells disintegrate or rupture; however, the molecular mechanism(s) employed by JCV to induce cell lysis and facilitate virion release remain elusive.

Viroporins are a group of proteins that participate in the promotion of release of viral particles from cells, and interact with cellular membranes modifying permeability. These proteins are not essential for the replication of viruses, but their presence enhances virus growth.

The genome of JCV encodes six major proteins including agnoprotein. Previous studies from our and other laboratories indicated that the JCV small auxiliary protein, agnoprotein, plays an important, though not fully understood, role in the propagation of JCV.

Here, we demonstrate that the JCV small integral membrane agnoprotein acts as a "viroporin" that is small viral polypeptides, interacts with cell membranes, and increases their permeability to ions and other low-molecular-weight compounds.

O-15 Towards imaging prion replication and spread

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Transmission of prion infections involves the spread of disease-associated prion protein (PrPres) within and between cells and tissues. Among mammalian protein misfolding diseases, only prion diseases are recognized as being transmissible between hosts under natural conditions. This may in part be attributed to enhanced spread of PrPres between cells relative to other pathological protein aggregates, thereby focusing attention on cellular mechanisms mediating the initiation, propagation, and intercellular spread of PrPres. To determine the mechanisms by which prions replicate and spread intercellularly, we developed IDEAL-labeling, a novel technique that allows specific fluorescent labeling of cell-surface proteins containing the tetracysteine (TC) tag. IDEAL-labeling has been used to detect fluorescent PrPres formation in living cells, providing the tools necessary for the first experiments aimed at imaging the propagation and intercellular spread of prions. Related studies aim to identify factors that contribute to the enhanced transmissibility of prion diseases relative to other protein misfolding diseases, focusing on the role of glycosylphosphatidylinositol (GPI)-anchored membrane association. To test whether GPI-anchoring can modulate the propagation and spread of protein aggregation, amyloid-forming domains from various proteins, such as Sup35NM, have been expressed as GPI-anchored fluorescent protein fusions in neuronal cells. GPI-anchored Sup35NM exhibits GPI anchor-dependent, inducible, self-propagating, prion-like aggregation that can spread between cells as visualized by live-cell imaging. Our data show GPI-anchoring facilitates the propagation and intercellular spread of protein aggregation and thus may enhance the transmissibility and pathogenesis of prion diseases relative to other protein misfolding diseases.

O-16 Effect of transplantation of bone marrow-derived mesenchymal stem cells on mice infected with prions.

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Backgrounds: Prion diseases are fatal neurodegenerative disorders of humans and animals, and no treatment is avail-

able so far. Bone marrow-derived mesenchymal stem cells (MSCs) have been reported to migrate to brain lesions in experimental models of neurodegenerative diseases, and to ameliorate functional deficits. In this study, we attempted to evaluate the therapeutic potential of MSCs for treating prion diseases.

Methods: Human MSCs (hMSCs) that stably expressed *LacZ* gene were used. The hMSCs were transplanted into the unilateral hippocampus or thalamus of mock- or prion-infected mice or via intravenous injection. Cryosections of the mice brains were prepared and the distributions of hMSCs were monitored by the expression of b-galactosidase. To investigate the mechanisms involved in the migration of hMSCs, their migration to the brain extracts was analyzed using a QCM^{TM} 96-well cell migration assay kit.

Results and Discussion: In mice infected with prions, hMSCs transplanted at 120 days post-inoculation were detected in the contralateral side at 2 days after transplantation and the cells existed there even at 3 weeks after transplantation. In contrast, hMSCs were hardly detected in the contralateral side of mock-infected mice. The hMSCs also migrated to brain lesions of prion-infected mice even via intravenous injection but not to brains of mock-infected mice. Therefore, hMSCs appeared to migrate by responding certain pathological changes caused by prion propagation, and their migration was correlated with the severity of neuropathological lesions including disease-specific prion protein deposition. The results in *in vitro* migration assay showed that several chemokines such as CCL3~5, 17 and CX3CL1 and their receptors, CCR4 and 5, and CX3CR1, respectively, are involved in the migration of hMSCs. Intra-hippocampal and intravenous transplantation of hMSCs prolonged the survival of mice infected with prions when hMSCs were transplanted at 90 or 120 dpi, although the effects were modest. A subpopulation of hMSCs transplanted in the brains of prion-infected mice produced various trophic factors such as BDNF, NT-3/4 and VEGF, and differentiated into cells of neuronal and glial lineages. Indeed, at the terminal stage, spongiform changes in the thalamus of mice transplanted with hMSCs appeared milder than those of non-transplanted control. These results suggest that MSCs have promise as a cellular vehicle for delivery of therapeutic genes to brain lesions associated with prion diseases, and furthermore, may help to protect the neuronal tissues from degeneration caused by prion propagation.

O-17 Progress towards an understanding of the broad host-range and pathogenesis of Henipaviruses

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Hendra and Nipah viruses are highly pathogenic paramyxoviruses that have recently emerged from flying foxes and have been classified within their own genus, Henipavirus. Both viruses have caused serious disease outbreaks in humans and livestock in Australia, Malaysia, Singapore and Bangladesh. They are distinguished from other paramyxoviruses by their unique genetic constitution, high virulence and wide host range. These features have resulted in them being designated as Biosafety Level 4 pathogens. Recent work within the laboratory has been focused at understanding why these viruses are able to infect a large range of host species. The factors which influence the ability of zoonotic viruses to effectively cross the species barrier from bats to other animal populations are poorly understood. Receptor specificity and evasion of host immune response are thought to be major determinants of host-range, tissue tropism and pathogenesis. To determine whether host-receptor molecules play a role in species-specific infection, cell surface receptors from a range of species were tested with no significant differences seen in receptor function from different species.

Hendra and Nipah viruses have both been demonstrated to encode proteins that are able to block the innate immune response in infected cells. Reverse genetics for these viruses will allow for the manipulation of these proteins in the context of a full infectious virus. Deletion and or mutation of these viral encoded proteins will allow for the role of individual proteins in different cell types or animal models to be assessed. A better understanding of both host range and pathogencity determinants of these emerging human and animal pathogens is essential for the development of effective vaccines and therapeutic countermeasures.

O-18 Suppression of rabies virus propagation in mice brain by intracerebral immunization of inactivated virus

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【Introduction】

Rabies is one of the classical viral zoonoses and lethal in many mammals including humans. The virus replicates in the central nervous system (CNS) and no effective treatment is available in rabid animals so far. The object of this study is to examine the efficacy of intracerebral (IC) immunization against for rabies virus propagation in mice brain.

【Materials and methods】

Mice were immunized with inactivated rabies viruses via subcutaneous (SC) or IC route, and lethal doses of live rabies virus, CVS strain, was inoculated into the brain of immunized mice. Clinical signs and body weights of mice were recorded. Measurement of antibody titers in blood, protein expression and histopathological analysis of brain were performed.

【Results】

Progressive paralytic neurological signs were observed in all control mice and 75% of SC immunized mice, whereas only 20% of IC immunized mice. The neutralizing antibody titer in blood plasma was significantly elevated in both SC and IC immunized group. Analysis of whole brain lysate of each mice showed that total immunoglobulin proteins were highly induced in IC immunized mice and they had virus neutralizing abilities. Histopathological examination of brain revealed severe encephalitis and disseminated virus antigens including nerve processes in control mice, but not in IC

immunized mice.

【Discussion】

It is clearly shown that IC immunized mice could induce preventive immune-response against intracerebrally inoculated rabies virus. Both systemic and intracerebral immune-responses are now under investigating which contribute to suppressing the virus propagation in mice CNS.

O-19 Virus specific T cells generated in the presence of calcineurin-inhibitor FK506 cause lethal disease: Implications for transplantation

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Lymphocytic choriomeningitis virus (LCMV) infection in humans typically causes a subclinical or mild self-limiting febrile disease with some individuals experiencing aseptic meningitis. However, recently reported cases of LCMV infection in transplant recipients that were under a calcineurin-inhibitor (FK506) based regimen of immunosuppression exhibited dramatically distinct clinical features from that seen in immunocompetent individuals with a case mortality rate exceeding 90%. Overall 13 patients in four different clusters received allografts that were infected with the virus and all patients developed clinical disease with 12 of them dying.

These findings were highly surprising since LCMV is the classic model of a non-cytolytic virus and raised the interesting question "how does a non-cytolytic virus cause acute lethal disease under condition of immunosuppression?" To address this question, we used the mouse LCMV model and found that similar to what was seen in the transplant recipients, infection of FK506 treated mice with LCMV resulted in a lethal disease characterized by high levels of viremia, lack of seroconversion and minimal lymphocytic infiltrates in the tissues. However, we found that despite the apparent absence of virus specific immune responses, this lethal LCMV disease in FK506 treated mice was T cell mediated. Surprisingly, FK506 did not prevent the proliferation of LCMV specific T cells but dramatically altered their differentiation so that these effector T cells lost their ability to control virus but were still capable of mediating disease. These "pathogenic" T cells orchestrated a cytokine storm characterized by high levels of TNF-α and IL-6 and depletion of T cells or blockade of these inflammatory cytokines prevented the lethal LCMV disease in these FK506 treated mice. Our study shows that T cell mediated viral disease can occur even under conditions of immunosuppression and identifies a strategy for treatment of transplant recipients who have acute complications of viral infection.

Oral Presentation Day 3: Aug. 21, 2009

O-20 The molecular determinants for the pathogenicity of H5N1 influenza A virus

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In 1997 in Hong Kong, highly pathogenic avian H5N1 influenza A virus was transmitted directly from birds to humans and resulted in the deaths of 6 of 18 infected people. In December 2003, another H5N1 avian influenza outbreak occurred in Asia, spread to the Middle East, Europe, and Africa, and resulted in huge economic losses to the poultry industries of the countries affected. To date, about 400 human cases have been reported, with over 250 deaths, raising serious worldwide concern of a catastrophic H5N1 influenza pandemic. Although efficient human-to-human transmission of this virus has yet to occur, the possibility exists. Accordingly, efforts continue to fully reveal the molecular basis for the efficient growth and transmission of the virus in humans.

The PB2 protein of segment 1 is a component of the RNA-dependent RNA polymerase of influenza A virus. This protein has a role not only in virus genome replication, but also in the pathogenicity and adaptation of influenza A virus in animals. Most authentic human influenza A viruses (e.g., H1N1 and H3N2 subtypes) possess the amino acid Lys at position 627 in the PB2 protein, whereas avian isolates generally have Glu at this position. PB2-627Lys has been linked to the high virulence of H5N1 viruses in mice, indicating the importance of Lys at this position for virus replication in mammals.

Here, we present the contribution of amino acid 627 of PB2 to efficient replication of H5N1 influenza viruses in the upper respiratory tracts of mice, and discuss why this residue may be a prerequisite for efficient human-to-human transmission.

O-21 Experimental mouse model of human influenza-associated encephalopathy in childhood

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[Introduction] Influenza virus-associated encephalopathy (IAE) affects mostly in young children, which is characterized by brain edema, disseminated intravascular coagulation (DIC) and multiple organ failure following clinical signs of flu. The pathological mechanisms are still unknown, although hypercytokinemia and clotting disorder have been suspected of contributing factors. Here, we tried to establish mouse model of IAE by using lipopolysaccharide (LPS) as a cytokine inducer.

