The 4th International Young Researcher Seminar for Zoonosis Control 2012

September 19 (Wed) ~ 20 (Thu), 2012

Graduate School of Veterinary Medicine, Hokkaido University

Front Cover
POPLAR AVENUE
Poplars were first planted in Hokkaido in mid-Meiji as a wind break. Many of these are far past their normal lifespan of sixty years, and despite the danger of them clashing down, they are an essential part of the Sapporo tourist's itinerary.

MODEL BARN
This cultural property was built in 1877 as the first farm for livestock farming in Hokkaido. Dr. William Smith Clark, the first President of Sapporo Agricultural College (Hokkaido University), named this facility "Model Barn" in the hope that it would be a model for agriculture in Hokkaido.

Back Cover
STATUE OF DR. WILLIAM S. CLARK

Photos by Dr. Motohiro Horiuchi, a program member of GCOE

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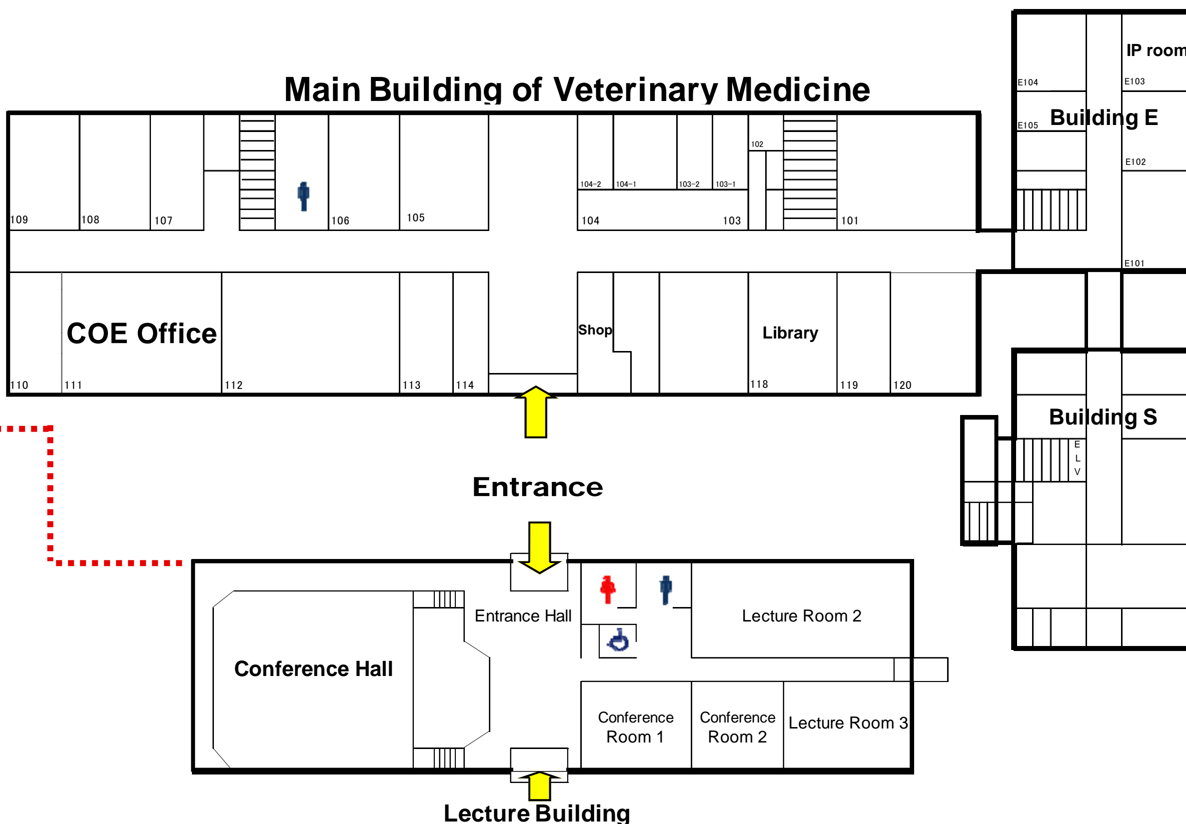
Venue:



The seminar will be held at Lecture Building of Veterinary Medicine, Hokkaido University

Entrance Hall: **Registration**

Conference Hall: **Presentation , Flash talk**

Lecture room 2 and Entrance Hall : **Poster session**

Conference Room 1 & 2: **Farewell party on Sept. 20**

Restaurant "Enreiso" at Faculty House Trillium:
Welcome reception from 18:30 to 20:30 on Sept. 19

Sapporo Aspen Hotel: **Accommodation for the invited speakers**
Address/ 5, Kita8-jyo Nishi4-chome, Kita-ku, Sapporo,
Phone +81 11-700-2111
FAX +81 11-700-2002
http://www.aspen-hotel.co.jp/english/

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 Conference Hall, Veterinary Medicine from **8:45 to 9:15** on September 19 (wed). You will have a programme & abstract, name card and information.

Accommodation in Sapporo for the invited speakers

Sapporo Aspen Hotel
Address/ 5, Kita8-jyo Nishi4-chome, Kita-ku, Sapporo, Hokkaido 060-0808 Japan
Phone +81 11-700-2111
FAX +81 11-700-2002
http://www.aspen-hotel.co.jp/english/

General Guideline for Oral presentation

Oral presentation

- The laptops running Windows 7 Professional operating system, with MS Office 2010 and Macintosh OS 10.7 operating system, with MS Office 2010 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.
- Please bring your presentation loaded in USB thumb drive (flash disk) or CD-ROM at the registration desk in the morning of Sept. 19.

【Keynote lectures】

Keynote presentations for Dr. Jonna Mazet and Dr. Takema Fukatsu are allocated 60 min including discussion.
(Dr. Jonna Mazet and Dr. Takema Fukatsu do not need to prepare poster presentation).

【9 Invited speakers (Oral presentation)】

Oral presentation for invited speakers is allocated 25 min including discussion.
Kindly prepare poster presentation too.

【10 speakers from Hokkaido University】

Oral presentation for 10 speakers from Hokkaido University is allocated 25 min including discussion.

Note: All participants (including invited and oral presenters from Hokkaido University except Keynote presenters) should prepare poster.
However, invited and oral presenters from Hokkaido University do not need prepare "One minute presentation (flash talk)".

General Guideline for Poster Presentation

Poster Presentation

- Each board size is 900 mm W x 2,000 mm H
- A0 size: 841 mm x 1189 mm may be appropriate if you prepare your poster as a single sheet.
- Boards with Poster No. card (147 mm W x 105 mm H) 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 from 8:45 to 9:15 in the morning of Sept. 19 at Poster presentation Venues as below.
- Poster is to be removed after the seminar on September 20.

900 mm
Poster
2,000 mm
Poster

Poster Presentation Venue: Entrance Hall

O-1	O-3	O-5	O-7	O-10
O-12	O-14	O-16	O-19	

Poster Presentation Venue: Lecture Room 2

P1-1	P1-2	P1-3	P1-4	O-8	O-4	P1-7	P1-8	P1-9	O-17
O-9	P1-5	P1-6	O-2	O-18	P1-10	P1-11	P1-12	P1-13	P1-14
O-11	P2-9	P2-10	P2-11	P2-12	O-15	P2-1	P2-2	P2-3	P2-4
		O-13	P2-13	P2-14	P2-5	P2-6	P2-7	P2-8	O-6

One minute presentation (flash talk) by 28 poster presenters from Hokkaido University

Flash talk will be held on September 19 (16:10-16:50) at Conference Hall .
The presentation should be within one minute. Please prepare one ppt slide by September 9 and send it to GCOE office by E-mail.

【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。)

Organizing Committee

Rie Hasebe
(Lab. of Veterinary Hygiene, Dept. of Applied Veterinary Sciences, Graduate School of Veterinary Medicine, Hokkaido University, e-mail: r-hasebe@vetmed.hokudai.ac.jp)

Yuji Sunden
(Lab. of Comparative Pathology, Dept. of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, e-mail: sunden@vetmed.hokudai.ac.jp)

Kentaro Yoshii
(Lab. of Public Health, Dept. of Environmental Veterinary Sciences, Graduate School of Veterinary Medicine, Hokkaido University, e-mail: kyoshii@vetmed.hokudai.ac.jp)

Masatoshi Okamatsu
(Lab. of Microbiology, Dept. of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, e-mail: okamatsu@vetmed.hokudai.ac.jp)

Tatsuya Sakurai
(Lab. of Parasitology, Dept. of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, e-mail: tsakurai@vetmed.hokudai.ac.jp)

Shiro Murata
(Lab. of Infectious Diseases, Dept. of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, e-mail: murata@vetmed.hokudai.ac.jp)

Organized by

Global COE Program, Hokkaido University
"Establishment of International Collaboration Centers for Zoonosis Control"
GCOE Office
Graduate School of Veterinary Medicine 1F Room No. 111
Hokkaido University
Kita-18 Nishi-9 Kitaku, Sapporo, Japan 060-0818
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gcoe@vetmed.hokudai.ac.jp
http://www.vetmed.hokudai.ac.jp/gcoe/

FYI
Sapporo city
http://www.welcome.city.sapporo.jp/english/index.html
Sapporo Visitor Attractions
http://www.congre.co.jp/iums2011sapporo/data/accom.html
Planners Guide for Sapporo
http://www.conventionsapporo.jp/planners/e/
Narita International Airport (Tokyo)
http://www.narita-airport.jp/en/index.html
New Chitose Airport (Sapporo)
http://new-chitose-airport.jp/en/
JR (timetable New chitose airport—Sapporo)
http://www2.jrhokkaido.co.jp/global/english/ttable/04.pd
Hokkaido University Campus
http://www.hokudai.ac.jp/en/pickup/accesstocampus.html

Program Day 1

September 19 (wed), 2012

Time		Program
8:45 ~ 9:15		Registration, Submitting presentation data and setting up poster
9:15 ~ 9:30		Opening speech (Hiroshi Kida, Leader of Global COE Program, Professor, Hokkaido Univ.) Orientation (Rie Hasebe, Organizing Committee)
9:30 ~ 10:30	**Keynote Lecture I** (Chair persons: Yuji Sunden, Masayoshi Isezaki)	
		Using a One Health approach to identify emerging infectious diseases at the human-domestic animal-wildlife interface **Jonna AK Mazet** (University of California, Davis, USA)
10:30 ~ 10:50		- Break -
10:50 ~ 11:40	**Oral presentation I** (Chair persons: Kenta Shimizu, Sawang Kesdangsakonwut)	
	O-1	Immune clearance of rabies virus results in neuronal survival with altered gene expression **Emily A Gomme** (Thomas Jefferson University, USA)
	O-2	Investigation on the interaction between rabies virus and intracellular protein transport system of the host cells **Yusuke Sakai** (Hokkaido University, Japan)
11:40 ~ 13:00		- Lunch -
13:00 ~ 13:50	**Oral presentation II** (Chair persons: Kentaro Yoshii, Mizuki Sakai)	
	O-3	New data on pathogenesis of experimental infection with tick-borne encephalitis virus in mice **Daniel Ruzek** (Academy Sciences of the Czech Republic, Czech Republic)
	O-4	Accumulation of ubiquitinated proteins is related to neuronal apoptosis induced by West Nile virus infection **Shintaro Kobayashi** (Hokkaido University, Japan)
13:50 ~ 14:40	**Oral presentation III** (Chair persons: Takeshi Yamasaki, Toru Ichihashi)	
	O-5	Ultrasensitive detection and quantitation of prion seeding activity by RT-QuIC and eQuIC assays **Christina D Orrù** (Rocky Mountain Laboratories, National Institutes of Health, USA)
	O-6	Lack of CD14 delays progression of prion disease by modulating inflammatory environment in the brain **Rie Hasebe** (Hokkaido University, Japan)
14:40 ~ 14:55		- Break -
14:55 ~ 16:10	**Oral presentation IV** (Chair persons: Takahiro Sanada, Takako Amada)	
	O-7	A 3D organotypic tissue model for hantavirus infection of the human airway mucosa **Karin B Sundström** (Karolinska Institutet, Sweden)
	O-8	The role of neutrophils in the induction of pulmonary edema during hantavirus infection in C.B-17Scid mice **Takaaki Koma** (Hokkaido University, Japan)
	O-9	Inhibition of Marburg virus budding by nonneutralizing antibodies to the envelope glycoprotein **Masahiro Kajihara** (Hokkaido University, Japan)
16:10 ~ 16:50	Flash talk	
16:50 ~ 17:50	Poster core time I (Odd numbers and oral presentations at day 1) at Lecture room 2 and the Entrance Hall	
18:30 ~ 20:30	Welcome reception at Enreiso (Faculty House)	

Program Day 2

September 20 (thu), 2012

Time	Program
8:45 ~ 9:45	**Keynote Lecture II** (Chair persons: Tatsuya Sakurai, Jung-Ho Youn) Symbiosis, evolution and biodiversity **Takema Fukatsu** (National Institute of Advanced Industrial Science and Technology, Japan)
9:45 ~ 10:05	- Break -
10:05 ~ 11:20	**Oral presentation V** (Chair persons: Masatoshi Okamatsu, Kyoko Hayashida) O-10 Human serum resistance in *Trypanosoma brucei gambiense* **Paul Capewell** (University of Glasgow, United Kingdom) O-11 A novel approach to identify potential pathogens in ticks **Ryo Nakao** (Hokkaido University, Japan) O-12 Astrovirus diversity in the cave nectar bats (*Eonycteris spelaea*) in Singapore **Ian H Mendenhall** (Duke-NUS Graduate Medical School, Singapore)
11:20 ~ 13:30	Group Photo at the entrance of the Lecture Building - Lunch -
13:30 ~ 14:20	**Oral presentation VI** (Chair persons: Ryo Nakao, Chandika Damesh Gamage) O-13 Genotypic analysis of extremely drug-resistant tuberculosis (XDR-TB) in Nepal **Ajay Poudel** (Hokkaido University, Japan) O-14 Metagenomic composition of the primate gut **Scott A Handley** (Washington University School of Medicine, USA)
14:20 ~ 15:10	**Oral presentation VII** (Chair persons: Shiro Murata, Manabu Hashimoto) O-15 Molecular characterization of immunoinhibitory factors PD-1/PD-L1 in bovine leukemia virus-infected cattle **Ryoyo Ikebuchi** (Hokkaido University, Japan) O-16 Bystander chronic infection negatively impacts the development of CD8 T cell memory **Erietta Stelekati** (University of Pennsylvania Perelman School of Medicine, USA)
15:10 ~ 15:25	- Break -
15:25 ~ 16:40	**Oral presentation VIII** (Chair persons: Naoki Yamamoto, Nilton Akio Muto) O-17 Brisk cytokine response against growth of highly pathogenic avian influenza virus leads systemic capillary leakage and sudden death in chickens **Saya Kuribayashi** (Hokkaido University, Japan) O-18 Heterosubtypic cross-reactivity of influenza virus hemagglutinin-specific antibodies **Mieko Muramatsu** (Hokkaido University, Japan) O-19 Signalling pathway involved in innate immune activation by avian influenza virus in chickens: potential application for vaccine development **Matthias Liniger** (Institute of Virology and Immunoprophylaxis, Switzerland)
16:40 ~ 17:40	Poster core time II (Even numbers and oral presentations at day 2) at Lecture room 2 and the Entrance Hall
18:00 ~ 20:00	Award & Closing Speech (Chihiro Sugimoto, Professor, Hokkaido Univ.) Farewell Party at Conference room

Profile of Invited speakers

Keynote lecture I

Jonna A.K. Mazet

Professor, Department of Medicine & Epidemiology
Director, One Health Institute & Wildlife Health Center
School of Veterinary Medicine
University of California, Davis
jkmazet@ucdavis.edu

ACADEMIC DEGREES:

BS	1990	Veterinary Science	University of California, Davis, California
DVM	1992	Wildlife Medicine	University of California, Davis, California
MPVM	1992	Infectious Disease Epidemiology	University of California, Davis, California
PhD	1996	Wildlife Epidemiology	University of California, Davis, California

BIOGRAPHY:

1994 – 1994 Adjunct Instructor, Department Population Health & Reproduction, UC Davis
1996 – 1998 Assist Adjunct Professor, Department Medicine & Epidemiology, UC Davis
1994 – 1998 Wildlife Veterinarian, California Department of Fish and Game
1998 – 2006 Assistant & Associate Professor of Epidemiology & Disease Ecology, UC Davis
2006 – Present Professor of Epidemiology and Disease Ecology, UC Davis
1998 – Present Director, Wildlife Health Center, School of Veterinary Medicine, UC Davis
2010 – Present Executive Director, One Health Institute, UC Davis

RESEARCH INTERESTS:

One Health problem-solving; identification of the source and mechanism of zoonotic pathogen pollution in coastal communities; zoonotic disease surveillance; disease transmission at human-animal-environment interfaces; conservation of endangered species through the application of novel and creative epidemiological and ecological methods

