Xth International Conference on HFRS, HPS and Hantaviruses

May 31 - June 3, 2016
Organ Recital Hall
University Center for the Arts
Colorado State University
Fort Collins, CO, USA

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University Center for the Arts
Colorado State University

Tuesday, May 31 - University Center for the Arts

17:00 Registration
18:00 **Connie Schmaljohn**, President ISH, Welcome to the Conference
18:15 **Tony Schountz**, Welcome and Meeting Logistics
18:30 Opening lecture **Antti Vaheri** (Finland) : *New pathogenicity markers, vascular leakage, tissue plasminogen activator and galectin-3 binding protein as therapeutic targets in hantavirus infections*

19:00-21:00 Reception at the University Center for the Arts

Wednesday, June 1

07:00 Registration and poster session I setup

08:00 Memorial Tribute to Prof. Song Gan: **Connie Schmaljohn (USA)**

08:15 **HO WANG LEE AWARD LECTURE**
Introduction of award recipient and presentation of plaque: **Connie Schmaljohn (USA)**
James W. LeDuc (USA): *The Power of We*

09:00-09:30 **Coffee/Tea/Networking BREAK**

09:30-11:30 **Ecology/Epidemiology 1**
Chair: **Anna Papa (Greece)**

09:30 **Roger Hewson (UK):** *Hantavirus infection in the UK: detection in wild rats, pet rats and renal patients.*
09:45 **Detlev Krüger (Germany):** *Human infections by rodent- and non-rodent associated hantaviruses in Africa*
10:00 **Barry Rockx (Netherlands):** *Tula virus surveillance in rodents in the Netherlands*
10:15 **Angela Luis (USA):** *The role of small mammal diversity in Sin Nombre virus transmission among deer mice: competing dilution and amplification effect*
10:30 **Lies Laenen (Belgium):** *Bruges virus, a newfound hantavirus in the European mole, contradicts host-specificity.*
10:45 **Boris Klempa (Germany):** *Hantaviruses got wings: Bat-borne hantaviruses in Africa*
11:00 **Claudia Filippone (Madagascar):** *First serological survey of hantavirus infection in human population from Madagascar*
11:15 **Heikki Henttonen (Finland):** *Ecology of Puumala hantavirus in Finland*

11:30-14:30 **Lunch and Poster Session I**
- Poster presenters attend posters from 12:00-13:30 (UCA 116)
- Box Lunch 11:30 (UCA 116)
- Advisory Council lunch meeting 12:30-14:30 (UCA 136)

14:30-16:00 **Ecology/Epidemiology 2**
Chair: Tatjana Avsic-Zupanc (Slovenia)

14:30 **Joerg Hofmann (Germany)**: *Hantavirus disease epidemiology in Germany*.
14:45 **Mifang Liang (China):** *HFRS and hantavirus in China 2006-2015*
15:00 **Lorraine McElhinney (Wales)**: *Increased detection of Seoul virus in the United Kingdom.*
1515 **Charlotte Robin (UK):** Pets, pests and pestilence: understanding social aspects of zoonotic disease transmission.
15:30 **Kumiko Yoshimatsu (Japan)**: *Hantavirus infection in Sri Lanka*
15:45 **Liudmila Yashina** (**Russia**): Co-circulation of three distinct Sorex-borne hantaviruses in far eastern Russia.
16:00 Recess

Brewery tour bus departs at 16:30 (UCA)

Thursday, June 2

07:45 Poster session II setup
08:00 Announcements and Program changes

08:15-10:15 Phylogeny and Replication
Chairs: Colleen Jonsson (USA) and **Detlev Krüger (Germany)**

08:15 **Nicole Tischler (Chile):** *Mechanism of the Andes hantavirus fusion process and inhibition by Gc domain III and stem peptides.*
08:30 **Rohit Jangra (USA)**: *Generation and characterization of a recombinant vesicular stomatitis virus (rVSV) expressing Hantaan virus glycoproteins*
08:45 **Jason Lanman (USA)**: *Structural studies of Bunyaviridae glycoproteins.*
09:00 **Adelaïde Dubois (France):** *Comparative genomics of bank vole sensibility/tolerance to Puumala hantavirus in France*
09:15 **Won-Keun Kim (Korea):** *Phylogeographic analysis of hemorrhagic fever with renal syndrome (HFRS) patients and natural hosts using multiplex PCR-based next generation sequencing*
09:30 **Satoru Arai (Japan):** *Whole genome analysis of Dakrong virus, a novel hantavirus harbored by the Stoliczka's Asian trident bat (*Aselliscus stoliczkanus*) in Vietnam*
09:45 **Richard Yanagihara (USA)**: *Partial Characterization of Nova Virus Isolated from the European Mole (*Talpa europaea*)*
10:00 **Matthias Schade (Germany):** *Packaging of hantavirus genomic segments studied in single cells and virions by multicolor fluorescence in situ hybridization*

10:15-10:45 Coffee/Tea/Networking BREAK

10:45-12:30 Pathogenesis and Immune Responses 1
Chair: **Jiro Arikawa (Japan)**

10:45 **Korva Misa (Slovenia):** *Evaluation of VEGF and sVEGFR2 dynamic in patients with haemorrhagic fever with renal syndrome*
11:00 **Carles Solà Riera (Sweden)**: *Hantavirus vs cytotoxic lymphocytes: do all hantaviruses act alike?*
11:15 **Matthew Simons (USA):** ANDV *N protein inhibits interferon induction by uniquely engaging TRIM21*
11:30 **Günther Schönrich (Germany):** *Immunopathology in a new model of hemorrhagic fever caused by hantavirus infection*
11:45 **Katerina Tsergouli (Greece):** *After crossing the barrier: immune response in Dobrava-Belgrade virus infections*
12:00 **Hiroaki Kariwa (Japan):** *Adaptation of Hantaan virus strain AA57 to Vero E6 cells affects the pathogenicity in mice*
12:15 **Peter T. Witkowski (Germany):** *Alimentary tract as entry route for hantavirus infection*

12:30- 1400 Lunch and Poster Session II
- Lunch served 12:30 (UCA 116)
- Poster presenters attend posters attend posters from 12:45-14:00 (UCA 116)

14:00-15:30 Pathogenesis and Immune Responses 2
Chair: **Jin-Won Song (Korea)**

14:00 **Myriam Ermonval (France):***Interaction of pathogenic and non pathogenic hantaviruses with their natural and human hosts.*
14:15 **Ivan-Christian Kurolt (Croatia):** *Micro RNAs in urine as potential biomarkers for severity of hemorrhagic fever with renal syndrome*
14:30 **Marina Garcia (Argentina):** *Virus specific and bystander-B cell activation account for a massive plasmablast response in Andes virus infected patients*.
14:45 **Anna Smed-Sörensen (Sweden):** *Rapid but transient depletion of human dendritic cells in blood during acute hantavirus infection*

15:00 **Erich Mackow (USA):** *ANDV activates RhoA directed endothelial cell permeability by engaging TSC2 and reducing TIAM1 and p190RhoGAP expression levels*
15: 15 **Daniel Bourquain (Germany):***Infection of human airway epithelial cells by different Dobrava-Belgrade virus subtypes reveals gene expression patterns corresponding to their virulence potenti*al
15.30 **Jan Clement (Belgium):** *Three pseudo-nephropathia epidemica (NE) retrospective cases after overt soricomorph exposure*

15:45-16:30 Dalrymple AWARD LECTURE
Introduction of award recipient and presentation of plaque: **Erich Mackow (USA)**
Irina Gavrilovskaya (USA): *Retrospective study of the etiology andpathogenesis of hantaviruses infection*

16:30 Recess

18:30 Gala Dinner (UCA G116)

Friday, June 3

08:00 Announcements and Program changes

08:15-10:00 Diagnostics and Clinical
Chair: Alemka Markotic (Croatia) and **Jan Clement (Belgium)**

08:30 **Tomas Strandin (Finland):** *Hantaviruses induce STAT1-dependent expression of tissue plasminogen activator*
08:45 **Jukka Mustonen (Finland):** P*roteinuria detected by albumin dipstick test predicts the severity of acute kidney injury in Puumala hantavirus induced nephropathia epidemica*
09:00 **Clas Ahlm (Sweden):** *Endothelial dysfunction during Puumala hantavirus infection*
09:15 **Kimia Maleki (Sweden):** *Analysis of plasma cytokines and inflammatory markers in PUUV-infected patients reveals a potential gastrointestinal involvement during HFRS*
09:30 **Johan Rasmuson (Sweden):** *Cytotoxic immune responses in the lungs correlate to disease severity in patients with hantavirus infection*
09:45 **Jan Clement (Belgium):** *Interstitial renal oedema is the purely mechanical mechanism of transient acute kidney injury (AKI) without sequelae in human hantavirus infections-A hypothesis*

10:00-10:30 Coffee/Tea/Networking BREAK

10:30-11:45 Clinical, Vaccines and Therapeutics
Chair: Dexin Li (China)

10:30 **Jonas Klingström (Sweden):** *Mechanisms behind, and consequences of, hantavirus-mediated inhibition of apoptosis*
10:45 **Cvetko Krajinović (Croatia):** *Early slowdown of the peripheral immune response triggered by Puumala virus infection*
11:00 **Greg Mertz (USA):** *Evaluation of antiviral, anti-inflammatory and supportive treatment for hantavirus cardiopulmonary syndrome in North America and Chile*
11:15 **Rebecca Brocato (USA):** *Transchromosomal bovine- and anseriform avian-based approaches to develop polyclonal antibody-based antivirals targeting hantaviruses*.
11:30 **Jay Hooper (USA**): *Hantavirus DNA Vaccine Update*
12:00 **Zhanqiu Yang (China)**: *Hantaan virus is the main etiologic agent responsible for hemorrhagic fever with renal syndrome*

12:15-14:15 Working Lunch
12:30 **Anna Pappa**: *Report of the Advisory Council meeting*
12:45 **Jan Clement (and panel members volunteering from AC):** *Roundtable Discussion: The Future of the ISH*
14:00 **Connie Schmaljohn** Introduction of Next EC and Closing Comments

14:15 Adjourn

Poster Session I - Wednesday, June 1, UCA room 116, 11:30-14:30

1. **Susceptibility of primary Syrian hamster and deer mouse pulmonary endothelial cells to zoonotic viruses.** Miedema K[1], Prescott J[2], Feldmann H[2], Schountz T[1]. [1]Arthropod-borne and Infectious Diseases Laboratory, Colorado State University; [2]Laboratory of Virology, Rocky Mountain Laboratories, NIAID. USA.

2. **Maporal hantavirus causes mild pathology in deer mice (*Peromyscus maniculatus*).** Amanda McGuire[1], Joseph Fauver[1], Amber Rico[1], Tawfik Aboellail[1], Kaitlyn Miedema[1], Sandra Quackenbush[1], Ann Hawkinson[2] and Tony Schountz[1]. [1]Arthropod-borne and Infectious Diseases Laboratory, Colorado State University[1]; Department of Biological Sciences, University of Northern Colorado[2]. USA.

3. **Markers of central nervous system damage in patients with hemorrhagic fever with renal syndrome (HFRS).** Viktoria Ivanis[1], Viktoria Verkhoturova[1], Larisa Pereverten[1], Evgeniy Tkachenko[2], Tamara Dzagurova[2]. Pacific State Medical University[1]; Chumakov Institute of Poliomyelitis and Viral Encephalitides[2], Russia.

4. **Long-term outcomes in patients suffering from HFRS**. Irina Artamonova , Guzel Muchetdinova, Raysa Faslyeva, Gulchagra Mirsaeva. Bashkir State Medical University, Russia.

5. **Dynamics of clinical and laboratory manifestations of HFRS caused by PUUV on the Middle Volga territory of the Russia.** Viacheslav Morozov[1], Alexei Suzdaltsev[2], Rinat Lukaiev[2], Tamara Dzagurova[3], Evgeniy Tkachenko[3]. Hepatolog LLC[1]; Samara State Medical University[2]; Chumakov Institute of Poliomyelitis and Viral Encephalitides[3], Russia.

6. **The polyneuropathy syndrome in HFRS.** Tamara Dzagurova[1], Evgeniy Dekonenko[1], Iskandar Zagidullin[2]. Chumakov Institute of Poliomyelitis and Viral Encephalitides[1]; Bashkir Medical State University[2], Russia.

7. **Initial biological characterization and pathogenicity of a novel subtype HTNV in Hubei province ,China.** Yan Zhong, Liang-jun Chen, Zhan-qiu Yang. State Key Laboratory of Virology, School of Medicine of Wuhan University, China.

8. **Understanding the ecology and host-switching potential of hantavirus in South America.** Gillian Eastwood[1], Yong Kyu Chu[2], Jeremy V Camp[3], Ryan McAllister[3], Vicente Javier Martínez Bruyn[4], Ashley Yu[1], Hai Yan[1], Jasper Lee[1], Evan P Williams[1], Robert D Owen[5], Colleen Jonsson[1]. University of Tennessee (Knoxville), USA[1]; University of Louisville, USA[2]. Universidad Nacional de Asunción, Paraguay[3]; Texas Tech University, USA[4]; Barrio Republicano, Asunción, Paraguay[5].

9. **Old World hantavirus invasion of human respiratory epithelial cells.** Giulia Torriani[1], Sylvia Rothenberger[1], Hajer Fritah[1], Nicole Tischler[2], Gert Zimmer[3], Olivier Engler[4], Stefan Kunz[1]. University of Lausanne, Switzerland[1]; Fundación Ciencia & Vida, Chile[2]; Institute of Virology and Immunology, Switzerland[3]; Spiez Laboratory, Switzerland[4].

10. **Modeling innate immune response of hantavirus infection in reservoir and nonreservoir hosts.** Annabel O. Meade[1], Colleen B. Jonsson[2], and Linda J.S. Allen[1]. Texas Tech University[1]; University of Tennessee, Knoxville[2], USA.

11. **Investigation of bat-borne hantavirus ecology and public health relevance in Côte d´Ivoire – an One Health approach.** Leonce Kouadio[1,2], Peter T. Witkowski[3]#, Kathrin Nowak[2] ,Sébastien Calvignac-Spencer[2], Emmanuel Couacy-Hymann[1], Chantal Akoua-Koffi[4], Detlev H. Krüger[3], Fabian H. Leendertz[2]. Laboratoire Central de la Pathologie Animal, Côte d'Ivoire[1]; Robert Koch Institut, Germany[2]; Charité Medical School, Germany[3]; Université Alassane Ouattara de Bouaké, Côte d'Ivoire[4].

12. **Pulmonary syndrome in HFRS.** Guzel Mukhetdinova[1], Raisa Fazlyeva[1] ,Venera Mustafina[2], Renata Fazlyeva[1]. Bashkir State Medical University, Russia[1]. Ministry of Health, Bashkortostan[2].

13. **Analysis of plasma kallikrein and FXII in patients infected with Puumala or Dobrava viruses.** Misa Korva[1], Shannon Taylor[2], Katarina Resman Rus[1], Connie Schmaljohn[2], Tatjana Avsic Zupanc[1]. University of Ljubljana, Slovenia[1]; United States Army Medical Research Institute of Infectious Diseases, USA[2].

14. **Development of a mouse model of hemorrhagic fever with renal syndrome and evaluation of the role of $CD4^+$ and $CD8^+$ T cells in the pathogenesis.** Shimizu K, Yoshimatsu K, Arikawa J. Hokkaido University Graduate School of Medicine, Japan.

15. **Antiviral immunity and the interaction of neutrophils with plasmacytoid dendritic cells.** Martin J. Raftery, Günther Schönrich. Charité-Universitätsmedizin, Germany.

16. **An immunosuppressed Syrian hamster model for New World hantavirus lethal disease.** Valentijn Vergote, Lies Laenen, Marc Van Ranst and Piet Maes. University of Leuven, Belgium.

17. **Descriptive analysis of Andes virus quasispecies in their natural reservoir *O. longicaudatus*.** Vial C[1], Perez R[1], Leon L[1], Cuiza A[1], Torres F[2], Calvo M[3], Vial P[1], Valdivieso F[1], Mertz G[4].Universidad del Desarrollo, Chile[1]; Pontificia Universidad Católica de Valparaíso[2]; 3 Universidad Austral de Chile[3]; University of New Mexico,USA[4].

18. **Vaccinia virus-free rescue of Fluorescent replication-defective vesicular stomatitis virus and pseudotyping with Puumala virus glycoprotein for use in neutralization tests.** Rommel Iheozor-Ejiofor[1], Lev Levanov[1], Jussi Hepojoki[1], Tomas Strandin[1], Åke Lundkvist[3], Alexander Plyusnin[1,3], and Olli Vapalahti[1,2,4]. University of Helsinki; Finland[1]; Helsinki University Hospital, Finland[2]; Uppsala University, Finland[3]; University of Helsinki, Finland[4].

19. **Aptamers against the hantavirus Andes nucleoprotein.** Renata Carvalho de Oliveira, Sotiris Missailidis, Alexandro Guterres, Jorlan Fernandes[1] Elba RS Lemos. Oswaldo Cruz Institute, Brazil.

20. **Occurrence of shrew- and mole-borne hantaviruses in Germany.** Lukáš Radosa[1], Peter T. Witkowski[1], Martina Ličková[2], Tomáš Szemeš[3], Sandra Essbauer[4], Rainer G. Ulrich[5], Lies Laenen[6], Piet Maes[6], Detlev H. Krüger[1], Boris Klempa[1,2]. Charité Medical School, Germany[1]; Slovak Academy of Sciences, Slovakia[2]; Comenius University, Slovakia[3]; Bundeswehr Institute of Microbiology, Germany[4]; Friedrich-Loeffler-Institut, Germany[5]; Rega Institute for Medical Research, Belgium[6].

21. **Production and characterization of the Juquitiba hantavirus nucleoprotein in *E. coli*.** Janaina Figueira Mansur[1], Renata Carvalho de Oliveira[2], Sotiris Missailidis[3], Elba Regina Sampaio de Lemos[2], Ronaldo da Silva Mohana Borges[1]. Federal University of Rio de Janeiro, Brazil[1]; 2.Oswaldo Cruz Institute, Brazil[2]; 3. Oswaldo Cruz Foundation, Brazil[3].

22. **Population and community ecology of hantavirus rodent hosts in southern Brazil.** Bernardo R. Teixeira[1], Liana Strecht[2], Nathalie M. Loureiro-da-Cruz[1], Rosana Gentile[1], Renata C. Oliveira[2], Alexandro Guterres[2], Jorlan Fernandes[2], Luciana H. B. V. Mattos[2], Sonia M. Raboni[4], Giselia B. G. Rubio[3], Claudia N. D. Santos[4], Cibele R. Bonvicino[1], Elba R. S. Lemos[2], Paulo S. D'Andrea[1]. Instituto Oswaldo Cruz/FIOCRUZ[1]; Lab. de Hantaviroses e Rickettsioses, Instituto Oswaldo Cruz/FIOCRUZ[2]; Secretaria de Estado de Saúde-PR[3]; Instituto Carlos Chagas/FIOCRUZ[4], Brazil.

23. **Analysis of hantavirus in Chile: Small mammals associated with peridomestic cases of human hantavirus disease.** Torres-Perez F[1], Palma RE[2], Boric-Bargetto D[1,] Ferres M[3], Vial PA[4], Yañez R[1], Mertz GJ[5]. Ten Years Instituto de Biología, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile[1]. Departamento de Ecología, Pontificia Universidad Católica de Chile, Santiago, Chile[2]. Centros de Estudios Médicos, Departamento de Enfermedades Infecciosas, Pontificia Universidad Católica de Chile, Santiago, Chile[3]. Facultad de Ciencias de la Salud, Universidad del Desarrollo, Santiago, Chile[4]. Division of Infectious Diseases, Department of Internal Medicine, University of New Mexico, Albuquerque, New Mexico[5].

24. **Development of a multiplexed immunoassay to detect IgG antibody responses against Pathogenic bunyaviruses within the Republic of Georgia.** Badger C, Voorhees M, Schmaljohn C. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD 21702, USA.

25. **Clinical and morphological analysis of renal changes in hemorrhagic fever with renal syndrom.** A.N. Evseyev, Department of Pathology and Forensic Medicine, Far Eastern Medical Institute, Khabarovsk.

26. **Optimized VSV-based Andes virus vaccine for adequate pre- and post-exposure protection.** Joshua Marceau[1,2], David Safronetz[1,3], Andrea Marzi[1], Kyle Rosenke[1], Heinz Feldmann[1] . Laboratory of Virology, Division of Intramural Research, National Institute for Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT, USA. Department of Biomedical and Pharmaceutical Sciences, The University of Montana, Missoula, MT, USA. Public Health Agency of Canada, Canada.

27. **Immunogenetic factors affecting susceptibility of rodents to hantaviruses at the macroevolutionary scale: Evidence from integrins and DAF genes.** Marie Pagès[1], Caroline Tatard[2], Maxime Galan[2], Bérénice Villegas[2], Joseph A. Cook[3], E. Fichet-Calvet[4], Peter T. Witkowski[5], Serge Morand[1], Nathalie Charbonnel[2]* [1]. Institut des Sciences de l'Evolution, Université Montpellier, Montpellier, France. [2]INRA, Centre de Biologie pour la Gestion des Populations, Montferrier sur Lez, France. [3]Museum of Southwestern Biology and Department of Biology, University of New Mexico, Albuquerque, USA. [4]Bernhard-Nocht Institute of Tropical Medicine, Hamburg, Germany. [5]Institute of Virology, Charité Medical School, Berlin, Germany.

28. **Why and how do we need to examine host microbiome in hantavirus studies?**Maxime Galan[1], Adélaïde Dubois[1,2], Guillaume Castel[1], Jean-François Cosson[3], Jean-Baptiste Pons[4], Séverinne Murri[1], Philippe Marianneau[2,] Nathalie Charbonnel[1]. [1]NRA, Centre de Biologie pour la Gestion des Populations, Montferrier sur Lez, France. [2]ANSES Unité de Virologie, Lyon, France. [3]INRA, VectoTic , Maison-Alfort, France. [4]CNRS, Laboratoire de Biométrie et Biologie Evolutive, Villeurbanne, France.

29. **Virological and immunological surveys during Puumala virus experimental infections of bank voles (*Myodes glareolus*) from endemic and non-endemic regions.** Adélaïde Dubois[1,2], Guillaume Castel[1], Coralie Pulido[3], Séverine Murri[2], Jean-Baptiste Pons[4], Laure Benoit[1], Anne Loiseau[1], Latifa Lakhdar[3], Maxime Galan[1], Philippe Marianneau[2], Nathalie Charbonnel[1]. [1]. INRA, Centre de Biologie pour la Gestion des Populations, Montferrier sur Lez, France. [2]ANSES, Unité de Virologie, Lyon, France. [3]ANSES, Plateforme d'Expérimentation Animale, Lyon, France. [4]CNRS, Laboratoire de Biométrie et Biologie Evolutive, Villeurbanne, France.

Poster Session II - Thursday, June 2, UCA room 116, 12:15-14:00

30. **Quantitative evaluation of reservoir potential of ecological hosts of Hantaviruses**. Tatyana Kushnareva. Institution of Epidemiology and Microbiology and Pacific Medical University, Vladivostok, Russian Federation.

31. **Prevalence of hemorrhagic fever with renal syndrome in Yiyuan County, China, 2005-2014**. Tao Wang, Yunping Zhou, Feng Cui, Ling Wang, Zhenshui Huang, Shenyong Zhai. Department of Infectious Disease Control and Prevention, Zibo Center for Disease Control and Prevention, Zibo, Shandong Province, P. R. China.

32. **Seoul virus infection in France**. Jean-Marc Reynes[1], Damien Carli[1], Jean-Baptiste Bour[2], Samir Boudjeltia[3], Anny Dewilde[4], Marie-Pierre Rapt[5], Véronique Jacomo [6],Alexandra Septfons[7], Pierre E. Rollin[8]. [1]Centre National de Référence des Hantavirus, Unité de Biologie des Infections Virales Emergentes, Institut Pasteur, Centre International de Recherche en Infectiologie, Lyon, France. [2]Département de virologie, Centre Hospitalier Universitaire Dijon-Bourgogne, Dijon, France. [3]Service de Néphrologie, Centre Hospitalier Universitaire Dijon-Bourgogne, Dijon, France. [4]Laboratoire de Virologie, Centre de Biologie Pathologie, Centre Hospitalier Universitaire, Lille, France. [5]Service de Médecine Interne et Pneumologie, Centre Hospitalier, Bar-le-Duc, France. [6]Laboratoire Biomnis, Lyon, France. [7]Département des maladies infectieuses, Institut de Veille Sanitaire, Saint-Maurice, France. [8]Special Pathogens Branch, Centers for Diseases Control and Prevention, Atlanta, Georgia, USA.

33. **Phylogeographic diversity and reassortment of Hantaan virus in nature, the Republic of Korea**. Jeong-Ah Kim[1], Won-keun Kim[1], Jin Sun No[1], Seung-Ho Lee[1], Sook-Young Lee[1], Ji Hye Kim[1], Jeong Hoon Kho[1], Daesang Lee[2], Dong Hyun Song[2], Se Hun Gu[2], Seong Tae Jeong[2], Man-Seong Park[3], Heung Chul Kim[4], Terry A. Klein [5], Jin-Won Song[1]. [1]Department of Microbiology, College of Medicine, Korea University, Seoul, Republic of Korea, [2]Agency for Defense Development, Daejeon, Republic of Korea. [3]Department of Microbiology, College of Medicine, the Institute for Viral Diseases, Korea University, Seoul, Republic of Korea. [4]5th Medical Detachment, 168th Multifunctional Medical Battalion, 65th Medical Brigade, Unit 15247, APO AP 96205–5247, USA. [5]Force Health Protection and Preventive Medicine, 65th Medical Brigade/US Army MEDDAC-Korea, Unit 15281, APO AP 96205–528, USA.

34. **Genetic diversity of Artybash virus in the Laxmann's Shrew (*Sorex caecutiens*)**. Satoru Arai[1], Hae Ji Kang[2], Se Hun Gu[2], Satoshi D. Ohdachi[3], Joseph A. Cook[4], Liudmila N. Yashina[5], Keiko Tanaka-Taya[1], Sergey A. Abramov[6], Shigeru Morikawa[1], Nobuhiko Okabe[1,7], Kazunori Oishi[1], and Richard Yanagihara[2]. [1]National Institute of Infectious Diseases, Tokyo, Japan; [2]University of Hawaii at Manoa, Honolulu, HI, USA; [3]Hokkaido University, Sapporo, Japan; [4]University of New Mexico, Albuquerque, NM, USA; [5]State Research Center of Virology and Biotechnology, Koltsovo, Russia; [6]Institute of Systematics and Ecology of Animals, Novosibirsk, Russia; [7]Kawasaki City Institute for Public Health, Kanagawa, Japan.

35. **HFRS outbreak caused by Sochi virus on the Russian Black Sea coast**. Tamara Dzagurova[1], Boris Klempa[2,3], Maria Balovneva[1], Natalia Korotina[1], Vyacheslav Morozov[4], Olga Pilikova[5], Yuliya Yunicheva[6], Viktoriya Bakhtina[7], Peter Witkowski[2], Evgeniy Tkachenko[1], Detlev Krüger[2]. [1]Chumakov Institute of Poliomyelitis and Viral Encephalitides, Moscow, Russia; [2]Institute of Virology, Charité Medical School, Berlin, Germany; [3]Institute of Virology, Biomedical Research Center, Bratislava, Slovakia; [4]Medical Company "Hepatolog" LLC, Samara, Russia; [5]Anti-Plague Station, Novorossiysk, Russia; [6]Anti-Plague Department, Sochi, Russia; [7]Infectious Disease Hospital, Krasnodar, Russia.

36. **PUUV active endemic areas patterns in Russia: Results of 40-years monitoring**. Alla Bernshtein[1], Natalia Apekina[1], Marina Ostanina[2], Elena Mutnykh[1], Evgeniy Tkachenko[1], Irina Gavrilovskaya[3]. [1]Chumakov Institute of Poliomyelitis and Viral Encephalitis, Moscow, Russia; [2]Udmurtia Republic Centre for Hygiene and Epidemiology, Izhevsk, Russia; 3Department of Molecular Genetics and Microbiology SUNY at Stony Brook, Stony Brook, USA.

37. **Presence of Hantaan virus RNA from anti-Hantaan virus IgG seronegative rodents in a highly endemic area, the Republic of Korea.** Jin Sun No[1], Won-keun Kim[1], Jeong-Ah Kim[1], Seung-Ho Lee[1], Sook-Young Lee[1], Ji Hye Kim[1], Jeong Hoon Kho[1], Daesang Lee[2], Dong Hyun Song[2], Se Hun Gu[2], Seong Tae Jeong[2], Heung-Chul Kim[3,] Terry A. Klein[4], Jin-Won Song[1]. [1]Department of Microbiology, College of Medicine, Korea University, Seoul 136-705, Republic of Korea. [2]The 5th R&D Institute, Agency of Defense Development, Yuseong P.O. Box 35, Daejeon 305-152, Republic of Korea. [3]5th Medical Detachment, 168th Multifunctional Medical Battalion, 65th Medical Brigade, Unit 15247, APO AP 96205-5247, USA. [4]Public Health Command District-Korea (Provisional), 65th Medical Brigade, Unit 15281, APO AP 96205-5281, USA.

38. **Inactivated purified hemorrhagic fever with renal syndrome vaccination based comprehensive intervention to prevent hemorrhagic fever with renal syndrome epidemiology effect evaluation in Weifang of Shandong Province.** Wang Zhiqiang,Zhai Wenji,Kang Dianmin. Shandong Center for Disease Control and prevention,Jinan,Shandong Province,P.R. China.

39. **Characterisation of the endonuclease activity of Hantaan virus L polymerase. Sylvia Rothenberger[1], Giulia Torriani[1], Stefan Kunz[1], Olivier Engler[2].** [1]Institute of Microbiology, University Hospital Center and University of Lausanne, Rue du Bugnon 48, CH-1011 Lausanne, Switzerland. [2]Spiez Laboratory, CH-3700 Spiez, Switzerland.

40. **Clinical assessment of a bivalent DNA vaccine for hemorrhagic fever with renal syndrome caused by hantavirus infections.** Connie Schmaljohn[1], Drew Hannaman[2], Kristopher Paolino[3], Jay W. Hooper[1]. [1]US Army Medical Research Institute of Infectious Diseases, Frederick, MD, [2]Ichor Medical Systems, San Diego, CA, [3]Walter Reed Army Institute of Research, USA.