[Materials and Methods] We inoculated H3N2 subtype influenza A virus (IAV) to 1 week-old ICR mice, and then

LPS twice (IAV+LPS mice). The animals were examined for histopathology, viral isolation by plaque assay, cerebral evans blue-leakage analysis, and plasma cytokine measurement by Cytometric Bead Array assay. Control mice were inoculated with the IAV or LPS only.

[Results] IAV+LPS mice showed decreased survival rate, compared with IAV or LPS mice. Histopathologically, LPS and IAV+LPS mice showed microhemorrhage, neutrophilic infiltration and astrocytic swelling in the brain, and these were more prominent in IAV+LPS mice. In IAV and IAV+LPS mice, virus was detected from the lungs and absent in the brain. Moreover, IAV+LPS mice showed increased cerebrovascular permeability and higher IL-6 and TNF-α.

[Conclusion] Increased cerebrovascular permeability, absence of the virus in the brain and elevated levels of plasma IL-6 and TNF-α in IAV+LPS mice were consistent with the characters of IAE patients. The origin of these cytokines and the cause of increased permeability of blood-brain barrier are now under investigation.

O-22 PB2 protein of a highly pathogenic avian influenza virus strain A/chicken/Yamaguchi/7/2004 (H5N1) determines its replication potential in pigs

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It has been shown that not all but most of the avian influenza viruses replicate in the upper respiratory tract of pigs (H. Kida *et al*., J. Gen. Virol. 75:2183-2188, 1994). It was shown that A/chicken/Yamaguchi/7/2004 (H5N1) [Ck/Yamaguchi/04 (H5N1)] did not replicate in pigs (N. Isoda *et al*., Arch. Virol. 151:1267-1279, 2006). In the present study, the genetic basis for this host range restriction was determined using reassortant viruses generated between Ck/Yamaguchi/04 (H5N1) and A/swine/Hokkaido/2/1981 (H1N1) [Sw/Hokkaido/81 (H1N1)]. Two in vivo-generated single-gene reassortant virus clones of the H5N1 subtype (virus clones 1 and 2), whose PB2 gene was of Sw/Hokkaido/81 (H1N1) origin and whose remaining seven genes were of Ck/Yamaguchi/04 (H5N1) origin, were recovered from the experimentally infected pigs. The replicative potential of virus clones 1 and 2 was further confirmed by using reassortant virus (rg-Ck-Sw/PB2) generated by reverse genetics. Interestingly, the PB2 gene of Ck/Yamaguchi/04 (H5N1) did not restrict the replication of Sw/Hokkaido/81 (H1N1), as determined by using reassortant virus rg-Sw-Ck/PB2. The rg-Sw-Ck/PB2 virus replicated to moderate levels and for a shorter duration than parental Sw/Hokkaido/81 (H1N1). Sequencing of two isolates recovered from the pigs inoculated with rg-Sw-Ck/PB2 revealed either the D256G or the E627K amino acid substitution in the PB2 proteins of the isolates. The D256G and E627K mutations enhanced viral polymerase activity in the mammalian cells, correlating with replication of virus in pigs. These results indicate that the PB2 protein restricts the growth of Ck/Yamaguchi/04 (H5N1) in pigs.

O-23 Characterization of a new mycobacterial species belonging to *Mycobacterium tuberculosis* complex isolated from monkeys in Bangladesh

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Background: *Mycobacterium tuberculosis* complex (MTC) is a group of causative agents for human and animal tuberculosis (TB) including *M. tuberculosis*, *M. bovis* and other closely related species and isolates. Although MTC members are highly similar to each other in DNA level, they differ widely in terms of phenotype, virulence and host tropism. All of the MTCs are considered to be pathogenic for humans, however, epidemiological studies about MTCs other than *M. tuberculosis* and *M. bovis* are rarely performed. Since horizontal gene transfer between bacterial cells has not been observed in MTC, chromosomal deletions and point mutations occurred in an ancestral cell are assumed to be fixed in progeny cells. These lacked regions, called regions of difference (RD), can be a good marker for the positioning of a strain on the phylogenetic tree of the MTC species. In current study, we performed a species identification analysis on MTC isolates derived from monkeys in Bangladesh using RDs and other genetic markers.

Methods: Samples: MTC isolates were obtained from lung TB lesions of two monkeys (*Rhesus Macaque*) dead in a zoo in Dhaka, Bangladesh.

Genetic analysis: Following genetic analyses were performed with the extracted DNA from the isolates; 1) RD detection PCRs, 2) SNP (single nucleotide polymorphism) analysis, 3) Spoligotyping.

Results: The isolates lacked RD9 and RD12, and possessed RD1 and RD4, suggesting they are to be *M. caprae*. However, the results of SNP analyses of *gyrB*, *mmpL6*, TbD1 and *PPE55*, accompanied by a specific spoligotyping pattern indicated that the isolates should be on the branch of "oryx bacillus" in the MTC phylogenetic tree. Two isolates showed same genetic characteristics.

Discussion: The isolates derived from monkeys in Dhaka were revealed to be mycobacteria closely related to the oryx bacillus according to a species segregation diagram drawn by Huard *et al.* (2006). The oryx bacillus strains were retrieved from two captured oryxes and determined to be in an exclusive phylogenetic position within the MTC by their distinctive genetic features. However, the oryx bacillus has not been registered as a new MTC species because of the small number of investigated isolates. In 2007, authors reported that four cattle suffering from lung TB in a farm in Dhaka were infected with a MTC having the same *gyrB* SNP and spoligotype as the oryx bacillus. A strain that had similar genetic characteristics was also isolated from a human in the Netherlands. This indicates the possibility of human infection with the oryx bacillus.

The monkey isolate, possibly the oryx bacillus, seems to have a wide host range. Furthermore, lung TB of the victims suggests that the bacterium might be an airborne infectious agent. To prevent human infection, continuous monitoring of animal TB should be needed in Bangladesh and surrounding areas.

O-24 The unique strategy of viral proteins for robustness and evolvability

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Natural selection shapes the sequence, structure, and biophysical properties of proteins to fit their environment. We hypothesize that highly thermostable proteins and viral proteins represent two opposing adaptation strategies. As demonstrated by proteins from thermophilic organisms, thermostable proteins are highly compact, and posses well-packed hydrophobic cores and intensely charged surfaces. By contrast, viral proteins, and RNA viral proteins in particular, display a high occurrence of disordered segments and loosely-packed cores. These features might endow viral proteins with increased structural flexibility and effective ways of interacting with the host's components. They could also be related to higher adaptability and the unusually high mutation rates observed in viruses, and thus represent a unique strategy for dealing with the deleterious effects of mutations, such that those who have little (interactions), have little to lose.

O-25 Prediction of *N*-glycosylation potential of influenza virus hemagglutinin:a bioinformatics approach

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The addition of carbohydrates to influenza virus hemagglutinin (HA) is believed to facilitate viral escape from neutralizing antibodies. It is well known that the number of carbohydrates in HA, especially in the globular head region, changes during circulation of the viruses in the human population. Retrospective sequence analysis confirmed a gradual increase in the number of *N*-linked glycosylation sequons (Asn-Xaa-Ser/Thr, where Xaa is any amino acid except Pro) in the amino acid sequences of human H3N2 viruses isolated from 1968 to 2006. We then analyzed the potential candidate codons that were not sequons, but able to become sequons with 1-3 nucleotide mutations (i.e., a set of three codons that required single, double, or triple nucleotide substitutions to produce sequons). All of the sites that acquired *N*-glycosylation sequons during the past 38 years were observed in these candidate codons in HA of the prototype strain, A/Hong Kong/1/68. These results suggest that the genetically destined potential for *N*-glycosylation in H3 HA could have been one of the key factors prerequisite for this virus to continuously circulate in the human population.

To determine the implicit potentials for acquisition of *N*-linked glycosylation, we analyzed the genetic background of 16 subtypes of avian influenza virus, some of which may be potential pandemic viruses in the future. We found a significant difference among HA subtypes in their genomic sequences to produce *N*-glycosylation sites. Notably, recently circulating avian influenza viruses of the H5 and H9 subtypes may have rather greater capacities to undergo mutations associated with glycosylation of HA than past pandemic viruses. We hypothesize that influenza viruses maintained in natural reservoirs could have different potentials for sustained circulation, depending on their HA subtypes, if introduced into the human population.

Memo

P-1 **Identification and molecular characterization of a novel stage-specific surface protein of *Trypanosoma congolense* epimastigotes**
Tatsuya Sakurai (Lab. of Parasitology, Dept. of Disease Control, Graduate School of Veterinary Medicine)

P-2 **Prevalence and Source of Trypanosome Infections in Field-Captured Vector Flies (*Glossina pallidipes*) in South-eastern Zambia**
Hirohisa Mekata (Lab. of Infectious Diseases, Dept. of Disease Control, Graduate School of Veterinary Medicine)

P-3 **Immunopathological and chemotherapeutic studies on Leishmaniasis**
Saruda Tiwananthagorn (Lab. of Parasitology, Dept. of Disease Control, Graduate School of Veterinary Medicine)

P-4 **High resolution melting analysis of sequence variation in EMA-1 gene and 18S rRNA gene within *Theileria equi* and differentiation from *Babesia caballi***
Bashir Salim (Dept. of Collaboration and Education, Research Center for Zoonosis Control)

P-5 **Molecular epidemiology of *Theileria Orientalis* and the emergence of new strains in Australia**
Joseph Kamau (Lab.of Protozology, Dept.of Collaboration and Education, Center for Zoonosis Control)

P-6 **Study for gene function analysis on larval stage of *Echinococcus multilocularis***
Chiaki Mizukami (Lab. of Parasitology, Dept. of Disease Control, Graduate School of Veterinary Medicine)

P-7 ***Echinococcus multilocularis* Tetraspanin Proteins Protect Mice against Primary Alveolar Echinococcosis**
Zhisheng Dang (Lab. of Protozoology, Dept. of Education and Collaboration, Research Center for Zoonosis Control)

P-8 **Development of molecular diagnostic tools for canine cestodiosis**
Maria Teresa Armua Fernandez (Lab.of Parasitology, Dept.of Disease Control, Graduate School of Veterinary Medicine)

P-9 **Developmental mechanisms of distinct sexual dimorphismin the ponerine ant *Diacamma* sp. (Hymenoptera: Formicidae)**
Satoshi Miyazaki (Lab. of Parasitology, Dept. of Disease Control, Graduate School of Veterinary Medicine)

P-10 **Does H9N2 avian influenza virus acquire high pathogenicity for chicken?**
Kosuke Soda (Lab. of Microbiology, Dept. of Disease Control, Graduate School of Veterinary Medicine)

P-11 **Factors responsible for susceptibility of pigs to infection with a highly pathogenic avian influenza virus, A/chicken/Yamaguchi/7/04 (H5N1)**
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P-1 Identification and molecular characterization of a novel stage-specific surface protein of *Trypanosoma congolense* epimastigotes

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The cattle pathogen *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 (EMF) life cycle stage of *T. congolense*. Culture supernatants of EMF but not of procyclic forms (PCFs) promoted adhesion of PCF parasites in an *in vitro* assay. Biosynthetic labeling experiments showed that these EMF culture supernatants contained a 100 kDa trypanosome-derived protein that was not present in supernatants from PCF. We named this molecule "*congolense* epimastigote-specific protein" (CESP). The gene encoding CESP was isolated from an EMF cDNA library after immunoscreening. The multicopy gene had a 2070-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 may be explained partially by glycosylation of the molecule which has six potential *N*-glycosylation sites and a predicted GPI anchor. Indeed, metabolic labeling of CESP with [^{3}H] ethanolamine revealed that CESP was a GPI-anchored protein. Confocal laser scanning microscopy showed that CESP was expressed only on the surface of the EMF stage of the parasite. The identification of CESP as a unique component of culture supernatants from EMF and that such supernatants can confer plastic-adhesive ability on PCF suggest that CESP is worth further investigation as an adhesion molecule that perhaps allows *T. congolense* EMF to adhere to the tsetse proboscis.