Keynote lecture II

Takema Fukatsu

Group Leader
Symbiotic Evolution and Biological Functions Research Group
Bioproduction Research Institute
National Institute of Advanced Industrial Science and Technology (AIST), Japan
t-fukatsu@aist.go.jp

ACADEMIC DEGREES:

B.Sc.	1989	Department of Zoology, University of Tokyo, Japan
M.Sc.	1991	Department of Zoology, University of Tokyo, Japan
Ph.D.	1994	Department of Zoology, University of Tokyo, Japan

BIOGRAPHY:

1995-1998	Researcher, National Institute of Bioscience and Human-Technology (NIBH), Japan
1998-2001	Appointed Researcher, National Institute of Bioscience and Human-Technology (NIBH), Japan
2001-2004	Senior Research Scientist, National Institute of Advanced Industrial Science and Technology (AIST), Japan
2004-present	Group Leader, National Institute of Advanced Industrial Science and Technology (AIST), Japan
2003-2011	Associate Professor, Graduate School of Life and Environmental Sciences, University of Tsukuba, Japan
2011-present	Professor, Graduate School of Life and Environmental Sciences, University of Tsukuba, Japan
2005-2010	Professor, Department of General Systems Studies, University of Tokyo, Japan
2008	Invited Professor, University Louis Pasteur, Strasbourg, France

EXPERTISE:
Evolutionary Biology; Microbiology; Entomology

Profile of Invited speakers

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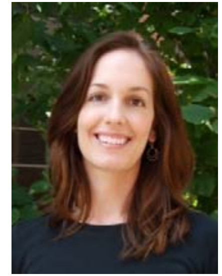

Emily A. Gomme

Post-Doctoral Fellow
Department of Molecular Genetics
The Wistar Institute, PA, USA
egomme@wistar.org

ACADEMIC DEGREES:

Ph.D.	2012	Thomas Jefferson University Immunology & Microbial Pathogenesis
B.S.	1997	Wilkes University Biology

BIOGRAPHY:

2012-Present	Post-doctoral Fellow, Wistar Institute, PA, USA Laboratory of Dr. Paul Lieberman
2006-2012	Ph.D. Candidate, Thomas Jefferson University, PA, USA Laboratory of Dr. Matthias Schnell
2004-2006	Assistant Scientist, GlaxoSmithKline Pharmaceuticals, PA, USA
2002-2004	Research Assistant, Wistar Institute, PA, USA

RESEARCH INTERESTS:

Pathogenesis of neurotropic viruses; molecular mechanisms of viral tumorigenesis

Daniel Ruzek

Research Scientist and Assistant Professor
Academy of Sciences of the Czech Republic
Biology Centre, Institute of Parasitology
Laboratory of Parasite Immunology
Branisovska 31
CZ-37005 Ceske Budejovice
Czech Republic
ruzekd@paru.cas.cz

ACADEMIC DEGREES:

BSc. 2003 Faculty of Biological Sciences, University of South Bohemia, Czech Republic (Biomedical Laboratory Techniques)
MSc. 2005 Faculty of Biological Sciences, University of South Bohemia, Czech Republic (Clinical Biology)
Ph.D. 2008 Institute of Parasitology, Academy of Sciences of the Czech Republic, and Faculty of Science, University of South Bohemia, Czech Republic (Molecular and Cellular Biology and Genetics)

BIOGRAPHY:

2008-2009 Postdoctoral Scientist, Texas Biomedical Research Institute, Department of Virology and Immunology, San Antonio, USA
2008- Assistant Professor, University of South Bohemia, Faculty of Science, Department of Medical Biology, Ceske Budejovice, Czech Republic
2009-2011 Postdoctoral Scientist, Academy of Sciences of the Czech Republic, Biology Centre – Institute of Parasitology, Ceske Budejovice, Czech Republic
2011- Research Scientist, Academy of Sciences of the Czech Republic, Biology Centre – Institute of Parasitology, Ceske Budejovice, Czech Republic

RESEARCH INTERESTS:

Pathogenesis and epidemiology of arboviral diseases (tick-borne encephalitis, Omsk haemorrhagic fever, West Nile fever, Tahyna virus disease, etc.).

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Christina Doriana Orru`

Post-Doctoral Fellow
Laboratory of Persistent Viral Diseases; Rocky Mountain Laboratories;
National Institute of Allergy and Infectious Diseases;
National Institutes of Health; Hamilton, MT USA
orruc@niaid.nih.gov

ACADEMIC DEGREES:
Master's Degree "summa cum laude" (Microbiology) - 2004 – University of Cagliari (Italy).

Ph.D. - 2007- University of Cagliari, Italy (Virology).

BIOGRAPHY:
2004 – 2007 - Teaching assistant University of Cagliari (Italy)
2007 - Visiting Post-Doctoral Fellow, National Institute of Health (USA)

AWARDS:
2011: Winner of the "NIH Director's Award" for the "research suggesting that early detection of prion disease is possible"
2011: Recognized number 3 science advance in the National Institute of Allergy and Infectious diseases (NIAID) and cited for "the advances on a sensitive and fast blood test to detect prion disease".

RESEARCH INTERESTS:
Pathogenesis of prion disorders, early diagnosis of prion diseases and mechanism of prion replication/propagation in the host.

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Karin Sundström

PhD student
Department of Microbiology, Tumor and Cell Biology
Karolinska Institutet, Sweden
Karin.sundstrom.2@ki.se

And
Swedish Institute for Communicable Disease Control, Sweden
Karin.sundstrom@smi.se

ACADEMIC DEGREES:
Master in Molecular Cell Biology 2006 University of Gothenburg

BIOGRAPHY:
Stockholm Research School in Molecular Life Sciences, Sweden
2007- PhD student, Department of Microbiology, Tumor and Cell Biology, Sweden, supervisor J. Klingström. Project: "Nephropathia Epidemica – An immunomediated disease?"

RESEARCH INTERESTS:
Pathogenesis of hantavirus caused diseases, innate immunity, adaptive immunity, model systems.

5

Paul Capewell

Research Associate
University of Glasgow, United Kingdom
paul.capewell@glasgow.ac.uk

ACADEMIC DEGREES:

BSc(Hons)	2006	University of Edinburgh
PhD	2010	University of Glasgow

BIOGRAPHY:

2010-2011	Research Assistant, Wellcome Centre for Molecular Parasitology, University of Glasgow, United Kingdom
2011-2012	Postdoctoral Fellow, Centre for Immunity, Infection and Evolution, University of Edinburgh, United Kingdom
2012-	Research Associate, Institute of Biodiversity Animal Health and Comparative Medicine, University of Glasgow, United Kingdom

RESEARCH INTERESTS:
Co-evolution of host, parasite & vector with a particular focus on the mechanisms used by the parasite Trypanosoma brucei to overcome the innate immunity to infection that has been inherited by humans and how the same parasite is able to thrive in the two disparate environments of the mammalian bloodstream and the Tsetse mid-gut

6

Ian Hewitt Mendenhall

Research Fellow
Laboratory of Virus Evolution
Program in Emerging Infectious Diseases
Duke-NUS Graduate Medical School
Ian.mendenhall@duke-nus.edu.sg

ACADEMIC DEGREES:

Ph.D.	2010	Parasitology. Department of Tropical Medicine, Tulane University
B.S.	1999	Entomology and Zoology. University of Wisconsin - Madison

BIOGRAPHY:

2000-2003	Laboratory Manager, Clarke Mosquito Control, Chicago, Illinois
2003-2010	Doctoral Student, Department of Tropical Medicine, Tulane University
2010-Present	Research Fellow, Program in Emerging Infectious Diseases, Duke-NUS Graduate Medical School

RESEARCH INTERESTS:

Evolutionary history of bat-borne viruses, ecological and environmental drivers of emerging infectious diseases, biological aspects of host and vector arbovirus transmission

7

Scott A. Handley

Research Instructor
Washington University School of Medicine
shandley@mrce.wustl.edu

ACADEMIC DEGREES:

Ph.D. 2006 Washington University School of Medicine

BIOGRAPHY:

2011 – Current	Research Instructor, Department of Pathology and Immunology, Washington University School of Medicine. Laboratory of Dr. Herbert W. Virgin, IV.
2005 – Current	Associate Director, Midwest Regional Center of Excellence for Biodefense and Emerging Infectious Diseases Research (MRCE)
2009 – 2011	Project Manager – Pfizer-Washington University Biomedical Agreement
1998 – 2000	Emerging Infectious Diseases Research Fellow, Centers for Disease Control and Prevention, Atlanta, GA. Laboratory of Dr. Russell Regnery.

RESEARCH INTERESTS:

Metagenomics, bioinformatics, pathogen discovery, emerging zoonotic diseases

8

Erietta Stelekati

Postdoctoral Researcher
Institute for Immunology,
Department of Microbiology,
University of Pennsylvania,
Philadelphia, PA, USA

erietta@mail.med.upenn.edu

ACADEMIC DEGREES:

Bachelor in Biology:	2002	Aristotle University of Thessaloniki, Greece
PhD in Immunology:	2008	Christian-Albrechts University of Kiel, Germany

BIOGRAPHY:

1998 - 2002	Graduate studies in Biology, Aristotle University of Thessaloniki, Greece
02/2002 - 09/2002	Diploma in Immunobiology, Department of Immunopharmacology, Utrecht University, The Netherlands
05/2004 - 01/2008	Ph.D., Department of Immunology and Cell biology, Research Center Borstel and Christian-Albrechts University of Kiel, Germany
02/2008 - 06/2008	Post-doctoral fellow, Department of Immunology and Cell biology, Research Center Borstel, Germany
09/2008 – 09/2009	Post-doctoral fellow, Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, U.S.A.
09/2009 – today	Post-doctoral fellow, Department of Microbiology and Immunology, The Wistar Institute and University of Pennsylvania, Philadelphia, USA

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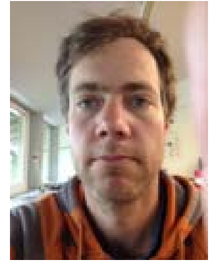

Matthias Liniger

Postdoctoral fellow
Institut für Viruskrankheiten und Immunprophylaxe (IVI)
Sensemattstrasse 293
CH-3147 Mittelhäusern, Switzerland
matthias.liniger@ivi.admin.ch

ACADEMIC DEGREES:
Ph.D. 2001 University of Bern, Institute of Cell Biology, Switzerland

BIOGRAPHY:
2001 - 2002 Postdoc at the University of Zurich, Institute of Molecular Biology, Switzerland
2002 - 2005 Staff scientist, live viral vectors, Berna Biotech LTD, Bern, Switzerland
2005 - 2007 Project Leader, Berna Biotech LTD / Crucell LTD / Etna Biotech S.p.A., Switzerland
2007 - 2012 Postdoc at the Institute of Virology and Immunoprophylaxis, Mittelhäusern, Switzerland

RESEARCH INTERESTS:
Avian influenza virus, Bluetongue virus, Innate immunity, Pathogenesis, Vaccine development

Keynote Lecture I
(Day 1: 9:30-10:30 on September 19)

Keynote lecture I

Using a One Health approach to identify emerging infectious diseases at the human-domestic animal-wildlife interface

Jonna AK Mazet
Wildlife Health Center and One Health Institute,
School of Veterinary Medicine,University of California, Davis, U.S.A.
jkmazet@ucdavis.edu

In order to predict, respond to, and prevent the emergence of novel infectious diseases, we must identify them at their source. Rapid human population growth and environmental changes have resulted in increased numbers of people living in close contact with animals. The resulting increased contact has altered the ecological balance between pathogens and their human and animal hosts. We have built a One Health team with a surveillance vision responsive to the fact that zoonotic pathogens account for the majority of emerging infectious diseases in people. Our approach employs integrated risk modelling, molecular diagnostics, and intensive field studies to detect novel diseases with pandemic potential early, giving health professionals the best opportunity to prevent emergence or control epidemics at the source of spill-over. It also targets important sentinel species at active transmission interfaces in hotspot regions to improve surveillance efficiencies. Our targeted and adaptive wildlife disease surveillance system includes the introduction of new technologies at the local level, as well as the use of cutting-edge information management and communication tools with the potential to bring the world closer to realizing an integrated, global approach to emerging zoonotic diseases. Our team is assisting in the development of global capacity to monitor diseases at the animal-human interface and has implemented a risk-based approach to concentrate efforts in surveillance, prevention, and response at the most critical points for disease emergence from wildlife. To date, we have sampled over 25,000 wild animals and discovered 150 novel viruses in families known to cause epidemics and pandemics with the potential to threaten human and animal health.

Keynote Lecture II
(Day 2: 8:45-9:45 on September 20)

Keynote lecture II

Symbiosis, Evolution and Biodiversity

Takema Fukatsu
Bioproduction Research Institute
National Institute of Advanced Industrial Science and Technology (AIST)
t-fukatsu@aist.go.jp

Many insects and other organisms constantly harbor microorganisms inside their body, which has been referred to as "endosymbiosis". Due to the close spatial proximity, extremely intimate biological interactions and inter-dependence are commonly found between the partners called host and symbiont. Novel biological properties are often generated through such associations. In many cases, host and symbiont are integrated into an almost inseparable biological entity. Here I present a series of our works on the evolutionary origin, biological function and ecological relevance of insect-microbe symbiotic associations.

References:

† Kondo N. *et al*. (2002) Genome fragment of *Wolbachia* endosymbiont transferred to X chromosome of host insect. ***PNAS*** 99: 14280-14285.
† Koga R. *et al*. (2003) Changing partners in an obligate symbiosis: a facultative endosymbiont can compensate for loss of the essential endosymbiont *Buchnera* in an aphid. ***Proc R Soc B*** 270: 2543-2550.
† Tsuchida T. *et al*. (2004) Host plant specializati
on governed by facultative symbiont. ***Science*** 303: 1989.
† Kutsukake M. *et al*. (2004) Venomous protease of aphid soldier for colony defense. ***PNAS*** 101: 11338-11343.
† Nakabachi A. *et al*. (2005) Transcriptome analysis of the aphid bacteriocyte, the symbiotic host cell that harbors an endocellular mutualistic bacterium, *Buchnera*. ***PNAS*** 102: 5477-5482.
† Hosokawa T. *et al*. (2006) Strict host-symbiont cospeciation and reductive genome evolution in insect gut bacteria. ***PLoS Biol*** 4: e337.
† Hosokawa T. *et al*. (2007) Obligate symbiont involved in pest status of host insect. ***Proc R Soc B*** 274: 1979-1984.
† Nikoh N. *et al*. (2008) *Wolbachia* genome integrated in an insect chromosome: evolution and fate of laterally transferred endosymbiont genes. ***Genome Res*** 18: 272-280.
† Aikawa T. *et al*. (2009) Longicorn beetle that vectors pinewood nematode carries many *Wolbachia* genes on an autosome. ***Proc R Soc B*** 276: 3791-3798.
† Kutsukake M. *et al*. (2004) Scab formation and wound healing of plant tissue by soldier aphid. ***Proc R Soc B*** 276: 1555-1563.
† Hosokawa T. *et al*. (2010) *Wolbachia* as a bacteriocyte-associated nutritional mutualist. ***PNAS*** 107: 769-774.
† Fukatsu T. (2010) A fungal past to insect color. ***Science*** 328: 574-575.
† Tsuchida T. *et al*. (2010) Symbiotic bacterium modifies aphid body color. ***Science*** 330: 1102-1104.
† Nikoh N. et al. (2011) Reductive evolution of bacterial genome in insect gut environment. ***Genome Biol Evol*** 3: 701-714.
†Koga R. *et al*. (2012) Cellular mechanism for selective vertical transmission of an obligate insect symbiont at the bacteriocyte-embryo interface. ***PNAS*** 109: E1230–E1237.
†Kikuchi Y. *et al*. (2012) Symbiont-mediated insecticide resistance. ***PNAS*** doi: 10.1073/pnas.1200231109.

Oral presentation I (Day 1: 10:50-11:15 on September 19)

O-1

Immune clearance of rabies virus results in neuronal survival with altered gene expression

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Rabies virus (RABV) is a highly neurotropic pathogen that typically leads to mortality of infected animals and humans. The precise etiology of rabies neuropathogenesis is unknown, though it is hypothesized to be due either to neuronal death or dysfunction. Analysis of human brains post-mortem reveals surprisingly little tissue damage and neuropathology considering the dramatic clinical symptomology, supporting the neuronal dysfunction model. However, whether neurons survive infection and clearance and, if so, whether they are functionally restored to their pre-infection phenotype has not been determined in vivo for RABV, or any neurotropic virus. This is due, in part, to the absence of a permanent "mark" on once-infected cells that allow their identification long after viral clearance. Our approach to study the survival and integrity of RABV-infected neurons was to infect Cre reporter mice with recombinant RABV expressing Cre-recombinase (RABV-Cre) to switch neurons constitutively expressing tdTomato (red) to expression of a Cre-inducible EGFP (green), permanently marking neurons that had been infected *in vivo*. We used fluorescence microscopy and quantitative real-time PCR to measure the survival of neurons after viral clearance; we found that the vast majority of RABV-infected neurons survive both infection and immunological clearance. We were able to isolate these previously infected neurons by flow cytometry and assayed their gene expression profiles compared to uninfected cells. We observed transcriptional changes in these "cured" neurons, predictive of decreased neurite growth and dysregulated microtubule dynamics. This suggests that viral clearance, though allowing for survival of neurons, may not restore them to their pre-infection functionality. Our data provide a proof-of-principle foundation to re-evaluate the etiology of human central nervous system diseases of unknown etiology: viruses may trigger permanent neuronal damage that can persist or progress in the absence of sustained viral antigen.