41. **Advanced Development of a Candidate Combination DNA Vaccine For Hemorrhagic Fever With Renal Syndrome (HFRS) Caused by Hantaviruses Using a Cross-Cutting Delivery Technology to Accelerate Protective Immune Responses.** Amy C. Shurtleff[1,2], Genevieve Long[2], Jay Hooper1, Robert Eackles[3], Julia Donnelly[3], Dan McLain[4], Drew Hannaman[5] and Connie S. Schmaljohn[1]. [1]US Army Medical Research Institute of Infectious Diseases (USAMRIID), Fort Detrick, Maryland, USA; [2]The Geneva Foundation, Tacoma, WA, USA; [3]US Army Medical Materiel Development Activity (USAMMDA), Fort Detrick, MD, USA; [4]Walker Downey & Associates, Inc., Verona, WI, USA; [5]Ichor Medical Systems, Inc., San Diego, CA USA.

42. **Rodent and shrew-borne hantaviruses in Germany.** Petra Strakova[1,2], Stephan Drewes[1], Maysaa Dafalla[1], Sabrina Schmidt[1], Ulrike M. Rosenfeld[1], Mathias Schlegel[1,3], Hanan Sheikh Ali[1], Rainer G. Ulrich[1]. [1]Friedrich-Loeffler-Institut, Institute for Novel and Emerging Infectious Diseases, Greifswald - Insel Riems, Germany. [2]Institute of Vertebrate Biology v.v.i., Academy of Sciences, and Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic. [3]Seramun Diagnostica GmbH, 15754 Heidesee, Germany.

43. **PUUV Active Endemic Areas Patterns in Russia: Results of 40-years Monitoring.** Bernshtein A[1], Apekina N[1], Ostanina M[1], Mutnykh E[1], Tkachenko E[1], Gavrilovskaya I[2]. [1]Chumakov Institute of Poliomyelitis and Viral Encephalitis, Moscow, Russia; Udmurtia Republic Centre for Hygiene and Epidemiology, Izhevsk, Russia; [2]Department of Molecular Genetics and Microbiology SUNY at Stony Brook, Stony Brook, New York. USA.

44. **Antiviral activity of arbidol hydrochloride in hantaan virus infection in vitro and in vivo.** Nian Ma, ZhanQiu Yang. State Key Laboratory of Virology/ Institute of Medical Virology, School of Medicine of Wuhan University, Wuhan 430071, P.R of China.

45. **Use of sequence independent single primer amplification next generation sequencing (SISPA NGS) for whole genome sequencing of Hantaan virus.** Dong Hyun Song[1], a, Won-keun Kim[2], Daesang Lee[1], Se Hun Gu[1], Jeong-Ah Kim[2], Seung-Ho Lee[2], Jin Sun No[2], Jin-Won Song[2], Seong Tae Jeong[1]. [1]The 5th R&D Institute, Agency for Defense Development, Yuseong P.O.Box 35, Daejeon, Republic of Korea 305-152. 2Department of Microbiology, College of Medicine, Korea University, Seoul, Republic of Korea**.**

46. **Hantavirus screening of peri-domestic rodents in the United Kingdom.** Ellen G. Murphy[1], Nicola J. Williams[2], Julian Chantrey[3], Malcolm Bennett[4] and Lorraine M. McElhinney[5]. [1]NIHR Health Protection Research Unit in Emerging Zoonotic Infections, NCZR, Leahurst Campus, School of Veterinary Science, Chester High Road, Neston, CH64 7TE, UK. [2]Zoonotic Infections of People, Pigs and Poultry Group, Department of Epidemiology and Population Health Institute of Infection and Global Health, Leahurst Campus, University of Liverpool, Neston, CH64 7TE, UK. [3]Institute of Integrative Biology, University of Liverpool, Biosciences Building, Crown Street, Liverpool, L69 7ZB, UK. [4]Faculty of Medicine & Health Sciences, Veterinary Clinical Building, Sutton Bonington Campus, Sutton Bonington, Leicestershire, LE12 5RD, UK. [5]Wildlife Zoonoses and Vector Borne Disease Research Group, Animal, Plant & Health Agency, Weybridge, Surrey, KT15 3NB, UK.

47. **Sin Nombre hantavirus nucleocapsid protein exhibits a metal-dependent DNA-specific endonucleolytic activity.** Elisabeth Möncke-Buchner, Michal Szczepek*, Marcel Bokelmann, Patrick Heinemann, Martin J. Raftery, Detlev H. Krüger, and Monika Reuter. Institute of Medical Virology, Helmut-Ruska-Haus, and *Institute of Medical Physics and Biophysics, Charité Medical School, Berlin, Germany.

48. **Seewis Virus in Eurasian Common Shrews (*Sorex araneus*) in Southwestern Poland.** Seung-Ho Lee[1], Ewa Gajda[2], Won-keun Kim[1], Joanna Hildebrand[2], Richard Yanagihara[3], Jin-Won Song[1]. 1Department of Microbiology, College of Medicine, Korea University, Seoul, Republic of Korea; [2]Department of Parasitology, Uniwersytet Wrocławski, Wrocław, Poland; [3] Department of Pediatrics, John A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, Hawaii, USA.

49. **Rodent-Borne Viruses Antibodies in Health Professionals Who Handle Wild Animals in Brazil.** Jorlan Fernandes[1], Renata Carvalho de Oliveira[1], Alexandro Guterres[1], Raphael Gomes[1], Monique Queiroz Lima[1], Felipe Moliterno[1], Márcio Neves Bóia[1], Silvana Levis[2], Elba Regina Sampaio de Lemos[1]. [1]Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, Brazil; [2]Nacional Institute of Human Viral Diseases, Pergamino, Argentina.

50. **Seroprevalence of hantavirus in bats from the Atlantic Forest, Brazil.** Renata Carvalho de Oliveira[1], Alexandro Guterres[1], Jorlan Fernandes[1], Pedro Cordeiro-Estrela[2], Roberto Leonan Morim Novaes[3], Emmanuel Messias Vilar[2], Ricardo Moratelli[3], Elba Regina Sampaio de Lemos[1]. [1]Oswaldo Cruz Institute, IOC-FIOCRUZ, Rio de Janeiro, Brazil. [2]Laboratory of mammals, DSE, CCEN, UFPB, João Pessoa, PB, Brasil. [3]Fiocruz Mata Atlântica, CFMA-FIOCRUZ, Rio de Janeiro, Brazil.

51. **Hantavirus Pulmonary Syndrome mimicking dengue in Rio de Janeiro, Brazil.** Renata Carvalho de Oliveira[1], João Bosco Júnior[2], Alexandro Guterres[1], Jorlan Fernandes[1], Liana Strecht Pereira[1], João Marcos Penna[2], Reynaldo de Jesus Oliveira Júnior[2], Cristina Giordano Dias[3], Stefan Vilges de Oliveira[4], Elba Regina Sampaio de Lemos[1]. [1]Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, Brazil. [2]Rio Claro Health Department, Rio de Janeiro, Brazil. [3]Rio de Janeiro State Health Department, Rio de Janeiro, Brazil. [4]Surveillance Service in Health, Ministry of Health, DF, Brazil.

52. **Phylogeny and Origins of Arena-, Bunya- and Filoviruses: Highlighting their relationships.** Alexandro Guterres[1], Renata Carvalho de Oliveira[1], Jorlan Fernandes[1], Elba Regina Sampaio de Lemos[1], Carlos Guerra Schrago[2]. [1]Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, Brazil. [2]Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.

53. **Serological Evidence of Hantavirus Infection in an Indigenous Reserve in the State of Mato Grosso, Brazil.** Ana Cláudia Pereira Terças[1,2], Leonir Evandro Zenazokenae[1], Vagner Ferreira do Nascimento[1], Thalise Yuri Hattori[1], Ariadne Cristinne Pereira de Moura[1], Liana Strecht[2], Renata Carvalho de Oliveira[2], Mariano Martinez Espinosa[3], Marina Atanaka Santos[3], Alba Valéria Gomes de Melo Via[4] and Elba Regina Sampaio de Lemos[2]. [1]University of the State of Mato Grosso, Tangará da Serra - MT. Foundation for the State of Mato Grosso Research - FAPEMAT. [2]Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro - RJ. [3]Federal University of Mato Grosso, Cuiabá - MT. [4]Department of Health of Mato Grosso, Cuiabá - MT.

54. **Hantavirus Seroprevalence in Gold-Mining Area in Mato Grosso, Brazil.** Ana Cláudia Pereira Terças[1,2], Elaine Cristina de Oliveira[3,4], Cor Jesus Fernandes Fontes[3], Rafael Gomes da Silva[2], Renata Carvalho de Oliveira[2], Marina Atanaka Santos[3], Elba Regina Sampaio de Lemos[2.] [1]University of the State of Mato Grosso, Tangará da Serra - MT. Foundation for the State of Mato Grosso Research - FAPEMAT, MT, Brazil. [2]Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro - RJ, Brazil. [3]Federal University of Mato Grosso, Cuiabá - MT, Brazil. [4]Department of Health of Mato Grosso, Cuiabá - MT, Brazil.

55. **A non-fatal case of hantavirus cardiopulmonary syndrome imported into the UK (ex-Panama), July 2014.** Barry Atkinson[1], Lisa J. Jameson[1], Begoña A. Bovill[2], Emma J. Aarons[3] Jodie Clewlow[3], Sarah Lumley[1], Jennie Latham[1], Megan H. Jenkins[2], Alasdair P. MacGowan[2], Andrew J. Simpson[3], Javeed Ahmed[4], Timothy J. Brooks[3,5] and Roger Hewson[1,5]. [1]Research Department, Microbiology Services Division, Public Health England, Porton Down, Salisbury, United Kingdom. [2]North Bristol National Health Service Trust, Bristol, United Kingdom. [3]Rare and Imported Pathogens Laboratory, Microbiology Services Division, Public Health England, Porton Down, Salisbury, United Kingdom. [4]Public Health Laboratory Bristol, Bristol, United Kingdom. [5]National Institute for Health Research, Health Protection Research Unit in Emerging and Zoonotic Infections, Liverpool, United Kingdom.

56. **Molecular characterization of hantaviruses circulating in Serbia in five years period.** Gorana Stamenkovic[1], Valentina Cirkovic[2], Marina Siljic[2], Bojana Bozovic[1], Ana Gligic[1], Maja Stanojevic[2]. [1]Department of Genetics, Institute for biological research “S. Stankovic”, University of Belgrade, Belgrade, Serbia. [2]Department of microbiology, Faculty of Medicine, University of Belgrade, Belgrade, Serbia. [3]National Center for Arboviruses and HF viruses, Institute of Virology, Vaccines and sera - Torlak, Belgrade, Serbia.

57. **Human hantavirus infections in the Czech Republic.** Hana Zelená[1], Jakub Mrázek[2]. [1]Institute of Public Health, Ostrava, Czech Republic. [2]University of Defence, Hradec Králové, Czech Republic.

58. **Ultrastructural changes in gastric mucosa in hemorrhagic fever with renal syndrome.** Evseyev AN, Plekhova NG, Evseyeva AA. Far Eastern State Medical University, Khabarovsk. Central Research Laboratory, Pacific Medical University, Vladivostok.

59. **Evolutionary history of Puumala Virus : analyse and comparison of different ancestral states inference methods.** Guillaume Castel[1,2], François Chevenet[3,2] and Olivier Gascuel[4,2]. [1]INRA, UMR 1062 CBGP, F-34988 Montferrier-sur-Lez, France. [2]Institut de Biologie Computationnelle (IBC), 34095 Montpellier, FRANCE. [3]UMR MIVEGEC, IRD, Montpellier, France. [4]Centre de Bioinformatique, Biostatistique et Biologie Intégrative (C3BI), Institut Pasteur, Paris, France.

60. **Complete genome, phylogeny and diversity of Puumala hantavirus isolates circulating in France.** Guillaume Castel[1], Mathilde Couteaudier[2], Elodie Monchatre-Leroy[3], Frank Sauvage[4], Franck Boué[3], Jean-Baptiste Pons[4], Adélaïde Dubois[5], Séverine Murri[5], Angelina Plyusnina[6], Jean-François Cosson[7], Dominique Pontier[4], Nathalie Charbonnel[1], Alexander Plyusnin[6], Philippe Marianneau[5] & Noël Tordo[8]. [1]INRA - UMR 1062 CBGP, F-34988 Montferrier-sur-Lez, France. [2]INRA - UR1282, Biologie Virus Aviaire, 37380 Nouzilly, France. [3]ANSES - Laboratoire de la rage et de la faune sauvage, Domaine de Pixérécourt - CS 40009 - 54220 Malzéville, France. [4]CNRS - Université Lyon 1, Laboratoire de Biométrie et Biologie Evolutive (UMR5558), F-69622 Villeurbanne, France & LabEx ECOFECT Ecoevolutionary Dynamics of Infectious Diseases, Lyon, France. [5]ANSES - Laboratoire de Lyon, Unité Virologie, 31 avenue Tony Garnier 69007, Lyon, France. [6]Department of Virology, University of Helsinki, Finland. [7]INRA - Bipar, 23 Av. Général de Gaulle, Maisons-Alfort, France. 8Antiviral Strategies Unit, Institut Pasteur, 25 rue du Dr. Roux, 75724 - Paris - France.

61. **Epidemiology and Emergence of Hantaviruses in Georgia.** Giorgi Babuadze, Nana Mamuchishvili, Giorgi Chakhunashvili, David Tsereteli, Tamar Chikviladze, Marina Chubinidze. National Center for Disease Control and Public Health, Tbilisi, Georgia.

62. **Development of minireplicon systems for Tula and Dobrava hantaviruses.** Kirill Nemirov[1], Alexander Plyusnin[2], Åke Lundkvist[3], and Noël Tordo[1]. [1]Antiviral Strategies Unit, Virology Department, Institut Pasteur, Paris, France. [2]Department of Virology, University of Helsinki, Haartman Institute, Helsinki, Finland. [3]Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden.

63. **Extracorporeal life support for severe hantavirus cardiopulmonary syndrome.** Tomicic V., Graf J., Umaña A., Abarca J., López R., Howard M., Castillo R., Cisterna S., Vial P. Departamento de Paciente Crítico, Clínica Alemana de Santiago, Santiago, Chile.

64. **Hantavirus infection confers resistance to Natural Killer cell-mediated killing and activates Natural killer cells through IL-15/IL15Rα expression.** Shawon Gupta[1,2], Carles Sola Riera[2], Monika Braun[2], Nicole Tischler[3], Malin Stoltz[1], Karin Sundström[1], Niklas Björkström[2], Hans-Gustaf Ljunggren[2] and Jonas Klingström[2]. [1]Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, 171 77 Stockholm, Sweden. [2]Center for Infectious Medicine, Department of Medicine, Karolinska Institutet, Karolinska University Hospital Huddinge, 141 86 Stockholm, Sweden. [3]Fundación Ciencia & Vida, 778 0272 Santiago, Chile.

65. **Spatial diffusion of Nova virus, a divergent hantavirus harbored by the European mole, in Belgium.** Lies Laenen[1], Simon Dellicour[2], Valentijn Vergote[1], Inne Nauwelaers[1], Sarah De Coster[1], Ina Verbeeck[1], Marc Van Ranst[1], Philipphe Lemey[2], Piet Maes[1]. [1]KU Leuven - University of Leuven, Department of Microbiology and Immunology, Laboratory of Clinical Virology, Rega Institute for Medical Research, Leuven, Belgium. [2]KU Leuven - University of Leuven, Department of Microbiology and Immunology, Laboratory of Evolutionary and Computational Virology, Rega Institute for Medical Research, Leuven, Belgium.

*66. **Cytokine and chemokine kinetics and their potential as biomarkers in patients with Puumala virus infection.** Petra Svoboda*[1], Lidija Cvetko Krajinović*[1], Petra Čikeš[1], Antea Topić[1], Martina Bosnar[2], Vesna Eraković Haber[2], Davor Jugović[1] and Alemka Markotić[1]. [1]University Hospital for Infectious Diseases “Dr. Fran Mihaljević”, Zagreb, Croatia; *equal contributions. [2]Fidelta Ltd, Zagreb, Croatia

67. **Hantavirus and Mammarenavirus infection in a single rodent host in in the area influenced by construction of Hydroelectric Power Plant in Brazil.** Jorlan Fernandes, Renata Carvalho de Oliveira, Alexandro Guterres, Thayssa Alves Coelho, Cibele Bonvicino, Paulo Sérgio D'Ándrea, Elba Regina Sampaio de Lemos. Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, Brazil.

*68. **Development of minireplicon systems for Tula and Dobrava hantaviruses.** Kirill Nemirov[1], Alexander Plyusnin[2], Åke Lundkvist[3], and Noël Tordo[1]. [1]Antiviral Strategies Unit, Virology Department, Institut Pasteur, Paris, France, [2] Department of Virology, University of Helsinki, Haartman Institute, Helsinki, Finland, [3] Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden.

69. **Sequence variability of Puumala virus strain cg1820.** Szemiel, A.M., Vatipally, S., Wilkie, G., and Elliott, R.M. MRC - University of Glasgow Centre for Virus Research, 464 Bearsden Road, Glasgow, G61 1QH, Scotland, UK

*Posters 66 and 68 will be part of the Wednesday session.

In Memoriam
Professor Song Gan

Prof. Song Gan left us early this year at the age of 95. This towering figure in Chinese hantavirology was in the midst of his long career a tragic and almost exemplary victim of the Cultural Revolution in China (1964-1976), during which he, as a respected researcher, had to perform manual work on the land, together with his team of students. Prof. Song said later that during this sad and long period, almost an entire generation of young intellectuals had been lost to science, and to hantavirus research in particular. Prof. Song Gan was the spirit behind the first isolation in 1981 of both HTNV and SEOV in his homeland China, and consequently the first recognition of the so-called “rat-type HFRS“ in the lab, with increasingly more important officially registered incidence of the so-called “second type” epidemics in his country. Obvious antigenic diversity between the two isolates was demonstrated by cross-neutralization, immunofluorescence blocking tests, and cross-ELISA, and described by Song Gan as first author in the December 1984 issue of J Infect Dis, under the title: “Antigenic difference between viral strains causing classical and mild types of epidemic hemorrhagic fever with renal syndrome in China”.This important virological and epidemiological finding led in turn and under his guidance, to the development of a bivalent inactivated golden hamster kidney cell (GHKC) in 1984, containing both the HTNV JR strain, and SEOV L99 strain. These combined vaccines are still in use, and millions of doses have been used up to today in China, with good success and few side-effects so far. Prof. Song was also among the first to describe preferential infection of the endothelium by hantaviruses, and vertical hantavirus transmission to the fetus in pregnant HFRS patients.

In summary, Prof. Song played a major role in nearly all aspects of hantavirology in his country, since his endeavors were encompassing classical virology, epidemiology, preventive and clinical medicine. Consequently, we honor him and remember him with our deepest respect.
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Ho Wang Lee Award
In recognition of
Outstanding Contributions to Hantavirus Research

Jim LeDuc, Ph.D. is the director of the Galveston National Laboratory (GNL) and a Professor in the Department of Microbiology and Immunology in the School of Medicine at the University of Texas Medical Branch (UTMB) in Galveston, Texas. Dr. LeDuc joined UTMB in late 2006 from the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, where he was the Influenza Coordinator. He also served as Director, Division of Viral and Rickettsial Diseases (2000-2005) and as the Associate Director for Global Health (1996-2000) in the CDC National Center for Infectious Diseases. As Medical Officer in charge of arboviruses and viral hemorrhagic fevers at the World Health Organization (1992-1996), he was the architect of the WHO program to address emerging infectious diseases and began the process to revise the International Health Regulations. He also held leadership positions during a 23-year career as a U.S. Army officer in the medical research and development command, with assignments in Brazil, Panama and at various locations in the United States, including the Walter Reed Army Institute of Research and the U.S. Army Medical Research Institute of Infectious Diseases. He is a Fellow, Infectious Diseases Society of America and a member of various professional organizations, has published over 200 scientific articles and book chapters, and is a recognized expert in virus diseases, biodefense and global health.

Joel M Dalrymple Award
In recognition of
Innovative Contributions to Hantavirus Research

I**rina Gavrilovskaya, MD, Ph.D. Dr. Sci.** Is currently an Emeritus Professor within the Department of Molecular Genetics and Microbiology in the School of Medicine at Stony Brook University. Dr. Gavrilovskaya came to Stony Brook in 1994 from a position as Professor at the Institute of Poliomyelitis and Viral Encephalitis AMS, in Moscow. Dr. Gavrilovskaya was a graduate student at the Institute from 1961-1966 studying Omsk Hemorrhagic Fever virus (OHFV) and from 1967 on Dr. Gavrilovskaya studied the etiology, epidemiology, and epizootology of Hemorrhagic Fever with Renal Syndrome (HFRS). Irina isolated HFRS viruses in Russia and collaborated with clinicians, epidemiologists and zoologista in Ukraine, Belorussia, Estonia, Bulgaria, Serbia, Finland, Udmurtia, Tataria and other countries. Dr. Gavrilovskaya's studies revealed information about HFRS virus distribution, HFRS cases and disease severity as well as the localization and transmission of HFRS viruses in host and human populations. Dr. Gavrilovskaya has studied the role of immunity in HFRS patients, immune influence on clinical manifestations, and she demonstrated the use of neutralizing human antibodies for HFRS treatment. Dr. Gavrilovskaya served as central coordinator of HFRS studies in Moscow and presented her results at domestic and international virology conferences and served as an ad hoc WHO adviser on HFRS. In 1970-90 Dr. Gavrilovskaya worked as the senior leading specialist and acting head of the laboratory of perspective studies. In 1994 Dr. Gavrilovskaya moved her studies to Stony Brook University where she discovered NY-1V and studied hantavirus interactions with human endothelial cells. She has identified hantavirus receptors on endothelial cells and potential mechanisms of capillary permeability directed by hantavirus infection. Irina's studies continue to seek therapeutic approaches to resolve HFRS and HPS diseases.

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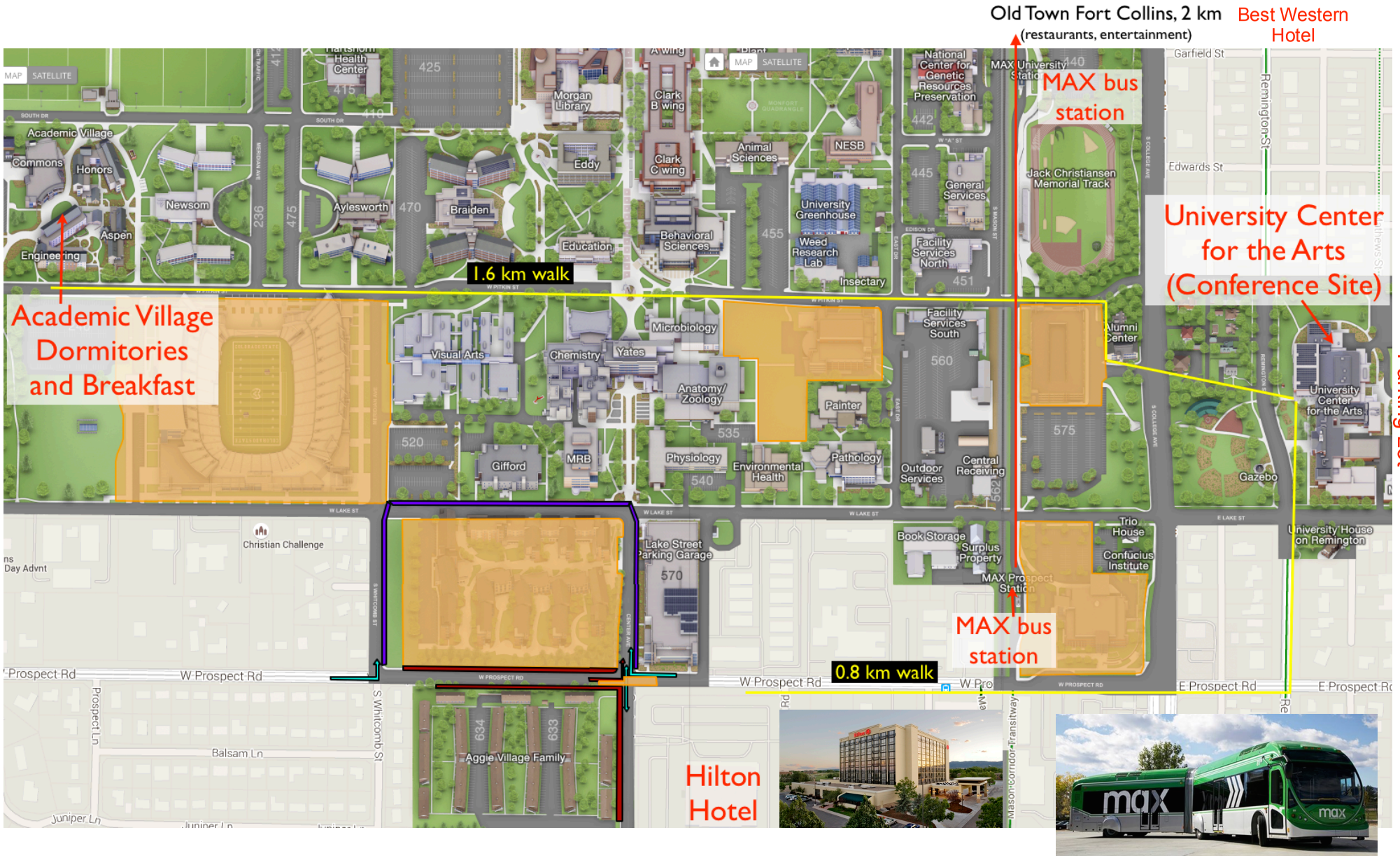
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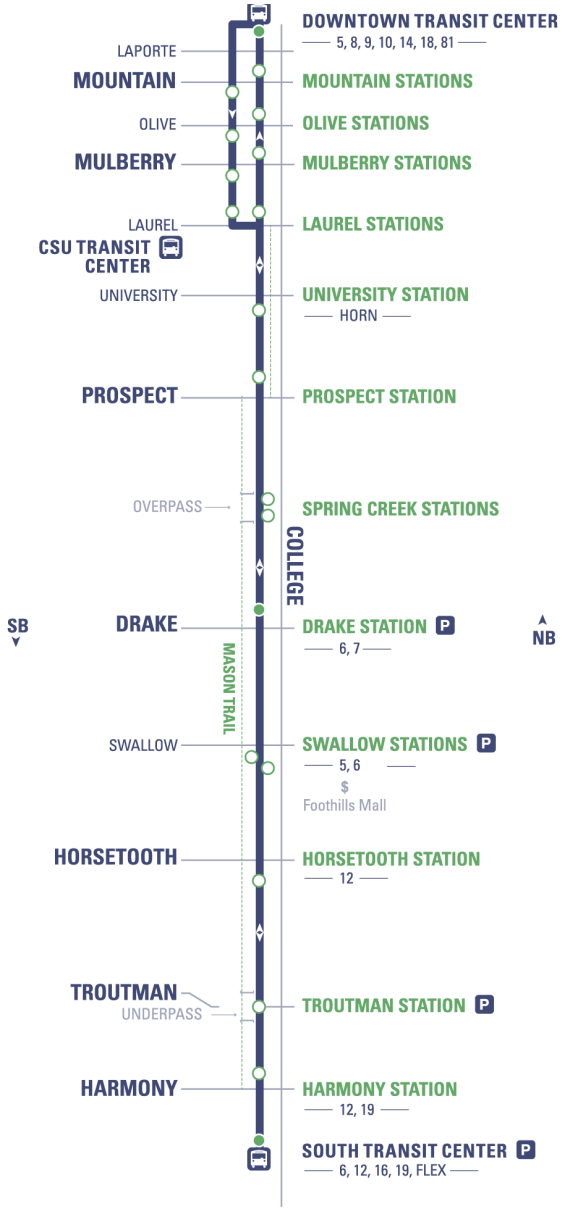

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:23	:31	:47
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:53	:01	:17
FROM 6:33A TO 6:53P		
:03	:11	:27
:13	:21	:37
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:43	:51	:07
:53	:01	:17
FROM 7:08P TO 9:38P		
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FROM 9:53P TO 11:23P		
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max SOUTHBOUND

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917	1510	1492
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FROM 5:10A TO 6:40A		
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:25	:37	:51
:40	:52	:06
:55	:07	:21
FROM 6:50A TO 6:50P		
:00	:12	:26
:10	:22	:36
:20	:32	:46
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- **Bus Route**
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Meeting Announcement

The 16th Annual
Rocky Mountain Virology Meeting
September 23 to 25, 2016
CSU Mountain Campus in Pingree Park

Keynote speaker:
Jonathan Towner, Ph.D.
"Filovirus replication in naturally and experimentally infected bats"
Centers for Disease Control, National Center for Emerging and Zoonotic Infectious Diseases, Division of High-Consequence Pathogens and Pathology Viral Special Pathogens Branch

Invited speakers
Heather L. True-Krob, Ph.D. **"Prion strains and amyloid polymorphism influence phenotypic variation"**
Associate Professor, Biology & Biomedical Sciences, Washington University in St. Louis

Jacquelin Dudley, Ph.D. **"Evasion of ERAD and the Immune Response by an Oncogenic Retrovirus"**
Professor, Department of Molecular Biosciences, The University of Texas at Austin

John Elder, Ph.D. **"Development of drug and vaccine strategies against FIV infection"**
Professor, Department of Immunology and Microbial Science, Scripps Research Institute

Charles Gross, MD. **"The pros and cons of virus-induced autophagy"**
Division Director, Department of Pediatrics, Division of Infectious Diseases
Professor of Pediatrics - Infectious Disease, Stead Family Department of Pediatrics, University of Iowa

Ann Hawkinson, Ph.D. **"Transcriptome profiling of a Pathogenic Viral Infection in Jamaican fruit bats (Artibeus jamaicensis) Experimentally Infected with Tacaribe Virus"**
School of Biological Sciences, University of Northern Colorado

John Parker, DVM, Ph.D. **"Virus-mediated compartmentalization of the host translational machinery"**
Associate Professor, Baker Institute for Animal Health, Department of Microbiology and Immunology, Cornell University

Jennifer Nyborg, Ph.D. **"Transcriptional deregulation by HTLV Tax protein"**
Professor, Department of Biochemistry and Molecular Biology, Colorado State University

The meeting starts with dinner on Friday evening and ends with lunch on Sunday. Information, pictures from past meetings, and links to proceedings are posted at our web site: **www.rockymountainvirologyclub.org.**

For more information, please contact Dr. Joel Rovnak, Joel.Rovnak@colostate.edu

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Oral Presentation Abstracts

Tula virus Surveillance in Rodents in the Netherlands.

Barry Rockx[1], Miriam Maas[1], Ankje de Vries[1], Annika van Roon[1] and Joke van der Giessen[1] 1.Center for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM).