P-2 Prevalence and Source of Trypanosome Infections in Field-Captured Vector Flies (*Glossina pallidipes*) in Southeastern Zambia

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The prevalence of trypanosome infections in tsetse flies, *Glossina pallidipes*, collected from Chiawa and Chakwenga in Zambia with endemic trypanosomosis was assessed by polymerase chain reaction (PCR). Out of the 550 *G. pallidipes*, 163 (29.6%) flies were found to harbor trypanosome DNA. Infection rates of tsetse flies with *Trypanosoma brucei, Trypanosoma vivax* and *Trypanosoma congolense* were 25.2% (139/550), 4.2% (23/550) and 6.9% (38/550), respectively. To determine the mammalian hosts of *T. brucei*, *T. congolense* and *T. vivax* infections from the tsetse flies, mammalian mitochondrion DNA of blood meal in these flies were analyzed by PCR and subsequent sequence analysis of the amplicons. Sequence analysis showed the presence of cytochrome b gene (*cyt b*) of 9 different mammalian species such as human, elephant, buffalo, goat, warthog, greater kudu, waterbuck, roan antelope and cattle. Goats which were main livestock in these areas were further examined to know the extent of its contribution in spreading the infection. The prevalence of trypanosome infections was examined in the domestic goat population in 6 settlements in Chiawa. Of the 86 goats sampled, 35 (40.6%), 4 (4.6%) and 11 (12.7%) were positive for *T. brucei*, *T. vivax* and *T. congolense*, respectively. These findings showed that the host-source of trypanosome infections in vector fly give an important information about the spread of the infection. The result of this study will certainly contribute to the epidemiology of trypanosomosis.

P-3 Immunopathological and chemotherapeutic studies on Leishmaniasis

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Chemotherapeutic study

Visceral Leishmaniasis (VL) is the most dangerous of three manifestations of leishmaniasis because the parasite migrates into the vital organs. VL is caused by *Leishmania donovani* complex Drug treatment today is restricted to a limited number of clinically useful drugs associated with the side-effects and increasing ineffectiveness. Alternative chemotherapeutic agents with cheaper, better activity and fewer side-effects are needed. One strategy for discovery of new drugs is to exploit medicinal plants used in traditional therapy. Quassinoids, extracts from *Brucea javanica*, are the degraded triterpenes which their C-20 types have been exhibited antibabesial and antitrypanosomal activities in our preceding studies. In this study, *in vitro* activities of free and liposome preparations of bruceine B, C and D against promastigotes of *L. donovani* have been evaluated. IC_{50} of amphotericin B as the standard drug was 0.07 μM. Only bruceine B and D revealed antileishmanial activities. The liposome preparation of bruceine B and D showed stronger antileishmanial activities (IC_{50} as 16.28 and 26.69 μM, respectively) than the free forms (IC_{50} as 27.37 and 32.92 μM, respectively).

Immunopathological study

Experimental VL in mice results in development of organ

specific immunity in the two main target tissues, liver and spleen. Liver is the site of an acute resolving infection associated with development of inflammatory granulomas around the infected Kupffer cells, and resistance to reinfection. On the other hand, spleen is an initial site for generation of cell mediated immune responses, but ultimately becomes a site of parasite persistence with associated immunopathological changes which contribute to the mmunocompromized status of host. In the present study, influence of secondary lymphoid tissues on host response to *L. donovani* infection will be investigated, especially for the mechanisms of the dissemination of parasites from the visceral organs to the skin. The parasitic burden in the peripheral blood, liver, spleen and other organs will be primarily evaluated in the alymphoplasia (*aly/aly*) mice. In addition, macroscopic and microscopic pathological changes and cytokine and antibody profile in sera will be successively examined.

P-4 High resolution melting analysis of sequence variation in EMA-1 gene and 18S rRNA gene within *Theileria equi* and differentiation from *Babesia caballi*

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A molecular epidemiological investigation was conducted in different geographical locations in Sudan on *Theileria equi* and *Babesia caballi* in horses, with a view to identifying strain differences among *T. equi* and differences between *T. equi* and *B. caballi*.

Twenty positive samples, out of 127 samples tested were further analyzed by high resolution melting, targeting a polymorphic region of the *EMA-1* gene. Samples were rapidly genotyped and single nucleotide polymorphisms (SNPs) were determined for *T. equi*. In addition, HRM analysis, targeting a region of the 18S rRNA gene, was carried out to differentiate between *T. equi* and *B. caballi*. Distinct melting curves, normalized curves and derivative plots showed distinct differences within *T. equi* isolates and between *T. equi* and *B. caballi*. Samples were subsequently sequenced to confirm the sequence heterogeneity, and the location of SNPs was identified.

Direct sequencing of twenty positive samples targeting the hypervariable region of the 18S rRNA gene revealed eight *T. equi* genotypes distinct from South African and Spanish isolates and only two genotypes were similar. Alignments demonstrated extensive sequence variation in the hypervariable region of the 18S rRNA gene within Sudanese *T. equi* isolates.

P-5 Molecular epidemiology of *Theileria Orientalis* and the emergence of new strains in Australia

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Theileriosis is usually considered to be a common, but, usually benign infection of cattle in many parts of Australia. However, in recent years, a number of reports have been received of a condition in cattle near the coast of the state of New South Wales (NSW) and Queensland (QLD) characterized by severe anaemia, heavy Theileria infections and sometimes death. In the present study, we carried out Theileria parasite detection using molecular methods. The genes encoding major piroplasm surface protein (MPSP) and rRNA were set for the molecular targets of highly sensitive polymerase chain reaction (PCR) detection. The finding reveals that the individual animals are either co-infected with two or all the three different genotypes of Benign Theileria orientalis (*T. buffeli* and *T. Chitose*), including *T. Ikeda* type which previously has not been reported to occur in Australia. Though errors due to PCR may have contributed, samples number 15 of individual animal from Gurya (NSW) share sequences similar to both Chitose and Ikeda-type genotype suggesting exchange of genetic material and possibility of a similar vector in parasite transmission.

P-6 Study for gene function analysis on larval stage of *Echinococcus multilocularis*

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Alveolar echinococcosis (AE) is a zoonotic parasitic disease caused by the active growth of the larval stage of the canid-tapeworm *Echinococcus multilocularis*. AE is attributed to tumor-like invasive growth of the larval parasite mainly in the liver of intermediate host such as small rodents or humans. Moreover, it is considered to be the most lethal helminthic disease of humans and is usually fatal if the infected patient does not receive appropriate treatment.

The present study is aimed to analyze the key genes on larval stage proliferation, differentiation and host-parasite interaction. For the parasites, to live inside of the host means to live just beside of host cells, in the condition filled with factors these cells releasing and to be exposed to host immune-system every time. The analysis of these genes is important to know the strategies how this parasite lives and survives through this environment, for example, by using these factors from the host as nutrients or useful signals to proliferate or differentiate, by controlling host immune-system with other factors which parasite-itself releasing and by having a suitable metabolic system to live in anaerobic condition, and so on. The knowledge from the analysis will be important to understand which gene affect strongly on pathological condition and would be useful to find target genes for treatment of AE.

cDNA library of larval stage of *E. multilocularis* was primarily established using vector-capping method and analysis of it by *in silico* comparing with genes of adult worm of *E. multilocularis* and close-related helminthes has been in progress. In addition, trials for gene suppression by RNA interference on the larval stage parasite such as protoscolex and primary cell, and immature adult have been conducted.

P-7 *Echinococcus multilocularis* Tetraspanin Proteins Protect Mice against Primary Alveolar Echinococcosis

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Echinococcus multilocularis causes an important zoonotic cestode disease, which progresses as a tumor-like proliferation in the liver of intermediate hosts-human and rodents. Tetraspanin (TSP) is a transmembrane protein abundant in the tegument or body wall of parasites of genera *Schistosoma*, *Taenia* and *Echinococcus*. Tetraspanins have been used as a vaccine against schistosomosis and as diagnostic antigen for cysticercosis. In this study, seven tetraspanins of *E. multilocularis*, designated as TSP1-TSP7, were evaluated for their protection potential against primary alveolar echinococcosis. Open reading frames of seven tetraspanins from full-length-enriched cDNA library of *E. multilocularis* metacestodes were cloned and expressed in *Escherichia coli*. Immunoblotting analysis with polyclonal antisera against 7 TSPs indicated that these proteins have very good antigenicity. By Reverse transcription-PCR, abundant existence of tetraspanins' transcripts in oncosphere and adult was detected. By Immunohistochemistry, the localization of these proteins on surface of *Echinococcus* was confirmed. In order to study protective potentials of these recombinant TSPs as vaccine against primary experimental infection in BALB/c mice, mice were infected with 200 eggs and cyst lesions in liver of vaccinated and non-vaccinated mice were counted one month post-infection. The results showed that the protection rates of 7 TSP proteins measured as the percentage reduction in cyst lesions in vaccinated vis-à-vis non-vaccinated mice were: 87.7%, 65.8%, 85.1%, 66.9%, 73.7%, 72.9% and 37.6%. All the results above suggested that these tetraspanins are very important proteins abundantly expressed in different stage of *Echinococcus* and have varying protective effect against primary alveolar echinococcosis, which could be the good candidates to be used in vaccine development.

P-8 Development of molecular diagnostic tools for canine cestodiosis

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Taeniid cestodes are parasites that require two kinds of hosts in order to complete their life cycle: an intermediate host which harbours the larval stage (metacestode), and a definitive host that harbours the adult stage. These parasites infect a variety of mammals, including humans, using them as either intermediate or definitive hosts. A number of them can affect the economy, causing industrial losses through the condemnation of meat and offal; for example, *Taenia ovis* affects muscle of sheep while *Taenia hydatigena* infects abdominal cavities and livers of pigs and sheep. Some of them cause severe illness in humans, such as cystic and alveolar hydatid diseases.

Unlike to many other parasitic infections, taeniid infections in definitive hosts cannot be diagnosed by faecal egg detection techniques, due to the similar morphological characteristics of the eggs among taeniid species (*Echinococcus* spp. and *Taenia* spp.). Nowadays, the advent of molecular techniques including the detection of coproantigens by ELISA (coproELISA) and coproDNA by multiplex PCR or PCR-RFLP, represents promise in achieving an accurate diagnosis. Nevertheless, coproELISA and multiplex PCR have been developed focusing on distinguishing *E. granulosus* and *E. multiloculoaris* from *Taenia* spp. By PCR-RFLP, it is possible to differentiate *Taenia* species but is not very suitable for applying to mass-screening. In epidemiological studies on definitive host, a target animal, a dog, can harbour a most important species of *Echinococcus* and *Taenia.* Therefore, it is necessary to develop a rapid test which can detect and differentiate all taeniid species presented in an infected animal.