Oral presentation I (Day 1: 11:15-11:40 on September 19)

O-2

Investigation on the interaction between rabies virus and intracellular protein transport system of the host cells

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Backgroud
Rabies is fatal disease of central nervous system and kills 55,000 humans annually in the world. Although rabies is important zoonosis, the molecular biology of its etiologic pathogen, rabies virus (RV), has not been well clarified. Thus, we investigated the molecular mechanism of RV life cycle especially interaction of RV and intracellular protein transport machinery of host cell.

It has been demonstrated that ubiquitin and heat shock protein (Hsp) 70 accumulate in Negri's body-like inclusions (NBLs) that is the site of viral proliferation and mainly consisted of RV proteins. Thus, the roles of ubiquitin and hsp70 in the development of IBLs were firstly investigated Additionally, the relation of RV and multivesicular body (MVB) mediated protein transport mechanism was investigated.

Materials and Methods
Inhibitors of Hsp70 or E1 ubiquitin ligase were used to analyse the role of Hsp70 or ubiquitin. NA cells, a murine neuloblastoma cell line, were concomitantly treated with inhibitor and infected with RV. The cells were cultured for 24 h in the presence of inhibitor and RV. After that, the cells underwent immunofluorescent staining of RBV nucleoprotein, RNA extraction or protein extraction. Expression vectors of MVB sorting proteins Vps4a and Vps4b, and their dominant negative mutant Vps4aΔ and Vps4bΔ were used to investigate the interaction of RV and MVB protein transport machinery. At 24 h after transfection, RV was added in the medium and, at 24 h post RV infection, the cells underwent immunofluorescent staining.

Results
Inhibition of ubiquitination impaired the formation of NBLs and consequently suppressed the replication of viral RNA and protein. On the other hand, inhibition of Hsp70 did not affect NBL formation or replication. However, distribution of RV protein was inhibited and, as a result, viral spread was significantly decreased. Immunoflorescent staining demonstrated the colocalization of MVB marker, Rab9, encircled or colocalized with NBLs suggesting that NBLs originated from MVB.

NBLs and distribution of RV protein in the cell that expressed Vps4 mutant proteins were similar to those observed in the Hsp70 inhibitor treated cells. Immunofluorescent staining of Vps4 proteins demonstrated the colocalization of Vps4 proteins and NBLs.

Conclusion and Discussion
Ubiquitination is required for the formation of NBLs and ubiquitin-dependent transport of the RV protein into MVB, also called late-endosome, seemed to be important for the formation of NBLs. Hsp70 plays a role in the event after viral replication such as assembly or budding of RBV.

Suppression of NBL segregation and distribution by transfection of dominant negative mutants of Vps4 proteins suggested that RV uses the MVB protein transport system to spread the infection.

Oral presentation II (Day 1: 13:00-13:25 on September 19)

O-3

New data on pathogenesis of experimental infection with tick-borne encephalitis virus in mice

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Tick-borne encephalitis (TBE) virus causes severe encephalitis with serious sequelae in humans. However, the pathogenesis of TBE remains poorly understood. TBE is a generalized infection. The virus invades the central nervous system after initial extraneuronal replication – virus replication is first detected within draining lymph nodes, this is followed by development of viremia and during the viremic phase virus enter into the brain. Our study was focused on the immunopathological features of the host immune system during TBE, on the interaction of the virus with neural cells, and on the role of the host blood-brain barrier (BBB) in the neuropathogenesis of TBE. Moreover, the role of host genetic background in the seriousness of TBE was investigated as well.

In TBE virus infected mice, we demonstrated immunopathological basis of TBE. The key mediator of the immunopathological reaction was the population of CD8+ T-cells. The results imply the inflammatory reaction significantly contributes to the fatal outcome of the infection.

Elevation of BBB permeability is generally observed and investigated in the context of a pathological CNS inflammatory response. It seems that increased permeability of the BBB appears to be a vital component of flaviviral encephalitis pathology. Changes in permeability of the BBB in two susceptible animal models (BALB/c, and C57Bl/6 mice), and in human primary brain microvascular endothelial cells (HBMVE), the main component of BBB, were investigated after infection with TBE virus. We demonstrated that TBE virus infection induces considerable breakdown of the BBB. The permeability of the BBB increased at later stages of TBE infection when high virus load was present in the brain (i.e., BBB breakdown was not necessary for TBE virus entry into the brain), and at the onset of the first severe clinical symptoms of the disease. The increased BBB permeability was in association with dramatic upregulation of proinflammatory cytokine/chemokine expression in the brain. Breakdown of the BBB was also observed in mice deficient in CD8+ T-cells, indicating, that these cells are not necessary for the increase in BBB permeability. HBMVE were infected with TBE virus and replication kinetics together with changes in the expression of key tight junction proteins and cell adhesion molecules was investigated.

We developed a unique mouse model for the study of the role of host genetic background in the development and course of flavivirus encephalitis. We found three mouse strains that represent high, medium and low sensitive hosts. We characterized extensively clinical and pathological changes occurring in these mice following TBE virus infection. Expression profiling of selected immunologically important genes was studied in these mouse strains after the infection and several important differences in the gene expression were revealed. IP-10 and MCP-1 expression correlated with the seriousness of the disease and could represent suitable markers for the development of the infection. The neutralizing antibody response differed markedly in the studied mouse strains following the infection, with the earliest and strongest antibody response observed in the mice with the lowest sensitivity to the infection.

Oral presentation II (Day 1: 13:25-13:50 on September 19)

O-4

Accumulation of ubiquitinated proteins is related to neuronal apoptosis induced by West Nile virus infection

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West Nile virus (WNV) belongs to the *Flaviviridae* family of viruses and has emerged as a significant cause of viral encephalitis in humans, animals, and birds. It has been reported that WNV replication directly induces neuronal injury, followed by neuronal cell death proven as apoptosis. Therefore, it is important to understand the mechanism of neuronal apoptosis caused by this virus to develop strategies to control its pathogenicity. Accumulation of ubiquitinated abnormal proteins has been reported to be associated with neuronal apoptosis in some pathological conditions. A lot of cellular stresses prevent cellular protein quality control mechanisms, resulting in the accumulation of ubiquitinated abnormal proteins. To obtain a better understanding of the mechanisms of WNV-induced neuronal apoptosis, we evaluated the accumulation of ubiquitinated proteins in the WNV-infected neuronal cells. We have observed that WNV infection caused massive neuronal injury in the brain of mice. Viral antigen was detected in the neuronal cytoplasm of the cells exhibiting neuronal apoptosis. Notably, ubiquitinated proteins were detected in WNV-infected neuronal cells. In addition, accumulation of ubiquitinated proteins was markedly enhanced in mouse neuroblastoma, Neuro-2a cells after WNV infection. Our histopathological and *in vitro* studies suggest that accumulation of ubiquitinated proteins in neuronal cells might be associated with the neuronal apoptosis caused by WNV infection.

Oral presentation III (Day 1: 13:50-14:15 on September 19)

O-5

Ultrasensitive detection and quantitation of prion seeding activity by RT-QuIC and eQuIC assays

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A major problem for the diagnosis and management of prion diseases is the lack of rapid high-throughput assays to measure low levels of prions. The real-time quaking induced conversion assay (RT-QuIC) involves prion-seeded conversion of bacterially expressed recombinant PrP^{C} to a beta sheet -rich amyloid fibrillar form. Analogous to end-point dilution animal bioassays and with similar sensitivity, the RT-QuIC allows testing of serial dilutions of samples and statistically estimating the seeding dose (SD) giving positive responses in 50% of replicate reactions (SD_{50}). Wilham and colleagues (Wilham et al., Plos Pathogens 2010) have used the RT-QuIC to quantify hamster 263K PrP^{res} in brain, nasal lavages and cerebrospinal fluid (CSF) samples and have reported sensitive detection of deer chronic wasting disease (CWD) and sheep scrapie PrP^{Sc} in brain samples. Furthermore, Peden and colleagues (Peden A. H. et al, Journal of General Virology 2012), have shown specific RT-QuIC detection of human sporadic Creutzfeldt-Jakob Disease (sCJD) PrP^{res} in brain samples with different PrP genotypes. Interestingly, both Atarashi and McGuire (Atarashi et al., Nature methods 2011; McGuire et al., Annals of Neurology 2012) have used this technique in a blinded study to identify human sCJD positive CSF samples. We have also used the RT-QuIC in a time course study to investigate prospects of early diagnosis of prion disease based on prion seeding activity in CSF (Orru` et al., Journal of Clinical Microbiology, 2012). We have integrated antibody 15B3-based immunoprecipitation with QuIC reactions to increase sensitivity and isolate prions from plasma samples (Orru` et al., mBIO 2011). This combined assay, which we call "enhanced QuIC" (eQuIC), detects attograms of human variant Creutzfeldt-Jakob disease (vCJD) proteinase K-resistant prion protein spiked into human plasma and discriminates between plasma and serum samples from scrapie-infected and uninfected hamsters. Further studies are aimed at developing and validating these sensitive techniques for the early diagnosis of human and livestock prion diseases.

Oral presentation III (Day 1: 14:15-14:40 on September 19)

O-6

Lack of CD14 delays progression of prion disease by modulating inflammatory environment in the brain

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Prion diseases are fatal neurodegenerative disorders in humans and animals. The diseases are characterized in the central nervous system by deposition of abnormal forms of prion protein, vacuolation of neural tissue, astrocytosis and microglial activation. Although microglia activation is observed before neuronal degeneration occurs, connection of microglial activation and development of the disease is unclear. To find genes expressed by microglia in the early stage of prion disease, we compared gene expression between scrapie-infected and mock-infected mouse brains by cDNA microarray. As a result, we found several immune-related genes including Cd14 were up-regulated in the infected mouse brains. To assess the role of CD14, CD14 knockout ($CD14^{-/-}$) mice were inoculated with scrapie Chandler strain. The $CD14^{-/-}$ mice survived significantly longer than wild type (WT) C57BL/6 mice, suggesting that CD14 is involved in progression of prion diseases. PrP^{Sc} detection by Western blotting showed that the amount of PrP^{Sc} was slightly reduced in the brains of $CD14^{-/-}$ mice in the middle stage of infection (90 and 120 days post infection, dpi). To confirm this, we performed PrP^{Sc}-specific immunohistochemical staining using mAb132 in combination with pre-treatment of frozen sections with 5M guanidine thiocyanate. At 60 dpi, PrP^{Sc} was detected in almost whole area of the thalamus of WT mice, however, in $CD14^{-/-}$ mice, PrP^{Sc} was localized in the ventral part of the thalamus. At that time, microglial markers CD11b and CD68 were detected more in the thalamus of $CD14^{-/-}$ mice than in WT mice. The distribution patterns of PrP^{Sc} and these microglial markers became indistinguishable between WT and $CD14^{-/-}$ mice after 90 (PrP^{Sc}) and 120 dpi (CD11b and CD68). These results suggest that the earlier activation of CD11b- and CD68-positive microgila may contribute to the delayed PrP^{Sc} deposition in the brains of $CD14^{-/-}$ mice by preventing PrP^{Sc} formation or by excluding PrP^{Sc}. Next, we analyzed distribution of Ym1, a marker for alternatively activated microglia. Ym1-positive cells were detected more in CD14-/- mice than WT mice at 90 dpi, although it was hard to distinguish the distribution of Ym1-positive cells between WT and $CD14^{-/-}$ mice after 120 dpi. Ym1-positive microglia was firstly appeared in callosum, then in hippocaumpus, fimbria of the hippocampus, internal capsule, cerebral peduncle and in a part of thalamus. Distribution of CD14-positive microglia in WT mice was similar to that of Ym1-posotive cells, except for hippocampus, suggesting that expression of Ym1 may be down-regulated by CD14 at least in the early to middle stage of the disease. Immunohistochemistry for anti-inflammatory cytokines showed that TGF-β- and IL-10-positive cells were detected more in $CD14^{-/-}$ mice than in WT mice at 60 and 75 dpi in these areas. The results of immunohistochemistry were confirmed by quantitative RT-PCR. These results suggest that brains of $CD14^{-/-}$ mice may be shifted to anti-inflammatory environment at the early stage after prion inoculation, and this may contribute to prolonged survival time of $CD14^{-/-}$ mice. Further analyses of microglial activation status function are needed to elucidate roles of microglia in the pathobiology of prion diseases.

Oral presentation IV (Day 1: 14:55-15:20 on September 19)

O-7

A 3D organotypic tissue model for hantavirus infection of the human airway mucosa

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In this study we use an *in vitro* 3D- model of the human lung to study hantavirus infection in man. So far, most *in vitro* studies on hantaviruses have been performed with cells in monolayer. Using this 3D-model we are able to study hantavirus infection in a lung microenvironment, including interactions between infected cells and immune cells. This model will render it possible to analyze how an infection influences e.g. the cytokine environment in the lungs, how infected cells affect their surroundings, and the involvement of for example monocytes during hantavirus infection.

Initial experiments shows that this model is highly susceptible for hantavirus-infection, and we will present data regarding specific as well as common responses after infection with HFRS and HCPS-causing hantaviruses.

Hantaviruses cause in two serious and often deadly zoonotic diseases in man: Hemorrhagic fever with renal syndrome (HFRS) and Hantavirus cardiopulmonary syndrome (HCPS). Humans are normally infected via inhalation of virus-contaminated rodent excreta, and the initial site of infection is consequently the lungs. The virus then spread systemically, and HFRS/HCPS is believed to be caused by increased vascular permeability. However, hantaviruses are not cytopathic, and it is currently not known if the virus directly cause the observed increased vascular permeability and/or if it is a consequence of virus-induced immune-pathogenesis. We believe that a better understanding of initial responses at the primary site for hantavirus infection will hopefully increase knowledge regarding mechanisms behind HFRS/HCPS.

Oral presentation IV (Day 1: 15:20-15:45 on September 19)

O-8

The role of neutrophils in the induction of pulmonary edema during hantavirus infection in C.B-17Scid mice

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Hantavirus causes two important rodent-borne viral zoonoses, hemorrhagic fever with renal syndrome (HFRS) in Eurasia and hantavirus pulmonary syndrome (HPS) in North and South Americas. Pulmonary involvement with edema has been reported in both HFRS and HPS patients. Most patients with pulmonary edema require mechanical ventilation. However, the mechanism of the induction of pulmonary edema is poorly understood.

We have developed a mouse pulmonary edema model by infection with Hantaan virus cl-1 (HTNV) in adult C.B-17Scid (SCID) mice. The pulmonary edema was histologically observed from 4 weeks after inoculation and peaked at around 5 weeks after inoculation. However, alveolar epithelial cells and vascular endothelial cells with pulmonary edema were intact, as observed in humans.

One of the typical clinical laboratory findings in patients of hantavirus infection is neutrophilic leukocytosis. The percentage of $CD11b^{+}Gr\text{-}1^{hi}$ neutrophils was increased in the lung and blood of the pulmonary edema model at 4 weeks after inoculation. To examine the relationship between the appearance of pulmonary edema and neutrophils, we depleted neutrophils in HTNV-infected SCID mice using rat anti-Gr-1 IgG antibody and performed histological evaluation by counting the number of edema-filled aveoli in more than 300 aveoli at 33 days after inoculation.

Administration of the anti-Gr-1 antibody resulted in depletion of more than 90 % of $CD11b^{+}Gr\text{-}1^{hi}$ neutrophils in blood. The lungs of HTNV-infected control mice were macroscopically transparent by wetting. On the other hand, the lungs of neutrophil-depleted HTNV-infected mice were macroscopically normal and were similar to those of uninfected control mice. Moreover, the percentage of edema-filled aveoli was reduced (91-98% reduction) by neutrophil depletion.

The results indicate that neutrophils might play an important role in induction of pulmonary edema after hantavirus infection in mice. Further studies are needed to determine whether HTNV-infected SCID mice with pulmonary edema are appropriate for a model of pulmonary edema in hantavirus infection.

We thank Dr. Noriyo Nagata and Dr. Hideki Hasegawa, Department of Pathology National Institute of Infectious Diseases for pathological analysis.