Objective: In Europe, five different rodent-borne hantavirus species have been detected: Dobrava virus (DOBV), Saarema virus (SAAV or DOBV-Aa), Seoul virus (SEOV), Puumala virus (PUUV) and Tula virus (TULV), all having their own main reservoir rodent species. PUUV, SEOV, SAAV and DOBV are known to cause hemorrhagic fever with renal syndrome (HFRS) in humans, whereas there is increasing evidence for the pathogenicity of TULV. PUUV is the most common and widespread hantavirus in Europe. In the Netherlands, there have been over 100 cases of hantavirus infection in humans since 2008, mostly attributed to PUUV. However, little is known about TULV circulation and prevalence in rodents in the Netherlands. The objective of this study was to determine the prevalence and genetically characterized TULV in rodents at different locations in the Netherlands.

Methods: A survey on TULV was performed in different geographic locations from the south of the Netherlands (Limburg) to the north of the Netherlands (province of Friesland). Common voles were trapped and tested for the presence of virus specific antibodies and/or the presence of TULV RNA in tissues by RT-PCR. Multiple sequence alignment of partial S-, M- and L-segment and complete S-segment sequences was performed.

Results: In the current study, over 300 common voles were trapped and tested. TULV RNA was detected in 36% of the animals but the prevalence varied by geographic region rom 10-44%. Phylogenetic analysis indicate that Dutch TULV strains are closely related to strains from Germany and Belgium. However, even within the Netherland, genetically distinct groups of TULV were identified, corresponding with their geographic origin.

Conclusions: TULV is present in rodent populations in the Netherlands but the prevalence varies at different locations. Interestingly, while TULV was present at high prevalences in two geographically distinct locations, no human cases of TULV have been reported in The Netherlands. Human diagnostics should include TULV specific assay to assess the pathogenic ability of TULV.

The role of small mammal diversity in Sin Nombre virus transmission among deer mice: competing dilution and amplification effects.

Angela D. Luis[1], Amy J. Kuenzi[2], James N. Mills[3]. [1]Department of Ecosystem and Conservation Sciences, University of Montana, Missoula, MT; [2]Department of Biology, Montana Tech of the University of Montana, Butte, MT. [3]Population Biology, Ecology, and Evolution Program, Emory University, Atlanta, GA. Email: Angela.Luis@umontana.edu.

Objectives: Studies have shown that in the Southwest US, Sin Nombre hantavirus (SNV) prevalence in deer mice is lower in communities with higher small mammal diversity– the so-called 'dilution effect'. The mechanism driving this relationship has not been understood. Dilution will occur if increased species diversity leads to 1) decreased deer mouse density or 2) decreased transmission rate. With this study we aim to examine the relative importance of deer mouse density and transmission rate in determining SNV prevalence in deer mice in communities of varying small mammal diversity (by Simpson's diversity index).

Methods: We have longitudinal datasets from CDC-funded studies of variable lengths at 10 sites in Arizona, New Mexico, and Colorado, from 1994-2006, and 7 sites in Montana from 1994-2015. These sites span a range of small mammal diversities and deer mouse population dynamics, allowing the opportunity to examine important questions about ecological drivers of SNV prevalence. We fit our established SI (Susceptible-Infected) epidemiological model to these sites, which predicts SNV prevalence based on deer mouse densities. We estimate parameters for each site and compare to small mammal diversity across sites.

Results: Our model predicts infection dynamics well for each site given mouse population density. We found that increased small mammal diversity leads to decreased deer mouse density, which leads to decreased SNV prevalence, but with time lags that make the relationship hard to detect (but which can be predicted using our mechanistic model). We found that the transmission rate varied between sites. In contrast to the dilution effect, we found that transmission rate was positively correlated with small mammal diversity- a component 'amplification effect'.

Conclusions: The decrease in SNV prevalence in deer mice in diverse small mammal communities ('dilution effect') is driven by reduced deer mouse density, and once this is taken into account (with a mechanistic model), there is a positive effect of small mammal diversity on transmission (a component 'amplification effect'). Experimental studies are needed to examine potential drivers of the increase in transmission rate.

Bruges Virus, A Newfound Hantavirus in the European Mole, Contradicts Host-Specificity.

Lies Laenen[1], Valentijn Vergote[1], Se Hun Gu[2], Liana E. Kafetzopoulou[1], Despoina Vassou[3], Joseph A. Cook[4], Dimitris Kafetzopoulos[3], Marc Van Ranst[1], Detlev H. Krüger[5], Richard Yanagihara[2], Boris Klempa[5, 6], Piet Maes[1] [1]KU Leuven - University of Leuven, Department of Microbiology and Immunology, Laboratory of Clinical Virology, Rega Institute for Medical Research, Leuven, Belgium. [2]Department of Pediatrics, and Department of Tropical Medicine, Medical Microbiology and Pharmacology, John A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, HI, USA. [3]Foundation for Research & Technology-Hellas, GR-71110 Heraklion, Greece. [4]Department of Biology, Museum of Southwestern Biology, University of New Mexico, Albuquerque, NM 87131, USA. [5]Institute of Medical Virology, Charité School of Medicine, Berlin, Germany. [6]Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovakia

Background: Hantaviruses have a complex evolutionary history with both virus-host codivergence and cross-species transmission playing important roles. While hantaviruses are generally host-specific, mole-borne hantaviruses have exhibited host switching during evolution. Recently, we detected a novel hantavirus, designated Bruges virus (BRUV), in renal tissue of a European mole (*Talpa europaea*), trapped near Bruges, Belgium. The discovery of a second hantavirus in the European mole, in addition to Nova virus (NVAV), raises questions about virus-host specificity.

Methods: Renal tissues of 275 European moles, captured in widely separated areas in Belgium, were analyzed for hantavirus RNA by RT-PCR and next generation sequencing technology.

Results: Sequencing confirmed BRUV infection in 8.7% of moles, which was significantly lower than that of NVAV (53.2%), suggesting that BRUV may have resulted from a more recent species-jumping event. Testing of additional samples indicated BRUV in European moles from Wandlitz, Germany, and Avon, United Kingdom, lending support to our hypothesis that two genetically distinct hantavirus species share the same reservoir host. Moreover, co-infection with NVAV was detected in renal tissues from 22 of 24 BRUV-infected European moles. Phylogenetic analysis of the complete genome of BRUV suggested that reassortment and host switching events may have shaped BRUV evolution.

Conclusions: This study revealed host sharing of 2 divergent hantaviruses in the European mole. Infection of the same host with two hantaviruses could potentially lead to genomic reassortment, in which viruses can acquire new antigenic properties. We hypothesize that the high genetic diversity between BRUV and NVAV makes the emergence of viable reassortants unlikely due to biological incompatibility of the virus proteins. Further investigation into possible reassortment of these viruses is planned. These results illuminate the importance of members of the family Talpidae in shaping hantavirus evolution and indicate that hantavirus diversity could be greater than previously surmised.

Hantaviruses Got Wings: Bat-Borne Hantaviruses in Africa

Peter T. Witkowski[1], Jan F. Drexler[2], Christian Drosten[2], Eric M. Leroy[3], Leonce Kouadio[4,5], Emmanuel Couacy-Hymann[4], Fabian H. Leendertz[5], Detlev H. Krüger[1], Boris Klempa B[1, 6]

1. Institute of Virology, Charité Medical School, Berlin, Germany; 2. Institute of Virology, University of Bonn Medical Center, Bonn, Germany; 3. Centre International de Recherches Médicales de Franceville, Franceville, Gabon; 4. Laboratoire Central de la Pathologie Animal, Bingerville, Côte d'Ivoire; 5. Robert Koch Institut, Berlin, Germany; 6. Institute of Virology, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia; Email: boris.klempa@savba.sk

Our view on hantavirus ecology and host range was fundamentally extended over the last decade; the dogmatic concept of hantaviruses as rodent-borne viruses was changed after 2007 when more than 20 new hantaviruses were described in shrews and moles. However, genetically distinct hantaviruses were recently found also in African and Asian bats. Magboi virus was identified in Slit-faced Bat (Nycteris hispida) from Sierra Leone and Mouyassué virus in Banana Bat (*Neoromicia nana*) in Côte d'Ivoire. Bats (order Chiroptera) are recognized as one of the most considered important reservoir hosts for emerging human pathogens. Here, we report on the identification of a novel hantavirus, provisionally named Makokou virus (MAKV), in Noack's Roundleaf Bat (*Hipposideros ruber*) in Gabon, Central Africa. Phylogenetic analysis of the genomic L-segment showed that MAKV was most closely related to other bat-borne hantaviruses and shared the a most recent common ancestor with the Asian hantaviruses Xuan Son and Laibin. Quantitative real-time RT-PCR analysis showed that MAKV resembled rodent-borne hantaviruses in its organ distribution in that it predominantly occurred in spleen and kidney. This provides the first insight into the infection pattern of bat-borne hantaviruses. Moreover, recent study in Côte d´Ivoire identified not only multiple Mouyassué virus infected Banana Bats but also an additional genetically distinct hantavirus in the very same bat species. Altogether, our data further supports the emerging concept of bats as previously overlooked hantavirus reservoir hosts. Moreover, ancestral state reconstruction based on a tree of L gene sequences combined with phylogenetic fossil host hypotheses testing indicated the mammalian superorder Laurasiatheria (including shrews, moles, and bats) as potential hosts of ancestral hantaviruses at most basal tree nodes.

First serological investigation of hantavirus infection in human population from Madagascar

Aina Harinirina Rabemananjara[1], Vololoniaina Raharinosy[1], Jean Pierre Ravalohery[1], Jean Théophile Rafisandrantantsoa[1], Soa Fy Andriamandimby[1], Minoarisoa Rajerison[2], Soanandrasana Rahelinirina[2], Fanjasoa Rakotomanana[3], Sandra Telfer[2-4], Christophe Rogier[5-6], Noël Tordo[7], Jean-Michel Heraud[1], Claudia Filippone[1]

[1]Virology Unit, Institut Pasteur de Madagascar, Antananarivo, Madagascar. Email: cfilippone@pasteur.mg-cla.filippone@gmail.com [2]Plague Unit, Institut Pasteur de Madagascar, Antananarivo, Madagascar. [3]Epidemiology Unit, Institut Pasteur de Madagascar, Antananarivo, Madagascar. [4]School of Biological Sciences, Zoology Building, University of Aberdeen, Tillydrone Avenue, Aberdeen, UK. [5]Director Institut Pasteur de Madagascar; [6]Current address: Ministry of Defense, Paris, France. [7]Antiviral Strategies Unit, Institut Pasteur, Paris, France; Institut Pasteur de Guinée, Conakry, Guinea.

Objective: In the last years increasing data have been produced on the distribution and animal reservoir host range of novel hantaviruses in Africa. The recent discovery of Anjozorobe hantavirus (ANJV), variant of Thailand virus (THAIV), in rodents (*Rattus rattus*, *Eliurus majori)* in Madagascar, resulted in a growing concern on the potential zoonotic risk to Humans and pathogenicity. In the framework of ongoing projects focused on a better understanding of zoonosis in Madagascar, the aim of this study was to evaluate the hantavirus seroprevalence among the Malagasy human population.

Methods: Sera have been collected in the last five years during a retrospective multi-site study set up at Institut Pasteur de Madagascar (ZORA – 'ZOonosis, Rodents and Arbovirus'). Sampling was carried out in 28 sites of Madagascar on 1,754 recruted individuals (867 females and 887 males; average age 37.6 years; range 18-99). The experimental approach was relied on a serological survey by enzyme immunoassays (EIA) (Reagena Ltd., Toivala, Finland), based on recombinant antigens corresponding to the nucleocapsid (N) protein from Dobrava-Belgrade virus (DOBV) and Hantaan virus (HTNV), both belonging to the Murinae-associated subfamily, as well as the Malagasy strain ANJV.

Results: Among the tested sera of the collection, 30 (1.7%) were found IgG positive and 31 (1.8%) showed a border line reactivity. On the 30 positive individuals, 16 (53.3%) were males and 14 (46.7%) were females, with an average age of 42.7 years (range 24-74 years). Interestingly, no difference across the country was observed. These seroprevalence results are in line with previous results observed among the general population in Africa where hantaviruses are known to circulate.

Conclusions: This exploratory study is the first survey investigating hantavirus human seroprevalence in Madagascar. Confirmation of these results will be carried out by using ELISA assay specific for both THAIV prototype and Anjozorobe virus. Importantly, this analysis will contribute to establish the zoonotic risk of hantaviruses among the human population and to implement public health programs in Madagascar for the control of viral infection as well as the occurrence of Haemorrhagic Fever with Renal Syndrome HFRS and other related pathologies in the community.

Ecology of Puumala hantavirus in Finland

Voutilainen, L.[1,2], Niemimaa, J.[1] & Henttonen, H.[1]

[1] Natural Resources Institute Finland, FI-01301 Vantaa, Finland[2] Dept Virology, University of Helsinki, FI-00014 University of Helsinki, Finland

Understanding the dynamics of zoonotic pathogens in their reservoir host populations is a prerequisite for predicting and preventing disease epidemics. The infection risk of humans by Puumala hantavirus (PUUV) is highest in northern Europe, where populations of the rodent host (bank vole, *Myodes glareolus*) undergo cyclic fluctuations. During 1995 -2015 about 35 000 human cases of NE (nephropathia epidemica) have been diagnosed in Finland. We conducted a 7-year longitudinal capture-mark-recapture study, mostly at monthly intervals, to monitor seasonal and multiannual patterns of the PUUV infection rate in bank vole populations exhibiting a 3-year cycle in the highly endemic area in boreal taiga in Central Finland. Infected bank voles were most abundant in mid-winter months during years of increasing or peak host density. Seroprevalence of PUUV infection in bank voles exhibited a regular, seasonal pattern reflecting the annual population turnover and accumulation of infections within each cohort. In autumn, the PUUV transmission rate tracked increasing host abundance, suggesting a density-dependent transmission. However, prevalence of PUUV infection was similar during cyclic increase and peak years despite a twofold difference in maximum host density. This may result from the high proportion of young individuals carrying maternal antibodies in summer of the peak year delaying transmission during the cycle peak years. This increase/peak dilemma is reflected in the human NE incidence in the region: even though the bank vole density is clearly higher in the peak year, the number of NE cases can often be similar or even higher in the vole increase year. As a comparison we analysed the annual NE incidence in various parts of Finland, from coastal areas to inland and from south to north, reflecting declining proportion of agricultural land and forest fragmentation. There was a trend for one year NE peaks in coastal and southern regions while two year peaks occurred inland, possibly indicating more restricted dispersal of host and virus in the increase phase in former ones. Our exceptionally intensive long-term dataset from the endemic region provides a solid basis on which to develop models to predict the dynamic public health threat posed by PUUV in northern Europe.

Hantavirus Disease Epidemiology in Germany

Jörg Hofmann, Detlev H. Krüger and the German Hantavirus Disease Consortium

Institute of Medical Virology, Charité University Medicine Berlin, Germany. E-mail: joerg.hofmann@charite.de

Objective: With an annual average of 886 cases (incidence 1.15/100,000) hantavirus disease is far from being an exotic illness in Germany. The years 2007, 2010, and 2012 were considered to be “outbreak years” with a maximum of 2,825 registered cases in 2012. Clinical cases occur after infection either by Puumala virus (PUUV) or Dobrava-Belgrade virus (DOBV). To assess and improve the hantavirus routine diagnostics we have established an external quality assessment (EQA) scheme.

Methods: Epidemiological and clinical survey. Molecular identification of hantaviruses in humans and rodents was performed by sequencing the S, L and/or M segments. The obtained sequence data were subjected to phylogenetic analyses.

Results: Detailed molecular phylogenetic analyses revealed the existence of at least 7 distinct molecular PUUV clades, with a high molecular identity between local human and rodent-derived virus strains. The different genetic clades clearly correspond to respective geographical regions in West and South Germany. In North-East Germany the Kurkino variant of Dobrava-Belgrade virus (DOBV) is transmitted to humans. Nucleotide sequences of this virus were amplified from ten patients as well as local *Apodemus* mice. Again, clustering of virus strains in dependence on the geographic place were found. As in the case of Puumala virus infections, most clinical cases occur to be mild to moderate, the case fatality rate is < 1%.

In contrast to molecular approaches, an exact serotyping by routine assays (PUUV vs. DOBV) is constrained as revealed by EQA’s (only 81–96% of correct results). When summarizing the about 3,200 test results from 90 laboratories from Germany and neighboring countries, the laboratories reached an over-all specificity of 96.7% and a sensitivity of 95% in their detection of a hantavirus infection at all. A correct distinction between acute and convalescent infections was forwarded in 90-96 % of answers.

Conclusions: Hantavirus outbreaks in Germany are caused by multiple outbreaks simultaneously occurring in the different geographical regions. A reliable typing, and moreover, a differentiation between the DOBV genotypes can only be achieved by molecular approaches. Regarding the serodiagnostic the EQAs revealed acceptable results for the participating laboratories but further improvement is still needed.

Human infections by rodent- and non-rodent associated hantaviruses in Africa

Patrick Heinemann [1#], Peter T. Witkowski [1#], Chantal G. Akoua-Koffi [2], Frieder Schaumburg [3], Lamine Koivogui [4], Jean-Jacques Muyembe-Tamfum [5], Brita Auste [1], Boris Klempa [1,6], Fabian H. Leendertz [7], Detlev H. Krüger [1]

[1] Charité Medical School, Institute for Virology, Berlin, Germany [2] Université Alassane Ouattara, Bouaké, Côte d’Ivoire [3] University Hospital Münster, Institute of Medical Microbiology, Münster, Germany [4] Institut National de Santé Publique, Conakry, Guinea [5] Department of Clinical Biology, Kinshasa University Medical School, Kinshasa, D.R. Congo [6] Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovakia [7] Robert Koch Institute, Berlin, Germany. [#] equal first authors; Email: detlev.kruger@charite.de

Question. Sangassou virus (SANGV) was isolated as the first indigenous African hantavirus from rodents in Guinea, West Africa. Recent studies revealed that also insectivores (shrews, moles) and bats can carry novel hantaviruses. There is an urgent need to investigate the human contact to hantaviruses carried by these hosts and to assess the public health relevance of hantaviruses in Africa.

Methods. Sera sampled in regions of Guinea, Côte d´Ivoire, and D.R. Congo (population-based surveys) as well as from febrile patients in Guinea were investigated with enzyme-linked immunosorbent assays (ELISA) and confirmatory assays [Western blotting (WB) and immunofluorescence (IF)] on the basis of antigens of several “classical” (rodent-borne) hantaviruses. Neutralizing antibodies were detected by FRNT. - For specific detection of infections by viruses associated with non-rodent hosts, we developed a panel of serological tests (ELISA, WB, IF) based on recombinantly expressed nucleocapsid proteins of African shrew-associated Bowé (BOWV) and Uluguru (ULUV) viruses. Cross-sectional human sera from Gabon as well as sera from patients with kidney or lung disorders from Ivory Coast were investigated by these assays.

Results. Seroprevalence rates of 1.2 % (8/649), 3.9 % (27/687) and 2.4 % (7/295), respectively, were found in Guinea, Côte d´Ivoire, and D.R. Congo. In the group of persons with fever of unknown origin from Guinea, 4.7 % (8/172) patients were IgG-positive including 1 patient exhibiting specific IgM and later on SANGV-neutralizing antibodies. - Using “Africa-specific” assays on the basis of antigens from BOWV, ULUV, and SANGV, 16/346 (4.6 %) sera from Gabon were generally hanta-positive; among them, 3 sera were found to be specifically BOWV-positive, 1 serum was ULUV-positive, and 3 sera were SANG-positive. Sera from patients (Ivory Coast) exhibiting acute renal or lung disorders contained 19/330 (5.8 %) hanta-positive samples, among them, 6 sera were specifically positive for BOWV antibodies and 1

sample was SANGV-positive. Acute infection was detected by BOWV-specific IgM-ELISA in 1 patient with febrile illness and kidney involvement.

Conclusion. Our study demonstrates a broad exposure of humans to hantaviruses in West and Central Africa. Here, hantavirus infections may be a significant unrecognized medical problem. The data strongly suggest that also non-rodent borne hantaviruses can infect humans.

Increased Detection of Seoul Hantavirus in the United Kingdom

Lorraine M McElhinney[1*], Denise A Marston[1], Kieran Pounder[2], Robert Smith[3], Tim Brooks[4], Anthony R Fooks[1], Charlotte Featherstone[5]

[1]Animal & Plant Health Agency (Weybridge), UK [2]Liverpool University, UK [3]Public Health Wales (Cardiff), UK [4]Public Health England (Porton), UK [5]Animal & Plant Health Agency (Thirsk), UK

*presenting author: lorraine.mcelhinney@apha.gsi.gov.uk

Objective: Hantavirus species are the etiological agents of haemorrhagic fever with renal syndrome (HFRS) and are responsible for thousands of human cases per year in Europe. The majority of HFRS cases in Europe are due to the bank vole associated hantavirus, Puumala Virus. The hantavirus associated with brown rats (Seoul Virus, SEOV) is responsible for increasing numbers of urban hantavirus infections worldwide. However, the prevalence of rodent and human cases associated with SEOV in Europe have long been considered to be low and speculated to be driven by the sporadic introduction of infected brown rats (*Rattus norvegicus*) via ports.

Methods: To investigate the prevalence of SEOV in two distinct port cities (Liverpool, UK and Lyon, France), brown rats were trapped and the lungs tested using a pan-hantavirus RT-PCR (polymerase gene).

Results: Between October 2010 and March 2012, 128 brown rats were caught at sites across the Lyon region in France. SEOV RNA was detected in the lungs of 18 brown rats (14%, 95% CI 8.6 – 21.3). In contrast, no SEOV RNA was detected in the lungs of 133 brown rats trapped in and around Liverpool. SEOV was previously believed to cause mild to moderate HFRS but has recently been linked to a number of severe HFRS cases with acute renal failure in the UK. In recent years, SEOV has been identified in both wild and pet rats in the UK, causing disease in individuals exposed to the rat excreta. However, in July 2015, an outbreak of SEOV associated HFRS in South Wales was traced to a number of domestic and commercial facilities breeding rats for reptile food. The outbreak data will be presented.

Conclusions: Our findings suggest that SEOV, whilst localized, may be more prevalent in European brown rats than previously believed and may contribute to a greater number of the reported HFRS cases in Europe.

Pets, pests and pestilence: understanding social aspects of zoonotic disease transmission.

Robin C[1,2], Watkins F[2,3], Perkins L[2,4], Christley, R[1,2].

Department of Epidemiology and Population Health, Institute of Infection and Global Health, School of Veterinary Science, University of Liverpool, Leahurst Campus, Neston, CH64 7TE, UK[1], NIHR Health Protection Research Unit, University of Liverpool, Ronald Ross Building, Liverpool, L69 7BE[2], UK, Department of Public Health and Policy, Institute of Psychology Health and Society, University of Liverpool, Liverpool, L69 3GL, UK[3], Health Services Research Department, Institute of Psychology, Health and Society, University of Liverpool, Liverpool, L69 3GL, UK[4]

Neglecting to explore social aspects of zoonotic disease transmission has led to public health interventions making assumptions about knowledge and that we, the scientific community know what is best for the general public. Individuals understand disease and risk differently; this concept is vital in understanding, and managing, transmission. When exploring zoonotic infections, a key construct is the relationship between humans and animals.

This study explores these constructions of risk, using hantavirus as a model. As rats are the predominant host in the UK, interactions between rats and humans were investigated in three at-risk groups; pet rat owners, farmers and pest control workers.

In-depth, semi-structured interviews were used to explore participants' understanding of health, illness and risk, in addition to their perceptions of rats. Interviews are being analysed using Grounded Theory, an iterative approach allowing new theories to be conceptualised.

Initial interviews with pet rat owners have revealed they not only experience stigma because of their pet, but may also have an affinity with this "outsider animal" that "draws people that might have had a bit of a messy life". Consequently, pet rat communities are a significant part of their social identity. A concept of fatalism in relation to hantavirus emerged; "it's just one of those things". Risk is constructed as "not significant", and although there is awareness of it being asymptomatic in rats, there is dissonance between this knowledge and believing pet rats are safe because "they live in a protected environment"; it is the outside world that poses the risk. Other zoonoses including leptospirosis are more acceptable; "a

completely kind of different risk, and more contained", whereas the uncertainty and lack of control surrounding hantavirus means management is "just not possible" and could lead to isolation from the pet rat community.

This study will provide a deeper understanding of hantavirus transmission and facilitate the effective communication of socially-targeted public health interventions, reducing the risk of hantavirus infection within these at-risk communities.

Hantavirus infection in the UK: detection in wild rats, pet rats and renal patients

Victoria Graham, Lisa Ottowell and Roger Hewson· Virology and Pathogenesis group, Public Health England, Porton Down, Salisbury, Wiltshire, SP4 0JG. UK E-mail: victoria.graham@phe.gov.uk roger.hewson@phe.gov.uk

Objective: To evaluate whether Hantaviruses are circulating within the UK rat populations. **Methods**: Three human cases of haemorrhagic fever with renal syndrome (HFRS) were epidemiologically linked to rodents: one individual residing on a farm in North Yorkshire and the Humber (a tidal estuary on the east coast of Northern England), the second a "fancy rat" breeder from Wales and the third an owner of pet rats. **Results:** Serological investigations in each patient showed evidence of hantavirus antibodies. The 3rd patient was a PCR positive follow up. Studies on rodents with RT-PCR showed evidence of SEOV which was confirmed with sequencing and phylogenetic analysis. Virus was also isolated from wild rats (*Rattus norvegicus*) around the farm of one of the patients (provisionally designated strain Humber) but not from pet agouti rats belonging to the second patient. Virus was not recovered from the human samples. To determine the level of hantavirus exposure and investigate the public health risk of a UK endemic hantavirus in the Northern England region an initial seroprevalence study was conducted in people living or working on farms. Of a total 119 individuals tested in Northern England, nine (7.6%) were seropositive for hantavirus antibodies. Seven of the seropositive samples showed a stronger reaction to Seoul and Hantaan compared to other clinically relevant hantaviruses. A further seroprevalence study of pet rat breeders showed that 33% were seropositive for hantavirus antibodies. Observation of rodents during the day, in particular mice, was associated with a reduced risk of seropositivity. In addition to one region known to be at risk following an acute case, five further potential risk areas have been identified. **Conclusions:** These are the first hantaviruses isolated from rats in the UK and confirms the presence of a pathogenic Seoul virus in Europe. The study also provides evidence that hantaviruses are likely to be of public health importance in regions of the UK.

HFRS and Hantavirus in China, 2006-2015

Shuo Zhang, Mifang Liang, Jiandong Li, Quanfu Zhang, Chuan Li , Dexin Li. National Institute for Viral Disease Control and Prevention, China CDC, Beijing 102206, E-mail: lidx@chinacdc.cn

Hemorrhagic fever with renal syndrome (HFRS) caused by Hantavirus is a serious public health problem in China. The National Disease Reporting System established by China CDC in 2004 supplied surveillance data of HFRS cases in the entire population in China. Here we analyzed the surveillance data of HFRS epidemics from 2006 to 2015 in China.

From 2006 to 2015, a total of 112177 HFRS cases and 1116 deaths were reported in China with the average annual incidence rate of 0.84/lakh and case fatality rate of 0.99%． The top 8 provinces with HFRS cases were Heilongjiang, Shanxi, Shandong, Liaoning, Jilin, Hebei, Hunan and Zhejiang，which accounted for above 75% of the total number of cases. HFRS cases were mainly reported in spring and autumn-winter seasons, with the peak in November． Reported cases were mainly clustered in age groups of 15-60 years and the incidence in males were over 3 times higher than females. Farmers were under the highest risk.

As rodent is the main host of HFRS, a total of 40 counties in 22 provinces were selected as surveillance sites and over 200,000 mousetraps were placed each year in both spring and autumn-winter seasons. The density of rodents was relatively high in the provinces of Heilongjiang, Jilin, Liaoning, Inner Mongolia, Henan, Sichuan, and rodents density was over 30% in some sites of Heilongjiang province. Analysis of rodent species indicated that Apodemus agrarius and rattus norvegicus were dominant in wild field and neighborhoods, respectively. Serological and molecular detection were practiced in samples from rodents, showing that the infection rate was much higher in the three provinces of Northeast area. Pathogen detection was also performed in collected rodent samples and several new Hantaviruses were isolated.

HFRS were still a natural focal disease with relatively high morbidity and fatality in China. Distribution and epidemic trend of the disease had also changed. Surveillance measures, together with prevention and control strategies should be improved and strengthened to reduce HFRS infection in China.

Hantavirus infection in Sri Lanka

Kumiko Yoshimatsu[1], Chandika D. Gamage[2], Yomani Sarathkumara[2], S.A.M Kularathne[3], Kanae Shiokawa[1], Kenta Shimizu[1], Yoshimi Tsuda[1], Jiro Arikawa[1]

[1]Department of Microbiology, Hokkaido University Graduate School of Medicine, Hokkaido University, Sapporo 060-8638 Japan. [2]Department of Microbiology, Faculty of Medicine, University of Peradeniya, Sri Lanka. [3]Department of Medicine, Faculty of Medicine, University of Peradeniya, Sri Lanka. E-mail: yosimatu@med.hokudai.ac.jp

Objective: Sri Lanka locates in South Asia and it has unique animal species. Frequent leptospirosis outbreak is one of the public health problems. Because leptospirosis and hantavirus infections show several clinical symptoms such as fever, renal dysfunction, and hepatic dysfunction. Thus it is difficult to differentiate both infectious diseases only from clinical symptoms. In this study, we examined both diseases in febrile patients and small animals for transmission of leptospira and/or hantavirus to human. **Methods:** Twelve febrile patients sera were obtained from Teaching Hospital Peradeniya, Kandy District Central Province, Sri Lanka. A total of 164 rodents and shrews were captured in Kandy city in 2014 and 2015. Sera were examined to detect IgM and IgG antibody in ELISA by using recombinant N antigen of Hantaan virus. IFA, Western blot and serotyping assay were also carried out. Serum antibodies and leptospiral genome were also examined. **Results:** Three male were antibody positive against Hantaan virus antigen in IgM ELISA, IgG ELISA, IFA and Western blot assay. They showed slightly higher reactivity against Thailand virus antigen in serotyping ELISA by using truncated N protein. Eight patients considered to be leptospirosis in Kandy city. The rest of one patient was not determined. Although rodents and shrews carried leptospira, anti-hantavirus antibody positive animals were not detected. **Conclusions:** In this study, serum antibodies from three patients were confirmed. They came from Central and North Central Provinces. In Kandy city area, rodents are not reservoir of hantaviruses, but they carry leptospires. Thailand-like and unknown hantavirus might be causative agent of febrile illness in rural areas in Sri Lanka.