Reverse line blot hybridization assay is a technique which enables simultaneous identification of different PCR products. In this technique, specific probes to different pathogens are first applied to parallel lines on a membrane using a mini-blotter. Then the membrane is rotated 90 degrees and set in the same mini-blotter, so that lines of the blotter and of probes cross. When PCR products of samples (i.e. DNA extracted from taeniid eggs) are applied to the lines of a blotter, PCR products hybridize with their corresponding probes located at different lines. Hybridization is visualized by substrate precipitating system using avidin-biotin-peroxidase complex (biotin labelling on probes).

The determination of mitochondrial DNA sequences has been used for the identification of many organisms, including parasites. Sequences of cytochrome oxidase subunit I (COI) and NADH dehydrogenase subunit 1 (ND1) gene are frequently used to study the genetic relationship which exists

between species. According to previous studies, ND1 showed sequential differences amongst *Taenia* species, ranging from 5.9% to 30.8%, thus allowing it to be a strong candidate for this study, and a reliable target in probe design.

Common primers for cestode ND1 sequence were designed. The sequences obtained were compared with the available sequences registered using BLAST search to find homology. The obtained sequences and the published ones were aligned and were used to design specie-specific probes of each taeniid species of interest in regions with the greatest inter-specific differences and without intra-specific polymorphisms. The specificity of all sequences of the probe candidates were predicted by alignment against all nucleotide collection present in the Genebank database. To check specificity and sensibility of probes candidates Dot Blot assay is being performed using known samples.

P-9 Developmental mechanisms of distinct sexual Dimorphism in the ponerine ant *Diacamma* sp. (Hymenoptera: Formicidae)

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Although, in Hymenoptera, both female and male share the same genome, many species exhibit conspicuous sexual dimorphisms. The developmental mechanisms of sexual dimorphism were examined in the ant *Diacamma* sp., which shows the distinct sex differences on body color. We focused the genetic and hormonal regulation.

Their sex differences were derived from whether black pigments (melanins) were produced or not, and insect melanin synthesis is suggested to be regulated by a single genetic pathway. Therefore, five pigmentation gene orthologs, constituting the melanin synthesis pathway, were cloned and their expression levels were estimated by real-time qPCR. The *yellow*, involved in the black DOPA melanin synthesis, was expressed at higher level in females than in males. It was also shown that *pale* and *tan* were differentially expressed depending on sexes. Furthermore, silencing of *yellow* by RNAi impaired the female black pigmentation.

In some insects, the body colors or sex differences were reported to be reguated by juvenile hormone. In order to confirm the JH action against their pigmentation, *Diacamma* sp. was treated with a JH analog (pyriproxyfen). In both sexes, the JH treatment impaired the pigmentation. Moreover, the responses of the five pigmentation genes to the JHA application were examined. Among those, the treatment reduced the *yellow* (coding an enzyme synthesizing black pigment) expression, while increased the *pale* expression (coding an enzyme synthesizing pigment precursors). In addition, for confirming the nature of JH action in the pigmentation processes during the pupal stage, JH titer and the expressions of *Methoprene-tolerant* (*Met*), which is a candidate molecule as a JH receptor, were profiled. Results showed that the JH titer was higher in males than in females, whereas the *Met* expressions were not sexually different.

In conclusion, the sexual color dimorphism is suggested to be regulated through the JH action, which down-regulates the *yellow* expression and black pigment synthesis.

P-10 Does H9N2 avian influenza virus acquire high pathogenicity for chicken?

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【**Objective**】H9N2 avian influenza virus strains have caused ourbreaks in Eurasian countries, giving serious economic losses. Since H9N2 viruses have been isolated not only from domestic birds but from pigs and humans, H9 virus is one of the candidates causing next pandemic in humans. It is presently believed that virus strains with H5 and H7 subtype hemagglutinins (HAs) become highly pathogenic during extensive infection in chicken population. It is known that highly pathogenic avian influenza viruses (HPAIVs) possess HA with insertion of more than a pair of di-basic amino acid (a.a.) residues at the cleavage site, resulting in cleavage into HA1 and HA2 subunits by ubiquitous proteases such as furin and PC6. In the present study, we investigated whether virus with H9HA become HPAIV strain for chicken by mutation to give a pair of di-basic a.a. residues at the cleavage site on the HA molecule.

【**Materials, methods and results**】We generated the mutants of A/chicken/Yokohama/aq-55/2001 (H9N2) (Y55) and A/duck/Hokkaido/Vac-1/2004 (H5N1) which acquired a series of basic a.a residues at the cleavage sites by site-directed-mutagenesis and reverse genetics. The a.a. sequences at the cleavage sites of their HAs are as follows: Y55sub (PARKKR/G), Vac1sub (PRRKKR/G), and Vac1ins (PQRERRKKR/G). These mutant viruses were experimentally inoculated intranasally or intravenously into chickens. There were no difference in pathogenicity between the original virus and mutant strains. However, all of the mutant viruses acquired high pathogenicity after 2-10 consecutive passages in air sac inoculation.

【**Discussion**】In the present study, H9 virus acquired high pathogenicity by introducing a series of basic a.a. residues at the HA cleavage site and passaging in chickens. Original Y55 strain was thought to be already well adapted to chickens since it was isolated from chicken meat imported from China. Therefore, artificial mutation in H9HA cleavage site must have been involved in conferring high pathogenicity on Y55. Because only the virus strains with H5 or H7 HAs have been actually observed as HPAIVs strains in nature until now, it is indicated that other subtype HAs have difficulty tolerating mutation at the HA cleavage sites.

P-11 Factors responsible for susceptibility of pigs to infection with a highly pathogenic avian influenza virus, A/chicken/Yamaguchi/7/04 (H5N1)

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[Introduction]
New subtypes of pandemic human type A influenza viruses have emerged three times in twenty century. It was shown that the H3 HA gene of the Hong Kong pandemic strain A/Hong Kong/1968 (H3N2) was of a migratory duck origin and was acquired as a result of reassortment with the precedent human H2N2 influenza virus in pig. It is also assume that the Asian influenza virus (H2N2) and the 1918 Spanish influenza pandemic strain has emerged similarly. These things indicate that pigs can support the growth of both avian and human influenza viruses and are therefore termed "mixing vessels". In the present study, the analysis of the factor in which it took part in infection in the pig of the H5N1 avian influenza virus with the possibility of appearing as a new influenza virus was tried.
[Methods]
Two pigs were coinoculated intranasally with A/chicken/Yamaguchi/7/04 (H5N1), which does not replicate in pigs, and A/swine/Hokkaido/2/81 (H1N1), which replicate in pigs. The nasal swabs were collected either for 7 days postinoculation (p.i.) from pigs. The infectivity titers in the nasal swabs of pigs were calculated in embryonated chicken eggs by the 50% end-point method and were expressed as EID50/ml of swab. The gene of the viruses collected from the nasal swab were analyzed, and compared with the gene of Ck/Yamaguchi/7/04 (H5N1). In order to assess the polymerase activity, a luciferase reporter gene construct was used.
[Result]
The result of gene analysis, the PB2 protein had aminoacid substitutions of glutamic acid to lysine at position 627 (E627K), glycine to aspartic acid at position 256 (D256G) and histidine to glutamine at position 566 (H566Q) respectively. The D256G, H566Q and E627K mutations enhanced viral polymerase activity in the mammalian cells.
[Discussion]
These findings suggested that the D256G, H566Q and E627K mutations must have played important roles in host adaptation. I want to investigate what role these amino acid substitutions play replication in the pig of the virus.

P-12 Characterization of H3N6 avian influenza virus isolated from a wild white pelican in Zambia

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Current information on avian influenza (AI) viruses circulating in wild birds in Africa is by far very limited compared to other regions such as North America, Europe and Asia. We started AI virus surveillance in wild aquatic birds in Zambia, Central Africa, to better understand the ecology and epidemiology of these viruses in this region. We report the characterization of an influenza virus, isolated from the Great White Pelican (*Pelecanus onocrotalus*). Phylogenetic analyses of all the eight viral RNA segments showed that they were of the Eurasian lineage. The HA, PB2, and NS phylogenies indicated that they were closely related to the H3N8 and H4N8 influenza viruses isolated from wild duck in South Africa in 2004. The NP, PB1, and M genes were closely related to influenza viruses isolated in Europe, particularly those of the H7 viruses found in Italian poultry between 1999 and 2005. The NA gene was closely related to an H4N6 influenza virus isolated in Germany in 2003. Interestingly, the PA gene clustered together with H5N1 highly pathogenic AI viruses isolated from ducks and chickens in China and those recently isolated from whooper swans in Japan. These data indicate that the gene segments appear to have evolved from distinct geographical regions in Europe, Asia and Africa, suggesting reassortment of AI virus genes maintained in the natural hosts whose flyways likely overlap across these continents. The present study underscores the need for continued monitoring of AI viruses in wild migratory birds in Eurasia and Africa.

P-13 Experimental study on antibody-mediated heterosubtypic immunity against influenza virus infection

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Influenza virus has two surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA), and 16 HA and 9 NA subtypes have been identified so far. HA and NA are the targets of antibodies that neutralize virus infectivity. HA is responsible for virus binding to host cell receptor, internalization of the virus, and subsequent membrane fusion. HA is initially synthesized as a precursor polypeptide, HA0, that requires proteolytic cleavage into disulfide-linked HA1 and HA2 to make the virus particle infectious. HA1 is essential for receptor binding and HA2 contains fusion peptide and membrane anchor. It has been known that amino acid sequence of HA2 region is more conserved than that of HA1 among HA subtypes.

It is generally believed that the neutralizing antibodies to HA are not broadly cross-reactive among HA subtypes. But we produced the monoclonal antibody (MAb) which showed a broad reactivity to all HA subtypes in enzyme-linked immunosorbent assay. This antibody (MAb S139/1) showed neutralizing and heamagglutination-inhibiting activities against some strains of H1, H2, H3, H13, and H16 subtypes. For example, this antibody neutralized A/WSN RG/33 (WSN) (H1N1), A/Adachi/2/57 (Adachi) (H2N2), and A/Aichi/2/68 (Aichi) (H3N2), but not A/PR/8/33 (PR8) (H1N1) and A/Memphis/1/96 (H3N2). To investigate the ability of MAb S139/1 to protect mice from influenza virus infection, mice were passively immunized with the antibody and challenged with WSN, PR8, Adachi, or Aichi. Protective efficacy was evaluated by titrating infectious virus in the lung tissues three days after challenge. WSN, Adachi, and Aichi titers in lungs, collected from mice treated with MAb S139/1 were significantly lower than those of control mice. On the other hand, PR8 titers were not significantly different between MAb S139/1-immunized and control mice. These results showed that neutralizing activity of MAb S139/1 ***in vitro*** was correlated with protective efficacy ***in vivo***.