Oral presentation IV (Day 1: 15:45-16:10 on September 19)

O-9

Inhibition of Marburg virus budding by nonneutralizing antibodies to the envelope glycoprotein

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The envelope glycoprotein (GP) of Marburg (MARV) virus is responsible for virus entry into host cells and known as the only target of neutralizing antibodies. Here we show that MARV GP-specific monoclonal antibodies AGP127-8 (IgG1) and MGP72-17 (IgM), which do not inhibit GP-mediated entry of MARV into host cells, drastically reduced budding and release of progeny viruses from infected cells. These antibodies similarly inhibited the formation of virus-like particles (VLPs) consisting of GP, the viral matrix protein and nucleoprotein, whereas the Fab fragment of AGP127-8 showed no inhibitory effect. Morphological analyses revealed that filamentous VLPs were bunched on the surface of VLP-producing cells cultured in the presence of the antibodies. These results demonstrate a novel mechanism of antibody-mediated inhibition of MARV budding in which antibodies arrest unformed virus particles on the cell surface. We hypothesize that such antibodies, like classical neutralizing antibodies, contribute to protective immunity against MARV.

Oral presentation V (Day 2: 10:05-10:30 on September 20)

O-10

Human serum resistance in *Trypanosoma brucei gambiense*

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The kinetoplastid *Trypanosoma brucei* is a major parasite of both livestock and humans in sub-Saharan Africa. The organism is traditionally delineated into three morphologically identical sub-species. The most prevalent sub-species is *T. b. brucei,* although it cannot infect humans due to sensitivity to trypanosome lytic factors (TLF) in human serum. Two sub-species have evolved mechanisms to overcome these lytic factors and extend their host range to include humans. *T. b. rhodesiesnse* achieves this via expression of the Serum Resistance Associated gene (*SRA*) that inhibits the lytic component of TLF, Apoliprotein L-1. However, *SRA* is not present in the predominant human-infective sub-species *T. b. gambiense* and so a novel resistance mechanism must be present. Matters are further complicated in that various studies indicate that there are two distinct types of *T. b. gambiense* that differ in population structure, pathology and genetics. Group 1 *T. b. gambiense* is a clonal organism found throughout the central and Western Tsetse belt of sub-Saharan Africa and causes more than 90% of human sleeping sickness cases. It has an invariant human serum phenotype and affects primarily humans, although some reservoirs have been described in domestic animals. Group 2 *T. b. gambiense* are more genetically diverse but have only been described in a few closely related foci in Côte d'Ivoire and Burkino Faso. This group shows a variable resistance to human serum in a manner superficially similar to *T. b. rhodesiense*.

Historically there has been little investigation of the mechanism of serum resistance in either group of *T. b. gambiense* due to the difficulty of working with the parasites in a laboratory setting. The development of improved culturing techniques and more laboratory amenable strains of *T. b. gambiense* has allowed several advancements to be made. Some of the more notable facets will be discussed in this presentation. These include preliminary data from both comparative transcriptomics and quantitative trait analyses identifying candidate genes involved in serum resistance in *T. b. gambiense*. An important resource for this study has been the creation of a *T. b. brucei* / *T. b . gambiense* genetic cross with a progeny library exhibiting differing degrees of human serum resistance. Additionally, several fundamental observations have been described for both groups of *T. b. gambiense* that impact future strategies for using recombinant TLF to combat the disease. A principal example that will be discussed is the decrease in both expression and a 20-fold decrease in the binding efficiency of a TLF receptor in group 1 *T. b. gambiense*. This trait is conserved across all studied *T. b. gambiense* foci and is due to a single polymorphism. This allows the sub-species group to avoid the majority of lytic particles in human serum. Conversely, no such TLF avoidance is exhibited by group 2 *T. b. gambiense*, suggesting that the two groups possess distinct lysis resistance strategies that have both evolved separately from *SRA* in *T. b. rhodesiense*.

Oral presentation V (Day 2: 10:30-10:55 on September 20)

O-11

A novel approach to identify potential pathogens in ticks

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Ticks can transmit a variety of viral, bacterial and protozoal pathogens, which are often zoonotic. The aim of this study was to reveal a diversity of tick microbiomes which may contain as-yet unidentified pathogens using a metagenomic approach. DNA prepared from bacteria-enriched fraction, obtained from seven tick species, was subjected to pyrosequencing after whole genome amplification. Resulting sequence reads were phylotyped using a Batch Learning Self-Organizing Map (BLSOM) program, which allowed phylogenetic estimation based on the similarity of oligonucleotide frequencies, and functionally annotated by BLASTX similarity searches. In addition to bacteria previously associated with human/animal diseases such as *Anaplasma*, *Bartonella*, *Borrelia*, *Ehrlichia*, *Francisella*, and *Rickettsia*, BLSOM analysis detected the microorganisms belonging to the phylum Chlamydiae in some tick species, which were further confirmed by pan-Chlamydia PCR and sequencing analysis. Gene sequences associated with bacterial pathogenesis were also identified, some of which were suspected to be involved in horizontal gene transfer. In conclusion, our efforts to construct a database of tick microbes may lead to the empowerment to predict emerging tick-borne diseases. Furthermore, a comprehensive understanding of the tick microbiomes will be useful to understand tick biology including their vector competency and interactions with pathogens and symbionts.

Oral presentation V (Day 2: 10:55-11:20 on September 20)

O-12

Astrovirus diversity in the cave nectar bats (*Eonycteris spelaea*) in Singapore

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The majority of emerging infectious diseases are zoonotic in origin and bats appear to play an important role as the primary reservoirs for a number of viruses that infect humans. Their population density, global distribution, flight range, migratory behavior, relatively long life span, and proclivity to socially roost may provide the means to maintain and transmit a number of viruses. Over 90 viruses from 16 families have been isolated from bats, including a number of recently emerged viruses such as Nipah, Hendra, Menangle virus, and mammalian astroviruses. After the SARS outbreak in 2003, bats were determined to be the natural hosts for the entire *Coronaviridae* family. Astroviruses have been isolated from a wide variety of animals, including bats, where previous studies have shown high rates of infection. This species studied here have been found infected with Group 2 coronaviruses. Though astroviruses have yet to be isolated from fruit bats, infection in this species has not been studied. Little is known about bat viruses in a highly urbanized setting such as Singapore, nor how the proximity of human and bat populations may influence interspecies transmission. Feces and urine were collected from *E. spelaea* across different locations in Singapore. Using both the RNA-dependent reverse polymerase gene and the capsid gene, we will establish the evolutionary-relatedness of these unique astroviruses.

Oral presentation VI
(Day 2: 13:30-13:55 on September 20)

O-13

Genotypic analysis of extremely drug-resistant tuberculosis (XDR-TB) in Nepal

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Background: Although Nepal has a high annual risk for TB cases and an increasing prevalence of multi drug-resistant tuberculosis (MDR-TB) cases, extensively drug-resistant TB (XDR-TB) has not yet been reported in Nepal. We present here the prevalence of drug-resistant associated mutations and population structure of XDR *M. tuberculosis* currently circulating in Nepal.

Methods: Testing for susceptibility to first-and second-line drugs was performed using proportional method on Löwenstein-Jensen medium. Subsequently, the strains identified as XDR *M. tuberculosis* were subjected to DNA sequencing, spoligotyping and variable numbers of tandem repeats (VNTR).

Results: Thirteen XDR isolates which were resistant to a broad spectrum of antituberculosis drugs were identified among 109 MDR-TB strains. Mutations predominant among XDR-TB isolates were *rpo*BS531L (92.3%), *katG*S315T (92.3%), *gyr*AD94G (53.9%) and *rrs*A1400G (61.5%). Spoligotyping revealed 76.9 % to be Beijing family of *M tuberculosis.* The VNTR typing grouped the isolates into 7 unique patterns and 2 clusters.

Conclusions: The isolation of XDR strains in Nepal is alarming. Our study provides information about the mutations that are common in XDR isolates from Nepal.
Furthermore, acquisition of extensive drug resistance appears to be the primary mechanism driving XDR-TB epidemics in Nepal. This emphasizes an urgent need of better TB control program to prevent the spreading of this potentially incurable disease.

Oral presentation VI
(Day 2: 13:55-14:20 on September 20)

O-14

Metagenomic Composition of the Primate Gut

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Progressive simian immunodeficiency virus (SIV) infection is associated with enteropathy which likely contributes to the development of AIDs, but the mechanisms responsible for intestinal epithelial injury are not understood. To identify candidate etiologies for SIV-associated enteropathy we defined the fecal virome and microbiome in SIV-infected rhesus monkeys that develop AIDs compared to SIV-infected African green monkeys that do not develop AIDs. SIV infection was associated with significant changes in the enteric virome and both the bacterial and phage components of the enteric microbiome in rhesus, but not African green, monkeys. Our analysis revealed the presence of at least 15 previously undescribed enteric viruses in SIV-infected rhesus monkeys. The presence of novel viruses was confirmed by culturing of five new adenoviruses and by confirmatory PCR testing for other viruses. Adenoviruses detected by deep sequencing caused unsuspected ileitis in necropsied SIV-infected rhesus monkeys. Analysis of bacterial and phage communities revealed SIV infection-associated dysbiosis which might also lead to intestinal disease. Thus the intestinal virome and microbiome are substantially altered by SIV infection of AIDs-susceptible rhesus monkeys, providing new candidate mechanisms for induction of epithelial damage, intestinal leakage, and systemic immune activation during progressive lentivirus infection. Studies of intestinal disease and mucosal immunity during lentivirus infection should include analysis of the intestinal virome and microbiome.

Highlights
- SIV-infected rhesus monkeys exhibit changes in the enteric virome and microbiome.
- SIV-infected African green monkeys do not exhibit changes in the enteric virome and microbiome.
- SIV-infected rhesus monkeys are infected with multiple novel enteric viruses.
- Unsuspected enteric virus infection is associated with intestinal pathology in SIV-infected rhesus monkeys.
- Metagenomic analysis of primate stool is a valuable approach for the identification of novel microbes

Oral presentation VII
(Day 2: 14:20-14:45 on September 20)

O-15

Molecular characterization of immunoinhibitory factors PD-1/PD-L1 in bovine leukemia virus-infected cattle

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An immunoinhibitory receptor, programmed dath-1 (PD-1), and its ligand, programmed death-ligand 1 (PD-L1), are involved in the immune evasion mechanisms for several pathogens causing chronic infections and tumors. The blockade of the PD-1/PD-L1 pathway by antibodies specific to either PD-1 or PD-L1 resulted in the reactivation of immune reactions, and is expected to be applied to new therapies for the chronic infectious diseases and tumors. However, few functional analyses of these molecules have been reported for domestic animals.

In the previous study, we cloned bovine PD-1 and PD-L1, and performed the expression analysis of PD-L1in bovine leukemia virus (BLV)-infected cattle. The proportion of PD-L1 positive cells in peripheral blood mononuclear cells (PBMCs) correlated positively with prediction markers for the progression of the disease, while it inversely correlated with the degree of IFN-γ expression. The blockade of PD-L1 *in vitro* by PD-L1-specific antibody upregulated the production of IL-2 and IFN-γ, and correspondingly, downregulated the BLV provirus load. These data suggest that PD-L1 induces immunoinhibition in disease-progressed cattle during chronic BLV infection. However, it is still unknown about PD-1 expression in BLV infected cattle. Thus, to investigate PD-1 expression and function, four anti-bovine PD-1 monoclonal antibodies (α-PD-1) which reacted with PD-1 expressing cells and activated PBMCs were established. PBMCs cultivated with either of the four α-PD-1 produced higher amount of INF-γ than those treated with isotype control antibody. Some α-PD-1 also reacted with activated lymphocytes of pig, horse, dog and cat. These results suggest that α-PD-1 blocks the PD-1/PD-L1 pathway and are useful for expression and functional analyses of PD-1 in BLV infection and other chronic infection of various types of animal.

Oral presentation VII
(Day 2: 14:45-15:10 on September 20)

O-16

Bystander chronic infection negatively impacts the development of CD8 T cell memory

Erietta Stelekati, Haina Shin, Travis Doering, Douglas Dolfi, Carly Zeigler, Daniel Beiting, Jennifer Liboon, David Wolski, Peter Katsikis, Hao Shen, David S. Roos, W. Nicholas Haining, Georg Lauer, E. John Wherry
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Accumulating epidemiological evidence suggests that chronic infections compromise immunity against antigenically unrelated infections, but the underlying mechanisms are unclear. Since chronic infections, including tuberculosis, malaria, persisting viruses and parasitic helminth infections, are known to negatively affect immunological responses to vaccination, we investigated whether antigenically unrelated "bystander" chronic infections impacted the development of memory CD8 T cells, the hallmarks of protective immunity against intracellular pathogens. We demonstrate that chronic bystander infection and inflammation substantially impair memory CD8 T cell differentiation in several mouse models with similar changes in non-human primates and in chronically HCV-infected humans. Type I IFN-induced molecular pathways were specifically dysregulated in memory CD8 T cells in inflammatory environments and induction of type I IFN by chronic poly(I:C) administration similarly impaired memory CD8 T cell development and function. Two transcriptional regulators of T cell development, Blimp-1 and T-bet, integrated the effects of inflammatory mediators in impairing optimal memory CD8 T cell development. Thus, exposure to chronic bystander infections deregulates the normal effector to memory CD8 T cell transition. These results have direct relevance concerning current therapeutic approaches and vaccination strategies for patients with persisting infections or chronic inflammatory conditions.

Oral presentation VIII (Day 2: 15:25-15:50 on September 20)

O-17

Brisk cytokine response against growth of highly pathogenic avian influenza virus leads systemic capillary leakage and sudden death in chickens

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Highly pathogenic avian influenza virus (HPAIV) causes lethal systemic infection in chickens. Although the aberrant cytokine response was observed in HPAIV infection in birds and mammals including humans, little is known about host factors involved in the pathogenicity of HPAIV in chickens. In order to elucidate how host cytokine affect the pathogenesis of highly pathogenic avian influenza in chickens, 106.0 EID50 of two HPAIVs, A/turkey/Italy/4580/1999 (H7N1) (Ty/Italy/99) and A/chicken/Netherlands/2586/2007 (H7N7) (Ck/NL/03) or low pathogenic avian influenza virus (LPAIV), A/chicken/Ibaraki/1/2005 (H5N2) (Ck/Ibaraki/05) were inoculated intranasally into chickens, and virus growth, cytokine response, and capillary permeability were examined. Ty/Italy/99 rapidly replicated in chickens, followed by excessive expression of mRNA of inflammatory (IFN-γ, IL-1β, and IL-6) and anti-viral (IFN-α) cytokines in the brain and spleen, and caused sudden death in chickens. Ck/NL/03 replicated slowly in chickens, followed by moderate expression of mRNA of cytokines in the brain and spleen, and the symptoms were milder compared with those of the chickens inoculated with Ty/Italy/99. By contrast, Ck/Ibaraki/05 caused local infection in chickens and the mRNA expression of cytokines in the tissues was not significant. The brisk response of cytokine, especially IL-6, was in proportion to the extensive virus growth. The capillary leakage assessed by Evans blue was found in the multiple organs, especially in the brain, of the chickens inoculated with Ty/Italy/99, while it was not found in any tissues of the birds inoculated with Ck/NL/03 and Ck/Ibaraki/05.

The present results suggest that sudden death of the chickens inoculated with HPAIV is attributed to multiple organ failure induced by brisk cytokine response against rapid replication of HPAIV.

Oral presentation VIII (Day 2: 15:50-16:15 on September 20)

O-18

Heterosubtypic cross-reactivity of influenza virus hemagglutinin-specific antibodies

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Influenza A viruses are divided into subtypes based on the antigenicity of two envelope glycoproteins, hemagglutinin (HA; H1-H17) and neuraminidase (NA; N1-N10). HA-specific neutralizing antibodies are critical for acquired immunity against influenza virus infection. However, it has been believed that HA-specific antibodies are predominantly specific for a single HA subtype. On the other hand, it was reported that mice and humans recovered from influenza virus infection showed resistance against infection with influenza viruses of different HA subtypes. It has been shown that this cross-protection is mediated by cytotoxic T cells recognizing conserved epitopes of viral internal proteins presented with MHC class I on the surface of infected cells. In contrast, contribution of virus-specific antibodies to the heterosubtypic protection has not been evaluated properly.

Recent reports indicate that some monoclonal antibodies specific to HA have neutralizing ability against multiple strains belonging to different HA subtypes. Thus, we hypothesized that some of antibodies induced by immunization with inactivated virus may have potential to neutralize influenza viruses of multiple HA subtypes. Therefore, we analyzed the cross-reactivity of HA-specific IgG and IgA antibodies induced by intranasal and subcutaneous immunization of mice.