Mechanism of the Andes hantavirus fusion process and inhibition by Gc domain III and stem peptides

Barriga, GP[1], Acuña, R[1], Bignon, E[1], Villalón-Letelier, F[1], Márquez, CL[1], Ross, BH[2] Monasterio, O[3], Mardones, GA[2] Vidal, SE[1], and Tischler, ND[1,4]

[1]Molecular Virology Laboratory, Fundación Ciencia & Vida, Santiago, Chile; [2]Laboratory of Structural Cell Biology, Department of Physiology, Universidad Austral de Chile, Valdivia, Chile;

[3]Laboratorio de Biología Estructural y Molecular, Facultad de Ciencias, Universidad de Chile, Santiago, Chile, [4]Facultad de Ciencias Biológicas, Universidad Andres Bello, Santiago, Chile.

E-mail: ntischler@cienciavida.org

To enter cells, hantaviruses fuse their envelope membrane with host cell membranes. Previously, we have shown that the Gc envelope glycoprotein is the viral fusion protein sharing characteristics with class II fusion proteins. The ectodomain of class II fusion proteins is composed of three domains connected by a stem region to a transmembrane anchor in the viral envelope. These fusion proteins can be inhibited through exogenous fusion protein fragments spanning domain III (DIII) and the stem region. **Objective**: To characterize and inhibit the fusion mechanism of hantaviruses. **Methods:** We established cell-free *in vitro* systems based on virus-like particles (VLPs)-liposome co-flotation, virus protein sucrose sedimentation and enzymatic digestion to characterize fusion activation and fusion intermediate states. Recombinant domains were synthesized in *E. coli* and purified by affinity and size exclusion chromatography. Cell-cell fusion and Andes virus (ANDV) infectivity assays were used to quantitate inhibition of fusion and infection. **Results**: Low pH triggered the interaction of VLPs with liposomes in absence of cellular factors. This interaction was dependent on a pre-fusion glycoprotein arrangement. Further, acidification induced Gc multimerization changes leading to non-reversible Gc homotrimers. These homotrimers were resistant to detergent, heat and protease digestion, suggesting characteristics of a stable post-fusion structure. Next recombinant Gc DIII and stem peptides were produced and tested for viral inhibition. The fragments blocked the infection of cells up to 60% when fusion of ANDV occurred within the endosomal route, and up to 95 % when fusion occurred with the plasma membrane. Furthermore, the fragments impaired ANDV glycoprotein-mediated cell-cell fusion and cross-inhibited the fusion mediated by the glycoproteins of Puumala virus (PUUV). The Gc fragments interfered in ANDV cell entry by preventing membrane hemifusion and pore formation, retaining Gc in a non-resistant homotrimer stage, as described for DIII and stem peptide inhibitors of class II fusion proteins. **Conclusions**: Hantavirus fusion is non-reversibly activated by acidification involving lipid interaction and Gc multimerization re-arrangements into a stable post-fusion homotrimer. The fusion activity was blocked with DIII and stem peptides suggesting that hantavirus Gc shares not only structural, but also mechanistic similarity with class II viral fusion proteins.

Generation and characterization of a recombinant vesicular stomatitis virus (rVSV) expressing Hantaan virus glycoproteins

Rohit K. Jangra1, Megan M. Slough1 Lara M. Kleinfelter1 & Kartik Chandran1

1 Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx NY 10461. Presenter's E-mail: rohit.jangra@einstein.yu.edu Corresponding author's E-mail: kartik.chandran@einstein.yu.edu

Objectives: Hantaviruses are classified as biosafety level-3 (BSL-3) agents. To study hantavirus entry and infection in BSL-2 setting, we aimed to generate and characterize a recombinant vesicular stomatitis virus (rVSV) expressing surface glycoproteins of Hantaan virus (HTNV), a prototypic Old World hantavirus that causes hemorrhagic fever with renal syndrome (HFRS).

Methods: DNA sequences for the open reading frame (ORF) of Hantaan virus (HTNV) GP from a cell culture-adapted strain, 76-118 (GenBank accession number NP_941978.1), were codon optimized for expression in human cells and synthesized. The VSV glycoprotein (G) sequences in the vector encoding the rVSV genome were replaced with HTNV GP-encoding sequences by using standard molecular biology techniques. The recombinant virus that expresses Hantaan virus glycoprotein (rVSV-HTNV GP) was generated by using a plasmid-based rescue system in 293T cells and the rescued virus was amplified on Vero cells.

Results: Replication and spread of the early passage rVSV-HTNV GP was very poor initially. However, passaging the virus on Vero cells (for about 4 weeks) selected for a virus that carried two key amino acid changes in the Hantaan viral glycoprotein (one each in Gn and Gc glycoproteins). Pseudotyping experiments confirmed that these two mutations increased rVSV- HTNV GP infectious virus production by up to a 1000-fold. We are currently analyzing effect of these mutations on hantavirus GP expression, sub-cellular localization, Gn-Gc interaction and eventual packaging into the rVSV virions.

Conclusions: We have identified key mutations in the HTNV GP that dramatically enhance the replication and spread of rVSV-HTNV GP, a replication-competent rVSV system for studying Old World hantavirus entry. Understanding how these mutations enhance rVSV-HTNV GP replication can pave the way for generation of model systems to study other members of the *Bunyaviridae* family.

Structural studies of *Bunyaviridae* glycoproteins

Jason Lanman[1], Amar Parvarte[1], and Colleen Jonsson[2]

[1]Department of Biological sciences, Purdue University Email: jlanman@purdue.edu. [2]Department of Microbiology, The university of Tennessee Knoxville

Hantaan virus is the prototypic member of the Genus *Hantavirus* within the family *Bunyaviridae.* The *Bunyaviridae* family consists of negative-sense RNA viruses with a three-part segmented genome - S (small), M (medium), and L (large). The genome segments, encapsidated by the nucleocapsid (N) protein to form ribonucleoproteins, are enclosed inside a lipid envelope that is decorated by spikes composed of glycoproteins. The M segment codes for a glycoprotein precursor which is co-translationally cleaved into the Gn 47 kDa) and Gc (51 kDa) glycoproteins. Structures of the glycoprotein complex of Hantaan, Tula virus and Orthobunyavirus have been solved to resolutions ranging from 3.6 to 2.5 nm which reveals only the overall shape and symmetry of the complex. However, in the absence of any high resolution structural data for the glycoproteins a detailed understanding of the mechanism for entry is limited. In these cryo-EM studies we have implemented the use of new imaging and reconstruction approaches to investigate the structure of the Hantavirus glycoprotein at higher resolution.

Comparative genomics of bank vole sensibility / tolerance to Puumala hantavirus in France

Adelaïde Dubois[1,2], Maxime Galan[1], Guillaume Castel[1], Laure Benoit[1], Audrey Rohfritsch[1], Bernhard Gschloessl[1], Jean-Baptiste Pons[2], Séverinne Murri[3], Philippe Marianneau[3], Nathalie Charbonnel[1]

[1]INRA, Centre de Biologie pour la Gestion des Populations, Montferrier sur Lez, France E-mail: maxime.galan@supagro.inra.fr [2]CNRS, Laboratoire de Biométrie et Biologie Evolutive, Villeurbanne, France [3]ANSES Unité de Virologie, Lyon, France

Objective: Nephropathia epidemica (NE) is a mild form of hemorrhagic fever with renal syndrome (HFRS) caused by the hantavirus Puumala (PUUV). In France, geographical variations in the incidence of reported human cases are observed that are not congruent with the distribution of PUUV reservoir host, the bank vole *Myodes glareolus*. We tested the hypothesis that variations in *M. glareolus* immune responses to PUUV could affect PUUV replication and excretion in the environment, what could ultimately shape NE incidence in France. Because PUUV infection in bank voles does not lead to high levels of pro-inflammatory responses nor immunopathological symptoms, we have proposed that some form of tolerance to the virus, at least in PUUV endemic areas, could have evolved in *M. glareolus.* **Methods**: We performed a genome scan study of bank vole populations sampled along two North/South transects (Ardennes ; Jura-Ain), both including PUUV endemic and non endemic areas. We combined candidate gene analyses (*Tnf* promoter) and high throughput sequencing of RAD (Restriction-site Associated DNA)

markers. We applied methods implemented in various softwares to emphasize F_{ST} outliers between PUUV endemic and non endemic areas and to identify genes with a putative role in the interaction with PUUV. This combination of methods allowed to consider several demographic scenarios with regard to bank vole population genetic structure as well as different environmental factors that could have contributed to the genome-wide pattern of local adaptation detected. **Results**: We emphasized signatures of selection along the bank vole genomes that could be linked to the adaptation to PUUV and we compared them between the two transects. Among these SNPs 'outliers', some included genomic regions associated with pro-inflammatory responses. These outliers could vary depending on the geographic region considered. **Conclusions**: Overall, this study provides new insight into the selective processes involved in *M. glareolus* / PUUV interactions and of the mechanisms underlying the strategies of defences of *M. glareolus* against PUUV infections.

Phylogeographic analysis of Hemorrhagic Fever with Renal Syndrome (HFRS) Patients and Natural Hosts using Multiplex PCR-based Next Generation Sequencing

Won-keun Kim[1], Jeong-Ah Kim[1], Dong Hyun Song[2], Daesang Lee[2], Yong Chul Kim[4], Sook-Young Lee[1], Seung-Ho Lee[1], Jin Sun No[1], Ji Hye Kim[1], Jeong Hoon Kho[1], Se Hun Gu[2], Seong Tae Jeong[2], Michael Wiley[3], Heung-Chul Kim[5], Terry A. Klein[6], Gustavo Palacios[3], Jin-Won Song[1]

[1]Department of Microbiology, College of Medicine, Korea University, Seoul, Republic of Korea 136-705 [2]The 5th R&D Institute, Agency for Defense Development, Yuseong P.O.Box 35, Daejeon, Republic of Korea 305-152 [3]The Center for Genome Science, U.S. Army Medical Research Institute of Infectious Disease, Fort Detrick, MD, USA 21702 [4]The Armed Forces Medical Center, Saemaeul-ro, 177 beon-gil, Seongnam-si, Gyeonggi-do, Republic of Korea 463-040 [5]5th Medical Detachment, 168th Multifunctional Medical Battalion, 65th Medical Brigade, Unit 15247, APO AP 96205-5247, United States of America [6]Public Health Command District-Korea (Provisional), 65th Medical Brigade, Unit 15281, APO AP 96205-5281, United States of America. Email: wkkim1061@korea.ac.kr

Objective: Emerging RNA virus infectious diseases pose a worldwide critical public health threat based on significant rates of mutation and transmission in the absence of effective antivirals. Next generation sequencing (NGS) is a robust technology to define genomic sequences and characteristics of viruses in endemic outbreaks. Of particular interest is the use of whole genome sequencing (WGS) to perform phylogeographic analysis, that allows the detection and tracking of the emergence of viral infections. Hantavirus infections cause hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS) in human with ~200,000 clinical cases reported annually and a fatality rate ranging from 1 to 35%. In combination with whole genome sequences of Hantaan virus (HTNV) from HFRS patients and natural hosts (*Apodemus agrarius)* in endemic areas, Republic of Korea (ROK), spatial phylogenetic analysis will provide a novel platform for the surveillance and genome-based diagnosis of HFRS incidents. **Methods:** HTNV positive specimens from HFRS patients, United States and ROK army, and rodent hosts from endemic areas were obtained. Multiplex PCR-based NGS applied to WGS of HTNV from the patient specimens using Illumina MiSeq, while conventional RT-PCR was performed to gather whole genome sequences of HTNV from natural hosts. Based on Maximum Likelihood method, the acquired genomes were phylogenetically analyzed to describe the spatial and temporal links between cases and their sources. Recombination analysis was performed using Recombination Detection Program (RDP). **Results:** Phylogenetic analyses of HTNV tripartite genomes recovered from HFRS patients showed geographic clustering of the strains with the HTNV circulating in rodents on each of their military exercising location, suggesting the most likely site and time of infection. Recombination analysis of the HTNV demonstrated a genome organization compatible with recombination of HTNV S segment. **Conclusions:** Multiplex PCR-based NGS is useful to obtain the whole genome sequence of HTNV from HFRS patients. Taken together with the genome sequences of HTNV from natural reservoirs, *A. agrarius*, the phylogeographic analysis using multiplex PCR-based NGS may provide a robust tool to develop risk analyses and preventive and therapeutic strategies against hantavirus-borne diseases.

Whole genome analysis of Dakrong virus, a novel hantavirus harbored by the Stoliczka's Asian trident bat (*Aselliscus stoliczkanus*) in Vietnam

Satoru Arai[1], Son Truong Nguyen[2], Vuong Tan Tu[2], Hoang Trung Thanh[3], Saw Bawm[4], Kyaw San Lin[4], Se Hun Gu[5], Satoshi D. Ohdachi[6], Keiko Tanaka-Taya[1], Yasuhiro Yoshikawa[7], Shigeru Morikawa[1], Richard Yanagihara[5], Kazunori Oishi[1]

[1] National Institute of Infectious Diseases, Tokyo, Japan; [2]Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, Hanoi, Vietnam; [3]Vietnam National University, Hanoi. Vietnam; [4]University of Veterinary Science, Nay Pyi Taw, Myanmar; [5]University of Hawaii at Manoa, Honolulu, HI, USA; [6]Hokkaido University, Sapporo, Japan; [7]Chiba Institute of Science, Choshi, Japan. E-Mail: arais@nih.go.jp

Background: The recent discovery of genetically distinct hantaviruses in multiple species of shrews and moles (order Eulipotyphla) prompted a further exploration of their host diversification by analyzing bats (order Chiroptera).

Methods: Total RNA, extracted from RNAlater®-preserved lung tissues of 449 bats representing 17 genera and 57 species in six families (Emballonuridae, Hipposideridae, Megadermatidae, Pteropodidae, Rhinolophidae and Vespertilionidae), captured in Vietnam and Myanmar during 2012-2014, was analyzed for hantavirus RNA by RT-PCR.

Results: Hantavirus RNAs were detected in one of 12 Stoliczka's Asian trident bats (*Aselliscus stoliczkanus*) (family Hipposideridae) and one of five least leaf-nosed bats (*Hipposideros cineraceus*) (family Hipposideridae) from Quảng Trị Province, Vietnam. Pair-wise alignment and phylogenetic analysis, based on the full-length coding regions of the S- (1339 bp), M- (3622 bp) and L-segments (6535 bp), using maximum likelihood and Bayesian methods, demonstrated that the newfound hantavirus in the Stoliczka's Asian trident bat, designated Dakrong virus (DKGV), belonged to divergent lineages, comprising other recently recognized bat-borne hantaviruses. The other hantavirus in the least leaf-nosed bat was very closely related to Xuan Son virus (XSV), previously identified in the Pomona round-leaf bat (*Hipposideros pomona*) in northern and central Vietnam. Tanglegrams, generated by TreeMap 2.0b, showed a high degree of concordance between bat-borne hantaviruses and their bat host species, suggesting that bats are natural reservoirs, much like rodents and shrews.

Conclusions: With the discovery of DKGV, the number of bat-borne hantaviruses now total eight (five in Asia and three in Africa). All eight are harbored by insectivorous bats, in keeping with the conjecture that primordial hantaviruses may have originated as insect viruses.

Partial Characterization of Nova Virus Isolated from the European Mole (*Talpa europaea*)

Se Hun Gu[1], Mukesh Kumar[1], Beata Sikorska[2], Janusz Hejduk[3], Janusz Markowski[3],

Marcin Markowski[3], Paweł P. Liberski[2], Richard Yanagihara[1]

[1] University of Hawaii at Manoa, Honolulu, Hawaii, USA; [2] Medical University of Łódź, Łódź, Poland; [3] University of Łódź, Łódź, Poland. E-mail: ryanagih@hawaii.edu

Background: A paucity of full-length genomes and a dearth of cell-culture isolates contribute to persistent uncertainties about the taxonomy and pathogenicity of hantaviruses. Although referred to as novel viruses, nearly all of the more than 30 hantaviruses identified recently in shrews and moles (order Eulipotyphla) and insectivorous bats (order Chiroptera) exist only as viral sequences. To date, only two non-rodent-borne hantaviruses have been isolated. The objective of this study was to isolate and characterize Nova virus (NVAV) from the European mole (*Talpa europaea*).

Methods: Lung tissue homogenates, prepared from NVAV-infected European moles captured in central Poland in 2013, were inoculated onto Vero E6 cell monolayers, and cells and culture media were analyzed for NVAV RNA by RT-PCR at two- to four-week intervals.

Results: After multiple failed attempts, NVAV RNA was detected in cells and culture media at 34 days after inoculation with tissues from one of four European moles. Typical hantavirus-like particles, measuring 80–120 nm in diameter, were found by transmission electron microscopy. Hantavirus genomic sequences of the isolate, designated NVAV Te34, were identical to that amplified from the original lung tissue, and phylogenetic analysis of the full-length L, M and S segments, using maximum-likelihood and Bayesian methods, showed that NVAV was most closely related to hantaviruses harbored by insectivorous bats, consistent with an ancient evolutionary origin. To determine if experimental NVAV infection in mice resembled that of Hantaan virus, infant Swiss Webster mice were inoculated with 200 pfu of NVAV by the intraperitoneal route. Mice developed weight loss and hyperactivity, beginning at 16 days, followed by hind-limb paralysis and death. High NVAV RNA copies were detected in lung, liver, kidney, spleen and brain by quantitative real-time RT-PCR. Neuropathological examination showed astrocytic and microglial activation and neuronal loss.

Conclusions: The long-awaited isolation of NVAV, as the first mole-borne hantavirus, will accelerate the acquisition of new knowledge about its infectivity and pathogenic potential in humans. Because European moles often reside near human habitation, individuals with known exposures, who develop febrile illnesses or unusual clinical syndromes, should be investigated for evidence of NVAV infection and disease.

Packaging of Hantavirus genomic segments studied in single cells and virions by multicolor fluorescence *in situ* hybridization

Matthias Schade[1]; Hannah Sperber[1], Beate Tenner[1], Malte Hilsch[1], Maik J. Lehmann[3], Detlev H. Krüger[4], Andreas Herrmann[1], Roland Schwarzer[2] , Peter T. Witkowski[4]

[1]Institute of Biology, Department of Molecular Biophysics, Humboldt-Universität zu Berlin, Germany; [2]Weizmann Institute of Science, Department of Biological Chemistry, Rehovot, Israel; [3]Institute of Virology, Department of Molecular Parasitology, Humboldt-Universität zu Berlin, Germany; [4]Institute of Virology, Charité Medical School, Berlin, Germany Email: matthiasschade.de@gmail.com

Objective: Visualizing the temporal and spatial organization of the segmented Hantavirus genome in infected host cells is essential for understanding the viral replication cycle and assembly process. To

assess vRNA packaging and any hierarchy involved, approaches at single cell and virus level are necessary. This enables quantitative characterization of the degree of segment co-localization and to identify structures of the host cell involved in genome assembly. To this end, we aimed to monitor all three Hantavirus genomic vRNA segments simultaneously in infected cells and in virions.

Methods: We established a multicolor FISH approach for Puumala virus in which all three viral genomic segments were distinguished simultaneously by specific sets of fluorescent oligonucleotides. In parallel, an immunofluorescence-based anti nucleocapsid protein staining was performed. Infected Vero E6 cells were fixed at different time points and an unbiased, automated image analysis of 1,000 cells, detecting 30,000 vRNA spots, was carried out. A cell-free single virus pull down was performed on a PEG-passivated glass-slide functionalized with glycoprotein-specific antibodies, followed by the aforementioned FISH assay.

Results: In infected cells, a comparison of all three vRNA segments resolved vRNA monomers, heterodimers, and heterotrimers at 3 dpi and at 11 dpi. There was no significantly preferred class of heterodimers at either time point. Over time, the fraction of monomers decreased and the fraction of trimers increased slightly up to 33-44% at 11dpi. In a cell-free Puumala virus pull down, co-localization of viral ribonucleic proteins (vRNP) of the two viral RNA segments M and L was evaluated. Only 38% of all spots containing at least one vRNP segment contained both segments.

Conclusions: The absence of a preferred class of vRNA heterodimers in infected cells points towards an assembly process lacking a specific segment assembly order. With over 62% of all detected mature virions lacking at least one of three vRNA segments, this result implicates a suboptimal, non-segment-specific assembly process of Hantaviruses.

Evaluation of VEGF and sVEGFR2 Dynamic in Patients with Haemorrhagic Fever with Renal Syndrome

Miša Korva[1], Katarina Resman Rus[1], Emil Pal[2], Nataša Kejžar[3], Franc Strle[3], Tatjana Avšič-Županc[1]

[1]Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Zaloška 4, Ljubljana, Slovenia. E-mail: misa.korva@mf.uni-lj.si [2]Murska Sobota General Hospital, Department of Infectious Diseases, Ulica dr. Vrbnjaka 6, Rakičan, Slovenia[3] Institute for Biostatistics and Medical Informatics, Faculty of Medicine, University of Ljubljana, Vrazov trg 2, Ljubljana, Slovenia [4]Department of Infectious Diseases, University Medical Centre Ljubljana, Ljubljana, Japljeva 2, Slovenia.

Objective: Hantavirus disease is a systemic illness targeting different organs and organ systems. The clinical spectrum is ranging from an asymptomatic infection to a severe course with a fatal outcome, depending, in part on the causative virus and host imunogenetics. Explanations for increased vascular permeability without disrupting the endothelium are elusive. One such elusive cytokine is the vascular endothelial growth factor (VEGF) and its soluble receptors. The aim was to investigate the dynamics of VEGF and sVEGFR2 in patients infected with PUUV or DOBV. We have also compared VEGF/sVEGFR2 concentrations with viral load and clinical/laboratory parameters. **Methods:** We have collected blood and urine samples of 88 HFRS patients (30 infected with DOBV and 58 infected with PUUV). A VEGF was measured with MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panel and sVEGFR2 concentration was measured with Quantikine® ELISA. **Results:** Most plasma VEGF concentrations measured in HFRS patients were not significantly higher than those in the control group. Contrary, sVEGFR2 plasma concentrations were lower in HFRS patients, when compared to the control group. The urinary concentrations of VEGF were increased only in DOBV-infected patients, where urinary concentrations of sVEGFR2 were significantly increased in both patients' groups, especially at the beginning of their hospitalization. Later on, urinary concentrations of sVEGFR2 decreased rapidly in PUUV-infected patients, but not in DOBV-infected patients. Analyzing urinary secretion of VEGF/ sVEGFR2 and clinical/laboratory parameters, we have found that both, platelet count and diuresis, are negatively linearly associated with VEGF and sVEGFR2. **Conclusions:** Our study suggests a dual role of VEGF in hantavirus pathogenesis; in kidneys, cells secrete VEGF in response to hypoxia, but elevated VEGF in plasma is detected during the recovery phase, which is presumably related with a vascular repair and improvement of clinical markers.

Hantavirus vs cytotoxic lymphocytes: do all hantaviruses act alike?

Carles Solà Riera, Shawon Gupta, Kimia Maleki, Niklas K. Björkström, Hans- Gustaf Ljunggren, Jonas Klingström[1]
Center for Infectious Medicine, Department of Medicine, Karolinska Institutet, Karolinska University Hospital Huddinge, Stockholm, Sweden

Objectives Hantaan virus (HTNV) and Andes virus (ANDV) inhibit apoptosis and infection of human endothelial cells triggers IL-15-dependent NK cell activation. In the present work, we compared the anti-apoptotic effects of various hantaviruses, including non-pathogenic ones. Further, we analyze the effect that various hantaviruses have on NK cell activation.

Methods Primary human umbilical vein endothelial cells (HUVECs) were infected with hantaviruses for 4 days. Cells were then stimulated with staurosporine and the level of apoptosis analyzed using TUNEL and

DCM staining. To analyze the effect on NK cells, infected HUVECs were co-incubated with naïve NK cell isolated from healthy individuals. To analyze the molecular anti-apoptotic mechanisms of hantaviruses, and the effect on NK cells, diverse methods such as flow cytometry, immunofluorescence, PCR and western blot were used.

Results Pathogenic and non-pathogenic hantaviruses inhibit staurosporine- induced apoptosis. Also, all tested hantaviruses induced IL-15/IL-15Rα expression on infected endothelial cells. The effect of hantaviruses on contact- dependent NK cell activation is further explored and analyzed.

Conclusions All tested hantaviruses are able to strongly inhibit apoptosis, indicating that this is an important feature of hantaviruses in general. Additional mechanism(s) behind hantavirus-mediated inhibition of apoptosis and NK cell activation are currently being investigated.

ANDV N Protein Inhibits Interferon Induction by Uniquely Engaging TRIM21

Matthew J. Simons[1,2], Lewis M. Brown[3], Shujuan Tao[3], Elena E. Gorbunova[1], Erich R. Mackow[1,2]

[1]Dept. of Molecular Genetics and Microbiology, [2]Molecular and Cell Biology Program, Stony Brook University, Stony Brook, NY.; [3]Quantitive Proteomics Center, Dept of Biological Sciences, Columbia University New York, NY.

Objective: Andes virus (ANDV) causes highly lethal hantavirus pulmonary syndrome (HPS) and is the only hantavirus known to spread person to person. The ANDV nucleocapsid (N) protein uniquely inhibits TBK1 phosphorylation and IFN signaling responses directed by RIG-I, MDA5, MAVS and TBK1. Analysis of mechanisms that mediate ANDV N protein inhibition failed to define interactions with TBK1, TANK or TRAF3 and suggested that N impacts ancillary regulators of TBK1 activation. **Methods:** We used lentivirus transduction approaches to persistently express a tandemly tagged ANDV N protein (N-TT) within primary human microvascular endothelial cells (ECs) and analyzed N interactions with EC proteins by an LC/MS/MS proteomics approach. Responses were validated by reciprocal immunoprecipitation of cellular targets with N protein and functional IFN-luciferase transcriptional reporter assays.

Results: Comparison of proteins precipitated from N-TT expressing vs nonexpressing ECs identified the E3 ubiquitin ligase TRIM21 as an N protein target with high certainty as the mean number of TRIM21 peptides detected in 4 LC/MS/MS runs was 13.8 (2.8 ppm RMS mass error). Of ~75 TRIM family members only TRIM21 was found to bind N protein. TRIM21 is known to regulate IFN induction and no other known IFN pathway regulatory proteins were observed to co-precipitate with ANDV N protein. Proteomics data was validated by finding ANDV N specifically co-precipitated with TRIM21, and by demonstrating that TRIM21 and ANDV N protein synergistically inhibit IFNβ induction.

Conclusions: These findings identify TRIM21 as a novel target of ANDV regulated IFN induction and provide a potential mechanism by which ANDV N protein uniquely inhibits TBK1 activation. A comparison with hantavirus N proteins that fail to regulate IFN responses suggests that novel ANDV residues form an IFN regulating virulence determinant.

Immunopathology in a new model of hemorrhagic fever caused by hantavirus infection

Martin J. Raftery[1], Lidija Kobak[1], Peter Witkowski[1], Detlev H. Krüger[1], Günther Schönrich[1]

[1]Institute of Medical Virology, Charité-Universitätsmedizin, Berlin, Germany

Objective: Zoonotic hantaviruses are a cause of renal and pulmonary failure in humans worldwide. The pathogenesis of hantavirus-induced human disease is a matter of debate, however. Although human pathology would suggest an immune component, rodent models of hantavirus infection indicate that CD8+ T cells are not involved. In order to resolve this point we have developed humanized mouse models of hantavirus infection based on NSG and NSG-HLA-A2 mice that harbour functional HLA-A2-restricted CD8+ T cells.

Methods: The course of infection in these mice was followed by qRT-PCR, flow cytometry and immunohistochemistry.

Results: In the absence of CD8+ cells lethal infection was accompanied by induction of cellular neutrophilic infiltrates. This is in accordance with patient data, where overproduction of neutrophil extracellular traps is a characteristic of clinical hantavirus infection. The presence of CD8+ T cells, however, induced a much stronger and more rapidly lethal infection, with widespread inflammatory infiltrates despite the same quantity of viral replication in organs. In addition, human but not murine platelets were depleted by the infection. Further experiments demonstrated that infection of hematopoietic cells was a central feature of pathogenic hantaviruses.

Conclusions: We propose that the capacity to activate neutrophils and to infect APC thereby inducing widespread activation of T cells is central to hantavirus-induced disease in humans.

After crossing the barrier: immune response in Dobrava-Belgrade virus infections

Katerina Tsergouli, Anna Papa
Department of Microbiology, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece E-mail: ktsergouli@gmail.com

Objective: Cytokines play a key role in pathogenesis of Hantavirus infection. Aim of the study was to investigate the cytokine patterns during the acute phase of Dobrava/Belgrade virus (DOBV) infection. **Methods:** The levels of 27 cytokines in 30 serum samples from 24 hospitalized patients with acute DOBV infection were evaluated. One case was fatal. Samples were grouped according to the course of the disease and the week of illness. A control group of 16 apparently healthy individuals was included. Quantification of 27 serum cytokine levels (IL-1b, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, Eotaxin, FGFb, G-CSF, GM-CSF, IFN-g, IP-10, MCP-1, MIP- 1a, MIP-1b, PDGF-bb, RANTES, TNF-a and VEGF) was performed using Bio- Plex Suspension Array system (Bio-Rad Laboratories, CA). **Results:** Compared to the control group, severe cases presented significantly higher IL-1ra, IL-6, IL-8, IL-9, IL-10, GM-CSF, IP-10, MIP-1b, TNF-α and VEGF levels, while only IL-13 and TNF-α levels were significantly higher in the non severe cases ($p<0.05$). In all groups IP-10 was increased and RANTES was decreased. Significant and time dependent differences among fatal, severe and non-severe cases were seen. The multivariate logistic regression analysis showed VEGF as positively associated with disease severity. A strong immune response was seen during the 1st week of illness, especially in the severe cases, while the response in the non-severe cases was weaker and delayed. It is of interest that the affected cytokines in severe cases during the first and second week of illness differed from those in the non-severe cases. Th1 response was high in non-severe cases and low in the fatal case, while a mixed Th1/Th2 immune response was seen in the survivors from a severe disease. Conclusion: Cytokine patterns differ between severe and non-severe HFRS cases caused by DOBV. The immune response is the result of numerous interactions and networks of several factors including cytokines. Further studies are needed for better understanding of the immune response in hantavirus (and other) infections, that may help to guide future studies for drug design.