To determine the epitope for MAb S139/1, escape mutants were selected and amino acid sequence of the parent strain and escape mutants were compared. We found the amino acid substitution at position 156, 158, or 193 (H3 numbering) in the mutants. A three dimensional analysis revealed that these three amino acids formed a conformational epitope on the HA1. These results indicate that MAb S139/1 neutralized the viral infectivity by blocking receptor binding of the virus. Although several studies reported some MAb broadly cross-reactive among HA subtypes, these antibodies did not inhibit receptor binding of virus and their epitopes are located in HA2. Thus, MAb S139/1 is a novel antibody which may play a role in heterosubtypic immunity against influenza A virus infection.

P-14 Molecular analyses of pathogenicity of influenza A virus to ducks

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Ducks are a natural host of the influenza A virus, and basically do not show any clinical signs when infected with influenza A viruses; however, some H5N1 highly pathogenic avian influenza viruses isolated after 2002 are lethal to ducks. To reveal the molecular basis of the pathogenicity of influenza A virus to ducks, A/Hong Kong/483/1997 (H5N1) [HK] low pathogenic and A/whooper swan/Mongolia/3/2005 (H5N1) [MON] highly pathogenic to ducks, and genetic reassortant viruses between HK and MON generated by reverse genetics (RG) were inoculated intranasally into ducks.

In experimental infection studies of RG HK and RG MON using 2-week and 4- week old ducks, all 2-week old ducks infected with RG MON died while two of three 4-week ducks survived, and difference in virus titers of organs between RG MON and RG HK was more significant in 2-week old ducks. These results indicate that 2-week old ducks are more susceptible to virus infection and can be used as an appropriate model to compare pathogenicity among the viruses. By inoculation of reassortant viruses into 2-week old ducks, it was revealed that 5 gene segments, PB2, PA, HA, NP and NS, are responsible for the pathogenicity to ducks. The present results showed that the pathogenicity of influenza A virus to ducks correlated with extensive replication in multiple organs. The mechanism how the proteins translated from these 5 gene segments contribute to increased pathogenicity of MON to ducks needs to be further investigated.

P-15 Characterization of monoclonal antibodies to Marburg virus glycoprotein

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Marburg virus (MARV) and Ebola virus, members of the family *Filoviridae,* cause outbreaks of severe hemorrhagic fever in primates. It was demonstrated that antibodies to particular epitopes on the surface glycoprotein (GP) of Ebola virus enhanced the virus infectivity in vitro. To investigate this phenomenon, so called antibody-dependent enhancement, in MARV infection, we produced mouse antisera and monoclonal antibodies by immunization with virus-like particle containing GP of MARV strain, Angola or Musoke. Neutralization and enhancement tests of the antisera and monoclonal antibodies were performed using vesicular stomatitis virus (VSV) pseudotyped with Angola or Musoke GP. We found that the infectivity of VSV pseudotyped with Angola GP in K562 cells was strongly enhanced in the presence of antisera to Angola, while only minimal enhancing activiy was seen in the antisera to Musoke, which is less pathogenetic in primates than Angola strain. In Vero E6 cells, neutralizing antibody was equally detected in these sera. At a clonal level, Angola and Musoke GPs similarly induced neutralizing monoclonal antibodies, while Angola GP induced larger population of enhancing antibody clones than Musoke GP. Our findings suggest that antibody-dependent enhancement of infectivity may partially account for the different pathogenicity between Angola and Musoke strains. We will determine both neutralizing and enhancing epitopes on GP molecules to fully clarify the conformational basis of antibody-dependent enhancement of MARV infection.

P-16 Elucidation of the inhibitory mechanisms for influenza A virus propagation by TNF-alpha

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Some of the zoonoses including influenza which severely damage to animals as well as human are caused by viral infection. To clarify the host antiviral response is important for the prevention and treatment for disease by viral infection. Recently, it is reported that tumour necrosis factor-alpha (TNF-α) which is a pleiotropic cytokine is induced by several virus infection, and can inhibit propagation of vesicular stomatitis virus, hantavirus, and influenza A virus (IFV). TNF -α stimulation activates several intracellular signalling pathways mediated by c-Jun-N-terminal kinase (JNK), nuclear factor-kappaB (NF-κB), and caspases resulting in the regulation of cytokine production or apoptosis induction. However, the detail mechanisms for antiviral effects of TNF-α are not clear.

To clarify the effects of TNF-α on IFV propagation, human lung carcinoma cell line A549 cells were treated with TNF-α for 24 h and then infected with IFV (H1N1, A/Puerto-Rico/8/34). IFV propagation was analyzed by plaque assay and it is observed that TNF-α pretreatment inhibited IFV propagation. Moreover, qPCR method for the detection of vRNA synthesis revealed that TNF-α played antiviral effect on the step of vRNA synthesis in viral life cycle. To clarify the detail of intracellular mechanism for antiviral response mediated by TNF-α, we examined the effects of inhibitors for several intracellular signalling, such as pro-apoptotic pathway, JNK or NF-κB pathway. Blockages for the induction of apoptosis or activation of JNK pathway could not affect on the antiviral effects of TNF-α. On the other hand, inhibitor

for NF-κB pathway completely abolished antiviral effect of TNF-α. Furthermore, constitutive activation of NF-κB signaling pathway markedly inhibited IFV propagation as well as its vRNA synthesis. These data indicate that activation of NF-κB signaling pathway is essential for the antiviral effect of TNF-α. Considering that NF-κB is a transcription factor, antiviral effect of TNF-α should be performed through regulation of gene expression. At present, we are exploring key molecules induced by NF-κB that inhibit IFV propagation.

P-17 PI3K-Akt signal transduction and its roles in influenza virus infection

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Influenza viruses are important pathogens that are contagious and cause pulmonary inflammation and exacerbate chronic lung diseases, because of an infiltration of inflammatory cells and an increased airway hyper-responsiveness.

Upon influenza virus infection of cell, a wide variety of antiviral and virus supportive signaling pathways are induced. Serine threonine kinase Akt is one of the core PI3 K effector which is activated by various various cytokines or growth factors. The PI3K-Akt signaling pathway is one of important signaling pathways that is known to be activated through the interaction of the various viral proteins.

Phosphorylation of Akt plays a central role in modulating diverse downstream signaling pathways associated with cell proliferation, migration, cell cycling, protein synthesis, differentiation, or anti-apoptosis. Hence, genomic mutations or alterations of the PI3K-Akt signaling pathway underlie various human diseases such as cancers, glucose intolerance, schizophrenia, and autoimmune diseases.

Recently, in addition to direct involvement in tumorigenesis by genetic alterations of human cancers, the PI3K-Akt signaling pathways also underlie the clinical manifestation of various stages of viral infection, such as latent infection, chronic infection, and malignant transformation of the Epstein-Barr virus, the Hepatitis C virus, the Hepatitis B virus, or the Human Immunodeficiency Virus (HIV).

We will try to address the role of Akt in influenza virus-infected cell as to how Akt-viral protein interaction possibly regulate the various cellular processes upon influenza viral infection. Modulation of the the PI3K-Akt activity will certainly help to understand various pathological conditions of viral infection for developing theraputic implications.

P-18 Epidemiology of Hantavirus infection in Indonesia

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Hemorrhagic fever with renal syndrome (HFRS) is a rodent-borne zoonotic disease caused by hantavirus infection. The number of HFRS cases is estimated to be about 60,000 annually, of which more than 90% occur in Asian countries. In this study, to clarify the situation of hantavirus infection in Indonesia, we investigated the prevalence of hantavirus antibody in rats captured in Thousand islands of Jakarta province in 2005 by IFA, ELISA and WB. A total of 170 rats (*Rattus norvegicus* and *R. tanezumi*) were captured in Panggang, Untung Jawa Tidung, Pari, Kotok and Rambut islands. Antibodies against Hantaan virus (HTNV) antigen were detected in 15.9% of rat sera. The prevalence of rats in Panggang, Untung Jawa and Tidung islands were 29.8%, 21.6% and 9.1%, respectively, whereas those in other islands were 0%. The prevalence of *R. norvegicus* in Panggang island and *R. tanezumi* in Untung Jawa island were especially high (40.6% and 20.8%, respectively) compared to the overall prevalence (28.4% and 6.3%, respectively). To determine the serotypes of the infected viruses, the sera were subjected to serotyping ELISA using truncated NP proteins of HTNV, Seoul virus (SEOV) and Thailand virus. Most sera reacted strongly to SEOV-specific antigen. On the other hand, some sera reacted to no or multiple antigens, implying the existence of novel type of hantaviruses. In conclusion, SEOV and possibly novel type of hantaviruses were prevalent in specific islands in Indonesia. Genetic characterization of the viruses and evaluation of the risk for human will be carried out in the next collaborative studies in June and July 2009.

P-19 Development of serotyping ELISA for new world Hantavirus infection

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Background: Hantavirus causes two important rodent borne viral zoonoses, hemorrhagic fever with renal syndrome (HFRS) in Eurasia by Old World hantaviruses and hantavirus pulmonary syndrome (HPS) in North and South Americas by New World hantaviruses. We have developed serotyping ELISA by using truncated recombinant nucleocapsid protein (rNP) which was deleted cross-reactive epitopes locating at N-terminal region of NPs. The aim of this study is application of the rNP-based ELISA for serotyping for New World hantavirus infection.

Methods: Amino acid (aa) similarities of internal variable region of NP (aa 230-302) of New World hantaviruses. The cDNA encoding NP of New World hantaviruses; Sin Nombre virus (SNV), Andes virus (ANDV), Lagna Negra virus (LANV) were provided by Dr. H. Feldmann of Canadian Science Center, that of El Moro Canyon virus (ELMCV) was provided by Dr. H. Kariwa of Hokkaido University. SNV, ANDV, LANV and ELMCV patient or rodent sera were used for evaluation of the ELISA.

Results: New World hantaviruses could be divided into five groups based on the aa similarity (about 70% and 80%) at internal variable region. Farther, the group 2 was divided into five subgroups. The variable region was considered to include type-specific epitopes. Therefore we chose four representatives from five groups, namely SNV, ANDV, ELMCV, and LANV. Entire rNP and truncated NP (trNP100) lacking 100 aa of N-terminal of them were successfully expressed in pET system and baculovirus expression system, respectively. MAb binding to the cross-reactive epitopes located to N-terminal region of NP did not react with the trNP100s. SNV, ANDV, LANV and ELMCV patients and rodent sera showed the highest OD values to homologous rNP100 antigens. The trNP-based ELISA might be useful for serotyping for New World hantaviruses.
Conclusion: The trNP-based ELISA could be a simple and safe alternative mthod for ordinarily neutralization test to differentiate virus type of New World hantavirus. Further studies are necessary for evaluation of applicability by using large numbers of antisera.

P-20 Study of replication and localization of hantavirus

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Hantavirus (HV) belongs to the genus *Hantavirus* within the family *Bunyaviridae* and is the pathogens of hantavirus infections in humans which are classified into two disease types, hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome. The major characteristics of hantaviruses is that each hantavirus infect persistently to their specific reservoir host. Some studies indicate that CD8+ T cell and regulatory T cell act as a cellular factor of this mechanism. But viral factor of persistent infection have not been analyzed yet. Recently, reverse-genetics system of some species of *Bunyaviridae* (Rift valley fever virus, Uukuniemi virus or so) and this system is used for the tool of virological studies of these viruses. So we studied the replication of hantavirus to construct the reverse-genetics system of this virus.