H1, H3, H5, H7, H9, and H13 subtypes were selected for immunization, as the representatives from each cluster of phylogenetic tree based on HA amino acid sequences. After intranasal or subcutaneous immunization of mice with formalin-inactivated virus, serum, trachea-lung wash (TW), and nasal wash (NW) were collected. By an enzyme-linked immunosorbent assay (ELISA) using recombinant HAs (rHAs) of H1-H16 HA subtypes as antigens, the cross-binding ability of IgG and IgA antibodies to each rHA was examined. We found that the heterosubtypic reactivity of antibodies to multiple rHAs was considerable in all immunized groups, while the cross-reactivity was mainly directed to particular HA subtypes of phylogenetically related viruses. Both subcutaneous and intranasal immunization induced antibodies which bound to rHAs of multiple subtypes, although IgA antibodies were not detected remarkably in subcutaneously immunized mice. The overall spectrum of cross reactivity of IgG and IgA did not vary depending on the samples (i.e., serum, TW, and NW) and route of immunization.

To evaluate whether these cross-reactive antibodies have ability to inhibit virus entry or budding *in vitro*, plaque-reduction assay was carried out using the serum, TW, or NW derived from H9 virus-immunized mice which showed a broad spectrum of cross-binding ability in ELISA. When the viruses (H1, H2, H5, H7, H8, H9, H11, or H12 virus) were mixed with the samples before inoculation into MDCK cells, these antibodies exhibited neutralizing activities only against the virus used for immunization (i.e., H9), indicating that the cross-reactive antibodies did not block virus entry. To assess the ability of antibodies to inhibit virus budding, MDCK cells were first infected with H3, H5, H9, or H12 virus, and then incubated for 48 hours in the presence of the serum, TW, or NW of mice immunized with H9 virus. We found that the plaque reduction was significant for H12 virus, which was shown to be recognized by cross-binding antibodies induced in mice immunized with H9 virus. Interestingly, the reduction of plaque size and numbers was significant in the serum of mice immunized intranasally. Since IgG levels were lower in mice immunized intranasally, cross-reactive IgA antibodies may play a major role in the heterosubtypic neutralizing activity. These results suggest that majority of the cross-reactive antibodies did not block virus entry into host cells, but might inhibit virus budding from host cells.

Oral presentation VIII (Day 2: 16:15-16:40 on September 20)

O-19

Signalling pathway involved in innate immune activation by avian influenza virus in chickens: potential application for vaccine development

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Avian influenza viruses (AIV) raise worldwide veterinary and public health concerns due to their potential for zoonotic transmission. While infection with highly pathogenic (HP) AIV results in high mortality in chickens, this is not necessarily the case in wild birds and ducks. We found that infection of chickens with HPAIV H5N1 induces exorbitant levels of bioactive type I interferon (IFN) in the lung, plasma and spleen (Moulin H.R., Liniger M. et al., 2011, Vet. Res. 42, 6). This contrasts with our recent findings *in vitro* with chicken macrophage HD-11 cells in which HPAIV (H5N1) controls the induction of type I IFN. Using reverse genetics, we found that the NS1 protein and the polymerase complex of HPAIV H5N1 act in concert to antagonize type I IFN secretion in HD-11 cells (Liniger M. et al., 2012, Virol. J. 9, 7).

In mammals, the retinoic acid-inducible gene I product (RIG-I) is the main cytosolic pattern recognition receptor (PRR) known to sense influenza A virus infection. However, chickens, unlike ducks, lack RIG-I. They do nevertheless express the melanoma differentiation‑associated gene 5 (MDA5) PRR. Recently we found that chicken cells employ MDA5 to sense AIV infections without specific recognition of triphosphorylated RNA. We further identified the chicken CARDIF and chicken LGP2 genes and demonstrated the functional relevance of their products in the chicken MDA5 signalling cascade (Liniger M. et al., 2012, J. Virol. 86, 705-17). Interestingly, expression of the caspase activation and recruitment domains of chicken MDA5 [chMDA5(1-483)] in chicken DF-1 fibroblast cells triggers type I IFN responses in the absence of pathogen-associated molecular patterns. We hypothesized that mimicking virus infection by chMDA5(1-483)-mediated type I IFN induction may enhance vaccine-induced adaptive immunity. In order to explore this, a plasmid DNA vaccine expressing haemagglutinin (HA) of H5N1 AIV was used to test the potential genetic adjuvant properties of chMDA5(1-483) *in vivo*. When the plasmid expressing chMDA5(1-483) was co-administered with a suboptimal quantity of plasmid DNA expressing HA, a 10-fold higher HA-specific humoral immune response was observed after two intramuscular immunizations. Compared with the non-adjuvanted vaccine, the chMDA5(1-483)-adjuvanted vaccine mediated significantly enhanced protection against a lethal H5N1 challenge infection in chickens, with reduced clinical signs and cloacal virus shedding. These data suggest that innate immune activation by expression of signalling domains of RIG-I-like receptors may be exploited to enhance vaccine efficacy.

P1-1 **Rapid and whole blood diagnostic test for detecting anti-hantavirus antibody in human and rodent**
Takako Amada

P1-2 **Application of SNV and LANV pseudovirions to an alternative method to PRNT assay**
Sanae Nishio

P1-3 **Development of an animal model of hemorrhagic fever with renal syndrome**
Kenta Shimizu

P1-4 **The N-terminus of the Montano virus nucleocapsid protein possesses broadly cross-reactive conformation-dependent epitopes conserved in rodent-borne hantaviruses**
Ngonda Saasa

P1-5 **Characterization of Hokkaido virus, *genus* hantavirus**
Takahiro Sanada

P1-6 **Treatment of rabid rabbits by intrathecal immunization**
Sawang Kesdangsakonwut

P1-7 **Evaluation of antigenic cross-reactivity of tick-borne encephalitis virus and Omsk hemorrhagic fever virus**
Chidumayo Nozyechi Ngulube

P1-8 **Identification of virulence factors in far-eastern subtype of tick-borne encephalitis virus**
Mizuki Sakai

P1-9 **The N^{pro}, E2, and NS4B of classical swine fever virus synergistically play a role for the acquisition of pathogenicity in pigs**
Tomokazu Tamura

P1-10 **Is the function of the HA other than receptor binding specificity responsible for the efficient replication of influenza virus of duck origin in chickens?**
Takahiro Hiono

P1-11 **Susceptibility of jungle crows (*Corvus macrorhynchos*), sparrows (*Passer montanus*), and black rats (*Rattus rattus*) to infection with influenza A/whooper swan/Hokkaido/4/2011 (H5N1)**
Masatoshi Okamatsu

P1-12 **The PB2 with lysine at position 627 of influenza virus A/Hong Kong/483/97 (H5N1) is responsible for the efficient virus replication in mouse cells**
Naoki Yamamoto

P1-13 **Inhibitory effects of an M2-specific monoclonal antibody on different strains of influenza A virus**
Nilton Akio Muto

P1-14 **A computational model for humoral immune responses against influenza virus**
Keisuke Ueno

Blue: Poster Core Time I (Day 1: 16:50-17:50 on September 19)

White: Poster Core Time II (Day 2: 16:40-17:40 on September 20)

P2-1 **Molecular characterization of an immunoinhibitory receptor Tim-3 in bovine leukemia virus-infected cattle**
Tomohiro Okagawa

P2-2 **Characterization of the protooncogene TCL1b as an Akt kinase co-activator**
Manabu Hashimoto

P2-3 **Phosphatidylserine micelle as T cell induction enhancing peptide carrier specifically ingested by professional antigen presenting cells**
Toru Ichihashi

P2-4 **Analysis of intracellular dynamics of inoculated PrP-res and newly generated PrP^{Sc} in Neuro2a cells**
Takeshi Yamasaki

P2-5 **Identification of target antigens with anti-tick vaccine potential**
Naftaly Githaka

P2-6 **Molecular characterization of expressed sequence tags for Dermanyssus gallinae for the development of a novel control method of the mite**
Masayoshi Isezaki

P2-7 **Molecular detection of tick-borne pathogens in ticks from Uganda**
Jesca Nakayima

P2-8 **Metagenomic analysis for tick microbial populations: implications for emerging tick-borne diseases**
Yongjin Qiu

P2-9 **A PCR-based survey of *Babesia bigemina*, *B. bovis* and benign *Theileria* group in cattle in the Republic of the Union of Myanmar**
Tatsuya Sakurai

P2-10 **Genome sequence of *Theileria orientalis* and comparative genome analysis with lymphocyte-transforming species, *T. parva* and *T. annulata***
Kyoko Hayashida

P2-11 **Is the knowledge on Leptospirosis significantly associated with attitude and practice? Results from a survey in Sri Lanka 2011**
Afona Habtemariam

P2-12 **A comparison of recombinant antigen base ELISA assays to diagnose carrier infection of leptospirosis in laboratory rats**
Chandika D. Gamage

P2-13 **Genomic analysis of *Leptospira* species using the next-generation sequencer**
Jung-Ho Youn

P2-14 **Development of loop-mediated isothermal amplification method for detection of *Legionella pneumophila***
Kanjana Changkaew

Abstract

Poster Core time I at Lecture room 2 (Day 1: 16:50-17:50 on September 19)

P1-1

Rapid and whole blood diagnostic test for detecting anti-hantavirus antibody in human and rodent

Takako Amada[1] Kumiko Yoshimatsu[1], Shumpei P. Yasuda[1], Takaaki Koma[1], Kenta Shimizu[1], Nobuhito Hayashimoto[2], Akira Takakura[2], Jiro Arikawa[1]*
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Objectives: Hantaviruses are associated with two rodent borne viral zoonoses, haemorrhagic fever with renal syndrome (HFRS) and Hantavirus pulmonary syndrome (HPS). Although ELISA and IFA have most widely been used as diagnoses of hantavirus infection, they require laboratory equipments and are time-consuming tests. Therefore, more simple, rapid, and accurate method for the detection of Hantavirus infection is needed. In this study we tried to develop an immunochromatography (ICG) test to diagnose hantavirus infection in humans and rodents.

Methods: Colloidal Gold WRGH2 (Wine red Chemicals) labelled with rabbit anti-rat IgG protein (Sigma), and Protein A-Colloidal Gold conjugate (EY Laboratories) were used for the visualization of antigen-antibody binding of rat and human sera, respectively. Seoul virus (SEOV), Puumala virus (PUUV) and Andes virus (ANDV) were selected as representative causative viruses of HFRS, NE (mild type HFRS in northern Europe) and HPS, respectively. N-terminal 103 amino acids of nucleocapsid protein (NP) of the three representative hantaviruses were expressed by *E. coli* (pET43.1) and used as ICG antigen. Serially collected rat sera from experimentally inoculated with SEOV were used as control positive rat sera. Serum specimens (1:75) showing clear band at test line was regarded as ICG test positive. Results by ICG test were compared with ELISA and IFA.

Results: Experimentally inoculated rat sera began to be positive by ICG test against SEOV on day 6, while those by ELISA on day 9. Serum specimens obtained at 49 days post infection showed IFA, ELISA and ICG titers of 10^3, 10^5 and 10^5, respectively. These results indicated that this ICG test has higher and similar sensitivity to detect antibody comparing to IFA and ELISA, respectively. A total of 340 sera, which composed of 19 experimentally inoculated rats, 38 naturally infected urban rats, 283 uninfected rats, were examined. The overall sensitivity and specificity of ICG compared to ELISA and/or IFA were 100% and 99.8%, respectively. Whole blood diluted with PBS (1:75) was also applicable to ICG test. A total of 28, 64 and 30 sera from HFRS, NE and HPS patients, respectively were examined by ICG test with SEOV, PUUV and ANDV antigens. The sensitivities of ICG test compared to ELISA were 100% for HFRS and HPS patient sera and 92.2% for NE patient sera. The multiplex ICG test which immobilized with 3 different antigens on one strip able to serotype HFRS, NE and HPS patient sera.

Conclusion: The ICG test was considered as rapid, simple and safe diagnoses of SEOV infection in rats as well as HFRS, NE and HPS patients. Applicability of whole blood specimens for ICG enables to utilize this ICG test for field survey and bedside diagnoses.

Poster Core time II at Lecture room 2 (Day 2: 16:40-17:40 on September 20)

P1-2

Application of SNV and LANV pseudovirions to an alternative method to PRNT assay

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Sin Nombre virus (SNV) , Laguna Negra virus (LANV) and Andes virus (ANDV) are members of the *Hantavirus* genus of the *Bunyaviridae* family, and causative agents of fatal disease, hantavirus cardiopulmonary syndrome (HCPS) . Since the fatality rate of them are different, the distinction of them has clinical importance. The standard diagnosis of HCPS is neutralization test, which need BSL3 facility. There are two groups in hantavirus, the New World hantaviruses which cause HCPS and the Old World hantaziruses which cause hemorrhagic fever with renal syndrome (HFRS) . the Old World hantaviruses involve Hantaan virus (HTNV) , Seoul virus (SEOV) , and so on. We have already established the pseudovirion systems of the Old World hantavirus. The aim of this study is to develop pseudovirion systems and as a neutralization test of the New World hantavirus.

The pseudovirions would have hantavirus glycoproteins and vesicular stomatitis virus (VSV) core in which the gene of G protein has been replaced by that of GFP. They would contribute for the study of hantavirus; safe and effective neutralization assay, investigation of virus tropism or mechanism of virus entry, development of effective vaccine. We have already prepared pseudotype VSV of ANDV. Now we are tring to clone cDNA of envelope glycoproteins of SNV and LANV. We will report present outcome.

Poster Core time I at Lecture room 2 (Day 1: 16:50-17:50 on September 19)

P1-3

Development of an animal model of hemorrhagic fever with renal syndrome

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Hemorrhagic fever with renal syndrome (HFRS) caused by hantavirus infection is characterized by fever, renal dysfunction and hemorrhage. To understand the pathogenesis and develop therapeutic measures, animal model that mimics the human disease is necessary. Here we developed an animal model showing renal hemorrhage like in HFRS patients. 5 clones of Korean hemorrhagic fever virus (KHFV) that was derived from a HFRS patient serum were obtained by plaque cloning and inoculated into 4-week-old female BALB/c mice. Mice inoculated intraperitoneally with the virus clones showed no symptom. In contrast, mice inoculated intravenously with the virus clones, except for KHFV clone 4, showed transient bodyweight loss from 6 to 9 days post-inoculation (dpi). Pathological examination demonstrated hemorrhage of renal medulla in KHFV clone 5-infected mice, but not in KHFV clone 4-infected mice, at 9 dpi. Sequence analysis showed that amino acid at position 417 in glycoprotein Gn was different between KHFV clone 4 and 5: lysine (K) and glutamic acid (E), respectively. These results suggest that amino acid at position 417 of Gn is important for the induction of renal hemorrhage in hantavirus infection. This animal model may be useful for analyzing the pathogenesis of HFRS.

Poster Core time II at Lecture room 2 (Day 2: 16:40-17:40 on September 20)

P1-4

The N-terminus of the Montano virus nucleocapsid protein possesses broadly cross-reactive conformation-dependent epitopes conserved in rodent-borne hantaviruses

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Hantaviruses are important human pathogens causing hantavirus pulmonary syndrome (HPS) and hemorrhagic fever with renal syndrome in the Americas and Eurasia, respectively. The hantavirus nucleocapsid (N) protein stimulates a strong and cross-reactive immune response in humans and rodents. A significant proportion of the response to N protein has been found to target the N-terminus coiled-coil domain. However, the exact nature of this bias towards the N-terminus has not yet been thoroughly examined.
Cross reactivity of six monoclonal antibodies (mAbs) raised against the N protein of Mexican hantavirus, Montano virus (MTNV) was evaluated for various hantaviruses by ELISA, Western blotting (WB) and indirect immunofluorescence (IFA).
ELISA and IFA results indicated that the epitopes of all the six mAbs were within the N terminus 100 amino acids. Five of the six mAbs recognized eight American and six European and Asian rodent-borne hantaviruses, but not the Soricomorpha-borne Thottapalayam hantavirus. Analysis of deleted mutants within the N-terminus 100 amino acids revealed that the N-terminal 13-51 amino acid residues were critical for recognition by 5 mAbs; while the epitopes of a single serotype-specific mAb was mapped to discontinuous 1-25 and 49-75 residues.
The N terminus of hantavirus N protein is known to assume a coiled-coil α-helix configuration. The results obtained by IFA and WB using deleted mutants indicated that the epitopes of the six mAbs were discontinuous and/or conformation-dependent in nature. Additionally, despite the extensive differences of up to 50% within the N-terminus 80 amino acid residues of the N protein of different hantaviruses examined, all the mAbs still retained reactivity. These findings therefore suggest that cross reactive epitopes at the N -terminus are structurally conserved, at least in rodent borne hantaviruses.