Adaptation of Hantaan virus strain AA57 to Vero E6 cells affects the pathogenicity in mice

Hiroaki Kariwa[1)], Masahiro Maki[1)], Takahiro Seto[1)], Hirofumi Kondo[1)], Kumiko Yoshimatsu[2)],

Jiro Arikawa[2)], and Kentaro Yoshii[1)]

[1)] Graduate School of Veterinary Medicine, [2)] Graduate School of Medicine, Hokkaido University, Sapporo, Japan,

Objective: Hantaviruses are the causative agents of hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS) in humans. Hantaan virus (HTNV) is one of the causative agents of HFRS in East Asia. Recently, it was found that infection of HTNV Galkino/AA57/2002 strain (AA57), causes severe pulmonary symptoms in juvenile mice. To know if the passage of AA57 in Vero E6 cells effects on virus pathogenicity in mice, mice were infected with AA57 of low and high passage histories in Vero E6 cells. **Methods:** Two-week old ICR mice were subcutaneously infected with AA57 having 3- and 30-time passage histories in Vero E6 cells (P3 and P30, respectively). The morbidity, mortality, the virus titers in lungs, and cytokine expressions were examined in P3- and P30-infected mice. **Results:** When mice were infected with P3, some mice weakened (body weight loss, labored breathing) and died at 8-11 days post infection (dpi). The morbidity and mortality of P3 were 63.3% and 36.7%, respectively. In mice infected with P30, morbidity and mortality were significantly lower (5% and 0%, respectively). The highest virus titer (8.8×10^4 ffu/gram) was observed at 5 dpi in the lungs of P3-infected mice, but only few viruses (lower than detection limit) was detected in P30-infected mice throughout the experiments. The expressions of inflammatory cytokines, IL-1β and TNF-α, were induced in the lungs of P3-infected mice at 7 dpi, but no cytokine expressions were observed in P30-infected mice. P30 grew significantly faster and greater than P3 in Vero E6 (African green monkey) and A549 (Human) cells. One nucleotide substitution was found in the S segment of P30 resulting in a substitution of amino acid position 43 in nucleocapsid protein compared to that of P3. In viral polymerase encoded by L segment, there were four amino acid substitutions at positions 200, 772, 1,662, and 2,096 in P30 compared to P3. In glycoprotein precursor encoded by M segment, no amino acid substitutions were observed. **Conclusions:** These results indicate that adaptation of AA57 to Vero E6 cells induced high virus multiplicity in cultural cells and reduced multiplicity in mice resulting in lower virulence.

Alimentary tract as entry route for hantavirus infection

Peter T. Witkowski[1]; Christian Jürgensen[2]; Annett Petrich[1]; Jörg-Dieter Schulzke[3]; Roland Bücker[3#]; Detlev H. Krüger[1#]
1. Institute of Virology; 2. Division of Hepatology and Gastroenterology; 3. Institute of Clinical Physiology, Charité Medical School, Berlin, Germany; Email: peter.witkowski@charite.de

[#] Authors contributed equally

Objective: Pathogenic hantaviruses are supposed to enter human body by inhalation of contaminated droppings. However, several studies have shown that the majority of patients exhibits haemorrhagic

gastropathy or at least noticeable histopathology of gut mucosa. Generally, a gastrointestinal hantavirus transmission route via contaminated food was never explicitly proposed or excluded. For this reason we deployed an *in vitro* study to investigate susceptibility of the human small intestine epithelium for hantavirus infection, based on the polarized CaCo-2 cell culture system, serving as a model for intestinal barrier function. Studies were performed with Puumala (PUUV) and Dobrava-Belgrade (DOBV) viruses – the main European hantavirus pathogens.

Methods: Firstly, hantavirus acid susceptibility was tested by titration of virus stocks exposed to human gastric juice. Secondly, a polarized human intestinal epithelial (Caco-2) cell culture infection model was established to simulate PUUV and DOBV transmigration in human gut. Viral growth was monitored by quantitative RT-PCR and virus titration. Transepithelial electric resistance (TER) was measured to track changes in integrity and permeability of infected tissue. Immunofluorescence was performed to illustrate changes in cell organization and rule out apoptotic processes.

Results: Hantavirus survived gastric juice for time periods sufficient for stomach passage. Hantavirus receptors ß1-integrin and CD55/DAF were shown to be expressed on Caco-2 cell surface. Infection of polarized cells resulted in viral entry as demonstrated by endosomal antigen 1 (EEA-1) expression co-localising with viral antigen, followed by viral replication and loss of epithelial barrier function. Cytoskeleton reorganization and depletion of tight junction protein ZO-1 was visible as result of cell infection, showing no signs of apoptosis, however cell rounding and cell detachment was observed.

Conclusions: Our data indicate that hantavirus entry into the organism via alimentary tract is possible. The results denote a novel and neglected aspect of hantavirus transmission and pathogenesis to be considered in epidemiological observations.

Interaction of pathogenic and non pathogenic Hantaviruses with their natural and human hosts

Myriam Ermonval[1], Florence Baychelier[1], Isabella Eckerle[2], Noël Tordo[1]

1. Unité des Stratégies antivirales, Institut Pasteur, 25 rue du Dr Roux, 75015 Paris, France E-mail : myriam.ermonval@pasteur.fr 2. Institute of Virology, Sigmund-Freud-Strasse 25, 53127 Bonn, Germany

Objective: Hantaviruses are persistent and asymptomatic in their rodent reservoir but can cause pathogenesis in humans. However, many questions remain about the way hantaviruses interact with their hosts in these different situations. In order to better understand their different outcomes, we are comparing the pathogenic Puumala (PUUV) and the non pathogenic Tula (TULV) and Prospect Hill (PHV) viruses. On that purpose, we investigate tropism, maturation and propagation of different hantaviruses and their relation with the innate immune system leading either to persistence or pathogenesis. **Methods:** Cell susceptibility to infection was assessed using different detection technics in cell lines derived from various tissues of different species and in human primary innate immune cells. In particular, effect of hantavirus infection on activation of epithelial cells and neutrophils was carried out by proteome array and flow cytometry analysis. **Results:** The VeroE6 and the HuH7 cell lines appeared highly permissive to the three types of hantaviruses, varying from 30 to 100% infected cells. For other cell lines, we obtained various levels of infection depending on host and tissue origin. It is striking that PUUV and PHV but not TULV, were able to infect cells (up to 50%) derived from *Myodes glareolus*, the natural host of PUUV. In contrast, a low percentage of human cells were sensitive to all three viruses. Our comparative studies of proteome carried out in VeroE6 and HuH7 cells (infected *versus* non-infected cells), showed an upregulation of various factors. Different pathways were activated either leading to recruitment of immune cells, among which neutrophils, or to pro-inflammatory responses. Finally, we highlighted that PUUV, unlike TULV and PHV, promoted survival of purified human neutrophils and led to a delay in apoptosis. **Conclusions:** Our results support a role of inflammatory processes in the immunopathogenesis of hantaviruses as reported in patients with nephropatia epidemica caused by Puumala virus. This also correlates with the increased survival of neutrophils obtained in response to PUUV. Our approach could be useful to identify potential factors which could be targeted to counteract direct or indirect effects of pathogenesis due to hantavirus.

Infection of human airway epithelial cells by different Dobrava-Belgrade virus subtypes reveals gene expression patterns corresponding to their virulence potential

Peter T. Witkowski[1†], Daniel Bourquain[2†#], Katrin Bankov[2], Brita Auste[1], Piotr W. Dabrowski[2], Andreas Nitsche[2], Detlev H. Krüger[1], Lars Schaade[2]

[1]Institute of Virology, Helmut-Ruska-Haus, Charité Medical School, Charitéplatz 1, 10117 Berlin, Germany. [2]Robert Koch Institute, Seestraße 10, 13353 Berlin, Germany, E-Mail: bourquaind@rki.de

† Authors contributed equally

presenting author

Objective: Virulence and case fatality rates of human Dobrava-Belgrade hantavirus (DOBV) infections are associated with virus genotype. While DOBV-Kurkino tends to cause a milder form of hantavirus disease, DOBV-Dobrava and DOBV-Sochi induce moderate to severe HFRS. However the reasons for

these differences are not well understood. To elucidate possible reasons for the varying virulence of the DOBV genotypes, we studied the impact of DOBV infection on the gene expression profiles of human lung epithelial cells. **Methods:** Analysis of the cellular gene expression profiles was conducted using whole-genome gene expression microarray technology and validation via quantitative real-time PCR. Gene expression profiles of human lung epithelial cells (A549) were measured at 12 h following infection with DOBV genotypes Dobrava, Kurkino, and Sochi, or with the low-virulent Tula virus (TULV). Significant changes of the cellular gene expression profile in response to infection were analyzed via Gene Ontology (GO) term enrichment analysis to identify affected biological processes. **Results:** Hantavirus infection induced slight but significant changes of the cellular gene expression, modulating the expression of a number of cellular transcripts ranging from 247 (DOBV-Dobrava) to 498 (DOBV-Kurkino). Changes to the cellular gene expression profiles induced by infection with different DOBV are largely virus-specific, despite their close genetic relationship. Major differences were observed in the regulation of immune response genes. A GO term-based cluster analysis revealed a statistical overrepresentation of genes functioning in type I interferon signaling among the genes modulated by highly-virulent DOBV-Dobrava and DOBV-Sochi, but not by less-virulent DOBV-Kurkino and TULV. An analysis of known interferon regulated genes (IRGs) furthermore revealed a greater similarity in their regulation during DOBV-Dobrava and DOBV-Sochi infection (which alter the expression of the same 39 IRGs) in comparison to DOBV-Kurkino and TULV. **Conclusions:** This work gives first insights into the differences of virus-host interactions of DOBV on the genotype level. Major differences between the individual DOBV genotypes and TULV concern the modulation of innate immunity.

Micro RNAs in urine as potential biomarkers for severity of hemorrhagic fever with renal syndrome

Kurolt Ivan-Christian[1], Lidija Cvetko-Krajinović[1], Alemka Markotić[1]

1. Research Unit, University Hospital for Infectious Diseases “Dr. Fran Mihaljević”, Zagreb, Croatia E-mail: ikurolt@bfm.hr

Objective: Micro RNAs (miRNAs) are a class of small RNAs, 18 – 25 nucleotides in length, that represent a way of posttranscriptional regulation of gene expression by binding to mRNAs and facilitating there inhibition or degradation depending on the degree of similarity. Besides intracellularly they have been detected in several bodily fluids, implicating a possible regulation of selected tissues or organs. Certain miRNAs found in urine can be predictors of disease outcome in various renal pathologies, e.g. glomerulonephritis, IgA or diabetic nephropathy. We measured, for the first time, levels of selected miRNAs in urinary samples of patients with hemorrhagic fever with renal syndrome (HFRS) after Puumala virus infection and compared them to patients with pyelonephritis and healthy controls. **Methods:** Midstream urinary samples were obtained upon hospitalization and before discharge from 30 patients with HFRS after Puumala virus infection and 15 patients with pyelonephritis. The control group consisted of 15 sex and age matched individuals. Urinary miRNA and a spike-in control RNA were isolated and transcribed into cDNA. A custom real-time PCR Array was designed for the detection of seven selected miRNAs (miR-21-5p, miR-24-3p, miR-27a-3p, miR-127-3p, miR-146a-5p, miR-155-5p, let-7e-5p). Laboratory and clinical parameters were correlated by descriptive statistics. Differences between two independent groups were calculated using non-parametric statistics, e.g. Mann-Whitney U test. Differences between more than two groups of numeric variables were determined through Kruskal-Wallis and ANOVA & Median test. The calculated correlations were evaluated with the Spearman rank correlation. **Results:** Except miR-146a-5p and miR-155-5p all have been readily detected in patients urine albeit low concentrations. In urinary samples of HFRS patients and patients with acute pyelonephritis, miR-21 and miR-27a were more abundant, while let-7e could be HFRS specific. In mild patients with HFRS only miR-21 and let-7e were deregulated, whereas in severe patients miR-24, miR-27a, and miR-127 were also altered. **Conclusions:** Here we show for the first time a distinct profile of miRNA abundance in urine of HFRS patients and patients with acute pyelonephritis, which could serve as HFRS biomarkers as they correlate significantly with urea and creatinine levels, which in turn are hallmarks of HFRS progression and severity.

Virus Specific and Bystander-B cell Activation account for a Massive Plasmablast Response in Andes virus Infected Patients

Marina Garcia[1], Ayelen Iglesias[2], Veronica Landoni[3], Carla Bellomo[2], Sabrina C. Bassi[2], Agostina Bruno[4], Maria Teresa Cordoba[4], Maria del Carmen Sasiain[1], Valeria P. Martinez[2], Jonas Klingström[5], Pablo L. Schierloh[1].

[1] Laboratorio de Inmunologia de Enfermedades Respiratorias, IMEX-CONICET-Academia Nacional de Medicina, C.A.B.A, Argentina. [2] Laboratorio Nacional de Referencia para Hantavirus, Servicio de Biologia Molecular, INEI-ANLIS “Dr. Carlos G. Malbran”, C.A.B.A, Argentina. [3] Laboratorio de Fisiologia de los Procesos Inflamatorios, IMEX-CONICET-Academia Nacional de Medicina, C.A.B.A, Argentina. [4] Laboratorio de Enfermedades Tropicales – Hospital San Antonio de Paul – Salta, Argentina. [5] Center for Infectious Medicine, Department of Medicine, Karolinska Institutet, Karolinska University Hospital Huddinge, Stockholm, Sweden. E-mail: marinagarcia.ar@hotmail.com

Objective: The presence of “immunoblast-like” cells in patients with Hantavirus Pulmonary Syndrome (HPS) is a regular finding during routine clinical blood exam. Nonetheless their cellular linage has not been established nor their relevance in the course of the disease. Therefore, we aimed to characterize the phenotype of those immunoblasts in HPS patients infected with Andes virus (ANDV). **Methods**: Anticoagulated blood samples were obtained from confirmed HPS patients ($ANDV^+$ n=18), seronegative patients with acute respiratory symptoms (ARS n=15) and healthy subjects (HS n=21). Samples were inactivated and stabilized and processed for flow cytometry and confocal microscopy. Additionally, the red blood cells from samples from $ANDV^+$, ARS, HS, influenza vaccinated HS (v-HS, n=12) and tuberculosis patients (TB, n=10) were lysed and intracytoplasmatic antibodies (cyAb) from leucocytes were obtained by non-anionic detergent extraction. CyAb were tested by indirect ELISA against ANDV specific antigens (Ags)/epitopes (n=4) and unrelated Ags (n=8). **Results:** In $ANDV^+$ acute samples, we observed high numbers of $CD19^+$ $CD27^{hi}$ $CD38^{hi}$ $CD20^{neg}$ IgD^{neg} $CD138^{+/neg}$ intracellular Igs^{hi} blasted ($FSC^{hi}SSC^{hi}$) lymphoid cells identified as circulating plasmablasts (PB), a B cell-derived population virtually absent on HS ($p<0.0012$). PB levels dropped in $ANDV^+$ late samples (n=6), resembling the ones observed in HS. Additionally, flow-sorted $ANDV^+$-derived PB exhibited intracellular Igs contents and bigger size than $CD38^{low/neg}$ B-lymphocytes ($p<0.05$). This phenotype resembled the “immunoblast-like” cell shape often described during cytology exams. The strong PB response was time dependent with peak values around one week after symptoms onset. A further analysis revealed that only $ANDV^+$ PB derived cyAb reacted with all 4 virus specific Ags ($p<0.0005$), as was expected. Interestingly, we also observed that most $ANDV^+$ PB derived cyAb also reacted with all the unrelated Ags tested. **Conclusions:** Our results show that $ANDV^+$ present a massive and transient PB response, corresponding to what has been previously morphologically defined as “immunoblasts”. Furthermore the strong immunoreactivity of cyAb against virus related and unrelated Ags indicates that this massive PB response could be explained by a polyclonal activation of B cells that takes place during the acute phase of HPS in $ANDV^+$ patients.

Rapid but transient depletion of human dendritic cells in blood during acute hantavirus infection

Saskia Thomas[1], Gregory Rankin[2], Shawon Gupta[3], Kimia Maleki[3], Faezzah Baharom[1], Sindhu Vangeti[1], Magnus Evander[4], Niklas Björkström[3], Hans-Gustaf Ljunggren[3], Anders Blomberg[2], Jonas Klingström[3], Clas Ahlm[4] and Anna Smed-Sörensen[1]

[1]Dept. of Medicine Solna, Immunology and Allergy Unit, Karolinska Institutet, [2]Dept. of Public Health and Clinical Medicine, Div. of Medicine/Respiratory Medicine, [3]Center for Infectious Medicine, Dept. of Medicine Huddinge, Karolinska Institutet, Stockholm, Sweden, [4]Dept. of Clinical Microbiology, Umeå University, Sweden. Email: anna.smed.sorensen@ki.se

Objective: Acute respiratory viral infections are the most frequent reason for medical consultations in the world and they are a major cause of morbidity and mortality also in Sweden. Puumala virus (PUUV) is the endemic hantavirus in Europe that causes hemorrhagic fever with renal syndrome. Hantavirus infection is known to impact the frequency and distribution of effector immune cells like NK cells and CD8 T cells. However, little is know on how the infection affects dendritic cells (DCs), professional antigen presenting cells that play a crucial role in recognizing and presenting viral antigens. Since the lung is continuously exposed to the air, it is equipped with an elaborate network of DCs sensing incoming foreign pathogens. We hypothesize that DCs migrate to the lung during acute respiratory PUUV infection in humans to initiate the adaptive effector immune responses required to clear infection.

Methods: We investigated lung biopsies using immunohistochemistry and blood samples from 17 patients with acute hantavirus infection using multicolor flow cytometry. In addition, 3-6 longitudinal blood samples from the same patients were collected during the convalescent phase of disease. The samples were compared with sex- and age-matched lung and blood samples from uninfected controls.

Results: We found a significant influx of CD11c+ myeloid DCs (MDCs) and CD123+ plasmacytoid DCs to the lung during acute infection, as compared to uninfected controls. In parallel, we found that the absolute numbers of MDCs were dramatically reduced in peripheral blood during acute infection. The MDC levels normalized during convalescence when virus levels were undetectable in plasma by PCR. Next, we asked if the loss of MDCs from blood could be a consequence of direct virus infection of the DCs. Indeed, we found that MDCs are susceptible to infection in vitro and that MDCs are direct targets of the virus.

Conclusions: Our results suggest DC recruitment to the airways during acute hantavirus infection and a possible role for DCs in the immune-mediated pathogenesis of the disease. A more detailed knowledge on why MDCs are depleted from blood and recruited to lung during acute infection will improve our understanding of the role of DCs in Hantavirus pathogenesis.

ANDV Activates RhoA Directed Endothelial Cell Permeability By Engaging TSC2 and Reducing TIAM1 and p190RhoGAP Expression Levels

Elena E. Gorbunova[1], Matthew J. Simons[1,2], Irina N. Gavrilovskaya[1], Erich R. Mackow[1,2]

[1]Department of Molecular Genetics and Microbiology, [2]Molecular and Cell Biology Program, Stony Brook University, Stony Brook, NY.

Objective: Andes virus (ANDV) predominantly infects endothelial cells (ECs) and nonlytically causes acute pulmonary edema termed hantavirus pulmonary syndrome (HPS). In HPS patients virtually every pulmonary EC is infected, ECs are enlarged and infection results in highly lethal vascular leakage (1 L/hr). The mechanism of EC dysfunction that permits hantavirus induced vascular permeability remains to be resolved. **Methods:** We analyzed the regulation of primary microvascular endothelial cell signaling pathways and responses to ANDV infection and following nucleocapsid protein expression. Nucleocapsid protein was persistently expressed in EC by lentivirus transduction and puromycin selection and transient co-expression approaches were similarly used to validate signaling responses and protein:protein interactions in HEK cells. **Results:** We observed that ANDV infection of primary human pulmonary microvascular ECs activates the vascular permeability inducer RhoA and increases cell size by preventing de- repression mTOR signaling responses. mTOR regulates hypoxia directed EC permeability while RhoA is a GTPase that directs the dissociation of VE-cadherin within EC adherens junctions. In contrast, activating Rac1 inhibits RhoA directed permeability by enhancing VE-cadherin assembly. We found that expressing the ANDV nucleocapsid protein in ECs activates mTOR and increases cell size by forming a complex with TSC2. Similar to ANDV infection, the nucleocapsid protein activated RhoA, and appears to accomplish this by reducing EC levels of both p190RhoGAP, which inhibits RhoA, and TIAM1, which activates Rac1. Consistent with this, compounds that inhibit these signaling responses dramatically reduced ANDV induced EC permeability. **Conclusions:** Our findings indicate that nucleocapsid protein interactions with TSC2 and Rac1/RhoA regulatory proteins control EC permeability responses. These findings implicate pathway specific mechanisms by which ANDV proteins enhance EC permeability and suggest potential therapeutic approaches for resolving hantavirus-induced vascular leakage.

Hantaviruses induce STAT1-dependent expression of tissue plasminogen activator

Tomas Strandin[1], Jussi Hepojoki[1], Outi Laine[2], Satu Mäkelä[2], Jonas Klingström[3], Åke Lundkvist[4], Ilkka Julkunen[5], Jukka Mustonen[2], Antti Vaheri[1]

[1]Department of Virology, Haartman Institute, University of Helsinki, Helsinki, Finland.

[2]Department of Medicine, University of Tampere School of Medicine and Department of Internal Medicine, Tampere University Hospital, Tampere, Finland.

[3]Center for Infectious Medicine, Department of Medicine, Karolinska Institutet, Karolinska University Hospital Huddinge, Stockholm, Sweden.

[4]Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden.

[5]Department of Virology, University of Turku, Turku, Finland and Department of Infectious Disease Surveillance and Control, National Institute for Health and Welfare, Helsinki, Finland.

Objective: Induced leakage of microcapillaries is the hallmark of all hantavirus-mediated diseases. Hantaviruses are known to infect human vascular endothelial cells *in vivo* and can infect isolated primary endothelial cells efficiently *in vitro*. However, hantaviruses are not directly cytopathic in these cells to explain the enhanced vascular leakage. Elevated levels of thrombin and fibrin degradation products are indicative of enhanced coagulation and fibrinolysis in the acute stage of Puumala hantavirus disease. In this study we analyzed the regulation of tissue-type plasminogen activator (tPA), best-known for its capability to induce fibrinolysis, and plasminogen activator inhibitor (PAI)-1, the main inhibitor of tPA, in the acute stage of Puumala hantavirus disease. **Methods:** The levels of tPA and PAI-1 were measured from of acute vs. convalescent phase plasma of Puumala-infected patients by ELISA. The regulation of tPA and PAI-1 mRNA levels in response to hantaviruses were studied also *in vitro* in infected blood microvascular endothelial cells by RT-qPCR. **Results**: We could detect enhanced levels of tPA in the acute vs. convalescent stage of Puumala infection. In contrast, the levels of PAI-1 were not regulated to similar extent, thus facilitating enhanced activity of tPA during acute Puumala infection. The enhanced levels of tPA positively correlated with many parameters describing the severity of the disease. We detected upregulated levels of tPA mRNA and protein also in hantavirus-infected human microvascular endothelial cells. In addition, we found that interferons (type I and II) were able to induce tPA in endothelial cells. Interferons regulate cell responses mainly by activating signal transducer and activator of transcription (STAT)-1. By downregulating STAT1 through short interfering RNA we could abolish the induction of tPA mediated by interferon treatment. Furthermore, by using chromatin immunoprecipitation of interferon-treated endothelial cells with STAT-1 antibody we could detect direct association of STAT-1 to the enhancer element of *tPA* gene. **Conclusions:** Taken together, we can confirm the previously suggested enhanced fibrinolysis in acute hantavirus infection and pin-point it to the induction of tPA by hantavirus-infected microvascular endothelial cells. This could lead to locally impaired hemostasis and enhanced bleeding through the microvascular beds which is a determinant of hantavirus pathogenesis.

Proteinuria detected by albumin dipstick test predicts the severity of acute kidney injury in Puumala hantavirus induced nephropathia epidemica

Paula Mantula[1], Tuula Outinen[1], Jan Clement[2], Heini Huhtala[3], Ilkka Pörsti[1,4,] Satu Mäkelä[1,4], Jukka Mustonen[1,4]

[1]Department of Internal Medicine, Tampere University Hospital, Finland, [2]National Reference Centre for Hantaviruses, University Hospitals Leuven, Belgium, [3]Tampere School of Health Sciences, University of Tampere, Finland, [4]Medical School, University of Tampere, Finland. E-mail: jukka.mustonen@uta.fi

Objective: Puumala hantavirus induced nephropathia epidemica (NE) is common in Finland and in several other European countries. Acute tubulointerstitial nephritis is the renal histologic lesion in NE. Acute kidney injury (AKI) in NE is self-limiting and has a favorable prognosis. Transient proteinuria (PU) is present in > 90% of the patients. Its pathogenesis is still obscure. Thrombocytopenia and increased capillary leakage are the other main manifestations of NE. Here we studied prognostic significance of PU detected by albumin dipstick test for the severity of AKI and other clinical and laboratory findings of NE, as well as time relationship of glomerular and tubular PU to AKI.

Methods: 205 patients with serologically confirmed acute NE treated in Tampere University Hospital, Finland during 1997-2014 were studied. The amount of PU detected by albumin dipstick test on hospital admission was graded in three categories: 0-1+ (n=54), 2+ (n=73), 3+ (n=78). In 70 patients 24-hour urinary protein excretion and overnight excretion of albumin as well as α-1-microglobulin (tubular PU), were also studied during three consecutive hospitalization days. The severity of AKI was defined by maximum plasma creatinine (crea-max). The other parameters studied were maximum leukocytosis (leuk-max), minimum platelet count (thromb-min), maximum C-reactive protein (CRP-max) and duration of hospitalization. The onset of NE was the day when fever commenced.

Results: Maximum 24-hour PU and overnight albuminuria were observed five to six days and crea-max seven to nine days after the onset of NE. There was no clear time peak of urinary excretion of α-1-microglobulin. 24-hour PU ranged from 0.14 to 17.6 (median 1.78) g. Increased urinary α-1-microglobulin levels (>7µg/min) were found in 90% of patients. Median crea-max values were significantly different in three categories of dipstick albuminuria: 0-1+ category 98 µmol/l (58-1499), 2+ category 139 µmol/l (71-829), 3+ category 363 µmol/l (51—1285) ($p<0.001$, Kruskal-Wallis test). Significant differences in the same categories were also found for leuk-max ($p<0.001$), CRP-max ($p=0.012$) and duration of hospitalization ($p=0.026$), but not for thromb-min ($p=0.232$).

Conclusions: The amount of PU detected by albumin dipstick test on hospital admission clearly predicts the severity of AKI in NE. It also associates with several other severity parameters of NE. This quick and easy assessment is highly useful in daily clinical work. Moreover, maximum excretion of glomerular PU occurs some days before the most severe phase of AKI. Association with tubular proteinuria is less clear.

Endothelial dysfunction during Puumala hantavirus infection

Anne-Marie Connolly-Andersen[1], Therese Thunberg[1], Julia Wigren[1], Greg Renkin[2], Clas Ahlm[1]

1. Department of Clinical Microbiology, Infectious Diseases, Umeå University, Umeå, Sweden. E-mail: clas.ahlm@umu.se
2. Department of Public Health and Clinical Medicine, Medicine, Umeå University, Umeå, Sweden

Objective: The endothelium plays a central role in the pathogenesis of viral hemorrhagic fevers. It regulates inflammation, adhesion of immune cells and platelets, and regulates vascular permeability. Previously, we have shown that approximately 30% of patients with Puumala hantavirus infection fulfill the criteria for disseminated intravascular coagulation (Sundberg et al. 2011 PLOS One) and also identified that the infection constitutes a significant risk factor for acute myocardial infarction and stroke (Connolly-Andersen et al., 2014, Circulation).

A specific compartment on the lumen side of the endothelium, the endothelial surface layer (ESL), has received increased attention. ESL consists of the endothelial glycocalyx (e.g. the transmembrane proteins syndecan-1). The primary consequences of ESL loss are increased vascular leakage, adhesion of leukocytes and platelets, and microthrombi. ESL cleavage is caused by excessive inflammatory stimuli and hypoxia/ischemia.
In the present study we aimed to investigate the endothelial activation and dysfunction during Puumala hantavirus infection.

Methods: Endothelial activation and dysfunction were analyzed during Puumala hantavirus infection. Various endothelial markers were studied; e.g. endothelial glycocalyx degradation (syndecan-1) and leukocyte adhesion molecules (soluble vascular cellular adhesion molecule, sVCAM-1, intercellular adhesion molecule 1, sICAM-1 and endothelial selectin, sE-selectin). Cytokines associated with vascular repair were also analyzed (vascular endothelial growth factor (VEGF), erythropoietin (EPO), angiopoietin (Ang-2), and stromal cell-derived factor 1 (SDF-1). In addition, a marker for hypoxia, insulin-like growth

factor binding protein 1 (IGFBP-1) was analyzed.

Results: The levels of studied endothelial markers were highest during the earliest phase of hantaviral disease and associated with clinical and laboratory surrogate markers for disease outcome. In particular, the marker for glycocalyx degradation, syndecan-1, was significantly associated with levels of thrombocytes, albumin, IGFBP-1, decreased blood pressure and disease severity.

Conclusions: Our results show endothelial activation, dysfunction and systemic glycocalyx degradation during hantaviral disease, which normalizes during the follow up period.
Disease outcome was associated with endothelial dysfunction. Consequently, the endothelium warrants further notice when investigating the pathogenesis and designing future therapeutic interventions.

Analysis of plasma cytokines and inflammatory markers in PUUV-infected patients reveals a potential gastrointestinal involvement during HFRS

Kimia Maleki[1], Clas Ahlm[2], Jonas Klingström[1]
[1]Center for Infectious Medicine, Department of Medicine Huddinge, Karolinska Institutet, Karolinska University Hospital, SE-141 86 Stockholm, Sweden. E-mail: kimia.maleki@ki.se
[2]Department of Clinical Microbiology, Division of Infectious Diseases, Umeå University, SE- 901 87, Umeå, Sweden.

Objective: Human hantavirus infection is characterized by a strong inflammatory response and patients often display various gastrointestinal symptoms. The mechanistic background to these symptoms and their significance in the disease pathogenesis is however unknown. In this study, we aimed to study the expression of pro-inflammatory cytokines and acute phase reactants, including markers of gastrointestinal inflammation, during acute and convalescent Puumala virus-caused HFRS. **Methods**: Longitudinal plasma samples from Swedish patients with confirmed Puumala virus-infection were analyzed for different inflammatory cytokines and acute phase reactants, using ELISA. Levels of the measured analytes were further correlated to clinical parameters. **Results:** Elevated levels of pro-inflammatory cytokines and inflammatory markers were, as expected, observed during the acute phase of HFRS. In addition, the levels of gastrointestinal inflammation markers were elevated. **Conclusions:** Our preliminary data confirms a strong inflammatory response during the acute phase of HFRS and indicates the existence of a potential gastrointestinal inflammation during the disease. The mechanisms behind the gastrointestinal involvement, as well as its consequences, remain to be elucidated.