Each gene of Seoul hantavirus (SEOV) proteins, nucleocapsid protein, glycoprotein and L protein are cloned into pCAGGS mammalian cell expression vector. Expression of each proteins are confirmed by using immnofluorescence assay. We first examined that L protein of SEOV is found near to golgi body. This result indicates that genome of hantavirus is replicated near to golgi body.

Now we are examinating T7 RNA polymerase – driven minigenome system by using hantavirus infected cell. And we plan to identify replication signal of hantavirus.

P-21 Development of diagnostic methods applicable to various hantavirus infections

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Hantaviruses exist worldwide in association with rodent carriers. Some of them cause severe human illnesses, such as hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). Although no human HFRS cases have been reported in Japan since 1984, brown rats (*Rattus norvegicus*) in several ports and field grey red-backed voles (*Myodes rufocanus*) in Hokkaido have been found to be hantavirus-positive. Therefore, there is a possibility of re-emergence of human hantavirus infections in Japan.

Diagnostic methods for hantavirus infections require serotype-specific reagents since the antigenic and genetic characteristics of hantavirus varies among different serotypes of the viruses.

To develop the diagnostic method applicable to various hantavirus infections with a single sets of reagents, the antigen detection ELISA (Ag-ELISA) was established by using a monoclonal antibody clone E5/G6 which reacts broadly with various hantavirus nucleocapsid proteins (NPs). By the Ag-ELISA, NPs of Hantaan, Seoul, and Puumala viruses were detected from hantavirus-infected cells and virus stock in a dose dependent manner. In addition, NPs were detected from lung tissues of Puumala-infected Syrian hamsters (*Mesocricetus auratus*) at 7 and 14 days post-infection.

The antibody detection ELISA (Ab-ELISA) was also developed by using three serotypes of recombinant NPs as antigens and peroxidase-conjugated protein G. By the Ab-ELISA, the antibodies against Hantaan, Seoul, Amur, Puumala, and Sin Nombre viruses were detected in the immunized laboratory animals. In sera from Puumala-infected Syrian hamsters, the antibodies were detected from 14 to 70 days postinoculation. In addition, the antibodies were detected by the Ab-ELISA in wild rodent sera from brown rats, field grey red-backed voles, striped field mice (*Apodemus agrarius*), and Korean field mice (*Apodemus peninsulae*) with the sensitivity of 90.9%, 100%, 60%, and 60%, respectively, and the specificity of 100%, 100%, 100%, and 94.7%, respectively, in comparison with IFA.

All these data suggest that Ag- and Ab-ELISAs are useful tools to detect hantavirus infections in rodents.

P-22 Analysis of the pathogenicity of influenza A virus

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After the infection with influenza A virus, severe pneumonia is occasionally induced by the excessive production of inflammatory cytokines, such as interleukin(IL)-1, IL-6 and tumor necrosis factor-α. However, it was also reported that blockage of expression or function of these cytokines showed little effect on survival rates of mice infected with influenza A virus. Therefore, we focused on the osteopontin (OPN) that has been shown to play a prominent role in the inflammatory process and immune system. In this study, we found that OPN had a function for the host defense against influenza A virus.

When mice were infected with influenza virus A/PR/8 strain (H1N1) or A/Aichi/2/68 strain (H3N2), which exhibits high or low lethality in mice, respectively, the expression of OPN in blood and lung tissue was increased only in the mice infected with A/PR/8. In addition, when OPN knockout mice were infected A/PR/8, the number of natural killer (NK) cells and virus titer in the lung tissue was reduced at 5 days after viral infection, and the survival rate was also reduced compared with the wild type mice. Therefore, it was suggested that OPN expression was associated with a protective role against influenza A virus infection.

Since, it has been reported that OPN was involved in recruitment of neutrophils into the inflammation sites, production of myeloperoxidase in neutrophils, and differentiation of NK cells, we intend to examine whether these functions of OPN are critical for the improvement in survival rate after A/PR/8 infection. In addition, we will also study the effect of recombinant OPN protein for the treatment after the infection with influenza A virus.

P-23 Molecular epidemiological study of infectious diseases derived from wild birds in Hokkaido

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Wild birds such as wild waterfowls play an important role in the epidemiology in many infectious diseases including the zoonosis as the infection sources such as the influenza and West Nile viruses (WNV). Therefore, in this study, epidemiological surveillance of the pathogens in various kinds of wild birds, such as wild geese and waterfowls, and domestic chickens was performed to clarify the distributions of various pathogens and their molecular biological properties.

As the first step for epidemiological survey, the molecular diagnostic methods (PCR method and RT-PCR method, etc.) were established to detect several viral genomes including WNV and Japanese encephalitis virus from feather tips of the wild birds because the sampling of feathers from birds is a very easy and convinient procedure. Feather tips were obtained at several time points from chickens experimentally infected with WNV, and RT-PCR was performed using viral RNA extracted from those feather tips. The WNV genome was detected from feather tips at 3 days after infection, suggesting that this RT-PCR method using feather tips is useful for the detection of the WNV genome from wild birds.

Next, the molecular epidemiological surveillance of these infectious diseases is performed by using the feather tips, blood and excrement samples gathered from the wild birds (wild geese, swans, crows, sparrows, starlings, bulbuls, and raptors, etc.) in Hokkaido, and the data base is constructed based on the pathogens detected. Moreover, the epidemiological surveillance of bacterial infections is scheduled to be done on the samples gathered from the wild bird, and the data base of the bacterial pathogens is constructed as well as the viruses. In addition, the pathogenicity of these pathogens to birds is examined by the experimental infection.

P-24 Cysteine residues of JC virus capsid protein, VP1 play an important role in capsomere formation

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Introduction

JC virus (JCV) belongs to the Polyomavirus family, which includes simian virus 40 (SV40). JCV capsid is about 45 to 50 nm in diameter, and its major component is VP1. The capsid is formed by the arrangement of 72 VP1 pentamers. VP1 is important in virion assembly, because it is able to self-assemble into virus-like particle (VLP) when expressed in either *Escherichia. coli,* baculovirus-insect cell, or yeast cell. In previous studies, disulfide bond from cysteine residues in SV40 VP1 is related to pentamer formation. There is a similarity in position of cysteine residues between JCV and SV40, however it has not been reported to examine the relationship between cysteine residues and pentamer formation in JCV assembly. In this study, we investigate the mechanism of pentamer formation using cysteine mutants of JCV-VP1.

Methods

Using an inverse PCR method, seven types of Cys-to-Ala mutants in pURE2-VP1 were generated. Each cystein residue at position 42, 80, 97, 200, 247, 260, and all the cysteine residues (designated as C42A, C80A, C97A, C200A, C247A, C260A, and 6X, respectively) were replaced with alanin. Wild-type and mutant VP1 proteins were synthesized by reconstituted cell-free protein synthesis system which can form disulfide bonds in cysteine residues of recombinant proteins. These proteins were fractionated by an SDS-denature-sucrose gradient centrifugation. Each fraction (22 fractions) was analyzed by SDS-PAGE and immunoblotted with anti-VP1 antibody. Signal intensities of the VP1 bands were digitalized and plotted against sucrose concentration. Subsequently, the rate of pentamer formation of the mutants was compared with that of wild-type.

Results and Discussion

Expression levels of wild-type and mutants VP1 are similar. SDS-denature-sucrose gradient centrifugation analysis demonstrated that two peaks of wild-type VP1 can self-assemble into pentamer. Subsequently, each mutant VP1 protein was analyzed by same assay. C42A, C97A, C200A, C247A, and C260A mutants formed pentamer, but the rate of pentamer formation of C80A and 6X mutants were significantly reduced compared to those of other mutants. These results show that cysteine 80 plays an important role in pentamer formation. The relationship between cysteine 80

and pentamer formation in virion assembly is now under investigation.

P-25 Large T antigen of JC virus promotes viral replication by inducing ATM- and ATR-mediated G_2 checkpoint signaling

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The human polyomavirus JC virus (JCV) is the causative agent of progressive multifocal leukoencephalopathy (PML), a fatal demyelinating disease of the central nervous system (CNS). JCV is a small virus with a double-stranded DNA (dsDNA) genome that encodes early proteins (large T antigen, small t antigen, and T′ antigen) and late proteins (VP1, VP2, VP3, and agnoprotein). Large T antigen (TAg) possesses DNA binding and helicase activities, which, together with various cellular proteins, are required for replication of the viral genome. We show that JCV-infected cells expressing TAg accumulate in G_2 phase of the cell cycle as a result of activation of ATM- and ATR-mediated G_2 checkpoint pathways. Transient transfection of cells with a TAg expression vector also induced G_2 checkpoint signaling and G_2 arrest. Analysis of TAg mutants with different subnuclear localizations suggested that association of TAg with cellular DNA and the nuclear matrix contributes to the induction of G_2 arrest. Moreover, inhibition of ATM and ATR by caffeine suppressed JCV proliferation. The observation that oligodendrocytes productively infected with JCV in vivo also undergo G_2 arrest suggests that ATM-ATR inhibitors such as caffeine are potential therapeutic agents for JCV infection.

P-26 Inhibition of the SDF-1α-CXCR4 axis by the CXCR4 antagonist AMD3100 suppresses the migration of human ATLL cells and murine lymphoblastoid cells from HTLV-I Tax transgenic mice

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Adult T cell Leukemia/lymphoma (ATLL) is a T-cell malignancy caused by human T-lymphotropic virus type I (HTLV-I), and presents as an aggressive leukemia with characteristic widespread leukemic cell infiltration into visceral organs and skin. The molecular mechanisms associated with leukemic cell infiltration are poorly understood. We have recently established a mouse model of ATLL by generating HTLV-I *tax* transgenic mice using the *lck* proximal promoter to restrict transgene expression to developing thymocytes. The mice developed leukemia and diffuse large cell lymphomas histologically identical to those in ATLL with extensive perivascular infiltration of many organs. Flow cytometric analysis demonstrated the cells to be a CD4- CD8- double negative, pre-T-cell phenotype. Transfer of splenic lymphomatous cells from transgenic to SCID mice rapidly reproduces a leukemia and lymphoma which is histologically identical to human disease. We have characterized the expression of chemokine and their receptors associated with cellular invasion in this model. It could be shown that lymphomatous cells exhibit specific chemotactic activity in response to SDF-1α. We also observed that CXCR4, the specific receptor of SDF-1α is expressed on the surface of the lymphomatous cells. Chemotaxis of the cells in the presence of SDF-1α was associated with rapid phosphorylation of intracellular ERK1/2. AMD3100, a specific CXCR4 antagonist, was found to markedly inhibit both SDF-1α-induced migration and phosphorylation of ERK1/2. Investigation of primary human ATLL cells revealed identical findings. By SDF-1α-immunostaining, positive signal was observed in epithelial cells of regenerative hepatic ducts surrounded with leukemic cell infiltration. These results suggest that expression of SDF-1α might be involved in leukemic cell invasion and CXCR4 antagonist can be applied to ATLL treatment.