Abstract

Poster Core time I at Lecture room 2 (Day 1: 16:50-17:50 on September 19)

P1-5

Characterization of Hokkaido virus, *genus* hantavirus

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[Introduction]
Hantaviruses belong to the *Bunyaviridae* family and cause two severe human illnesses, hemorrhagic fever with renal syndrome (HFRS) and hantavirus cardiopulmonary syndrome (HCPS). More than 30 viruses have been reported in the genus *Hantavirus* and are carried by the specific rodent host. Although VeroE6 cell line originating from African green monkey kidney is used widely in hantavirus research, hantaviruses do not grow well even in VeroE6 cells, and isolation is not always successful. Therefore, to generate a novel cell line that shows a greater susceptibility to hantaviruses and from which hantavirus could be isolated easily, we established new cell line, designated as "MRK101", derived from a kidney of gray red-backed vole (*Myodes rufocanus bedfordiae*), the natural host of Hokkaido virus (HOKV). Using this cell line, we isolated HOKV Kitahiyama128/2008 strain. In this study, to analyze the newly isolated HOKV in more detail, we analyzed the viral growth of HOKV in VeroE6 and MRK101cells and we determined the full-length sequences of S, M, and L segments.
[Materials and Methods]
To assess the growth of HOKV, the HOKV Kitahiyama128/2008 strain was inoculated to MRK101 and VeroE6 cells. Culture fluids and infected cells were collected at 1, 3, 5, 7, 10, and 14 dpi. The collected fluids were subjected to focus-forming assays, and the presence of viral antigens in infected cells was evaluated by Western blotting. To determine the full-length sequences of S, M, and L segments of the newly isolated HOKV strain, each segment of the HOKV was amplified using specific primers and then sequenced directly.
[Results]
In MRK101 cells, the expression of N protein was confirmed at 14 dpi. The level of progeny virus increased gradually until 14 dpi. In contrast, propagation of HOKV in VeroE6 cells was unsuccessful; neither expression of N protein nor infectious virus was detected. The S segment sequence of this isolated HOKV was almost identical to previously reported HOKVs (97–98.7% nt and 99.8–100% aa identity). HOKV was the most closely related to PUUV (S, 81.9–83.8% nt and 94.7–95.8% aa identity; M, 78.1–79.9% nt and 90.6–91.6% aa identity; L, 79.7–80.7% nt and 95.3–95.6% aa identity) which is the etiological agent of HFRS. Phylogenetic analysis based on the nucleotide sequence of the coding region of the S, M, and L segments supported the close relationship between HOKV and PUUV.
[Discussion]
This is the first report on hantavirus which propagates in a cell line that originated from the natural host but not VeroE6 cells. At present, the reason for the inability of HOKV to propagate in VeroE6 cells is not known, but analysis of this phenomenon would provide us important information regarding cell adaptation and host specificity in hantavirus infection.

Poster Core time II at Lecture room 2 (Day 2: 16:40-17:40 on September 20)

P1-6

Treatment of rabid rabbits by intrathecal immunization

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Rabies is a progressive fatal encephalitis causing more than 55,000 cases annually. No effective treatment is available so far for humans and animal affected with rabies. We haves reported that intrathecal (IT) immunization, a direct introduction of the vaccine into the subarachnoid space, induces high antigen-specific antibody response both in the serum and cerebrospinal fluid (CSF), and the immunization completely protected the mice against pseudorabies and rabies virus challenge via peripheral route. In addition, 80% of IT immunized mice survived after intracerebral rabies virus inoculation. Recently, we also demonstrated that the CSF antibody induced by IT vaccination derived from both serum antibody and de novo antibody locally produced in the central nervous system (CNS). In additon, subcutaneous (SC) immunization prior to IT immunization induced higher and more prompt antibody response in the CSF than IT immunization alone.

In this study, we investigated the therapeutic effects of IT immunization for experimentally induced rabid rabbit. Seven, New Zealand white rabbits received SC immunization 3 days before intramuscular challenge with CVS (fixed) strain of rabies virus in the hind limbs. When the rabbits showed clinical signs of rabies, the rabbit was given IT (SC/IT group; 4 rabbits) or SC (SC/SC group; 3 rabbits) immunization as soon as possible. Clinical signs and body weight changes were recorded daily. Three rabbits (Non-treatment group) were challenged with rabies virus without any vaccination and served as positive control. Three rabbits (SC group) were given SC vaccination and challenged with rabies virus. After that, no treatments were given to the rabbits of the SC group. All the dead and euthanized rabbits were necropsied and tissue samples including the CNS were collected for pathological examination and viral antigen detection.

All rabbits showed rabid signs such as unstable and incoordination of the hind limb within 4-8 days post inoculation (dpi). Then, the rabbits progressively deterioated at 8-10 dpi. All the 3 rabbits in Non-treatment group, 2 of 3 rabbits of SC group and all the 3 rabbits of SC/SC groups died within 8-12 dpi. On the other hand, all the rabbit in SC/IT group and one rabbit in SC group recovered from the terminal stage and started eating and drinking from 12-18 dpi and survived until the end of the experiment. Pathologically, died rabbits revealed the necrosis of many neurons with occasional neuronophagia throughout the CNS with the presence of RV antigen in the nerve cells. These changes were most prominent in the cerebral cortex of parietal lobe, thalamus and hypothalamus, ascending neuclei and reticular formation of the brain stem, cerebellar vermis, dorsal horn and intermediate substance of gray matter of the spinal cords, and lumbar and sacral dorsal ganglions. Proliferation and hypertrophy of Iba-1$^+$ microglia and diffuse, perivascular and meningeal infiltrations of lymphocytes consisting mainly of CD3$^+$ T lymphocytes were also remarkable in these tissues. On the other hand, descending, motor and pyramidal nerve routes such as cerebral basal nuclei, hippocampus, cerebellar hemisphere and ventral horn of the spinal cord were relatively spared from the pathological changes. Survived 5 rabbits showed prominent nerve cell loss in cerebellar vermis (Purkinje cell and granular cell layers), brain stem (pontine reticular nuclei and tegmental areas), gray matter of the spinal cord (dorsal horn and intermediate substance), and small malacic foci were sometimes formed in these areas. These change accompanied mild lymphoplasmacytic infiltration in the meninges, perivascular space and nerve tissues, mild hyperplasia of Iba-1$^+$ microglial cells and swelling of astrocytic nuclei. CD3$^+$ T lymphocytes were mixed but were not dominant among the lymphocytes. These tissue damages of the nervous tissue were more severe in one survived rabbit of SC group in compare with 4 rabbits of SC/IT group. RV antigen was rarely found both in the CNS and peripheral nervous tissues of all the survived rabbits. Interestingly, both antibody titers measured by ELISA and neutralizing antibody of serum and CSF increased gradually after the virus challenge and those antibody titers were not significantly different among the rabbits of the four groups at the terminal stages (8-12 dpi).

Abstract

Poster Core time I at Lecture room 2 (Day 1: 16:50-17:50 on September 19)

P1-7

Evaluation of antigenic cross-reactivity of tick-borne encephalitis virus and Omsk hemorrhagic fever virus

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Background: Omsk hemorrhagic fever virus (OHFV) belongs to the tick-borne encephalitis serocomplex of flaviviruses. The viruses in this serocomplex have a close genetic relationship; however, unlike other tick-borne encephalitis viruses in the serocomplex, which cause neurological disease, OHFV causes a hemorrhagic fever syndrome. Limited studies have been performed on the immunogenic relationship between OHFV and other tick-borne encephalitis viruses. This study investigated the cross-reactivity of Tick-borne encephalitis virus (TBEV) and Omsk hemorrhagic fever virus.

Methodology: Virus neutralization tests were used to analyze neutralizing antibodies of C57BL/6 mice inoculated with either TBEV Sofin strain and OHFV Guriev strain. To further broaden our knowledge of the cross-reactivity between these two viruses, the efficacy of the TBE vaccine to protect against OHFV infection was assessed by analyzing the immune response of humans and C57BL/6 mice vaccinated with a formaldehyde inactivated whole virion vaccine based on the European subtype of TBEV. In addition, to determine the efficacy of the vaccine *in vivo*, mice were vaccinated and challenged with a lethal dose of OHFV.

Results and discussion: All the mice inoculated with the live viruses produced cross-reactive antibodies. However, mice inoculated with TBEV had high neutralization antibody titres against both OHFV and TBEV. In contrast, mice inoculated with OHFV had moderate neutralization antibody titres against TBEV but high neutralization antibody titres against OHFV.

In humans, the TBE vaccine induced a pronounced immune response against TBEV and OHFV with a seroconversion rate of 74% and 68% respectively after two vaccinations. Following three vaccinations, the seroconversion rate increased to 94% and 82% for TBEV and OHFV respectively. Similarly, the vaccine was able to induce an immune response in mice with a seroconversion rate of 71% and 57% against TBEV and OHFV respectively. Furthermore, the TBE vaccine provided 100% protection against a lethal challenge of OHFV.

These results indicate that TBEV and OHFV are highly cross-reactive and that the European TBE vaccine can protect against OHFV infection.

Poster Core time II at Lecture room 2 (Day 2: 16:40-17:40 on September 20)

P1-8

Identification of virulence factors in far-eastern subtype of tick-borne encephalitis virus

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【Background】
Tick-borne encephalitis virus (TBEV) is a flavivirus of wide geographic distribution and the causative agent of tick-borne encephalitis (TBE). TBEV is believed to cause about 10,000 cases of encephalitis in Europe and Asia annually. However, the mechanisms underlying the pathogenicity of TBEV remain unknown. In 1993, a human case of TBE was reported in Japan. The virus strain Oshima 5-10 was subsequently isolated from a sentinel dog in the same area in 1995. Although Oshima 5-10 was identified as a member of the Far-Eastern subtype of TBEV, a subtype known to be highly pathogenic to humans. However, in the mouse model, it is less virulent than Sofjin-HO, a prototype of the Far-Eastern subtype, the homology of amino acids between Oshima 5-10 and Sofjin-HO is 98.6%, with differences of only 44 amino acids. Therefore, identification of the virulence factors in the two strains may allow us to understand the mechanisms of the pathogenicity. In this study, we constructed chimeric viruses between the strain Sofjin-HO and Oshima 5-10 and assessed the pathogenicity.

【Materials and Methods】
We used cDNA clones of the two strains and the structural protein region of the cDNA clones of the two strains were substituted. The mRNAs were transcripted from the chimeric cDNA clones and transfected into BHK-21 cells. The recombinant viruses were then harvested from the supernatant. To examine their pathogenicity, 5 week old female C57BL6 mice were infected subcutaneously with 1,000 pfu of the viruses. We then constructed Oshima-based viruses with substitution of the non-structural protein region for those of Sofjin-HO.

【Results and discussion 】
The substitution of the coding region of most of the structural protein did not affect the pathogenisity.
The mice infected with the Oshima-based virus with C-terminal of the non-structural protein NS5 and 3'-untranslated region(UTR) (nt9829-11100) of Sofjin-HO showed similar clinical sign, average of survival time and survival rate to Sofjin-HO infected mice.
These results suggest that the NS5 and/or 3 '-UTR are important in the pathogenicity of the Far-Eastern subtype of TBEV. Therefore, we are investigating how these factors affect the virulence in the process of clarifying the mechanisms of pathogenesis of the viruses.

Abstract

Poster Core time I at Lecture room 2 (Day 1: 16:50-17:50 on September 19)

P1-9

The N^{pro}, E2, and NS4B of classical swine fever virus synergistically play a role for the acquisition of pathogenicity in pigs

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Classical swine fever virus (CSFV) is the causative agent of classical swine fever (CSF), a highly contagious disease of pigs. The live attenuated vaccine strain GPE^- was established through multiple passages of the virulent ALD strain in cells of swine, bovine, and guinea pig origin. To identify the determinants responsible for the attenuation, we examined whether GPE^- recover pathogenicity after serial passages in pigs.

The tonsil homogenates of pigs inoculated intramuscularly with GPE^- was used for serial passages in pigs. Passaged viruses were analyzed for their pathogenicity in groups of 5 pigs. The pigs inoculated with GPE^-/P-11 isolated from tonsils after 11 *in vivo* passages showed leucopenia, thrombocytopenia, and long-term viremia. One of 5 pigs died 11 days after inoculation and 3 of 5 pigs showed gross pathological lesions typical of CSF. Comparison of nucleotide sequence of the genome of GPE^-/P-11 with that of GPE^- revealed 3 amino acid substitutions, T830A in E2, and V2475A and A2563V in NS4B of the GPE^-/P-11 virus. On the basis of the sequence data, mutant viruses were generated by site-directed mutagenesis of the GPE^- backbone. Experimental infection of pigs with these mutant viruses obtained by reverse genetics confirmed that these 3 amino acid substitutions are responsible for the acquisition of pathogenicity. *In vitro* studies indicated that the single substitution in E2 influenced virus spreading, and that the double substitutions in NS4B enhanced the viral RNA replication.

We, then, examined whether restoring the counteraction of interferon-α and -β (IFN-α/β) induction by N^{pro} in the GPE^- virus carrying the 3 amino acid substitutions T830A, V2475A, and A2563V would further enhance pathogenicity. The virus carrying N136D substitution in N^{pro} of the triple mutant virus ($vGPE^-$/T830A; V2475A; A2563V) backbone was generated. The production of IFN-α/β was diminished in the cells infected with the $vGPE^-$/N136D; T830A; V2475A; A2563V virus, but not with $vGPE^-$/T830A; V2475A; A2563V. Experimental infections of pigs with these 2 viruses showed that the diminution of IFN-α/β induction by functional N^{pro} enhance pathogenicity of CSFV.

In conclusion, the present study identified amino acid residues in N^{pro}, E2, and NS4B of CSFV that are responsible for efficient replication of virus and pathogenicity in pigs.

Poster Core time II at Lecture room 2 (Day 2: 16:40-17:40 on September 20)

P1-10

Is the function of the HA other than receptor binding specificity responsible for the efficient replication of influenza virus of duck origin in chickens?

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Wild ducks are the natural host of influenza A viruses. Although it is explained that duck influenza viruses are transmitted to chickens via domestic water and terrestrial birds, the mechanism of the selection of the virus that can be transmitted to and replicate efficiently in chickens is not known. In the present study, virus factors responsible for the efficient replication of influenza viruses of duck origin in chickens were studied.

Duck influenza virus, A/duck/Mongolia/54/2001 (H5N2) (Dk/Mongolia), chicken influenza virus, A/chicken/ Ibaraki/1/2005 (H5N2) (Ck/Ibaraki) and their genetic reassortants were generated by reverse genetics and inoculated into 4-week-old chickens intranasally. Viruses with the hemagglutinin (HA) gene derived from Ck/Ibaraki replicated in chickens, whereas viruses with the HA gene from Dk/Mongolia did not.

Virus with the HA gene derived from Dk/Mongolia and other gene segments from Ck/Ibaraki (rgHA Mon/Iba) were inoculated into the air sacs of 3-day-old chicks. Viruses were recovered from the respiratory tract of infected chicks and designated as rgHA Mon/Iba P1. While rgHA Mon/Iba did not grow in 4-week-old chickens, rgHA Mon/Iba P1 did a little. Sequencing of the HA gene of the original virus and rgHA Mon/Iba P1 revealed an amino acid substitution, N79D of the HA2 subunit. The optimal pH of the original virus for fusion is 4.8, whereas that of rgHA Mon/Iba P1 is 5.2 respectively.

Thus, it was revealed that the HA is a determinant for the efficient growth of influenza virus in chickens and suggested that fusion activity may be involved in the efficient virus replication in chickens. Analysis of fusion efficiency of rgHA Mon/Iba P1 and the original virus is under way.

Abstract

Poster Core time I at Lecture room 2 (Day 1: 16:50-17:50 on September 19)

P1-11

Susceptibility of jungle crows (*Corvus macrorhynchos*), sparrows (*Passer montanus*), and black rats (*Rattus rattus*) to infection with influenza A/whooper swan/ Hokkaido/4/2011 (H5N1)

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H5N1 highly pathogenic avian influenza viruses were isolated from wild birds and domestic chickens in Japan during 2010 to 2011. To assess the role of the animals in transmission of the H5N1 viruses into chicken flocks, the susceptibility of jungle crows (Corvus macrorhynchos), sparrows (Passer montanus), and black rats (Rattus rattus) to infection with A/whooper swan/Hokkaido/4/2011 (H5N1) was examined. After confirming that the animals did not have the specific antibodies, 106.0 EID50 of the virus was intranasally inoculated into the animals and they were monitord daily for clinical signs. The animals were sucrificed on 3 days post inoculation (dpi) and virus titers in their oral and cloacal swabs and tissues were determined. All jungle crows inoculated with the virus did not show any clinical signs for 14 days and the virus was recoverd from the lungs, kidneys, and colon of one of the 4 crows. All sparrows inoculated with the virus died by 8 dpi and the virus was recovered in high titers from the brain, lungs, and colon. All black rats inoculated with the virus did not show any clinical signs for 14 days and the virus was recovered from the kidneys and rectum of one of the 5 rats. The present resultes indicate that sparrows are higly susceptible to infection with the H5N1 virus but jungle crows and black rats are not. Since sparrows usually stray into chicken farms, it is important for disease control of highly pathogenic avian influenza to keep the sparrow away from chicken flocks. The present study was supported by Yubari City, Hunting Association of Nagano Prefecture, and Ikari Corp.