Cytotoxic immune responses in the lungs correlate to disease severity in patients with hantavirus infection

Johan Rasmuson[1], Jamshid Pourazar[2], Nahla Mohamed[1], Kristina Lejon[1], Magnus Evander[1], Anders Blomberg[2], Clas Ahlm[1]

1. Department of Clinical Microbiology, Umeå University, Umeå, Sweden.
E-mail: johan.rasmuson@umu.se
2. Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden

Objective: Hantavirus infections cause pulmonary involvement and sometime even life-threatening lung failure. Similar to respiratory viruses, hantaviruses are transmitted by the airway route, but there is still limited information regarding the pulmonary immune responses and viral load, and their impact on hantavirus disease severity. Significant lung involvement is common in hantavirus disease and a main cause of death in infections caused by American hantaviruses. There is an urgent need for effective treatment against hantavirus infections. Herein, we present the results of a new study where we investigated the association between viral load and immune responses in the lungs, and their relation to disease severity.

Methods: Bronchoscopy with sampling of bronchoalveolar lavage (BAL) fluid was performed in 17 patients with acute Puumala hantavirus infection and 16 healthy volunteers acting as control group. Lymphocyte subsets, granzyme concentrations, and viral load were determined by flow cytometry, ELISA and quantitative RT-PCR, respectively.

Results: Our main finding was the correlation between pulmonary immune response and clinical severity. Analyses of BAL fluid revealed significantly higher numbers of activated CD8+ T cells and NK cells, as well as higher concentrations of the cytotoxins granzyme A and B, compared to controls. Puumala hantavirus RNA was detected in 88% of BAL cell samples. The expansion of activated cytotoxic CD8+ T cells in the lungs was inversely associated with viral load. The magnitude of the pulmonary cytotoxic lymphocyte response correlated to severity of disease, in terms of need for supplemental oxygen treatment, hypotension, and laboratory data indicating renal failure, cardiac dysfunction, vascular leakage and cell damage. Regulatory T cell numbers were significantly lower in patients, compared to controls, and may reflect inadequate immune regulation during hantavirus infection.

Conclusions: Our results indicate that the magnitude of the pulmonary immune response was associated with disease severity in hantavirus infection. Patients with a pronounced CD8+-response were more

severely ill in terms of impaired alveolar gas exchange and systemic organ dysfunction, implicating the significance of these cells in the pathogenesis. This is the first study reporting simultaneous investigations of pulmonary immune responses and viral load in relation to clinical parameters and severity in hantavirus disease.

Three pseudo-nephropathia epidemica (NE) retrospective cases after overt soricomorph exposure: vingt(-six) ans après.

Jan Clement[1], James LeDuc[2], Jiaxin Ling[3], Marc Van Ranst[1], Olli Vapalahti[3], Antti Vaheri[3]. [1]Hantavirus NRC, Gasthuisberg Univ. Hospital, Leuven, Belgium. [2]Galveston National Laboratory, UTMB, Galveston, TX, USA. [3]Dept Virology, Medicum, Faculty of Medicine, Univ. of Helsinki, Finland.

Introduction: We discovered in our archives three 1990 cases (A, B, and C), considered then serologically and clinically as quintessential NE, but each conspicuously occurring after overt soricomorph exposure. Moreover, 1990 PRNT appeared negative for PUUV, HTNV and SEOV, despite high levels found for HTNV in HDPA, and particularly for PUUV in IFA.

Exposure Stories: A) A 49-years old Belgian game keeper in the Ardennes developed NE symptoms on December 29th, 1989 (Day 0), 5 days after a bite by a water shrew (*Neomys fodiens*). A KDIGO grade 3 but transient AKI (Table) prompted dialysis on Days 11 and 12. Concomitant but transient RX lung infiltrates and severe O_2 desaturation (P_aO_2 29.3 mmHg) also developed. **B)** A 29-years old British military (SHAPE, Belgium) fell ill on February 2nd, 1990, 7 days after manipulating a dead mole (*Talpa europaea*) and molehills. **C)** A 45-years old American military (SHAPE, Belgium) fell ill on February 10th, 1990, 5 days after killing a mole with a spade, whereby the animal's blood splashed on his face and eyes.

Symptoms

	Fever (°C)	Lumbalgiae	Bradycardia	Myopia	Oliguria	Polyuria
Case A	38	No?	No	D 4-5	200ml (D6)	3.8 L (D16)
Case B	37.8	+++	55 bpm	No	None?	7 L (D11)
Case C	40	+	No	No	NR	5.6 L (D9-11)

Lab Abnormalities

	Initial Proteinuria (g/L)	Nadir Platelets (/µL)	Peak LDH (IU/L)	Peak Creatinine (mg%)	Serum sodium (mmol/L)	Liver Trans-aminases
Case A	3.8	45000	923	12.5	123	Normal
Case B	+++	45000	265	3	NR	increased (+)
Case C	+++	93000	N.R.	6.4	NR	Normal

Serology & 80 % PRNT

	HDPA	IFA HTNV	IFA PUUV	NT PUUV	NT HTNV	NT SEOV
Case A	2560	512	**4096**	**20**	10	5
Case B	2560	2048	**4096**	**40**	20	5
Case C	1280	256	**16384**	**20**	5	5

NR: not reported. D: day. HDPA: high density particle agglutination (IgM+IgG), based on HTNV 84/105. (neg.= <40)
IFA: immuno fluorescence assay. NT: plaque-reduction (80%) neutralization test

Conclusions: 1) Given the peculiar and invasive degree of soricomorph exposure, and prior negative results of "conventional" PRNT, these 3 so-called NE cases may retrospectively constitute the first indirect, but highly suggestive proof of human pathogenicity of European soricomorph hantaviruses. Further indirect evidencing by competition ELISA's is now in progress; however, conclusive PRNT necessitates specific live viruses, already possible now by the very recent NVAV isolation from a *Talpa europaea*. 2) If confirmed, these results suggest that soricomorph infections could hitherto have been misinterpreted as seroconfirmed "NE" in Europe. 3) Similar AKI/ALI in unexplained "PUUV+" infections, as recently found in India, Sri Lanka, and Africa, might have been caused by local soricomorph hantaviruses.

Interstitial renal oedema is the purely mechanical mechanism of transient acute kidney injury (AKI) without sequelae in human hantavirus infections-A hypothesis.

Jan Clement[1], Dirk Kuypers[2], Marc Van Ranst[1], Norbert Lameire[3].

[1] Hantavirus NRC, Clinical Virology, and [2] Nephrology, Gasthuisberg University Hospital, Leuven, Belgium. [3] Nephrology, University Hospital of Ghent, Belgium. E-mail: jan.clement@uzleuven.be

Introduction: The exact mechanism of AKI in all forms of "hantavirus fever" (HTVF) remains obscure. We formulate a novel but simple mechanism, supported point by point by the clinical features, unique for hantaviral AKI. Indeed, complex interactions of pro-inflammatory cytokines, or multiple factors promoting capillary leakage with generalized exudate formation, offered hitherto no straightforward explanation for the following often encountered symptoms:

Clinical Features: 1) HTVF AKI is often preceded by severe bilateral lumbalgiae. 2) Glomerular microhaematuria and massive, unselective proteinuria precede by a few days the onset of AKI. 3) AKI is very rapidly progressing, but transient and always self-remitting within days. 4) On kidney biopsies, light-microscopic glomerular lesions are completely lacking, but interstitial oedema is always present, sometimes together with mononuclear interstitial infiltrate, suggesting acute tubulo-interstitial nephritis. 5) Electron-microscopy often shows glomerular podocyte foot effacement. 6) One or two icatibant doses SC can resolve very rapidly dramatic HTVF AKI cases. 7) Prior HTVF AKI does not result in renal histologic sequelae, nor in residual arterial hypertension.

Clinical Deductions or Explanations: 1) Lumbalgiae are caused by intense interstitial oedema, causing stretching of the renal capsule, which contains sensitive nerve fibres. In ultrasound, renal parenchymal swelling is corroborated by (transient) nephromegaly. In several Finnish studies, increased echogenicity associated with AKI severity. 2) Initial (but transient) urine anomalies are due to temporary breaching of the podocyte slit diaphragm, which normally has a barrier function for big molecules, and for corpuscles like red blood cells (RBC). Glomerular RBC passage is suggested by the dysmorphic RBC aspect in urine examination. Urine protein electrophoresis equals serum electrophoresis in HTVF, and resembles multi-organ protein-rich exudate formation, such as in the lungs. Podocyte foot effacement (point 5) in other nephropathies often also results in massive proteinuria. However, disassembly of the slit diaphragm, nor of the endothelial tight junctions, can fully explain AKI with oliguria, even less with anuria. 3 & 4) Rapidly increasing interstitial oedema results in elevated intrarenal pressure, including elevated hydrostatic pressure in Bowman's space (normally about 15 mmHg). The latter is the main glomerular hemodynamic force counteracting the hydrostatic intracapillary pressure gradient, resulting in a net force K_f, driving ultrafiltration. Normal K_f is only about 10 mmHg, and relatively small changes can temporarily diminish or stop ultrafiltration, and thus glomerular filtration rate (GFR). 6) Remittance of oedema, installing spontaneously or after icatibant SC, diminishes again intracapsular hydrostatic pressure, normalizing K_f, with gradual remittance of ultrafiltration and of GFR. 7) After this normalization, no residual renal lesions remain, and GFR returns to pre-HTVF values.

Conclusions: Interstitial renal oedema is the only common factor explaining all above clinical anomalies. This hypothesis might also explain transient AKI in other "cytokine storm" emerging infections.

Mechanisms behind, and consequences of, hantavirus-mediated inhibition of apoptosis

Shawon Gupta[1], Carles Sola Riera[1], Kimia Maleki[1], Niklas K. Björkström[1], Hans-Gustaf Ljunggren[1], and Jonas Klingström[1]

[1] Center for Infectious Medicine, Department of Medicine Huddinge, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden

Objectives Hantaviruses are emerging zoonotic viruses that cause hemorrhagic fever with renal syndrome (HFRS) in Eurasia and hantavirus pulmonary syndrome (HPS) in the Americas, with case-fatality rates of up to 10% for HFRS and up to 40% for HPS. Currently, no FDA-approved vaccine exists and no specific treatment is available. A better understanding of the diseases hantaviruses cause in humans, and a better understanding of the mechanisms behind hantavirus pathogenesis, is needed for future development of specific treatments of HFRS/HPS and for possible long-term negative effects of human hantavirus-infection.

Methods We use various methods to study the effect hantaviruses have on infected cells, the mechanisms behind these effects, and how this in turn affect uninfected bystander cells and their functions and responses. We perform epidemiological studies in order to identify possible long-term effects of human hantavirus-infection.

Results Hantaviruses have strong general anti-apoptotic effects and also protect infected cells from being killed by cytotoxic lymphocytes. We have identified four different and specific mechanisms that hantaviruses use to inhibit apoptosis. These will be further discussed. Interestingly, a consequence of hantavirus-mediated anti-apoptotic mechanisms are deregulated inflammatory responses, linking the hantavirus-mediated anti-apoptotic effects to inflammation. These mechanisms will be discussed. Strong

inhibition of apoptosis and inflammation are hallmarks of cancer: indeed, HFRS-patients show increased risk for certain forms of cancer.

Conclusions Hantaviruses are equipped with several anti-apoptotic mechanisms, whereof some might be unique for hantaviruses. A surprising consequence of these anti-apoptotic mechanisms is deregulated inflammatory responses, which affects bystander cells and potentially contributes to the strong inflammatory state observed in patients. The findings also indicates that hantaviruses have possible direct and indirect effects on carcinogenesis, which might explain the increased risk for lymphoma observed in Swedish HFRS-patients.

Early slowdown of the peripheral immune response triggered by Puumala virus infection

Lidija Cvetko Krajinović[1], Heidi Spratt[2], Ante Tadin[1], Antea Topić[1], Rok Čivljak[1], Allan R. Brasier[2], Slobodan Paessler[2], Alemka Markotić[1]

[1]University Hospital for Infectious Diseases “Dr Fran Mihaljević”, Zagreb, Croatia. [2]University of Texas Medical Branch, Galveston, TX, USA

Objective: Pathogenic hantaviruses in Europe cause hemorrhagic fever with renal syndrome (HFRS). They significantly differ by their pathogenicity resulting in broad spectra of clinical presentations. Infections with hantaviruses are not lytic, and it is still not clear why infections in humans cause the disease. It is considered that pathogenesis of HFRS is mainly mediated by immune response. The aim of this study was to explore the components of innate and adaptive immunity important in the peripheral immune response as well as the possible regulatory effect of miRNA on early immunoreactions during the hantaviral infection. Based on this, biological pathways relevant for the immunopathogenesis of HFRS have also been searched. **Methods:** Using real-time PCR array technology, the relative expression of immune response genes and miRNAs was measured in peripheral blood mononuclear cells of patients infected with Puumala virus (PUUV). **Results:** The results showed down regulation of genes coding the synthesis of pattern recognition receptors, chemokines and their receptors, cytokines, transcription factors, as well as some signalling molecules. Functional analysis showed that differential expression of described set of genes modulates inflammatory response by interfering with various cell-signalling pathways. For the first time, the biological importance of miRNA in the regulation of immune response during HFRS was shown. Changes detected at the immune response level have been associated with disease severity but not with the viral load in the blood. **Conclusions:** The results showed initial suppression of the early immune response to PUUV which was more pronounced in more severe form of the disease.

Evaluation of Antiviral, Anti-inflammatory and Supportive Treatment for Hantavirus Cardiopulmonary Syndrome in North America and Chile

Gregory Mertz[1], Pablo Vial[2]

[1]Department of Internal Medicine, University of New Mexico Health Sciences Center, Albuquerque, NM USA E-mail: gmertz@salud.unm.edu, [2]Instituto de Ciencias e Innovación en Medicina, Facultad de Medicina - Clinica Alemana Universidad del Desarrollo, Santiago, Chile E-mail: pvial@udd.cl

Objective: Hantavirus cardiopulmonary syndrome (HCPS) has a case fatality rate (CFR) of 35% in North America and Chile. Results of clinical trials and retrospective case series in patients with HCPS have been reported, including placebo-controlled trials of intravenous (IV) ribavirin and methylprednisolone (MP), an an open trial of immune plasma that had been collected by plasmapheresis, and a retrospective case series in patients treated with extracorporeal membrane oxygenation (ECMO). **Methods:** Published trials and retrospective case series involving antiviral, anti-inflammatory or supportive treatment administered in the cardiopulmonary stage were reviewed. **Results:** A placebo-controlled trial of IV ribavirin in patients with Sin Nombre virus (SNV) infection in North America was terminated prematurely based on a futility analysis, but there was no trend suggesting benefit. High-dose IV MP was evaluated in a placebo-controlled trial in Chile that reached full accrual of 60 patients with confirmed HCPS. MP appeared safe but was ineffective. Results of a retrospective case series of 51 patients with HCPS with a predicted CFR close to 100% who were treated with ECMO at the University of New Mexico suggested that the risk of death could be reduced by more than 60%. Treatment with ABO-typed immune plasma with known Andes virus (ANDV) neutralizing antibody titer was evaluated in an open trial in Chile. Mortality 4/29 (14%) was significantly lower than mortality (63/199, 32%) in non-randomized, concurrent, untreated cases throughout Chile (P=0.049, OR=0.35, CI=0.12, 0.99) but not when compared to mortality (18/66, 27%) in untreated, concurrent patients at the same centers (P=0.15, OR=0.43, CI=0.14, 1.34) or when compared to mortality (20/60, 33%) in all patients treated in the MP study (P=0.052, OR=0.32, CI=0.10, 1.00). **Conclusions:** ECMO appeared to reduce the risk of death in persons with severe HCPS. However, ECMO is very expensive and carries risk of morbidity and mortality, and most patients with HCPS do not have access to centers with ECMO. Parenteral ribavirin and methylprednisolone are ineffective. Passive administration of neutralizing antibody appears promising and deserves further evaluation, ideally with a product with potential for commercial development and with neutralizing activity against both SNV and ANDV.

Transchromosomal bovine- and anseriform avian-based approaches to develop polyclonal antibody-based antivirals targeting hantaviruses

R. L. Brocato[1], J. Schiltz[2], E. J. Sullivan[3] J. Ballantyne[4], J. W. Hooper[1],

[1] Virology Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD, USA. E-mail: jay.w.hooper.civ@mail.mil [2]Avianax, LLC, Grand Forks, ND, USA. [3] SAb Biohtherapeutics Inc., Sioux Falls, SD, USA. [4] Aldevron, LLC, Fargo, ND, USA.

Objective: Hantaviruses can cause severe disease in infected humans. Disease syndromes include hemorrhagic fever with renal syndrome and hantavirus pulmonary/cardiac syndrome. There are no FDA approved medicines to prevent or treat hantavirus disease. Andes virus (ANDV) and Sin Nombre virus (SNV) are among the most lethal hantaviruses, having a case-fatality of ~35%. We have developed candidate DNA vaccines encoding the Gn/Gc glycoproteins of ANDV and SNV. These vaccines, delivered by a variety of different delivery technologies, elicit neutralizing antibodies in laboratory animals. We are currently advancing the ANDV/SNV DNA vaccines through preclinical testing towards a Phase 1 clinical trial. At the same time, we initiated research investigating the use of these vaccines to develop candidate polyclonal antibody-based antivirals targeting hantaviruses. **Methods/Results:** In one approach, we demonstrated that it was possible to use DNA vaccines to produce high-titer neutralizing antibodies in anseriform avian species (i.e., ducks and geese). Anseriforms produce IgY and a truncated form of IgY lacking an Fc. These immunoglobulins can be purified from egg yolks and are predicted to be nonreactogenic in mammals, including humans. We demonstrated that purified egg yolk-derived IgY protected hamsters against lethal disease following ANDV intramuscular and intranasal challenge when administered up to five days post-exposure. Similarly, IgY produced in pathogen-free geese and purified using quality systems, spray-dried, and then reconstituted, protected hamsters against lethal ANDV challenge. In an alternative approach, we vaccinated transchromosomal bovines (TcB) with both the ANDV and SNV DNA vaccines. TcB are bovines that have been engineered to produce fully human IgG. We demonstrated that TcB plasma-derived IgG had potent neutralizing activity against both ANDV and SNV. Purified TcB plasma-derived fully human IgG protected hamsters and immunosuppressed hamsters against lethal disease following ANDV and SNV intramuscular challenge, respectively. **Conclusions:** Our findings that anseriform- or Tc-bovine-derived polyclonal neutralizing antibodies are effective at protecting in animal models of lethal hantavirus disease provides a partial proof-of-concept that these novel platforms can be used to develop candidate next-generation polyclonal immunoglobulin-based medical products without the need for human donors, despeciation protocols, or inactivated/attenuated vaccine antigen. A full proof-of-concept will require Phase 1 testing in humans.

Hantavirus DNA Vaccine Update.

J. W. Hooper. Virology Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD, USA. E-mail: jay.w.hooper.civ@mail.mil

Objective: Hantaviruses can cause severe disease in infected humans. Disease syndromes include hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary/cardiac syndrome (HPS). There are no FDA approved vaccines targeting HFRS or HPS. One approach to the development of hantavirus vaccines is the use of DNA vaccine technology. DNA vaccines based on the full-length M genome segment elicit neutralizing antibodies targeting the hantavirus GnGc envelope glycoproteins, and protective immunity in animal models. Passive transfer experiments have demonstrated that antibodies produced in immunized animals are sufficient for protection. Currently the lead HFRS vaccine consists of plasmids encoding the Hantaan virus (HTNV) and Puumala virus (PUUV) envelope glycoproteins, and the lead HPS vaccine consists of plasmids encoding the Andes virus (ANDV) and Sin Nombre virus (SNV) envelope glycoproteins. The HFRS vaccine has been tested in two Phase 1 clinical trials and is currently being evaluated in a Phase 2a dose ranging study. The first Phase 1 evaluated a HTNV vaccine, PUUV vaccine, and combined HTNV/PUUV vaccine delivered by gene gun (2 mg DNA/vaccination). The second Phase 1 evaluated the same vaccines by intramuscular electroporation. The ongoing Phase 2a is evaluating the bivalent HTNV/PUUV vaccine delivered by intramuscular electroporation (IM-EP). There are currently plans to evaluate the HTNV, PUUV, and HTNV/PUUV DNA vaccines using alternative delivery technology including intradermal electroporation and needle-free disposable syringe jet injection (DSJI) technology. The ANDV, SNV, and ANDV/SNV DNA vaccines delivered using DSJI are currently in preclinical toxicity testing in rabbits. **Methods/Results**: An overview of the hantavirus DNA vaccine effort will be provided with particular emphasis on the most recent HFRS vaccine Phase 1 and ongoing Phase 2a clinical trials. In addition, animal data demonstrating proof-of-concept of using DSJI technology to deliver the HFRS and HPS DNA vaccines will be presented. **Conclusions:** Both the HFRS and HPS DNA vaccines delivered by various technologies including gene gun, IM-EP, and DSJI elicit high titer neutralizing antibodies in animals, including nonhuman primates. The HTNV and PUUV DNA vaccines delivered by IM-EP are safe and immunogenic in humans. The seroconversion rate (neutralizing antibodies) in the Phase 1 testing the HFRS DNA vaccine delivered by IM-EP was sufficiently high to warrant a Phase 2a dose ranging study (in progress). Alternative delivery technologies remain an area of active research.

Poster Session I - Wednesday, June 1, UCA room 116, 11:30-14:30

1. **Susceptibility of syrian hamster and deer mouse pulmonary endothelial cells to zoonotic viruses.** Miedema K[1], Prescott J[2], Feldmann H[2], Schountz T[1]. [1]Arthropod-borne and Infectious Diseases Laboratory, Colorado State University; [2]Laboratory of Virology, Rocky Mountain Laboratories, NIAID. USA.

Background: Zoonotic viruses that infect endothelial cells are a public health concern and it is imperative to gain a better understanding of the interaction between these viruses and cells. A number of viruses that are pathogenic in humans are endothelial cell-tropic, including hantaviruses, arenaviruses, henipaviruses and filoviruses. The Syrian golden hamster (*Mesocricetus auratus*) is an attractive small animal model to study the pathology caused by a number of endothelial cell-tropic viruses, and deer mice (Peromyscus maniculatus) infected with Sin Nombre virus is a valuable model to study the response of reservoir hosts.

Methods: To develop an in vitro system to study viral infections of the endothelium, a protocol was modified to generate pulmonary microvascular endothelial cells (PMECs) from Syrian hamsters and deer mice. Lungs were harvested from euthanized seven-day-old hamster or deer mouse pups and were digested to obtain pulmonary cells. Cells were then sorted for CD31 (PECAM) using magnetic beads to ensure a homogeneous population of pulmonary microvascular endothelial cells.

Results: Hamster microvascular pulmonary endothelial cells were susceptible to Maporal hantavirus (MAPV), Nipah virus (NiV) and Cedar virus (CedPV). Deer mouse pulmonary endothelial cells were susceptible to MAPV and Andes hantavirus (ANDV).

Conclusions: Using PMECs in vitro will facilitate understanding of the mechanisms used by endothelial cell-tropic viruses in both pathology and reservoir animal models. email: tony.schountz@colostate.edu

2. **Maporal hantavirus causes mild pathology in deer mice (*Peromyscus maniculatus*).** Amanda McGuire[1], Joseph Fauver[1], Amber Rico[1], Tawfik Aboellail[1], Kaitlyn Miedema[1], Sandra Quackenbush[1], Ann Hawkinson[2] and Tony Schountz[1]. [1]Arthropod-borne and Infectious Diseases Laboratory, Colorado State University[1]; Department of Biological Sciences, University of Northern Colorado[2]. USA.

Background: Hantavirus cardiopulmonary syndrome (HCPS) is a rodent-borne zoonosis caused by over a dozen New World viruses. The disease is characterized by a prominent inflammatory response without conspicuous damage to the microvasculature, the target organ of infection, suggesting HCPS is, in part, an immunopathology. Only two hantaviruses cause disease in small animal models, Andes virus (ANDV) or Maporal virus (MAPV) infections of Syrian hamsters (*Mesocricetus auratus*). ANDV causes HCPS and, thus, requires animal biosafety level-4 (ABSL-4) containment; however, MAPV is not a known human pathogen, permitting ABSL-3 precautions. We are interested in the differential immune responses of reservoir hosts and Syrian hamster HCPS models that could provide mechanistic explanations for persistence or immunopathology. While deer mice (*Peromyscus maniculatus*), the natural reservoir of Sin Nombre hantavirus (SNV), are experimentally susceptible to ANDV, both viruses require ABSL-4 containment.

Methods: We therefore examined deer mice for susceptibility to MAPV.

Results: Following inoculation with MAPV, viral RNA was detected in multiple organs of all deer mice during the 56-day experiment. Deer mice seroconverted as early as 13 days post infection, and generated both nucleocapsid-specific and neutralizing antibodies. Minimal to mild histopathologic lesions were present and primarily observed in lung, heart and liver. Cytokine gene expression at low to moderate levels was detected in spleens and lungs of infected deer mice, and deer mouse primary pulmonary cells were susceptible to MAPV. The deer mice appeared healthy for the duration of the experiment, suggesting that MAPV is not pathogenic in a heterologous reservoir host.

Conclusions: These features resemble those observed in deer mice infected with SNV and suggest the course of infection with MAPV is substantially similar. The development of this model will permit direct comparison of two ABSL-3 rodent models with differential outcomes to MAPV infections, and may clarify how hantaviruses evade sterilizing immune responses. email: tony.schountz@colostate.edu

3. **Markers of central nervous system damage in patients with hemorrhagic fever with renal syndrome (HFRS).** Viktoria Ivanis[1], Viktoria Verkhoturova[1], Larisa Pereverten[1], Evgeniy Tkachenko[2], Tamara Dzagurova[2]. Pacific State Medical University[1]; Chumakov Institute of Poliomyelitis and Viral Encephalitides[2], Russia.

Objective: Target organs of Hantavirus are considered to be the lungs and kidneys, although viremia, the processes of dissemination of the pathogen and precedence of immune responses in the pathogenesis of the disease make regular involvement in the infection process of all organs, including the central nervous system. Several researchers at replicating virus detected in brain capillary endothelium and neurons are subject to direct virus damage of brain cells. At autopsy, severe infection in the brain marked by a variety of lesions from vasogenic and cytotoxic edema, focal hypoxic damage to neurons and glial cells to partial necrosis and massive hemorrhage, which is the morphological basis of neurological disorders, coma, and may be the direct cause of deaths. **Methods:** Clinical and laboratory study of 20 patients in the acute phase of HFRS (age 28,1 ± 1,5 years). In serum levels of neuronal markers: Protein S-100, Neuron

specific Enolase (NSE) were determined solid-phase immunoassay (ELISA). **Results:** The clinical picture of the acute period of HFRS was typical: fever, a mild hemorrhagic syndrome and acute renal failure. Signs of nervous system damage were recorded in all patients and manifest cerebral symptoms: headache, vomiting at the height of headaches, dizziness, fatigue, sleep disturbance. 4 patients had: stunning, hallucinatory-delirious syndrome, transient organic symptoms (anizorefleksiya, horizontal nystagmus), tendinous hyporeflexia. One patient on 7th day of illness developed meningoencephalitis with changes on computer tomography of brain (edema, diffuse small foci demyelization). Research S-100 in serum of 20 patients with HFRS showed increased levels of protein in 13 times (p≤0,001). NSE content in the serum of 11 patients turned increased 5 times (p≤0,01). In cerebrospinal fluid of a patient with meningoencephalitis enolase was doubled (p≥0,05). **Conclusions:** Thus, the levels of protein S100, NSE are informative markers of encephalopathy in patients with HFRS. Pathogenetic basis of neurological disorders is not a direct effect of the virus, but the typical immunopathological reactions in the tissue structures of the central nervous system, vegetative nervous system, the vascular endothelium.

4. **Long-term outcomes in patients suffering from HFRS**. Irina Artamonova , Guzel Muchetdinova, Raysa Faslyeva, Gulchagra Mirsaeva. Bashkir State Medical University, Russia. E-mail: vadirina@yandex.ru

Objective: To explore the possibility of developing chronic kidney disease, arterial hypertension, disorders of carbohydrate metabolism in patients suffering from HFRS.

Methods: A prospective study of 108 patients in the period from 3 months to 3 years after HFRS. The control group consisted of 30 healthy subjects. We evaluated: GFR, albumin excretion rate, blood pressure, fasting glucose, serum insulin, index HOMA-IR, HbA1C at 3,6,12,24,36 months after HFRS.

Results: 3 months after HFRS 27.8% of patients had CKD (C1A2-5,6%, C2A2-8,3%, C3aA1-11,1%, C3bA1-2,8%). After 36 CKD was diagnosed in 16.5% (C1A2 - 4,1%, C2A2 - 8,3%, C3aA1 -4,1%). After 3 months it was found first grade arterial hypertension in 29.6% of patients, second grade in 8.3%. After 3 years first grade arterial hypertension was found in 8.3% of patients. ACE inhibitors or ARBs were given to control arterial hypertension or in the presence of albuminurea (category A2 and higher) without hypertension. The median fasting glucose level in patients suffering from HFRS in 3 months was 4.8 [4.3, 5.6], p <0.001 and in 6 months- 4.75 [4.2, 5.1], p <0.001. After more than 12 months of observation we were no significant differences between the medians of glucose levels in both groups. After 3 months of the HERS the median serum insulin of patients amounted 24.3 [23.2; 25.5], p <0.001, normalizing its level at 6 months. However it remained significantly different compared with the control group during the first 12 months. The median index HOMA-IR after 3 months was 4.8 [4.2, 5.4], p <0.001 and after 6 months 2.9 ([2.7, 3.7], p <0.001. Its normalization occurred at 12 months of observation, but within 24 months it exceeded the appropriate level in the control group. HbA1C after 3 months was 6.1 [5.5, 6.7], p <0.001 and after 6 months 6.0 [5.4, 6.5], p = 0.01. After 12 months and more no significant differences for the analyzed parameter groups were revealed.

Conclusion: In patients suffering HFRS chronic kidney disease, arterial hypertension, transient insulin resistance, hyperinsulinemia and hyperglycemia develop in some cases. In our analysis the stage of CKD in patients suffering HFRS mainly related to persistent albuminuria of category A2 and not GFR. Early renal protection treatment can reduce the risk of CKD in patients suffering from HFRS.

5. **Dynamics of clinical and laboratory manifestations of HFRS caused by PUUV on the Middle Volga territory of the Russia.** Viacheslav Morozov[1], Alexei Suzdaltsev[2], Rinat Lukaiev[2], Tamara Dzagurova[3], Evgeniy Tkachenko[3]. Hepatolog LLC[1]; Samara State Medical University[2]; Chumakov Institute of Poliomyelitis and Viral Encephalitides[3], Russia. E-mail: viacheslavmorozov@yandex.ru

Objective: Clinical and laboratory data of HFRS caused by PUUV in the Samara region of European Russia for the period of 1997–2002 were compared with those in 2012.