P-27 Friend murine leukemia virus A8 regulates Env protein expression through an intron sequence

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The murine leukemia virus (MLV) belongs to the simple retrovirus family. The genome of MLV contains 5' Long Terminal Repeat (LTR), 5' leader sequence, *gag*, *pol*, *env*, and 3' LTR. The *gag* gene encodes the structural proteins of the virion, and the *pol* gene encodes enzymes, including a reverse transcriptase. The *env* gene encodes the Env protein, which consists of a surface domain and a transmembrane domain. A splice donor site is located in the 5' leader sequence and a splice acceptor site is located in the 3' end of the *pol* gene. Gag and Pol proteins are translated from the unspliced full-length viral mRNA, and the Env protein is translated from the spliced *env*-mRNA. MLV has no regulatory genes, such as those that control the gene expression of lentiviruses, including splicing events. In Moloney-MLV, it was reported that the CA-encoding region within the *gag* gene was a negative *cis*-regulatory element and the region immediately upstream of the splice acceptor site was a positive *cis*-regulatory element regulating expression of the unspliced RNA. In any case, the molecular mechanisms regulating MLV gene expression, including splicing, are not well understood.

A neuropathogenic variant of Friend-MLV (Fr-MLV) clone A8 induces spongiform neurodegeneration without inflammatory infiltrates when infected into neonatal rats. Studies with chimaera constructed from the A8 virus and the non-neuropathogenic Fr-MLV clone 57 identified the *env* gene of A8 as a primary determinant for the induction of spongiform neurodegeneration in the brains of infected rats, and the 0.3-kb KpnI-AatII fragment containing the R, U5 and 5' leader sequence of A8 as necessary for neuropathogenicity. In previous studies we showed that the A8-Env protein expression level is correlated with neuropathogenicity.

In the present study, we focused on the role of the intron sequence containing the 5' leader sequence and the *gag-pol* region of MLV on Env protein expression. The role of the intron region on gene expression is well studied in eukaryotes. The intron region regulates the efficiency of polyadenylation, mRNA export from the nucleus to the cytoplasm, and the decay and stabilization of mRNA, which occurs mainly through the splicing process. We first analyzed the influence of *cis*-regulatory elements in the intron of the Fr-MLV gene on Env expression using vectors with serially truncated introns, and we found the positive and negative regulatory regions that control Env expression at the level of *env*-mRNA expression by controlling the splicing efficiency. Furthermore, our results indicated that the splicing process is important to acquire stable *env*-mRNA and to promote translation of the Env protein. They also suggested possible mechanisms for regulation of Env expression in MLV.

P-28 Molecular characterization of Marek's disease virus isolated from domestic and wild birds

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Marek's disease (MD) is an important neoplastic disease of poultry due to its economical threat. MD has been controlled since Marek's disease virus (MDV), a herpesvirus, was identified and live vaccines using apathogenic MDV were developed. However, the details of its pathogenic mechanism are still unknown. In addition, recent field isolates of MDV show tendency to increase their virulence, and the feasibility of future outbreaks cannot be ignored. Moreover, an MD case was reported in a white-fronted goose (precious natural treasure), migrating from Russia, at lake Miyajimanuma, Hokkaido in 2001. Therefore, wild geese could play a role as reservoir or carrier of MDV in transmission of MDV to domestic poultry, and outbreak in future may also occur in wild geese because of the increased virulence of MDV.

Currently, strains of MDV are classified into four categories based on their virulence, mild, virulent, very virulent, and very virulent+ MDV groups. It was also reported that distinct diversity and/or point mutations are present in the *meq* gene, a candidate oncogene of MDV, among highly virulent MDV strains. Therefore, we have established a novel method to detect the MDV genome in wild birds, a PCR detection of the *meq* gene using feather tips. This method also can distinguish virulent from mild or apathogenic MDV strains. the *meq* gene was detected in many white-fronted geese captured in Japan and Russia (28-46.7%), whereas no bean geese (*Anser fabris*) were positive for the *meq* gene. These results indicate that virulent MDV1 is widely spread in the population of white-fronted geese migrating between Japan and the Far East region of Russia. Recent researches reveled that other MDV genes and their gene products are also important for the disease progression of MD. Among them, a virokine, viral interleukin-8 (vIL-8), has been identified, and shown to play an important role during the cytolytic phase of the MDV infection. Presumably, vIL-8 could be a chemoattractant for T cells which infiltrate into nerve and muscle tissues and finally form tumors in the susceptible chickens, and vIL-8 knocked-out MDV showed less virulent to chickens compared to control wild type MDV. Thus, to further characterize the MDV recently detected in domestic and wild birds, conventional or nested PCR was done to amplify the *vIL-8* gene of MDV strains, and the amplified *vIL-8* gene fragments were sequenced. Polymorphisms were also identified in the introns of the *vIL-8* gene. Since the region encoded by the exon 1 is a determinant of the biological activity of vIL-8, these polymorphisms could alter the vIL-8 activity and might contribute to the increase in the virulence of MDV.

P-29 Studies on the intracellular localization of PrP^{Sc} in prion-infected neuroblastoma cells

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Background and Objectives: Prion diseases are fatal neurodegenerative diseases characterized by the accumulation of an abnormal isoform of prion potein (PrP^{Sc}), in the central nervous system. Although the propagation of prion in neurons is believed to be tightly associated with neuronal degeneration, the molecular mechanism of prion propagation has not been fully understood yet. Therefore, we extensively analyzed the intracellular localization of PrP^{Sc} that was the key information to clarify the mechanisms of prion propagation.

Methods: Subclone of Neuro2a mouse neuroblastoma cells (N2a) that was persistently infected with prion 22L strain, was used. To detect PrP^{Sc} with an indirect immunofluorescence assay, cells were pretreated with guanidinium thiocyanate and stained with anti-PrP mouse monoclonal antibodies (mAbs). For the analysis of the intracellular localization of PrP^{Sc}, double staining was performed with following various organelle markers: PDI, Giantin, Tgn38, Rab5, Rab7, Rab9, Vps26, Vps35, Snx1, Clint1, Ap1g1, clathrin heavy chain, Lamp1 and Shiga toxin subunit B.

Results and Discussion: We found that PrP^{Sc} could be specifically detected by using mAb132 that recognizes the region adjacent to the most amyloidgenic region of PrP (aa112-119). As a result of the double staining with mAb132 and organelle markers, some of the granular staining of PrP^{Sc} was found to be co-localized with Lamp1 as reported previously. However, the most prominent finding was the presence of PrP^{Sc} at peri-nuclear regions that was not co-localized with Lamp1 but existed juxtaposed to trans-Golgi network (TGN). Interestingly, the PrP^{Sc} was co-localized with some components of retromer and Rab9, which are associated with retrograde transports from early or late endosomes to TGN. The peri-nuclear PrP^{Sc} disappeared by the introduction of siRNA against Rab9. Furthermore, when the cells were cultured at 20˚C to prevent retrograde transports to TGN, PrP^{Sc} was scattered to cytoplasm. After subsequent incubation at 37˚C for 30 min, PrP^{Sc} signals returned to peri-nuclear regions. Interestingly, the returned peri-nuclear PrP^{Sc} signals disappeared after the additional 30 min incubation at 37˚C. The peri-nuclear PrP^{Sc} signals then appeared again after the additional

60 min incubation. This finding suggested that PrP^{Sc} was recycled between plasma membrane and the peri-nuclear regions. Taken together, these results propose a novel idea that retrograde transports from early or late endosomes to TGN may be involved in recycling of PrP^{Sc}.

P-30 A case control study on the risks of leptospirosis in Sri Lanka

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Introduction
Leptospirosis is a zoonosis of worldwide distribution, endemic mainly in countries with humid subtropical or tropical climates. Over the past decade or more leptospirosis also remains endemic in Sri Lanka with intermittent outbreaks. The country experienced the largest ever recorded outbreak of leptospirosis in 2007. When compared with the year 2006, the increase in the number of cases surpassed 400%. Based on the notification of suspected cases, the incidence of leptospirosis in Sri Lanka in 2007 is 35.7 per 100,000 populations (approx. 7,000 cases/year).
In 2008, there were 204 deaths with a case fatality rate of 2.9%.
Hypothesis
Are certain risk factors associated with human leptospirosis in Sri-Lanka?
Objectives
- To compare potential risk factors and other characteristics of the cases to those of the controls
- To suggest control measures and further studies in order to identify the potential reservoirs.

Methods
Leptospirosis is a reportable disease in Sri Lanka. Severe cases are referred to designated hospitals in all the provinces. A case of leptospirosis is defined as of the following manifestations (Appendix1), confirmed by serological testing (PCR and/or MAT). The cases will be drawn from 6 designated hospitals from July - November 2009.
The controls will be recruited matching on gender and age (±5 years) from the inpatients of the same hospitals that show no manifestations of suspected or confirmed leptospirosis.
The structured questionnaires (after pre-testing in Sinhalese and Tamil local languages) will be administered at the hospital to both cases and controls in order to obtain sociodemographic, environmental and other potential risk characteristics for leptospirosis.
Results
The questionnaire is under review. The study site and hospitals have been identified and study team has been organized consisting of both the University of Peradeniya and Hokkaido University in collaboration with the Provincial Health Department and the Veterinary Research Institute in Kandy under the Ministry of Livestock.
Implications
The results obtained from this study will provide information that would help in the development of better control strategies and interventions for the disease as well as create a basis for further researches including a molecular epidemiological study.
Appendix 1:
Surveillance case definition used in the Epidemiological Unit of the Ministry of Health, Sri Lanka
Acute febrile illness with headache, myalgia and prostration associated with increase in any of the following symptoms:
- conjunctival suffusion / conjunctival haemorrhage, meningeal irritation, anuria or oliguria / proteinuria / haematuria, jaundice, haemorrhages, purpuric skin rash, cardiac arrhythmia or failure
- a history of exposure to infected animals
- an environment contaminated with animal urine

P-31 A preliminary study on molecular epidemiology of Leptospirosis in Sri Lanka

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Introduction
In tropical regions of the world, leptospirosis is a widespread public health problem. The disease is endemic wherever open sewers or agricultural practices lead to contamination of water with infected animal urine. Although most human leptospirosis infections are self-limited, complications are common, involving hepatorenal failure, pulmonary hemorrhage, and death in 10%–50% of severe cases. WHO has estimated that approximately 10-100 cases per 100,000 people are infected annually worldwide; In Sri Lanka an increasing trend of the disease was noted between 1991 and 2008. In 2008, 7099 cases and 204 deaths were reported to the Epidemiology Unit of the Ministry of Health. Hence, this pilot study was conducted to evaluate feasibility of applying molecular methods to early diagnose Leptospirosis and identify circulating leptospria species among the hospitalized suspected leptospirsis patients.
Methods and Materials
A total of 142 suspected leptospirosis blood samples was received from the Teaching Hospital of Peradeniya, University of Peradeniya, which were collected in 2008 during routine diagnostic purpose. Samples were stored under -80^{o}C until process for molecular analysis PCR at the Department of Pathobiology, Faculty of Veterinary Medicine and Animal Science. DNA extracted from suspected serum by using DNeasy® Blood & Tissue Kit (Qigan,Hilden, Germany). The extracted DNAs were subjected to nested PCR for the detection of leptospira flab gene (flab-PCR). Sequencing of the amplicons and phylogenic distances calculation were performed at the National Institute of Infectious Diseases, Japan.
Result
The mean age of patients was 42 years (SD 14.7) and 86% were males. Out of 142 samples, 3 samples were positive for *flab*-PCR with MAT titer was 0, 100 and 100. Sequenced amplicons revealed that leptospira species were deduced to be *Leptospira interrogans* and *Leptospira kirschneri*.
Discussion
The results indicate that *Leptospira interrogans* and *Leptospira kirschneri* are circulating in the study area but future analysis is needed for more samples of both humans and ani-

mals.