Poster Core time II at Lecture room 2 (Day 2: 16:40-17:40 on September 20)

P1-12

The PB2 with lysine at position 627 of influenza virus A/Hong Kong/483/97 (H5N1) is responsible for the efficient virus replication in mouse cells

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Influenza virus polymerase complex consisting of the PB2, PB1, and PA plays a role in both transcription and replication of the viral genes. Replication efficiency of the virus is determined by the activity of viral polymerase, hence, responsible for the pathogenicity of the virus in the host. It was reported that pathogenicity of A/Hong Kong/483/97 (H5N1) (HK483-K) with lysine (K) at position 627 of the PB2 was much higher than that of A/Hong Kong/486/97 (H5N1) with glutamic acid (E) at the position in mice (Hatta *et al.,* 2001).

In the present study, to clarify the reason why the pathogenicity of HK483-K is higher than that of the virus which has the PB2 with E at position 627 in mice, mutant viruses were generated by substituting K at the position of the PB2 with E, alanine (A), arginine (R), aspartic acid (D), and valine (V) (HK483-E, HK483-A, HK483-R, HK483-D, and HK483-V, respectively). Growth potential of HK483-K was much higher than those of HK483-E, HK483-A, HK483-R, HK483-D, and HK483-V in mouse cells. The large amounts of the PB2, PB1, PA, NP, and M1 mRNAs and proteins were detected in the cells infected with HK483-K compared with those of the cells infected with HK483-E. The present results indicate that the PB2 with K at position 627 is responsible for efficient synthesis of viral RNA, leading to high growth of the virus in mouse cells and high pathogenicity in mice.

Poster Core time I at Lecture room 2 (Day 1: 16:50-17:50 on September 19)

P1-13

Inhibitory effects of an M2-specific monoclonal antibody on different strains of influenza A virus

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New approaches to the treatment of influenza have been designed based on the highly conserved antigenicity of the M2 envelope protein among influenza A virus strains. The present study examined the anti-viral activities of an anti-M2 ectodomain (M2e) monoclonal antibody (clone rM2ss23), which binds to the M2 proteins of the influenza A virus strains A/Aichi/2/68 (H3N2) (Aichi) and A/PR/8/34 (H1N1) (PR8). The results showed that rM2ss23 bound to both Aichi and PR8 M2 proteins expressed on the cell surface. While the antibody did not prevent virus entry into cells, it significantly inhibited plaque formation by the Aichi strain in a dose-dependent manner when infected cells were cultured in the presence of the antibody. By contrast, the growth of PR8 (H1N1) was not affected by the antibody. A reverse genetics approach revealed that the inhibitory effect of rM2ss23 on the Aichi virus was abolished by replacing the genes encoding the HA and/or M proteins with those of the PR8 strain. It is suggested that inhibitory effect of rM2ss23 is related to HA protein as well as M2 protein of influenza virus. Further studies on the interaction between HA and M2 proteins will be necessary to fully elucidate the mechanism of viral growth inhibition by anti-M2e antibody.

Poster Core time II at Lecture room 2 (Day 2: 16:40-17:40 on September 20)

P1-14

A computational model for humoral immune responses against influenza virus

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Artificial immune systems for modeling and simulation have been inspired from the biological immune systems with impressive capabilities of perception, pattern recognition and memory formation. In actual immune systems, the affinity maturation that produces antibodies binding an antigen with high affinity based on the antigenic structure is essential for proper immunity against pathogens. However, the causality between changes in antigenic structures and production of antibodies is unknown. Here we show a computational model of affinity maturation accounts for hemagglutination inhibition (HI) titers depending on antigenic changes of influenza virus. We found in the affinity maturation model that the statistical nature of structural space of antigenic sites rather than their own structures has the remarkable significance of predicting HI titers. For the HI titers of H1N1 and H3N2 subtypes, these results in two notable features observed in actual data: the different homologous titers among antiserums and the different heterologous titers when exchanging the pair of antiserum and virus strain. Furthermore, we found that the antigenic sites near the receptor-binding site were more correlated to HI titers than the others. Our model demonstrates how the structural changes of influenza viruses are likely to affect resistance to the infections, simulating the affinity maturation of antibodies as well as quantifying the differences from the antigenic structures of the past strains. We anticipate our model to be a starting point for more sophisticated vaccination models of influenza virus. For example, the multivalent vaccine and the repeated vaccination could be modeled, considering vaccine efficacy even for "hypothetical" sequences. Furthermore, antigenicity is a major determinant of the evolution of influenza virus, and a well-defined and quantitative model for herd immunity will be relevant for rapid detections of newly emerged epidemic strains of influenza virus.

Abstract

Poster Core time I at Lecture room 2 (Day 1: 16:50-17:50 on September 19)

P2-1

Molecular characterization of an immunoinhibitory receptor Tim-3 in bovine leukemia virus-infected cattle

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The immunoinhibitory receptor, T cell immunoglobulin domain and mucin domain-3 (Tim-3), and its ligand, galectin-9 (Gal-9), are involved in the immune evasion mechanisms for several pathogens causing chronic infectious deiseases. Previous studies showed that the blockade of the Tim-3 pathway by anti-Tim-3 antibody enhanced the production of anti-viral cytokines, proliferation of antigen-specific $CD8^+$ T cells and reduction of viral load. Moreover, the combined blockade of the Tim-3 and programmed death-1 (PD-1) pathways enhanced these effects. For these reasons, both Tim -3 and PD-1 could be a new potential therapeutic target against chronic viral infections. Thus, many studies have been performed on human and mouse Tim-3 pathway. However, there is no reports on the role of Tim-3 pathway in diseases of domestic animals. In this study, therefore, cDNAs encoding for bovine *Tim-3* and *Gal-9* were cloned and sequenced, and their expressions and roles on immune reactivation were analyzed in cattle infected with bovine leukemia virus (BLV).

Full-length ORF cDNA clones of bovine *Tim-3* and *Gal-9* were identified. For Gal-9, two kinds of transcription products, Gal-9 long and short, were obtained. Predicted amino acid sequences of Tim-3, Gal-9 long and Gal-9 short shared high homologies with human and mouse homologues, respectively. Functional domains, including tyrosine kinase phosphorylation motif in the intracellular domain of Tim-3, were highly conserved among cattle and other species.

Quantitative real-time RT-PCR analysis showed that bovine *Tim-3* mRNA is mainly expressed in T cells such as $CD4^+$ and $CD8^+$ cells, while *Gal-9* mRNA is mainly expressed in monocyte lineage cells and T cells. *Tim-3* mRNA expression on $CD4^+$ and $CD8^+$ cells was upregulated with disease progression of BLV infection. In addition, the *Tim-3* expression level on these cells correlated with proviral load and *IL-10* mRNA in infected cattle.

The expressions of *IFN-γ* and *IL-2* mRNA were upregulated when peripheral mononuclear cells from BLV-infected cattle were cultured with COS-7 cells expressing Tim-3 to block the Tim-3 pathway. Moreover, the combined blockade of the Tim-3 and PD-1 pathways significantly promoted *IFN-γ* mRNA expression compared to the blockade of the PD-1 pathway alone.

These results suggest that Tim-3 is involved in the suppression of T cell function during BLV infection. Further investigations are required to develop a novel vaccine and therapeutic method against BLV infection by the use of anti-Tim-3 antibody or recombinant Tim-3 Fc fusion protein.

Poster Core time II at Lecture room 2 (Day 2: 16:40-17:40 on September 20)

P2-2

Characterization of the protooncogene TCL1b as an Akt kinase co-activator

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We have demonstrated that protooncogene *TCL1* (T cell leukemia 1), implicated in human T cell prolymphocytic leukemia (T-PLL), interacts with Akt, enhances its kinase activity, and functions as an Akt kinase co-activator. The TCL1 protein family consists of three members—TCL1, MTCP1 and TCL1b—which consists of the114,106, and 128 amino acids, respectively—with relatively high sequence and structural homology. In human T-PLL, however, both *TCL1* and *TCL1b* genes are activated by juxtaposition onto the T cell receptor α or β loci, secondary to chromosomal translocations t(14:14) (q11: q32), t(7:14)(q35: q32), or inversion (14)(q11: q32). Therefore, it remains unclear that whether TCL1b, independent of TCL1, bears oncogenicity.

In this study, using biochemical, bioinformatics, and in vivo approaches we investigated whether and how TCL1b might have similar function as Akt kinase coactivator underlain the pathogenesis of human neoplastic diseases. In co-immunoprecipitation assays using 293T cells, TCL1b interacted with Akt. In vitro Akt kinase assays, TCL1b enhanced Akt kinase activity. Bioinformatics approach using Agilent expression DNA Array Analysis of TCL1b,TCL1, and Myr-Akt, a constitutive active form of Akt, for cluster analysis, KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway mapping, and Gene ontology demonstrated that TCL1b showed highly homologous gene induction signature as Myr-Akt or TCL1. In vitro soft agar colony transformation assay demonstrated that TCL1b showed oncogenic potency over Myr-Akt or TCL1.Two lines of β-actin promoter driven TCL1b-transgenic mice resulted in hemangiosarcoma from intestinal origin. Consistently, four cases of human angiosarcoma stained positively by both anti-TCL1b and phospho-Akt antibodies. In human cancer tissue arrays, 69 out of 146 cases stained positively by anti-TCL1b antibody, of which 46 (67%) were positive by anti-phospho-Akt antibody.

These observations together establish that TCL1b indeed functions as an Akt kinase co-activator, and possibly plays an active role in oncogenicity in vivo underlain various neoplastic diseases in humans associated with viral infection.

Poster Core time I at Lecture room 2 (Day 1: 16:50-17:50 on September 19)

P2-3

Phosphatidylserine micelle as T cell induction enhancing peptide carrier specifically ingested by professional antigen presenting cells

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To induce potent epitope specific T cell immunity, epitope peptides must be delivered efficiently to professional antigen presenting cells (APCs) *in vivo*. Therefore, an appropriate peptide carrier to deliver peptides to APCs is required for the development of effective peptide vaccine. In this study, we explored new peptide carriers which show enhancement in cytotoxic T lymphocytes (CTLs) induction capability. Among candidate materials, phosphatidylserine (PS) micelle revealed potent adjuvant effect in the process of screening by epitope specific *in vivo* CTLs assay. Further analyses show that PS micelle-conjugated antigens are preferentially and efficiently captured by professional APCs compared to conventional liposomes or unconjugated protein, especially the stimulatory function of $CD11c^{+}CD11b^{+}MHC\text{-}II^{+}$ conventional DCs considered to be significant for CTL induction. In addition, PS also demonstrated peptide specific helper T cell stimulatory capacity *in vivo*. Overall, this work is clearly indicating that PS micelle is an efficient carrier which specifically delivers antigen to professional APCs and induce both helper and cytotoxic T cells responses *in vivo* effectively. Thus, PS micelle should be novel promising adjuvant for the application of T cell inducing peptide vaccine.

Poster Core time II at Lecture room 2 (Day 2: 16:40-17:40 on September 20)

P2-4

Analysis of intracellular dynamics of inoculated PrP-res and newly generated PrP^{Sc} in Neuro2a cells

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Intracellular events that are required for the establishment of prion infection in cells after the challenge of prions are poorly understood. Extensive analyses of the intracellular dynamics of the inoculated PrP^{Sc} and de novo generation of PrP^{Sc} after the challenge of prions to cells are required for the elucidation of the mechanism by which prions establish infection in cells. To analyze the intracellular dynamics of the inoculated PrP-res and newly generated PrP^{Sc} after the inoculation, PrP-res was purified from brains of mice infected with the 22L prion strain, and purified PrP-res was labeled with amine reactive fluorescent dye, Alexa Fluor 555 succinimidyl ester. PrP^{Sc}-specific staining using mAb 132 was used for the detection of inoculated PrP-res and newly generated PrP^{Sc}. Combination of Alexa Fluor 555-labeled PrP-res with the PrP^{Sc}-specific detection using mAb 132 enabled us to distinguish the newly generated PrP^{Sc} (detected as green) from the inoculated PrP-res (detected as red and/or yellow) in immunofluorescence assay. Six hours after the uptake of PrP-res into N2a-3 cells, the majority of the inoculated PrP-res was detected at late endosomes (positive for exogenously introduced low-density lipoprotein) and/or lysosomes (positive for exogenously introduced 10 kD Dextran), suggesting that the most of the inoculated PrP-res was directed to the endo-lysosomal pathway possibly for degradation. On the other hand, newly generated PrP^{Sc} could be detected at plasma membrane, early endosomes (positive for EEA1) and late endosomes (positive for Rab7), but not at lysosomes within 24 hours after the inoculation. During the following 48 hours, the newly generated PrP^{Sc} increased remarkably at early endosomes and recycling endosomal compartments (positive for Rab11a) that are on the endocytic -recycling pathway, and thereafter, PrP^{Sc} became continuously detected at the peri-nuclear regions similarly to cells persistently infected with prions. The generation of PrP^{Sc} after the inoculation of PrP-res was partly inhibited by the overexpression of wild type of and Rab22a as well as dominant-negative mutant of Rab11a which are known to be involved in the membrane trafficking along the endocytic recycling pathway. Additionally, the de novo generation of PrP^{Sc} was partly inhibited by the overexpression of wild type of Rab9 as well as dominant-negative mutant of Rab7 which are known to be involved in the membrane trafficking from early to late endosomes and late endosomes to peri-nuclear regions. Detection of the inoculated PrP-res in the organelle that are involved in the endo-lysosomal pathway after the inoculation but the appearance of newly generated PrP^{Sc} mainly in organelle that are involved in the endocytic recycling pathway, as well as the inhibition of de novo generation of PrP^{Sc} by affecting membrane trafficking to late endosomes and from late endosomes to peri-nuclear region, suggesting that the transfer of inoculated PrP-res from the endo-lysosomal pathway to the endocytic recycling pathway is important for the initiation of prion propagation after the inoculation of prions.

Poster Core time I at Lecture room 2 (Day 1: 16:50-17:50 on September 19)

P2-5

Identification of target antigens with anti-tick vaccine potential

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Ticks are blood-feeding arthropods that parasitize terrestrial vertebrates, transmitting pathogens such as protozoa, viruses, bacteria and fungi, besides causing physical damages through blood sucking. Ticks are distributed globally, causing economic losses estimated at billions of dollars. At the present, control against tick infestation, and by extension, tick -borne diseases, is largely reliant on application of hazardous chemical acaricides that are expensive, pollute the environment, and many have become ineffectual due to rising tick resistance. Thus, alternative methods for tick control are urgently required.

Since ticks are obligate blood-feeders, interrupting this process through vaccination could reduce tick infestation. Tick vaccines derived from Bm86, a gut antigen of *Boophilus microplus* are commercially available. However, vaccination with these Bm86 vaccines has only been successful with a limited number of related tick species, limiting their deployment to other regions that are endemic for tick-borne diseases. Therefore, identifying tick antigens that can confer protection against heterologous tick challenge is subject of vigorous research presently.

Ferritins, iron-storage proteins that are conserved in ticks were recently identified as candidate antigens for tick vaccine development. Specifically, recombinant forms of ferritin 2 from *Boophilus microplus* and *Ixodid ricinus* were shown to confer protection level of 76% and 98%, respectively, against homologous challenges. Presently, we have identified three additional ferritin 2 homologues, two from *Ixodes persucatus* and *I. ovatus* from Japan, and one from *Rhipicephalus appendiculatus* from Africa. Recombinant expression and vaccination experiments are underway to evaluate the vaccine potential of the new antigens.

Poster Core time II at Lecture room 2 (Day 2: 16:40-17:40 on September 20)

P2-6

Molecular characterization of expressed sequence tags for Dermanyssus gallinae for the development of a novel control method of the mite

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The poultry red mite, Dermanyssus gallinae, is regarded as the most serious and economically important ectoparasite for the poultry industry all over the world. In addition, the red mite has been suggested as a potential vector for several pathogens including zoonotic agents. Recently, acaricide resistance is a major problem for the control of the mite in poultry farms. However, genetic information of this mite was scarcely available for candidates of vaccine antigens and for the development of novel acaricide. Hence, in this study, analysis of expressed sequence tags (ESTs) has been performed.

A plasmid cDNA library was constructed from the red mite collected from a poultry farm. A total of 2,466 cDNA clones were randomly picked and 1,147 cDNA clones except for shorter inserts were sequenced. These sequences were compared to those accumulated in NCBI databases, 373 sequences were identified as those known of functions. Some of these clones showed high similarity to drug-metabolizing enzymes (e. g., GST, cytochromeP450, microsomal epoxide hydrolase) and vaccine candidates (e. g., peroxiredoxins, type II allergen). Then, we focused on homologues of peroxiredoxins (Prx4 and Prx2) and type II allergen, which are suggested as possible vaccine candidates for other ticks, and their functions were analyzed by using recombinant proteins. Currently, functional analyses of other cDNA clones are in progress to identify new vaccine candidates and target molecules for the clarification of acaricide-resistant mechanism.