Methods: The comparative analysis was conducted in two groups of HFRS patients: Group 1 consisted of 434 patients, infected in Samara region in 1997–2002; Group 2 consisted of 91 HFRS cases, registered in 2012. Immunofluorescence assay (IFA) was used for serological diagnosis.

Results: Catarrhal and hemorrhagic syndromes were detected much rarer in group 2 (35.2% and 3.3%, respectively) than in group 1, while painless variants of the disease occurred more frequently in group 2 (39.6%). Decreased urine output was registered in 76% of group 1 patients compare to 50% of group 2 patients. Urea and creatinine levels were also more often recorded in group 1 of patients. At the same time, level of serum ALAT was registered much more frequently in group 2. Pathological changes in the urine of HFRS patients in group 1 were characterized by a greater frequency of proteinuria than in group 2. A significantly higher number of complicated HFRS forms were observed in group 1 also.

Conclusion: Significant differences of clinical manifestations of HFRS in Samara region were revealed over the past 10-15 years. In recent years, such HFRS syndromes as hemorrhagic, catarrhal, pain syndrome and decreased urine output were registered much rarer. In 2012 ALAT level in blood serum of HFRS patients increased in more than half of the cases.

6. **The polyneuropathy syndrome in HFRS.** Tamara Dzagurova[1], Evgeniy Dekonenko[1], Iskandar Zagidullin[2]. Chumakov Institute of Poliomyelitis and Viral Encephalitides[1]; Bashkir Medical State University[2], Russia.

Objective: Here we report on rare case of PUUV-associated HFRS accompanied by the acute Guillain-Barré's Syndrome.

Methods: Clinical studies were based on medical records. Immunological methods: IgM, IgG antibodies identification by ELISA and IFA with PUUV, DOBV, HTNV, SEOV antigens.

Results: A 40 years old women, developed malaise, chills, general weakness, muscle, joint and back pain. Fever (39 0C) kept for 7 days despite taking Paracetamol (she did not seek medical advice), during this period noted decreased urine output. That time she lived in a summerhouse in Moscow suburb. A week before the disease onset, when came to house, she engaged in cleaning: sweeping, mopping the floors and noted a large amount of mice feces. On day 9 of the disease, the patient felt better and visited the clinic. HFRS diagnosis was suspected according to the clinical and epidemiological data, that was serologically confirmed (IFA antibodies against PUUV – 1/2048, DOBV, HTNV , SEOV < 1/64; ELISA IgM antibodies 1/8192 and < 1/128 respectively). Weakness, ageusia, numbness of tongue, nasolabial triangle and distal part of legs and arms appeared 3 days later. Hereafter a low-grade fever and unsteady gait appeared and on day 16 of disease the patient was hospitalized. On presentation patient had single hemorrhages on the skin, moderately hyperemic conjunctiva. Neurological examination revealed bilateral paresis of facial muscles, reducing sonority and clarity of phonation, slurred speech. Gag reflex was reduced. Patient had tetraparesis: range of motions in the upper extremities - 4 points, in the lower extremities - 3 points. Data on the propagation of excitation along hands and feet motor nerves indicated axonal-demyelinating nature of the process. Protein in the cerebrospinal fluid was 0.99 g/l (0,33 g/l). Four rounds of plasmapheresis with concomitant symptomatic therapy quickly led to a significant positive effect.

Conclusions: Rare case of acute Guillain-Barré's Syndrome in the course of moderate form HFRS, caused by PUUV has been described. Hantavirus trigger of polyneuropathy should be considered in differential diagnosis of neurological disorders.

7. **Initial biological characterization and pathogenicity of a novel subtype HTNV in Hubei province ,China.** Yan Zhong, Liang-jun Chen, Zhan-qiu Yang. State Key Laboratory of Virology, School of Medicine of Wuhan University, China. E-mail: yangzhanqiu@163.com

Objective: Hubei Province, located in central-south of China, used to have the largest HFRS patients in the whole country. We have previously isolated a novel subtype Hantaan virus (designated as HV004) from Jianghan plain in the central east of Hubei province, which can be taken as representative for hantavirus causing HFRS in this geographical region.The occurrence of novel hantaan virus may pose potential danger to control and prevention of HFRS in China.In order to getting more information about HV004, initial biological characterization and pathogenicity of HV76-118,HV114 and HV004 was performed in susceptible cells and in vivo.

Methods: Three susceptible cells were inoculated with different HTNV viruses and cells were harvested at indicated time and subjected to quantitative real time polymerase chain reaction (qRT-PCR) and immunofluorescence assay (IFA) respectively. Suckling mice were intracranially inoculated with viral suspension except normal control group. Weight of each suckling mice were recorded each day for 18 consecutive days post inoculation. Brain, lung, and kidney were aseptically dissected from the animals. Total RNA was extracted from the lung, brain, heart and kidney using TRIzol.

Results:We found that HV004 replicated with the highest viral copy number in all three cells than HV114 and HV76-118 during the observed period and HV004 provoked differential induction of antiviral responses in the established cell lines with a similar pattern of HV114.In vivo,the clinical features of mice infected with HV004 first became apparent 8 day after inoculation.The first deaths occurred 10 days after infection In contrast, mice inoculated with HV76-118 and HV114 began to lose weight 10 days after infection and died 12-14 day post inoculation. The viral RNA copies of HV004 were higher than those of HV76-118 and HV114 in brain on days 3,5, and 7 post infection.

Conclusions: These results indicated that HV004 had similar but higher infectivity and pathogenicity to that of HV76-118 and HV114.

8. **Understanding the ecology and host-switching potential of hantavirus in South America.** Gillian Eastwood[1], Yong Kyu Chu[2], Jeremy V Camp[3], Ryan McAllister[3], Vicente Javier Martínez Bruyn[4], Ashley Yu[1], Hai Yan[1], Jasper Lee[1], Evan P Williams[1], Robert D Owen[5,] Colleen Jonsson[1]. University of Tennessee (Knoxville), USA[1]; University of Louisville, USA[2]. Universidad Nacional de Asunción, Paraguay[3]; Texas Tech University, USA[4]; Barrio Republicano, Asunción, Paraguay[5].
E-mail: geastwoo@utk.edu

Objective: Ecological and environmental factors play a role in the prevalence and spillover of hantaviruses. For rodent-borne zoonotic viruses, extrinsic pressures may include climate and land-use affecting resource availability (e.g. water), habitat (e.g. fragmentation), and/or rodent community structure (e.g. population density). In Eastern Paraguay, *Akodon montensis* and *Oligoryzomys nigripes* are the

reservoir hosts of co-circulating Jabora and Juquitiba virus respectively. In field studies, we manipulate two extrinsically-driven factors: (1) increased resources, (2) decreased predators. We test hypotheses predicting that these factors, coupled with either natural, or agriculturally-disturbed habitat, affect hantavirus prevalence in wild rodent reservoirs and non-reservoirs.

Methods: In a baseline study in 2014, rodents were collected along 22 transects within the Mbaracayú Reserve, each with 50 Sherman traps. Rodent species identifications were confirmed morphologically and genetically using Cytb sequencing. Blood samples were screened by IFA for the presence of antibody, and lungs were screened for the presence of viral RNA by RT-PCR and sequencing. Urine and saliva were collected to assess viral shedding. Based on prevalence in this initial survey, we have focused monitoring at six grid sites of varying vegetation quality since 2015. On three of these grids, we have increased food resources to simulate anthropological change in land-use.

Results: Of the 432 mice captured in 2014, *A. montensis* was the most frequently captured rodent; overall, mouse species diversity increased with forest degradation. Twenty-one mice had antibodies against hantavirus, and viral RNA was detected in 11 mice (seven also being seropositive). Sequencing confirmed both Juquitiba and Jabora virus at the Reserve, and, so far, each virus has come from the expected rodent host reservoir. Hantavirus-positive prevalence occurred at 11 regions of the forest. Food supplementation during 2015-16 has increased the frequency of *Oligoryzomys nigripes* captures.

Conclusions: We evidence recent circulation of hantavirus (two strains) at Mbaracayú, involving at least two wildlife hosts. There is primary intra-species genetic diversity in both reservoir and potential spillover mice species which could affect individual susceptibility to hantavirus infection. We suggest disturbed habitat has the potential to change rodent population structure and contact frequency, increasing the prevalence of hantaviruses in both the wild reservoir and spillover host.

9. **Old World hantavirus invasion of human respiratory epithelial cells.** Giulia Torriani[1], Sylvia Rothenberger[1], Hajer Fritah[1], Nicole Tischler[2], Gert Zimmer[3], Olivier Engler[4], Stefan Kunz[1]. University of Lausanne, Switzerland[1]; Fundación Ciencia & Vida, Chile[2]; Institute of Virology and Immunology, Switzerland[3]; Spiez Laboratory, Switzerland[4].

E-mail: Giulia.Torriani@chuv.ch

Objectives: The aim of the present study is to characterize the glycoproteins Gn and Gc of hantaviruses and to use these characterized recombinant glycoproteins to establish a viral pseudotype platform. Using the prototypic Hantaan virus (HTNV), we investigate the largely unknown mechanisms underlying cell entry of Old World hantaviruses into polarized human epithelial cells. **Methods:** Since hantavirus cell attachment and entry are mediated exclusively by the viral envelope, we have developed a pseudotype platform based on a recombinant vesicular stomatitis (VSV) reporter virus. The pseudotyped viruses are unable to complete their replication cycle, making them suitable for research and diagnostics under BSL2 conditions. **Results:** We could show that by the process of pseudotyping, the HTNV glycoproteins, Gn and Gc, provided in trans were incorporated into replication-deficient vesicular stomatisis virus (VSV) vectors that contain EGFP and luciferase reporter genes. The specificity of the HTNV pseudotypes was assessed by a neutralizing antibody against VSV G. As models, we are using human alveaolar epithelial cells (A549) and bronchiolar epithelial cells (16HBE14o) which represent classical models for polarized human respiratory epithelia and that have been extensively used to study the mechanisms of airborne virus transmission. We measured the susceptibility of these two cell lines relative to BHK21 cells to infection with HTNV and VSV pseudotypes. Conclusion: We successfully established a pseudotype platform for hantaviruses that will be used to define the site(s) of entry of HTNV into polarized human respiratory epithelial cells, to address the roles of the known candidate HTNV receptors and dissect the endocytotic pathways hijacked by the virus.

10. **Modeling innate immune response of hantavirus infection in reservoir and nonreservoir hosts.** Annabel O. Meade[1], Colleen B. Jonsson[2], and Linda J.S. Allen[1]. Texas Tech University[1]; University of Tennessee, Knoxville[2], USA. E-mail: annabel.offer@ttu.edu

Objective: The early innate immune response during hantavirus infection of a natural reservoir or a nonreservoir host often determines the final outcome of the infection process. A mathematical model tests conditions for viral persistence or extinction in an individual host when the immune response is regulated by proinflammatory and anti-inflammatory cytokines. The model dynamics illustrate three potential outcomes of infection: (1) persistence of infection with no disease (2) acute infection with viral clearance or (3) severe pathology and disease. The first outcome is typical of reservoir hosts, the second, infection of a nonreservoir host with a nonpathogenic hantavirus and the third, infection of humans with a pathogenic hantavirus.

Methods: A preliminary mathematical framework for hantavirus infection in lung tissue is formulated to model these three outcomes. The dynamics of healthy and infected endothelial cells and macrophages, proinflammatory and anti-inflammatory cytokines, and viral antigen are followed over time. Proinflammatory cytokines such as interferon (IFN) protect nearby cells from becoming infected, a bystander effect. Hantaviruses affect the induction of proinflammatory cytokines which in turn impact the number of cells that become infected.

Results: The model illustrates the three infection outcomes. Restriction of early induction of IFN by a pathogenic hantavirus results in a large number of infected cells and a high viral load, outcome (3). With no restriction, the virus is cleared or viral load is low, outcome (2). Finally, intermediate levels of IFN and anti-inflammatory cytokines lead to outcome (1). Estimation of model parameters for SNV, BCCV, and PHV from in vitro experiments with lung tissue from deer mice and humans will test the validity of model outcomes.

Conclusions: Application of models and data on timing and magnitude of the immune response in reservoir and nonreservoir hosts provides essential information for designing interventions and therapeutics for treatment of hantavirus pulmonary syndrome.

11. **Investigation of bat-borne hantavirus ecology and public health relevance in Côte d´Ivoire – an One Health approach.** Leonce Kouadio[1,2], Peter T. Witkowski[3]#, Kathrin Nowak[2] ,Sébastien Calvignac-Spencer[2], Emmanuel Couacy-Hymann[1], Chantal Akoua-Koffi[4], Detlev H. Krüger[3], Fabian H. Leendertz[2]. Laboratoire Central de la Pathologie Animal, Côte d'Ivoire[1]; Robert Koch Institut, Germany[2]; Charité Medical School, Germany[3]; Université Alassane Ouattara de Bouaké, Côte d'Ivoire[4]. Email: peter.witkowski@charite.de

Objective: All so far recognised bat-borne hantaviruses in Africa represent occasional detections in single animals, hampering further genetic or ecological analyses, and even raising doubts about their bat origin or zoonotic potential. We conducted a serological screening of the human population in Taï forest of Cote d´Ivoire (CI) to ascertain public health relevance of hantaviruses. In areas with high human seroprevalence we investigated the presence of indigenous hantaviruses in chiropteran hosts as possible agents causing human infections.

Methods: We performed a cross-sectional serological study amongst 687 Taï Forest inhabitants; ELISA, Western Blot and immunofluorescence techniques were carried out serially. Following this serostudy, more than 250 bats were trapped in villages with high human seroprevalence, dissected in the field, and frozen in liquid nitrogen. After RNA extraction and testing for hantavirus L segment, PCR products were sequenced and a molecular phylogenetic analysis was performed.

Results: A seroprevalence of 3.9% was found in the Taï forest human population. Moreover, six insectivorous bats (*Neoromicia nana*) were found PCR-positive in villages comprising a small area of approximately 130 km², where seropositive persons had been found. Phylogenetic analyses indicated that sequences from five animals resembled Mouyassué virus, previously identified in CI, while the sequence divergence in sample no. CIV437 (27% difference on nt level, 10% on aa level) indicated the presence of a novel hantavirus species (tentatively called Ponan virus).

Conclusions: Here we present the first study of bat-borne hantavirus infections under the „One Health" aspect. The discovery of several bats carrying Mouyassué-like virus confirms *N. nana* as its natural host. Moreover, a putative novel hantavirus – Ponan virus – has been molecularly detected in *N. nana*, suggesting that two different hantavirus species can occur in this host sympatrically.

Bats can be found roosting in human dwellings where hantavirus seroprevalence amongst the inhabitants is shown to be relatively high. Thus bat-borne hantaviruses possess a high potential for further emergence and continuing efforts should be made to understand their ecology. Currently we are analysing more bat samples from CI, while collection of serological and molecular samples from humans and the environment of bat infested houses are ongoing.

12. **Pulmonary syndrome in HFRS.** Guzel Mukhetdinova[1], Raisa Fazlyeva[1] ,Venera Mustafina[2], Renata Fazlyeva[1]. Bashkir State Medical University, Russia[1]. Ministry of Health, Bashkortostan[2].

Objective: We have observed 220 patients with HFRS: 52 (23.6%) patients with a mild form of HFRS, 112 (50.9%) patients with a moderate form of HFRS, 56 (25.5%) patients with a severe form of HFRS.

Methods: Statistical analysis of clinical data, results of chest radiography, pulse oximetry, immunological research methods.

Results: The main clinical symptoms of lung disease in patients with HFRS characterized by a dry cough and dyspnea. The frequency of their development depended on the form of the disease: in patients with a moderate form predominated cough, with a severe form of the disease has been more often observed dyspnea. In 2 patients with a severe form of HFRS has been observed hemoptysis. In 3 patients with a severe form of HFRS has been observed clinical pulmonary edema. X-ray examination of the chest revealed three major syndromes: pulmonary pattern increasing (39%); lung tissue infiltration (36.4%) and fluid accumulation in the pleural cavity (9.1%). The severity of radiographic changes depended on the severity of the disease. During the evaluation of arterial blood saturation with oxygen by the pulse oximetry method (SrO2), depending on the form and period of the disease, our results demonstrated the development of arterial hypoxemia in the initial and oliguric periods in patients with moderate and severe forms of the disease. It should be emphasized that the oxygen saturation in the group of patients with a moderate form without clinical signs of lung lesions was lower than the control value ($p = 0.002$), what means that this method can be used in patients with HFRS for early diagnosis of respiratory failure.

In all 220 patients has been revealed the presence of specific antibodies only for serotype Puumala.

Conclusion: in HFRS, etiologically caused by a virus Puumala, observed lung damage, mostly in patients with moderate and severe forms. The dependence, that we have identified, of the severity and duration of X-ray changes in the lungs on the severity of the disease, in our opinion, is a reflection of the extent of vascular permeability disorders in patients with HFRS.

13. **Analysis of plasma kallikrein and FXII in patients infected with Puumala or Dobrava viruses.** Misa Korva[1], Shannon Taylor[2], Katarina Resman Rus[1], Connie Schmaljohn[2], Tatjana Avsic Zupanc[1]. University of Ljubljana, Slovenia[1]; United States Army Medical Research Institute of Infectious Diseases, USA[2]. E-mail: misa.korva@mf.uni-lj.si

Objective: Hemorrhagic fever with renal syndrome (HFRS) patients generally experience mild coagulation abnormalities and vascular leakage. Unfortunately, the mechanisms of coagulation abnormalities and vascular leakage are poorly understood, especially since the vascular endothelium remains intact during hantavirus infection and there are no apparent cytopathic effects to explain leakage and edema. In vitro studies have revealed increased binding of FXII to Hantaan virus (HTNV)-infected cells and subsequent increased activation of plasma kallikrein (PK). To further examine whether these results correlate to findings in vivo, we performed studies on plasma samples obtained from patients infected with Dobrava virus (DOBV) or Puumala virus (PUUV).

Methods: We analyzed acute plasma samples of patients infected with DOBV or PUUV and examined total levels of FXII and plasma prekallikrein (PPK) as well as their activated forms (FXIIa and kallikrein) utilizing chromogenic substrate assays. A detailed medical chart was collected for each patient. Additionally, plasma samples of healthy donors were included in the testing.

Results: Our results indicate that the total levels of FXII and PPK were significantly lower in DOBV- and PUUV-infected patients when compared to the control group, suggesting that plasma factors are being consumed due to activation of coagulation pathways. Additionally, we observed the lowest levels of FXII and PPK in DOBV-infected patients with severe form of the disease. We also measured activated FXII (FXIIa) and plasma kallikrein (PK) and determined that FXIIa was higher in DOBV- and PUUV-infected patients when compared to the control group, but these results were not statistically significant. We did not observe any differences in PK activity when DOBV- and PUUV- infected patients were compared to the control group.

Conclusions: Our results suggest that reduced plasma FXII and PPK and elevated FXIIa are relevant coagulation abnormalities recorded in HFRS patients. Importantly, coagulation factors measured in our study were also associated with the severe clinical form, especially in DOBV-infected patients in which bleeding manifestation are observed.

14. **Development of a mouse model of hemorrhagic fever with renal syndrome and evaluation of the role of $CD4^+$ and $CD8^+$ T cells in the pathogenesis.** Shimizu K, Yoshimatsu K, Arikawa J. Hokkaido University Graduate School of Medicine, Japan.

Objective: Hemorrhagic fever with renal syndrome (HFRS) caused by hantavirus infection is characterized by renal dysfunction and hemorrhage. Host immunity is thought to be involved in the pathogenesis of HFRS. However, lack of animal model mimicking the human disease hampers elucidation of the mechanism in vivo. In this study, we aimed to develop a mouse model showing renal symptoms characteristic of HFRS and clarify whether CD4+ and CD8+ T cells are involved in the pathogenesis in the mouse model.

Methods: Hantaan hantavirus strain Korean hemorrhagic fever virus (KHFV) clone (cl) 1 to 5 were obtained by plaque cloning. The pathogenisicy of the virus clones were evaluated by using 6-week-old female BALB/c mice. Sequence analysis of the viral genome was performed by the conventional methods. To evaluate the role of CD4+ and CD8+ T cells in the pathogenesis, they were depleted by intraperitoneal inoculation of rat anti-mouse CD4 or CD8 antibody 2 days before inoculation of KHFV cl-5.

Results: Mice intravenously inoculated with KHFV cl-1, -2, -3 and -5 developed symptoms such as transient bodyweight loss, ruffled fur and renal hemorrhage. In contrast, mice inoculated with KHFV cl-4 showed no symptoms. Pretreatment of inoculum with immune serum of Hantaan virus inhibited the manifestation of symptoms. Sequence analysis of viral genes revealed that only one amino acid at position 417 in the glycoprotein Gn is different between avirulent KHFV cl-4 and virulent KHFV cl-5. The CD4+ T cell-depleted mice developed symptoms including renal hemorrhage as in non-depleted mice after KHFV cl-5 infection. In contrast, the CD8+ T cell-depleted mice showed no symptoms. Quantities of viral RNAs in kidneys and serum IgG antibody titers in the CD8+ T cell-depleted mice at 12 dpi were comparable with those of non-depleted mice.

Conclusions: A mouse model showing renal hemorrhage as in HFRS patients was developed. In spite of the presence of viral RNAs and virus-specific IgG antibodies, CD8+ T cell-depleted KHFV cl-5-infected mice showed no symptoms, suggesting that cell-mediated immunity induced by CD8+ T cells are involved in the development of renal hemorrhage in this mouse model.

15. **Antiviral immunity and the interaction of neutrophils with plasmacytoid dendritic cells.** Martin J. Raftery, Günther Schönrich. Charité-Universitätsmedizin, Germany.

Objective: Neutrophilic granulocytes represent the most common immune cells and were thought to react to virally-infected cells exclusively by cytotoxic attack and phagocytosis. It is now recognised that neutrophils can also be induced to release dsDNA in the form of neutrophil extracellular traps (NETs) in response to DNA viruses and RNA viruses including hantaviruses. These complexes of dsDNA and neutrophil granule proteins are known to be antimicrobial and are released after signalling through β2 integrins. Their role in viral infections is less well documented.

Methods: The course of infection with DNA and RNA viruses in vitro was analysed by means of cell culture, viral titer assays, ELISA, flow cytometry and qRT-PCR

Results: We show that NETs appear to assist local antiviral defence principally by virion immobilisation, rather than induction of type I interferon. However, immune activation via DNA-specific pattern recognition receptors such as TLR-9, in particular that expressed by plasmacytoid dendritic cells, induces an antiviral immune response distal to the infection. We demonstrate that NETs are also subject to viral immunevasion by a number of different mechanisms.

Conclusions: Induction of dsDNA release from neutrophils is controlled by the interaction between β2 integrins, integrin ligands and pattern recognition receptors. The interaction of neutrophils and plasmacytoid dendritic cells represents an important axis both in the control of viral infections and the induction of autoimmune responses.

16. **An immunosuppressed Syrian hamster model for New World hantavirus lethal disease.** Valentijn Vergote, Lies Laenen, Marc Van Ranst and Piet Maes. University of Leuven, Belgium.

Hantavirus, the haemorrhagic causative agent of two clinical diseases, is found worldwide with variation in severity, incidence and mortality. The most known lethal hantaviruses are found in the American continent where Sin Nombre virus and Andes virus are known to cause hantavirus pulmonary syndrome. New World hantavirus infection of immunocompetent hamsters results in an asymptomatic infection except for Andes virus; the only hantavirus causing a lethal disease in immunocompetent Syrian hamsters mimicking HPS in humans patients. In the present study, we show that immunosuppression of hamsters followed by infection of a New World hantavirus results in an acute disease that precisely mimics both HPS disease in humans and Andes virus infection of hamsters.Hamsters, immunosuppressed with dexamethasone and cyclophosphamide, and infected intramuscularly with different New World hantavirus strains (Bayou virus, Black Creek Canal virus, Cano Delgadito virus, Choclo virus, Laguna Negra virus, Maporal virus and Sin Nombre virus) have a mean day-to-death of 16 days. Infected hamsters showed a significant loss in body weight and showed specific clinical disease symptoms. During the study, hamsters were euthanized on specific time points for the collection of blood and internal organs, which were used for further analysis. Moreover, histologic analysis of lung tissue showed signs of pulmonary oedema and inflammation within alveolar septa. In this study we were able to infect immunosuppressed hamsters with different New World hantaviruses reaching a lethal outcome with symptoms mimicking human infections.

17. **Descriptive analysis of Andes virus quasispecies in their natural reservoir *O. longicaudatus*.** Vial C[1], Perez R[1], Leon L[1], Cuiza A[1], Torres F[2], Calvo M[3], Vial P[1], Valdivieso F[1], Mertz G[4].Universidad del Desarrollo, Chile[1]; Pontificia Universidad Católica de Valparaíso[2]; 3 Universidad Austral de Chile[3]; University of New Mexico,USA[4].

Objective: Describe and compare the Andes virus (ANDV) S segment quasispecies (QS) from tissues of *O. longicaudatus*, captured in Southern Chile.

Methods: Five rodents were captured at sites where a patient with hantavirus cardiopulmonary syndrome was probably infected. The ANDV S segment from heart and lung was sequenced using amplicons of the viral S segment, and the library for deep sequencing was prepared with Nextera XT kit (Illumina). All samples were sequenced in a MiSeq sequencer, and FASTQ files were used to identify QS. The bioinformatic pipeline was as follows: (A) Mapping and alignment were done using Burrows-Wheeler Aligner. (B) PCR duplicates were marked and BAM files were sorted using PICARD. We reconstructed the quasispecies of the S segment using Shorah software. In order to compare QS, the alignment was done with ClustalW. The phylogenetic tree was constructed with neighbor-joining, and reliability of nodes estimated by bootstrap analysis after 1000 pseudo-replicates.

Results: S segment sequences were obtained with 400X of coverage. After reconstructing QS, a range of 9 to 20 QS per mouse tissue was found. Interestingly, more QS were detected in heart than in the lung (t test p=0.049). When aligning all QS, none were identical in two mice. However, QS were very similar, and QS from a same mouse group together in a phylogenetic tree regardless of the tissue they come from.

Conclusions: We identified and compared quasispecies from ANDV S segment by deep sequencing; we believe is the first successful attempt for a hantavirus. QS were present in every mouse, with a larger

number in heart than in lung. No quasispecies were identical in two mice, supporting the hypothesis that ANDV has large mutation rate.

18. **Vaccinia virus-free rescue of Fluorescent replication-defective vesicular stomatitis virus and pseudotyping with Puumala virus glycoprotein for use in neutralization tests.** Rommel Iheozor-Ejiofor[1], Lev Levanov[1], Jussi Hepojoki[1], Tomas Strandin[1], Åke Lundkvist[3], Alexander Plyusnin[1,3], and Olli Vapalahti[1,2,4]. University of Helsinki; Finland[1]; Helsinki University Hospital, Finland[2]; Uppsala University, Finland[3]; University of Helsinki, Finland[4]. E-mail: rommel.iheozor-ejiofor@helsinki.fi

Objective: Puumala virus, is considered a BSL-3 agent in Finland. The current “gold standard” for hantavirus serotyping i.e. the orthodox focus reduction neutralization test (oFRNT) is cumbersome (performed under BSL-3 conditions) and time consuming (the viruses grow slowly). In order to overcome these issues, we sought to: 1) set-up rescue of a replication-defective rVSVΔG*EGFP using vaccinia virus T7-free system, 2) restore the M segment of the PUUV prototype strain Sotkamo adapted to Vero E6-cells to the consensus PUUV M segment and 3) produce replication-defective VSV pseudotypes bearing PUUV Sotkamo strain glycoproteins and to use them as a rapid and safe substitute for native virus in FRNT (pFRNT).

Methods: A replication-defective recombinant vesicular stomatitis virus (VSV) in which the envelope protein gene is replaced with enhanced green fluorescent protein gene (rVSVΔG*EGFP) was rescued using BSRT7/5 cells which express T7 RNA polymerase and encephalomyocarditis virus (EMCV) Internal Ribosomal Entry Site (IRES)-enabled rescue plasmids in a vaccinia virus T7-free system. The system was used to produce pseudotype viruses bearing PUUV glycoproteins. The PUUV pseudotypes were harvested from the culture supernatant with titers in the range of 105-108 fluorescent focus forming units/ml and were used in (pFRNT) with neutralizing monoclonal antibodies and patient sera. The results were compared with those from oFRNT using native virus and the same samples.

Results: We identified three amino acids necessary for the expression PUUV glycoprotein which also affected the neutralizing antibody binding. These residues were found to be mutated in the Vero E6 cell culture adapted PUUV Sotkamo strain sequence. We showed that we could achieve a strong positive correlation (rs=.82) between the pFRNT and oFRNT using our system.

Conclusions: PUUV pseudotypes can be used as a substitute to native virus in neutralization tests to determine hantavirus serotypes. Vero E6 cell culture adapted PUUV has adopted 3 amino acid substitutions on their M segments which likely enables its enhanced growth in Vero E6 but however these mutations affect their antigenic properties.

19. **Aptamers against the hantavirus Andes nucleoprotein.** Renata Carvalho de Oliveira, Sotiris Missailidis, Alexandro Guterres, Jorlan Fernandes[1] Elba RS Lemos. Oswaldo Cruz Institute, Brazil. Email: reoliveira@ioc.fiocruz.br

Objectives: Our objective has been to generate aptamers against the nucleoprotein of the Andes Hantavirus, for diagnostic assay development.

Methodology: Aptamers have been selected against the nucleoprotein of the Hantavirus Andes utilizing previously published methodologies based on affinity chromatography. More specifically, 0.35mg of protein have been immobilised in a 1ml N-activated sepharose column and incubated with the aptamer library at room temperature for 1hr. Following washes with PBS, PBS 500mM, 750mM and 1M NaCl, to eliminate low or non-specific aptamer binders, elution was achieved using 1.5M NaCl and 3M NaSCN. Eluate was desalted and amplified by PCR for subsequent selection rounds. Ten rounds were performed and aptamers were cloned using a TopoTA cloning kit and sequenced. Selected aptamers were validated using Fluorescent quenching (FQ) and ELISA. FQ was based on the reduction of the fluorescent signal of tryptophan residues within the protein upon aptamer binding and Stern-Volmer and binding constants were plotted. ELISA assays validated the ability of the aptamers to identify the antigen in a serological assay or compete with antibodies against the antigen in a competition assay.

Results: Twenty aptamers were selected, with little homology between them, but with common structural patterns involving a double loop and a stabilising hairpin structure, as indicated by MFold, and conserved G-rich regions at specific points of the loops. FQ experiments allowed us to decipher aptamer affinity constants against the nucleoprotein. Experiments were previously validated using rN protein of Araraquara Hantavirus. Similarly, the ability of the selected aptamers to compete with natural antibodies, or bind in allosteric sites that do not interfere with antibody binding was analysed by sandwich and competition ELISA.