P-32 Studies on tick-borne zoonotic rickettsiosis

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Development of loop-mediated isothermal amplification (LAMP) assay for detection of *Ehrlichia ruminantium*

E. ruminantium is the causative agent of heartwater, an important tick-borne disease of livestock in Africa and the Caribbean. Recently, this pathogen was associated with several cases of human illness in Africa, raising the possibility of undiagnosed zoonotic infections in endemic countries. The polymerase chain reaction (PCR) assay targeting the pCS20 gene of *Ehrlichia* sp. has been widely used in the epidemiological studies of heartwater. However, this assay is reported to be cross-reactive with other ehrlichial agents such as *E. chaffeensis* and *E. canis.* Here we developed LAMP assay for the detection of *E. ruminantium*. By comparing the sequences of house keeping genes among strains of *E. ruminantium*, super oxide dismutase B (sodB) gene was found to be highly conserved among strains. LAMP primers were constructed based on the sequence of *sodB* and no cross-reactivity with *E. chaffeensis* and *E. canis* was observed with this primer set. Sensitivity of the LAMP assay was evaluated with serially diluted plasmid DNA, which contains LAMP amplified region, and the detection limit of the assay was 5 copies per reaction. Also the prevalence of *E. ruminantium* both in vector ticks and blood of cattle, which were collected in sub-Saharan countries, was assessed with this assay.

A metagenomic survey of microbes in pathogen-transmissible ticks

Ticks are known to have variety of microbes including pathogenic or non-pathogenic rickettsia. Increased awareness of rickettsiosis, combined with the development of molecular biological detection techniques, has resulted in the discovery of novel rickettsial agents in ticks in the last few decades. There are several reports suggesting gene conversion between rickettsia and other microbes in ticks, causing antigenic diversity within species. Therefore, analysis of microbial communities in ticks may lead to identification of novel rickettsial agents as well as better understanding of the mechanisms for the antigenic variation of rickettsia. In this study, ticks were collected in Miyazaki, Japan and pooled bacterial DNA was extracted without contamination of tick DNA by filtration and DNase treatment. As a preliminary experiment prior to sequencing with next generation sequencer, 16S rDNA of bacteria was amplified by PCR and cloned. Rickettsia closely related to *Rickettsia honei* and *R. tamurae* were identified in *Haemaphysalis longicornis* and *Amblyomma testudinarium*, respectively. For further identification of microbes in ticks, metagenomic analysis using next generation sequencer is under progress.

P-33 Prevalence of Lyme disease among Hokkaido wildlife and ticks, and dilution with greater biodiversity

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Lyme disease is an emerging zoonotic disease of wildlife, which is found in parts of Asia, Europe, and North America. In the United States alone, over 27,000 human cases were reported in 2007, and cases are increasing annually worldwide. Whereas the reservoir species has been fully identified in the U.S., the reservoir species in Japan have not been definitively determined. We hope, through our research, to definitively identify the reservoir species and carrier animals, as well as the type of ticks which carry the infectious agent (*Borrelia spp*.). Moreover, we hope to determine prevalence of the infectious agent in ticks and wildlife of Hokkaido, and elucidate whether there is a dilution effect on prevalence with greater biodiversity.

P-34 Immune response to intracerebral vaccination against pseudorabies virus in mice

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In previous study, intracerebral (IC) immunization of inactivated pseudorabies virus (PRV) was more effective at inducing a protective immune response against PRV than subcutaneous (SC) immunization. In this study, we tried to investigate the mechanism IC immunization showed more efficient protection than SC immunization.

Humoral immunity, in particular secreted neutralizing antibodies, is of central importance to protect the body against acutely cytopathic viruses. Long-term antibody production is one of the hallmarks of effective vaccination and is an important characteristic of immunological memory. The cells produce antibody are antibody secreting cells (ASCs) known as plasma cells. Therefore, we studied about the immune response to IC immunization focusing on ASCs.

We compared immune response in both peripheral system and central nervous system (CNS). We checked the level of serum anti-PRV antibodies (Abs) with ELISA and neutralization test, and the cytokine levels produced by splenocytes with RT-PCR. The levels of IgG and PRV within CNS were also analyzed with real time RT-PCR and immunohistochemistry.

Anti-PRV antibodies and neutralizing antibodies induced by IC immunization were relatively higher than SC immunization. When I stimulated the splenocytes by inactivated PRV, higher level of Th1-type cytokine (IFN-r) and Th2-type cytokines (IL4, IL10) were produced in IC immunized mouse than SC immunized mouse. The real time RT-PCR and IHC for IgG showed ASCs existed in CNS after IC immunization, but not SC immunization. Furthermore, IC immunized mouse conferred more efficient protection than SC immunized mouse when challenged with the live PRV by IC inoculation after 2nd immunization. The former had more IgG and less PRV than the latter in CNS, when we analyzed the IgG and PRV levels after virus challenge. These data

suggest IC immunization induced stronger immune response not only in peripheral system but also within CNS. I consider ASCs recruited into CNS contributed to clearance of PRV in IC immunized mouse.

P-35 Development of CTL inducing peptide vaccine against Influenza A virus

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[Purpose] Although the antibody inducing vaccine against the surface proteins, hemagglutinin and neuraminidase, of influenza A virus is effective, drift variants of these proteins reduce protection efficacy of the vaccine in most cases. Therefore, the antibody inducing vaccine for flu needs to be updated annually. To develop the virus subtype-independent influenza vaccine, we focused on the role of cytotoxic T lymphocyte (CTL), one of the major immune cell, in the control of virus infection, because CTL is able to recognize not only variable virus surface proteins but also conserved internal viral proteins. In this study, we established peptide-based CTL-inducing influenza A vaccine system with several computed epitope prediction programs. In addition, we constructed human CTL evaluation system using HLA, human major histocompatibility complex (MHC) molecule, class I transgenic mouse.
[Methods] Several epitope peptides were predicted by CTL epitope peptide prediction programs from avian influenza virus strain, A/Hong Kong/483/97(H5N1), HK483, internal proteins, then 48 high-scored peptides were synthesized. To verify immunodominancy of the peptides, in vivo cytotoxicity assay was used. To evaluate protective effect of CTL-peptide vaccine in human CTL immune system, human MHC HLA-A*2402 transgenic mice (A24Tg) were immunized subcutaneously or intranasally with immuno-dominant peptides, then challenged with several influenza A virus strains. After virus infection, the survival rate and the changes in body weight were daily monitored. In addition, lung virus titer at day 5 after virus infection was measured.
[Result] Most of CTL inducing peptide vaccine inoculated A24Tg mice were survived after influenza A virus infection independent of virus subtypes. Furthermore, body weight loss of virus infected immunized mice were mild or not observed. Intranasal administration of peptide vaccine was more effective than subcutaneous administration in survival and body weight reduction. Lung virus titers at infection day 5 of peptide immunized groups were significantly lower than those of unimmunized groups.
[Conclusion] Flu antigen specific CTL induced by peptide vaccine provided protection against influenza A infection. The efficiency of virus protection was dependent on the route of vaccine administration. Our study suggests the important result to practical use of usable CTL inducing peptide vaccine development for human use.

P-36 Further characterization of NKT-cell hybridomas with invariant T-cell antigen receptor

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Invariant NKT (iNKT) cell (mouse Vα14Jα18/Vβ8, 7, 2; human Vα24Jα18/ Vβ11 NKT cells) are CD1d-restricted lymphocytes that play important roles in regulating immune responses against infections, tumors, allergens, and auto-antigens through recognition of endogenous or pathogen-derived glycolipids. iNKT cells react with α-galactosylceramideα-GC) presented by CD1d, and these cells are readily detectable with α-GC-loaded CD1d (α-GC/CD1d) multimers. One hallmark of iNKT cells is the rapid production of copious amounts of immunoregulatory cytokines such as IL-4 and IFN-γ following stimulation, suggesting that these cells provide an important link between the innate and adaptive immune systems, and serve as cellular adjuvant in various infectious diseases. However, functional analysis of NKT cells is accompanied by difficulty due to the fact that NKT cells are usually too small in number to examine.

To circumvent the difficulty, two natural killer T (NKT)-cell hybridomas were established by fusing sorted NKT cells with BW1100 thymoma – one is $CD4^+8^-$, another $CD4^{low\sim-}8^-$. The $CD4^+8^-$ hybridoma, 1B6, and the $CD4^{low}8^-$ one, 2E10, both expressed invariant T cell antigen receptor, and were readily detected with α-galactosylceramide-loaded CD1d:Ig fusion protein (α-GalCer/CD1d dimer). Sequence analyses of α- and β-chain of the T cell receptor V genes revealed that 1B6 and 2E10 expressed Vα14Jα18/Vβ8.2Dβ2Jβ2.7 and Vα 14J α 18/V β 8.1D β 1J β 1.1, respectively. When these hybridomas were stimulated with immobilized anti-CD3ε monoclonal antibody, α-GalCer/CD1d dimer, or α-GalCer in the presence of antigen presenting cells, these cells produced IL-4 and IFN-γ. The expressions of CD69, CD154, and CD178 were concomitantly induced on both hybridomas upon stimulation. We would like to present, in the present study, cytokines other than IL-4 and IFN-γ and chemokines produced by the hybridomas upon stimulation with TCR cross-linking. We also would like to compare antigen-presenting capacity of CD1d1 or CD1d2 molecules obtained from SL/Kh mice with the hybridomas.

Both hybridomas may provide a useful platform to study a variety of functions of NKT cells.

P-37 Functional analysis of murine flavivirus resistance gene *Oas1b*

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Oas1b, induced by type 1 interferon (IFN), play a role in the early antiviral innate immunity by inhibiting the replication of viruses. In mice, Oas1b confer resistance to flaviviruses including West Nile virus. The laboratory mice have been used to study pathogenesis of these important viruses, however, almost all laboratory mice lost *Oas1b* gene

during selective breeding. In addition, it has been reported that wild-derived mice strains have an intact *Oas1b* gene and show resistant phenotype to this viruses.

In previous study, we generated congenic strains, which *Oas* loci of the MSM/Ms mice was introduced to the most widely used strain, C57BL/6J mice. *Oas* congenic mice showed resistance to West Nile virus but not to influenza virus and sendai virus infection. Although this gene is induced by the virus RNA, Oas1b show high antiviral specificity in mice possessing the same genetic background. These data raise the possibility that murine Oas1b inhibits flavivirus replications specifically, however, the reason why mice carrying functional Oas1b show resistance to flavivirus specifically remains unknown.

To explore this possibility, we first aimed to establish *in vitro Oas1b* expression system to evaluate murine Oas1b activity on flavivirus replicatioin using West Nile virus- virus like particle. Cells expressing Oas1b exhibit reduction of virus like particle. This suggest that this system is available for investigating the function of murine Oas1b.

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