Poster Core time I at Lecture room 2 (Day 1: 16:50-17:50 on September 19)

P2-7

Molecular detection of tick-borne pathogens in ticks from Uganda

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Tick-borne diseases (TBDs) are a major constraint to livestock production throughout the tropics. World-wide, an estimated 600 million cattle are exposed to anaplasmoses and babesioses and 200 million cattle are exposed to theilerioses. TBDs cause economic losses to individual farmers and governments. Such losses can be classified into direct and indirect production losses, losses through costs incurred for controlling the disease and costs for providing research, training and extension services pertaining to the disease. The most important tick-borne diseases of livestock in sub-Saharan Africa are East coast fever (caused by *Theileria parva*), babesioses (caused by *Babesia bigemina* and *B. bovis*), anaplasmosis (caused by *Anaplasma marginale*) and heartwater (caused by *Ehrlichia ruminantium*). Despite their economic importance, information on the epidemiology of these diseases in many countries is often lacking or inadequate, resulting in inappropriate disease control strategies being implemented. The availability of specific, sensitive and cost-effective diagnostic methods is important in the design and implementation of effective disease control strategies. A molecular epidemiological investigation will be undertaken using ticks collected from indigenous cattle in Uganda to determine the prevalence of pathogens of veterinary and public health importance. Tick species endemic in Uganda will be identified morphologically and by molecular methods using 28S rDNA / 12S rRNA/ Cytochrome c oxidase subunit I (COI) mitochondrial gene sequence analysis. Pathogens to be screened in ticks will include piroplasmids (B. bovis and B. bigemina), A. marginale, Borrelia theileri, Rickettsia spp., Theileria parva/spp., and Ehrlichia spp. Ticks positive for pathogen DNA by PCR using species specific primers will be selected to assess the genetic diversity of tick pathogens by cloning and sequencing. Population dynamics of tick pathogens will be evaluated using phylogenetic analysis and population genetic analysis. These results will provide an improved understanding of the epidemiology of TBDs in Uganda.

Poster Core time II at Lecture room 2 (Day 2: 16:40-17:40 on September 20)

P2-8

Metagenomic analysis for tick microbial populations: implications for emerging tick-borne diseases

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[Background]
Distribution of blood-sucking arthropods has been expanding due to global warming and social fluidity at regional, national and global levels (Roseberg, et al., 2011, *Emerg Infect Dis*). Among them, ticks are one of the important vectors for zoonotic disease. Recently novel Rickettsia and Bunyavirus transmitted by ticks were found as agents of emerging diseases. Therefore, it is possible to imagine that ticks still have unidentified pathogens. The analysis of tick microbes might allow to predict emergence of new infectious diseases and to assess their potential risk. As metagenomic analysis is able to genetically analyse bacterial populations without the processes of culture, this approach has been successfully applied to analyze bacterial flora in environmental samples. In this study, metagenomic analysis was applied to characterize tick microbiome which may contain unidentified and hidden pathogens.
[Material and Methods]
Ixodes ovatus is one of the most common tick species in Japan. They are known to transmit *Rickettsia japonica* which is the agent of Japanese spotted fever. Ticks were collected using the flagging method in six different areas in Hokkaido. After morphological identification, 10 ticks were pooled and washed with a 70% ethanol containing 1% povidone iodine followed by rinsing with PBS(-). The ticks were homogenized and treated with achromopeptidase to lyse a broad range of bacteria. DNA was extracted by using DNeasy blood & tissue kit (QIAGEN). All the preparation processes were carried out in a biosafety cabinet to avoid contamination with environmental bacteria, and prevent laboratory infection. Universal primers which were tagged with multiplex identifier were used for PCR amplification of the V1-V2 region of bacterial 16S rRNA genes. The tag-encoded pyrosequencing was performed on 454 GS Junior (Roche). Sequence reads were identified to the genus level with the help of the Ribosomal Database Project Classifier.
[Result and Discussion]
From each sample, 4,373~18,381 sequences were obtained, 80% of which were assigned to the genus levels. Bacteria belonging to the genera associated with known zoonotic diseases, such as *Borrelia*, *Rickettsia* and *Ehrlichia* were detected. In total 113 different genera were detected in this study. There was a difference of bacterial populations between male and female ticks. For example *Coxiella* was predominant genus in female ticks, but not in male, which may be reflected by preference of *Coxiella* for female genital organs. Male ticks were shown to harbour more varieties of bacterial populations than female. Our approach is considered to be useful for analysis of tick microbes which might contain hidden pathogens to humans and animals.

Poster Core time I at Lecture room 2 (Day 1: 16:50-17:50 on September 19)

P2-9

A PCR-based survey of *Babesia bigemina*, *B. bovis* and benign *Theileria* group in cattle in the Republic of the Union of Myanmar

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Bovine piroplasmosis, babesiosis and theileriosis caused by *Babesia* and *Theileria* parasites, respectively, are important tick-borne protozoan diseases in tropical and subtropical countries. In the livestock industry, *Babesia bigemina* and *B. bovis* cause enormous economic losses, whereas the benign *Theileria* group (*T. sergenti/buffeli/orientalis*) occasionally causes serious economic losses. Previously, a wide distribution of bovine piroplasmosis in Southeast Asia was reported. In Myanmar, however, the prevalence of the disease has been poorly investigated. In this study, cattle (Holstein, Zebu and their crossbreed) blood (n=521) and *Haemaphysalis* sp. (n=46) and *Rhipicephalus* (*Boophilus*) *microplus* ticks (n=96) from 6 cities in northern, central and southern parts of Myanmar were collected, and DNA was extracted from them. Thereafter, the prevalence of *B. bigemina*, *B. bovis* and benign *Theileria* group was analyzed by PCR targeting *Spe*I-*Ava*I restriction fragment, *rhoptry-associated protein-1* (*rap-1*) gene and *major piroplasm surface protein* (*MPSP*) gene, respectively. The results showed that, in cattle blood DNA samples, 191 (36.7%), 108 (20.7%) and 257 (49.3%) samples were PCR-positive for *B. bigemina*, *B. bovis* and benign *Theileria* group, respectively. In addition, in *Rhipicephalus* (*Boophilus*) *microplus* tick samples, 20 (30.2%) and 29 (20.1%) samples were PCR-positive, respectively, for *B. bigemina* and *B. bovis*, whereas *Theileria* MPSP gene was not detected from any *Haemophysalis* sp. tick samples. These results are the first to demonstrate that bovine piroplasmosis are highly prevalent in a broad area of Myanmar.

Poster Core time II at Lecture room 2 (Day 2: 16:40-17:40 on September 20)

P2-10

Genome sequence of *Theileria orientalis* and comparative genome analysis with lymphocyte-transforming species, *T. parva* and *T. annulata*

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Theileria are tick-borne intracellular protozoan parasites belonging to the phylum Apicomplexa, which also contains many important zoonotic parasites including *Toxoplasma* or *Plasmodium*. Although infection by most of the *Theileria* species is asymptomatic or persists as a chronic infection, *T. parva* and *T. annulata* are highly pathogenic to cattle. These two species are among the “transforming *Theileria*” due to their ability to transform and induce indefinite proliferation of infected host lymphocyte- or macrophage/monocyte-lineages. In contrast, *T. orientalis*, an intraerythrocytic parasite in cattle causes anemia and icterus, but usually not fatal disease. *T. orientalis* does not induce uncontrolled proliferation of infected leukocytes and classified as ”non-transforming *Theileria* group”. In this study, we sequenced the whole genome of a non-transforming *Theileria* species, *T. orientalis* and compared it to the published sequences of two transforming species, *T. parva* and *T. annulata*. The main goals of this analysis were to provide supportive data on existing candidates and/or identify novel candidate genes that enable transformation of bovine leukocytes upon infection with *T. annulata* and *T. parva*.

The nuclear genome is approximately 9.0 Mbp in size, which is larger by 8 % than those of *T. parva* and *T. annulata*, which consist of four chromosomes. Overall syntenies among homologous chromosomes of three *Theileria* species are well conserved while subtelomeric structures of each chromosome differ substantially as *T. orientalis* lacks the tandemly arrayed gene family, subtelomeric variable secreted proteins (SVSP). In addition, transforming *Theileria* lineage-specific gene expansions, most notably TashAT/TpHN and Tar/Tpr gene family expansion in *T. parva* and *T. annulata* by gene duplication event were also found. Furthermore, 30 transforming *Theileria* parasites specific gene families which were not found in *T. orientalis*, *Babesia bovis* or *Plasmodium* were also selected. These gene families can be considered as transformation candidates. These genetic background variations between *Theileria* species with different pathogenicities will provides insight into the unique characteristics inducing host cell transformation and immortalization by lymphocyte-transforming *Theileria* species.

Poster Core time I at Lecture room 2 (Day 1: 16:50-17:50 on September 19)

P2-11

Is the knowledge on Leptospirosis significantly associated with attitude and practice? Results from a survey in Sri Lanka 2011

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Objective: To investigate and evaluate whether attitude and practice on Leptospirosis in the general population of the country are directly correlated with their awareness and precaution.
Background: Leptospirosis is endemic mostly to marshy areas and is particularly related with heavy rain fall and flooding localities, with which Sri Lanka well coincides. The disease affects not only health of the local residents, but also their economical statues by infecting their livestock. This makes even more challenging predominantly for the most affected vulnerable communities. In Sri Lanka, the number of reported leptospirosis cases showed an overall gradual increase until 2008.
Methodology: In this survey which was conducted from December 2011 to January 2012 in one of the districts of western province, Kegolle district of the country, participants with a written consent of age greater than 15 were recruited.
Results: Our survey showed that the awareness of the general population to the method of transmission and prevention of leptospirosis from animals was found to be generally satisfactory. For example, 88.5% of the participants responded positive for animal urine as a major source of contamination. In addition, their consciousness about the symptoms and significance of antibiotics to prevent leptospirosis were 82.0% and 81.8%, respectively. Majority (87.0%) of the participants accepted leptospirosis as a preventable disease. Though vast number of the representative of the general population responded with a much motivating awareness of the disease, but their attitude towards the probability of getting infected by the disease somehow seems to be more or less categorized in to two as those who believe that they will get the disease and those who are confident enough that there might be an incidence of being infected in the future. The first group accounts for 44.0% and the second for 44.8% of the respondents with some independent feedback from both. The usage of antibiotics among the participants consisted a small proportion (17.5%) only.
Conclusion: The majority of the respondents showed their readiness to participate in future prevention programs which need a special attention and planning, taking into account these findings.

Poster Core time II at Lecture room 2 (Day 2: 16:40-17:40 on September 20)

P2-12

A comparison of recombinant antigen base ELISA assays to diagnose carrier infection of leptospirosis in laboratory rats

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Introduction
Leptospirosis is a globally significant serious zoonotic disease with veterinary and public health impacts. Laboratory diagnosis of the disease is based on either demonstration of antigen or antibodies in diseased humans or animals. However serology is the most suitable for the rapid testing of a large number of samples in resource limited settings. Thus, in this study we have evaluated the diagnostic utility of four recombinant antigens in enzyme-linked immunosorbent assays (ELISAs) for serodiagnosis of carrier infection of leptospirosis.
Materials and methods
WKAH/hkm 6-week old female 24 rats were inoculated with *Leptospira interrogans* serovar Manilae and 6 rats were included as controls. Blood samples were collected on post infection days of 3, 6, 9, 12, 14, 21, 30, 45 and 60. Serum samples were analyzed using microscopic agglutination test (MAT) and ELISA based on recombinant antigens. Antigens were made by PCR amplifying of whole sequence of LipL32 and three segments of the LigA gene. Those sequence segments were designated as K, C, and V, and subcloned in to pET-43.1a. The recombinant plasmid was transformed in to *E.coli* BL21 and both fusion (NUS) proteins expression was induced by ITPG. MAT conducted using 4-5 days old *Leptospira interrogans* serovar Manilae culture. Samples collected between PID 9 - 60 were considered as immune phase samples. Each antigen's cutoff point optical density (OD) value for ELISA was determined by considering the control group's OD values.
Results
Out of 18 infected rats 15 had anti-leptospiral antibody titers of 400 or greater in MAT. Cutoff point OD values of ELSA assays were determined as 1.03 for LipL32; 0.12 for both LigA-K and LigA-V and 0.09 for LigA-C. ELISA using recombinant antigens showed 100% specificity. Sensitivities of these tests were 100% for both LipL32 and LigA-K antigens. On the other hand, the sensitivities of assays comprised LigA-C and LigA-V were 16.67% and 0%, respectively. A statistically significant correlation was revealed between MAT titers and the OD values of ELISA-LipL32 and ELISA-LigA-K assays (Correlation coefficient 0.59 and 0.63 respectively, $P < 0.01$).
Conclusion
The results indicate that recombinant antigens of LipL32 and LigA are useful for the laboratory diagnosis of carrier infection of leptospirosis in rats.

Poster Core time I at Lecture room 2 (Day 1: 16:50-17:50 on September 19)

P2-13

Genomic analysis of *Leptospira* species using the next-generation sequencer

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[Background]
The most widespread zoonotic disease worldwide is leptospirosis which is a health concern to human as well as to animals. Although 50% of more than 230 serovars among pathogenic leptospiras belonging to the species interrogans and borgpetersenii, only 3 full genome sequences of *L. interrogans* serovars are available until now. Therefore, comparative genomic analyses across serovars and species are limited and for the characterization of *Leptospira* serovars requires a large number of specific antisera. With the next-generation sequencing technology, a faster and especially an affordable DNA-sequencing is given which allows us a detailed analysis of the genomes of *Leptospira* isolates related to pathogenesis as well as provide a new method of genotyping in place of the conventional serotyping. To date, numerous studies regarding serovar typing and pathogenesis of *Leptospira* in ongoing but they are very inaccurate, costly, laborious, and determination of genes or factor associated with pathogenesis is still not fully revealed.
[Materials & methods]
Whole genome of eight *Leptospira interrogans* isolates (seven domestic and one foreign isolate) belonging to six different serogroups were sequenced. CLC Genomics Workbench 4 program was used to map the reads of each sample to genome sequence of the reference strain *L. interrogans* serovar Lai str. 56601 and to analyze the genetic background related to pathogenicity, single nucleotide polymorphism (SNP), genomic (pathogenic) island, O-antigen gene clusters (*rfb*) and genes for outer membrane proteins (OMPs). With the reconstructed genome information, a dendrogram of the 8 genome sequences was drawn in order to determine phylogenetic relationships between strains and correlations between their genotypes and serovars.
[Result and discussion]
From genome-wide comparisons between stains, two huge and five minor regions existing in strain Lai but missing in some of other strains were observed. The localizations of the two huge missing stretches were found to correspond to three known gene clusters - the *rfb* gene cluster (from LA1576 to LA1672) and the genomic island B (GI B: LA1750 to LA1851). Analyses of other missing regions which seemed to be candidates of new genomic islands/islets. The neighbor joining phylogenic tree was constructed based on concatenated sequence of SNP of the eight isolates (37,651 bp, random 200, 100, 50, 20) under the criteria frequency 100% and coverage >=5. The result demonstrated close relation between same serogroup isolates to each other. In case of the SNP alignment of OMP genes, results revealed similarities even between same but also different serogroups. These findings suggest gene recombination of isolates within the same species. This study provides deep insight into molecular bases of pathogenicity in leptospirosis from the evolutional viewpoint and a new molecular method of serovar typing.

Poster Core time II at Lecture room 2 (Day 2: 16:40-17:40 on September 20)

P2-14

Development of loop-mediated isothermal amplification method for detection of *Legionella pneumophila*

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Background: *Legionella pneumophila* is intracellular of free living protozoa which causes legionellosis in humans. These bacteria are commonly found in natural freshwater, moist soil and also present in human-made water systems. The infection is usually caused by inhalation of aerosolized water containing the bacteria. Legionellosis is considered as preventable illness. This has resulted in a number of guidelines and new control strategies which include development of rapid and highly specific detection technique. Loop-mediated isothermal amplification (LAMP) is an established nucleic acid amplification method offering rapid, accurate, and cost-effective diagnosis of infectious diseases. The most significant advantage of LAMP is the ability to amplify specific sequences of DNA under isothermal conditions between 63°C and 65°C, thereby obviating the need for a thermal cycler.

Methods: In this work, we aim to develop a novel LAMP assay for detection of *L. pneumophila* by targeting macrophage infectivity protentiator (*mip*) gene which is specific to this species. The assay sensitivity and specificity will be compared with the results from Loopamp *Legionella* detection kit and nested PCR for detecting *L. pneumophila*.

Results: We designed 5 sets of primers using PrimerExplorer V4 software and evaluated these primers by Loopamp real-time turbidimeter. Result showed that 2 primer sets could amplify the *mip* gene within 60 min. Now we are trying to optimize LAMP condition and will further examine sensitivity and specificity of the assay.

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