Conclusion: We were able to select aptamers against the Andes nucleoprotein and analyse them for their affinity and specificity. Aptamers that presented the highest affinity constants, specificity and ability to bind to allosteric sites without competing with natural antibodies would have the ideal properties as lead compounds for a Hantavirus diagnostic assay development.

20. **Occurrence of shrew- and mole-borne hantaviruses in Germany.** Lukáš Radosa[1], Peter T. Witkowski[1], Martina Ličková[2], Tomáš Szemeš[3], Sandra Essbauer[4], Rainer G. Ulrich[5], Lies Laenen[6], Piet Maes[6], Detlev H. Krüger[1], Boris Klempa[1,2]. Charité Medical School, Germany[1]; Slovak Academy of Sciences, Slovakia[2]; Comenius University, Slovakia[3]; Bundeswehr Institute of Microbiology, Germany[4]; Friedrich-Loeffler-Institut, Germany[5]; Rega Institute for Medical Research, Belgium[6]. Email: boris.klempa@savba.sk

Since 2007, more than 20 new hantaviruses associated with insectivores were described worldwide. New hosts as bats, shrews and moles completely changed our view on hantavirus ecology, formerly believed to be only rodent-borne viruses. In previous years, our extensive molecular screening of insectivores and subsequent sequence analyses revealed the presence of two shrew-borne hantaviruses in Central Europe, Seewis virus (associated with Eurasian common shrew, Sorex araneus) and Asikkala virus (associated with Eurasian pygmy shrew, Sorex minutus), and showed the broad geographic distribution, strong geographic clustering, and high genetic divergence of both viruses.

In addition, a small scale study focused on the European common mole (Talpa europaea) in Germany led to detection and genetic characterization of the mole-borne Nova virus (NVAV). Sequence comparisons with NVAV strains from Hungary and France showed a high degree of sequence diversity on nucleotide level (86.6-87.3% sequence identity) but nearly identical amino acid sequences (98.4-98.9% sequence identity). Phylogenetic analyses confirmed that the German strains form a separate clade within the monophyletic group of NVAV sequences. Moreover, opportunistic testing of a single specimen of T. europaea mole found dead in the vicinity of Wandlitz village near Berlin/Germany followed by phylogenetic analysis showed that moles can carry yet another hantavirus. The virus is highly divergent from NVAV and more related to recently discovered shrew-borne hantaviruses. Most recent analyses indicated that the virus is closely related to the Bruges virus identified in Belgium.

In summary, our studies showed the presence and revealed first genomic sequence data for two shrew-borne and two mole-borne hantaviruses in Germany. Further steps will aim for development of new diagnostic tools and evaluation of the public health relevance of these new insectivore-borne viruses.

21. **Production and characterization of the Juquitiba hantavirus nucleoprotein in *E. coli*.** Janaina Figueira Mansur[1], Renata Carvalho de Oliveira[2], Sotiris Missailidis[3], Elba Regina Sampaio de Lemos[2], Ronaldo da Silva Mohana Borges[1]. Federal University of Rio de Janeiro, Brazil[1]; 2.Oswaldo Cruz Institute, Brazil[2]; 3. Oswaldo Cruz Foundation, Brazil[3]. Email: reoliveira@ioc.fiocruz.br

Objective: This work aimed at the production of the nucleoprotein from Juquitiba Hantavirus (JUQV) in *E. coli* and its characterization by biochemical and structural approaches.

Method: The cDNA codifying for JUQV nucleoprotein was cloned into plasmid pET21a(+) to express the nucleoprotein with a six-histidine tag on its C-terminus. This construct was used to transform into BL21DE3 strain from E. coli and the protein expression was induced by addition of 1mM IPTG. After the cell lysis, the pellet containing inclusion bodies was dissolved at buffer containing 8M urea. The cleared lysate was loaded onto a HP column connected to the Äkta Purifier HPLC. Proteins were eluted with a linear gradient of imidazole (10 – 500Mm) at the buffer containing 8M urea and the purified nucleoprotein was refolded by dialysis.

Results: This protein was obtained with high purity and yield. The nucleoprotein was analyzed by 15% SDS-PAGE and as expected, a unique band about 48kDa was observed. The refolding process of the nucleoprotein was evaluated by fluorescence spectroscopy by monitoring the intrinsic fluorescence from tryptophan at buffer containing 8M urea and after dialyses. After refolding, the protein showed a shift in the maximum fluorescence from 340nm in buffer containing 8M urea, to 330nm, suggesting that protein was not completely unfolded even at this high concentration of denaturant agent. The effects of the temperature and pH on the secondary and tertiary structure of the recombinant nucleoprotein were analyzed by Circular Dichroism (CD) and fluorescence spectroscopy. The CD spectra of the nucleoprotein showed a characteristic profile of α-helix proteins, with negative peaks about 208 and 222nm. The temperature increase promotes a reduction of the CD signal at 222nm, indicating loss of secondary structure. The nucleoprotein showed a Tm of about 56°C and thermal plasticity. A reduction of the fluorescence emission was also observed as result of the increase at the exposition of the tryptophan to the solvent, promoted by the thermal denaturation. **Conclusions**: JUQV nucleoprotein was successfully expressed and purified. The protein is neutral with α-helical secondary structure, consistent with that observed in other hantavirus nucleoproteins and resistant to chemical and thermal denaturation.

22. **Population and community ecology of hantavirus rodent hosts in southern Brazil.** Bernardo R. Teixeira[1], Liana Strecht[2], Nathalie M. Loureiro-da-Cruz[1], Rosana Gentile[1], Renata C. Oliveira[2], Alexandro Guterres[2], Jorlan Fernandes[2], Luciana H. B. V. Mattos[2], Sonia M. Raboni[4], Giselia B. G. Rubio[3], Claudia N. D. Santos[4], Cibele R. Bonvicino[1], Elba R. S. Lemos[2], Paulo S. D'Andrea[1]. Instituto Oswaldo Cruz/FIOCRUZ[1]; Lab. de Hantaviruses e Rickettsioses, Instituto Oswaldo Cruz/FIOCRUZ[2]; Secretaria de Estado de Saúde-PR[3]; Instituto Carlos Chagas/FIOCRUZ[4], Brazil. E-mail: bernardoteixeira@gmail.com

Objective: We studied population dynamics and community structure of hantavirus rodent hosts in Southern Brazil, in order to analyze prevalence of infection over two years, micro-habitat relationships and associate biotic and abiotic variables with prevalence of infection.

Methods: Rodents was sampled in different areas every three months from Dec/2009 to Dec/2011. Animals were identified by morphology and karyotype and their serum and tissue samples were submitted to ELISA and RT-PCR, respectively. Prevalence of infection, population size, frequency of reproductively females and age structures based on body weight were analyzed by species and year/season. Habitat preference was analyzed by logistic regression regarding 14 quantitative variables. A Spearman correlation matrix was doneto detect spatial segregation or overlap of rodent species. A generalized linear model (GLM) was performed to evaluate the influence of biotic (species, sex, age, reproduction and scars) and abiotic (season, rainfall and temperature with 1-month time lag, richness and abundance) variables on hantavirus infection.

Results: Fourteen rodent species were captured. The main host species were Akodon montensis and Oligoryzomys nigripes wich are associated with the Jaborá Virus (JABV) and the Juquitiba virus (JUQV), respectively. We found a higher hantavirus prevalence in seasons with higher rodent reproductive activity, greater number of adults and lower population size for the hantavirus infected species (A. serrensis, A. montensis, A. paranaensis, O. nigripes). Spillover infections of A. montensis/JUQV, O. judex/JUQV, A. serrensis/JABV, A. paranaensis/JABV and A. paranaensis/JUQV were observed. The spillover infection between A. serrensis and A. montensis is related to micro-habitat preferences of these species. Both species showed a spatial segregation indicating spatial competition. The GLM indicates that JABV infection is related to a lower abundance of A. serrensis and the occurrence of adult males of A. montensis.

Conclusion: The spillover infection on secondary hosts plays an important role in maintaining JABV and JUQV in the hantavírus sylvatic cycle. JABV infection may be related to spatial competition between A. serrensis and A. montensis as in areas where A. serrensis was the most abundant species, the overall prevalence was lower than in areas where its abundance was smaller or similar to A. montensis.

23. **Ten year analysis of hantavirus in Chile: Small mammals associated with peridomestic cases of human hantavirus disease.** Torres-Perez F[1], Palma RE[2], Boric-Bargetto D[1,] Ferres M[3], Vial PA[4], Yañez R[1], Mertz GJ[5]. Instituto de Biología, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile[1]. Departamento de Ecología, Pontificia Universidad Católica de Chile, Santiago, Chile[2]. Centros de Estudios Médicos, Departamento de Enfermedades Infecciosas, Pontificia Universidad Católica de Chile, Santiago, Chile[3]. Facultad de Ciencias de la Salud, Universidad del Desarrollo, Santiago, Chile[4]. Division of Infectious Diseases, Department of Internal Medicine, University of New Mexico, Albuquerque, New Mexico[5].

Andes hantavirus is a major ethiologic agent of HCPS, and the Sigmodontinae *O. longicaudatus* (the pigmy rice rat) is the reservoir. This rodent occurs in Chile along a wide latitudinal range that spans contrasting geographic features and landscapes. Small mammals in southcentral and Patagonian Chile were analyzed to evaluate the spatial dynamics of seropositive rodents to Andes strain Hantavirus. Seventy nine sites in peridomestic and countryside areas were evaluated in 10 years of sampling. Rodents were captured in live traps according to standard protocols as previously described and followed established safety guidelines for rodent captures and processing. Rodent antibody against ANDV were detected in blood samples using a strip immunoblot assay (SIA). Comparison analyses included estimation of relative density and relative seropositivity. The species *Abrothrix longipilis*, *A. olivaceus* and *O.longicaudatus* were the most frequently trapped, with the latter having the highest seropositive rate. In the Mediterranean region, *O. longicaudatus* was almost the sole seropositive rodent. Mediterranean region appears with high values of relative seropositivity. The reservoir of Andes virus in Chile is frequently found in peridomestic areas, including the Mediterranean region (ecoregion with high human population density). Significant differences in the relative prevalence of anti-ANDV antibodies in rodent samples also were found across the ecoregions. High relative seropositive in the Mediterranean region is of high concern. We discuss the implications of the geographic distribution of the host and the hantavirus disease in Chilean human populations.

24. **Development of a multiplexed immunoassay to detect IgG antibody responses against Pathogenic bunyaviruses within the Republic of Georgia.** Badger C, Voorhees M, Schmaljohn C. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD 21702, USA. E-mail: catherine.v.badger.civ@mail.mil

Objective: HFRS has been reported in the Republic of Georgia; however, there is limited information about the prevalence of disease or of the circulation of hantaviruses in rodents. We have initiated a project to develop and implement a MagPixtm-based multiplex system for ecology and epidemiology studies in Georgia. In addition to an assay for hantaviruses, we also developed a MagPixtm assay for Crimean Congo hemorrhagic fever virus (CCHFV), which has caused several outbreaks throughout Georgia.

Methods: A capture sandwich immunoassay was developed using purified recombinant viral nucleocapsid proteins (NP) generated in either a bacterial expression system (hantaviruses) or a baculovirus-insect cell expression system (CCHFV). Microspheres and amine coupling kits were purchased from Luminex. Capture antibodies directed against the viral nucleoproteins were chemically conjugated to carboxylated microspheres. After incubation with recombinant antigen, test sample was added followed by an anti-human PE-conjugated detection antibody. The median fluorescence intensity (MFI) from the output.csv file of the Luminex xPONENT software was copied into an Excel file containing simple formulas for data analysis.

Results: A checkerboard assay was performed to determine optimal monoclonal antibody pairs to be used as capture antibodies. To evaluate assay sensitivity, the limit of detection (LOD) was determined for suitable antibody candidates. LODs were also performed using a variety of diluents such as serum, whole blood and urine to rule out excessive background as might be encountered with clinical samples.

Conclusions: We have developed a multiplexed immunoassay that measures virus-specific IgG antibodies. This assay has been determined to be sensitive and specific, and uses a lower sample volume than a traditional ELISA. Further, this assay can be adapted to detect serum viremia using optimized monoclonal antibody pairs. Sera from febrile patients will be tested to confirm samples initially identified by ELISA as having antibodies against hantaviruses or CCHFV. These data will be instrumental in determining the seroprevalence of these two pathogenic bunyaviruses in the Republic of Georgia.

25. **Clinical and morphological analysis of renal changes in hemorrhagic fever with renal syndrom.** A.N. Evseyev, Department of Pathology and Forensic Medicine, Far Eastern Medical Institute, Khabarovsk.

Objective, comparison of morphological findings in kidneys of HFRS patients using immunohistochemical and morphometric methods, study of proliferation processes in tubular epithelium, and assessment of clinical and laboratory findings.

Materials. Renal biopsy samples of 41 HFRS patients at an acute stage with acute renal failure (ARF) and of 17 convalescents at 6 months to 3 years after the discharge were studied. Methods. Morphometric, immunohistochemical (IM) and electron microscopic methods were used in the study of biopsied kidneys. With IM, Ki-67 was calculated for positive nuclei in epithelial cells of convoluted tubules (CTE). Specific volumes (%) of peritubular capillaries (Vcap) and stroma (SSV) were calculated. Renal biopsy samples of 20 male patients aged 18 to 27 with microproteinuria were taken as controls.

Results. At an acute stage (10 days after onset) (20 cases), glomerular anemia and focal proliferation of mesangial cells were observed. Tubular epithelium presented with degenerative and necrotic changes, while stroma presented with edema, hemorrhage foci, and lymphoid macrophage infiltrations. At a stage of 11-20 and 21+ days after onset (21 and 17 cases), productive sclerotic changes in glomerules and stroma with capillary bed exsanguinations, and focal atrophic changes of tubular epithelium were observed. Analysis of proliferation processes in tubular epithelium showed that HFRS patients at an acute stage presented with increase in Ki-67 of positive nuclei in tubular epithelium ($P<0.05$; $t=0.005$) versus the comparison group. At an acute stage, negative correlation between specific stromal volume (SSV), microvessel volume (MV) and Ki-67 value ($r=-0.8223$; $p=.000$; and $r=-0.9428$; $p=0.000$, respectively), and positive correlation between those indicators and hematuria value ($r=0.7230$; $p=.000$; and $r=0.4427$; $p=0.000$) were found.

Conclusions. Structural changes in kidneys in HFRS are characterized by a combination of alteration of all elements of nephron most pronounced in the tubular region of nephron with damaged stromal vascular component and simultaneous increase in proliferation activity of convoluted tubular epithelium. At a stage of 21+ days after onset, interstitial sclerosis, degenerative and atrophic changes are observed. Condition of stroma and vessels was found to influence processes of renal tubule regeneration in HFRS.

26. **Optimized VSV-based Andes virus vaccine for adequate pre- and post-exposure protection.** Joshua Marceau[1,2], David Safronetz[1,3], Andrea Marzi[1], Kyle Rosenke[1], Heinz Feldmann[1] . Laboratory of Virology, Division of Intramural Research, National Institute for Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT, USA. Department of Biomedical and Pharmaceutical Sciences, The University of Montana, Missoula, MT, USA. Public Health Agency of Canada, Canada.

Objective: Among human pathogenic hantaviruses, Andes virus (ANDV) is associated with high case fatality rates and potential human-to-human transmission. This is important for case patient management and a vaccine could be of particular public health benefit. Here we compared the efficacy of monovalent and bivalent live-attenuated vaccine vectors in pre- and post-exposure protection of Golden Syrian hamsters against lethal ANDV challenge.

Methods: The vaccine vectors were generated by replacing the glycoprotein (G) of vesicular stomatitis virus (VSV), Indiana serotype, with either the Zaire ebolavirus (EBOV) glycoprotein (gp), the ANDV glycoprotein precursor (gpc) or both using reverse genetics technology. Two monovalent (rVSVΔG-ANDVgpc and rVSVΔG-ZEBOVgp) and two bivalent (rVSVΔG-ANDVgpc-ZEBOVgp and rVSVΔG-ZEBOVgp-ANDVgpc; differing in the order of the two foreign genes/immunogens) vaccine vectors were rescued and compared for their protective efficacy following immunization by two routes, intraperitoneal or intranasal, at different time points pre- and post- challenge.

Results: The two bivalent vectors were equally efficacious and superior over the monovalent ones in pre-exposure vaccination independent of the route of immunization. The bivalent vector rVSVΔG-EBOVgp-ANDVgpc was most potent in post-exposure administration with intraperitoneal immunization as the superior route.

Conclusions: The replacement of VSVG by EBOVgp in the design of rVSV vaccine vectors seems to provide a benefit likely due to specific immune cell targeting mediated through EBOVgp. This in particular if used for peri-exposure prophylaxis for which intranasal immunization seems less efficacious.

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27. **Immunogenetic factors affecting susceptibility of rodents to hantaviruses at the macroevolutionary scale: Evidence from integrins and DAF genes.** Marie Pagès[1], Caroline Tatard[2], Maxime Galan[2], Bérénice Villegas[2], Joseph A. Cook[3], E. Fichet-Calvet[4], Peter T. Witkowski[5], Serge Morand[1], Nathalie Charbonnel[2]* [1]. Institut des Sciences de l'Evolution, Université Montpellier, Montpellier, France. [2]INRA, Centre de Biologie pour la Gestion des Populations, Montferrier sur Lez, France. [3]Museum of Southwestern Biology and Department of Biology, University of New Mexico, Albuquerque, USA. [4]Bernhard-Nocht Institute of Tropical Medicine, Hamburg, Germany. [5]Institute of Virology, Charité Medical School, Berlin, Germany. E-mail: marie.pages@univ-montp2.fr

Objective: In Europe, hantaviruses are one of the main agents of emerging infectious diseases that cause hemorrhagic fever with renal syndrome, including nephropathia epidemica. Understanding the processes driving infection and clearance of this pathogen in natural populations is a major objective of infectious disease research. In this study, we assessed whether interspecific molecular differences in the membrane proteins β1, β3 and DAF could be associated with differences in rodent susceptibility to hantaviruses.

Methods: We tested the hypothesis that amino acid variation in these genes could provide a key to discriminate a priori hantavirus reservoirs from non-reservoir species. By conducting site-by-site and lineage specific models of evolution we compared sequences among 25 species of wild rodents and tested whether some amino acid positions in these genes had evolved under positive selection.

Results: We detected significant negative selection for most amino acid sites in β1 and DAF, suggesting that both genes have been mainly subjected to purifying selection along their evolutionary history. Our analyses further revealed that two SNPs within different domains of β1 and at least one SNP in the S/T-rich domain of DAF evolved under significant positive selection in some reservoir rodent species only. By comparing reservoir and non-reservoir species, we also found distinctions between some lineages with respect to variation at these sites that were consistent with their reservoir status.

Conclusions: Our findings suggest that there may not be any "universal" key to determine a priori which rodent species can and could carry human pathogenic hantaviruses. Future mutagenesis studies related to hantavirus infection will help us gain a deeper understanding of the mechanisms of virus entry into rodent cells.

28. **Why and how do we need to examine host microbiome in hantavirus studies?**Maxime Galan[1], Adélaïde Dubois[1,2], Guillaume Castel[1], Jean-François Cosson[3], Jean-Baptiste Pons[4], Séverinne Murri[1], Philippe Marianneau[2,] Nathalie Charbonnel[1]. [1]NRA, Centre de Biologie pour la Gestion des Populations, Montferrier sur Lez, France. [2]ANSES Unité de Virologie, Lyon, France. [3]INRA, VectoTic , Maison-Alfort, France. [4]CNRS, Laboratoire de Biométrie et Biologie Evolutive, Villeurbanne, France. E-mail: maxime.galan@supagro.inra.fr

Objective: These last years, our understanding of disease emergence and host susceptibility to pathogens has been improved by shifting the paradigm from 'pathogens' to 'pathobiome'. Coinfections strongly affect the conditions of hosts but also their immune balance, what in turn may modify individual ability to respond to new infections. Only few studies have examined how reservoir host pathobiome could influence susceptibility to hantaviruses. In this study, we developed a metagenomic study to emphasize potential links between bacterial infections and the circulation of Puumala virus in bank voles.

Methods: We analyzed and compared the bacterial pathobiome of 180 bank voles sampled in 2014 in six localities from Eastern France, four being endemic for nephropathia epidemica (Puumala hantavirus, PUUV), and the two others being non endemic to this disease. We applied a 16S rRNA amplicon sequencing approach developed in the lab to the multiplex analyses of a large number of samples within a high-throughput sequencing run (Illumina MiSeq). Several negative and positive controls were included to filter sequence datasets, especially with regard to potential contaminations due to the high sensitivity of this approach. Bacterial taxonomic diversity and composition were identified using mothur software and the Silva ribosomal database.

Results: We listed 12 pathogenic bacteria (Operational Taxonomic Units, OTUs) in these bank vole populations, including the genera Anaplasma, Bartonella, Bordetella, Borrelia, Mycoplasma, Candidatus neoehrlichia, Rickettsia, Spiroplasma and Orientia. Surprisingly, we also identified the presence of the protozoal pathogen Sarcocystis muris. Prevalence of bacterial pathogens ranged from 0% to 73%, while PUUV seroprevalence ranged from 0% to 13% (PUUV endemic localities). About 80% of the bank voles exhibited at least one bacterial infection, and half of them were coinfected by several bacterial OTUs. These proportions remained similar in the PUUV endemic and non-endemic areas. Nevertheless, the bacterial community structure differed with bank vole populations from the non endemic area being more infected by a particular Mycoplasma OTU and Candidatus_Neoehrlichia.

Conclusions: These preliminary results are particularly interesting because some of these bacteria might influence the probability of further PUUV infections, through immune modulation or ecological processes.

29. **Virological and immunological surveys during Puumala virus experimental infections of bank voles (*Myodes glareolus*) from endemic and non-endemic regions.** Adélaïde Dubois[1,2], Guillaume Castel[1], Coralie Pulido[3], Séverine Murri[2], Jean-Baptiste Pons[4], Laure Benoit[1], Anne Loiseau[1], Latifa Lakhdar[3], Maxime Galan[1], Philippe Marianneau[2], Nathalie Charbonnel[1]. [1]. INRA, Centre de Biologie pour la Gestion des Populations, Montferrier sur Lez, France. [2]ANSES, Unité de Virologie, Lyon, France. [3]ANSES, Plateforme d'Expérimentation Animale, Lyon, France. [4]CNRS, Laboratoire de Biométrie et Biologie Evolutive, Villeurbanne, France.

Objective: The bank vole Myodes glareolus is the natural reservoir of the hantavirus Puumala (PUUV), the etiological agent of nephropatia epidemica (NE) in humans. In Europe, there is considerable geographical heterogeneity in NE occurrence, despite the continuous distribution of bank vole populations. This discrepancy could be explained by the complexity of factors implied in PUUV and NE dynamics, including virological characteristics, human activities or environmental conditions. Host population features could also be different and underlie variations in vole sensibility / resistance to PUUV infection. Because we currently know a few about PUUV / bank vole interactions, we compared the response to PUUV infection of bank voles from two regions of eastern France.

Methods: We infected bank voles sampled in 2015 in the Jura (endemic for NE) and Ain (non- endemic for NE). Bank voles were subcutaneously inoculated with PUUV Sotkamo strain. Serological analyses and qRT-PCR were applied on sera, saliva, urine and feces sampled once a week and on organs once voles were sacrificed. Haptoglobin concentrations were measured in sera and used as a marker of inflammatory response.

Results: All voles seroconverted between J14 post-infection (PI) and J28 PI. For the two regions, presence of viral RNA was very transient in bank vole sera, and viral RNA was totally absent in the other excreta. We found viral RNA in the liver, kidney, salivary glands and bladder of bank voles from the two regions. Levels of viral RNA were higher for bank voles from the Jura, but at seven days PI and in lungs only.

Conclusions: Except in the early stages of infection, bank voles from endemic and non-endemic regions seem to have similar susceptibility to PUUV infection. Only viral replication seemed to be stronger in the lungs of bank voles from the Jura compared to Ain.

Poster Session II - Thursday, June 2, UCA room 116, 12:15-14:00

30. **Quantitative evaluation of reservoir potential of ecological hosts of Hantaviruses**. Tatyana Kushnareva. Institution of Epidemiology and Microbiology and Pacific Medical University, Vladivostok, Russian Federation.
E-mail: tatyana.kushnareva@inbox.ru

Objective: Markers of possible development of HFRS epidemical situation were designated according to quantitative evaluation of carriers-rodents in reservoir potential dynamics of hantavirus natural foci. In Primorye Krai pathogenic AMRV and HTNV (FE) circulate in Apodemus peninsulae and A.agrarius, and not pathogenic HOKV and VLAV in Myodes rufocanus and Microtus fortis respectively.

Methods: Monitoring (2001-2013) conducted in forests where A.peninsulae and M.rufocanus are dominants and forest-steppes where A.agrarius and M.fortis are dominants. Reservoir potential of hantavirus natural hosts was evaluated by RP (reservoir potential) and IRP (index of reservoir potential) [http://link.springer.com/article/10.1134/S1995425514010090].

Results: Annual and seasonal RP and IRP for hantavirus ecological hosts were calculated. RP was highest for A peninsulae in forest and for A.agrarius in forest-steppe landscapes. M.rufocanus and M.fortis had low RP. A.peninsulae RP was much higher during the early summer period than in the fall (r=0,001), and A.agrarius RP was higher in the fall, than in summer (r=0,022). Mean annual IRP for Apodemus drew up 0,83. The share of A.peninsulae and A.agrarius drew up 0,55 and 0,28 respectively (r=0,017). Average annual IRP varied for A.peninsulae from 0,08 to 0,84 and A.agrarius from 0,04 to 0,96. Years with dominating only one epidemic species A.agrarius or A.peninsulae were designated. At years with low of number of HFRS cases *M.rufocanus* had a significant reservoir role (IRP 0,61 and 0,55). According to the analysis of Apodemus IRP dynamics and HFRS cases the epizootological indicator of possible development of epidemic danger was designated: A. peninsulae IRP≥0,7 during the spring and A. agrarius IRP≥0,8 during summer season.

Conclusion: Quantitative estimation of reservoir potential dynamics of carriers-rodents in the hantavirus natural focus gives an opportunity to predict the increase of people infection risk in regions where circulate a few pathogenic/not pathogenic hantaviruses.

31. **Prevalence of hemorrhagic fever with renal syndrome in Yiyuan County, China, 2005-2014**. Tao Wang, Yunping Zhou, Feng Cui, Ling Wang, Zhenshui Huang, Shenyong Zhai. Department of Infectious Disease Control and Prevention, Zibo Center for Disease Control and Prevention, Zibo, Shandong Province, P. R. China. E-mail: dawangtao@126.com

Objective: Hemorrhagic fever with renal syndrome (HFRS) is highly endemic in mainland China, where human cases account for 90% of the total global cases. Yiyuan County is one of the most serious affected areas in China. Therefore, there is an urgent need for monitoring and predicting HFRS incidence in Yiyuan to make the control of HFRS more effective.

Methods: The study was based on the reported cases of HFRS from the National Notifiable Disease Surveillance System. The demographic and spatial distributions of HFRS in Yiyuan were established. Then we fit autoregressive integrated moving average (ARIMA) models and predict the HFRS epidemic trend. **Results:** There were 362 cases reported in Yiyuan during the 10-year study period. The human infections in the fall and winter reflected a seasonal characteristic pattern of Hantaan virus (HTNV) transmission. The best model was ARIMA (2, 1, 1)×(0, 1, 1)12 (AIC value 516.86) with a high validity.

Conclusions: The ARIMA model fits the fluctuations in HFRS frequency and it can be used for future forecasting when applied to HFRS prevention and control.

32. **Seoul virus infection in France**. Jean-Marc Reynes[1], Damien Carli[1], Jean-Baptiste Bour[2], Samir Boudjeltia[3], Anny Dewilde[4], Marie-Pierre Rapt[5], Véronique Jacomo [6],Alexandra Septfons[7], Pierre E. Rollin[8]. [1]Centre National de Référence des Hantavirus, Unité de Biologie des Infections Virales Emergentes, Institut Pasteur, Centre International de Recherche en Infectiologie, Lyon, France. [2]Département de virologie, Centre Hospitalier Universitaire Dijon-Bourgogne, Dijon, France. [3]Service de Néphrologie, Centre Hospitalier Universitaire Dijon-Bourgogne, Dijon, France. [4]Laboratoire de Virologie, Centre de Biologie Pathologie, Centre Hospitalier Universitaire, Lille, France. [5]Service de Médecine Interne et Pneumologie, Centre Hospitalier, Bar-le-Duc, France. [6]Laboratoire Biomnis, Lyon, France. [7]Département des maladies infectieuses, Institut de Veille Sanitaire, Saint-Maurice, France. [8]Special Pathogens Branch, Centers for Diseases Control and Prevention, Atlanta, Georgia, USA.

Objective: Surveillance of hantavirus infection in France before 2012 was based on results of serological diagnostic assays performed by clinical laboratories and the National Reference Center (NRC) for Hantavirus. In 2012, molecular assays were used at the NRC in order to identify the virus species responsible for these infections, leading to the virological confirmation of a Seoul virus (SEOV) infection in a pregnant woman. We report here two additional SEOV cases detected in 2014.

Methods: Samples from patients tested positive for IgM and/or IgG against hantavirus by clinical laboratories were sent for result confirmation to the NRC where they were tested using home-made

ELISA and IF assays. Samples collected within 7 days after onset were also tested by real-time RT-PCR and Nested RT-PCR, allowing detection of Puumala virus (the main prevalent virus in Europe), and hantaviruses respectively.

Results: Two SEOV infections were detected in February and September 2014. Patients presented a classical hemorrhagic fever with renal syndrome. Liver blood parameters were elevated. Exposure for one case was suspected to have occurred during building restoration work, while exposure for the other case was clearly attributed to close contact with a pet rat (Rattus norvegicus), bought about one month before the onset of the disease, and also found to be infected by the same SEOV strain. All strains detected belonged to the main phylogroup including strains from Asia and from the rest of the World.

Conclusion: In 2012, we reported the first detection of SEOV in humans in France and even in Europe. The detection of SEOV in those two cases plus the report of a SEOV associated case in 2014 published elsewhere indicate that SEOV infection in humans are uncommon but not rare in France. Attention should be given to this virus at least in Europe where surveillance is focused on Puumala and Dobrava hantaviruses.

We recently also reported the detection of a Tula virus infection in a French patient. The diagnostic of these infrequent TULV and SEOV infections indicate that molecular diagnostics should be promoted to discriminate between hantaviruses involved in human diseases and consequently to adapt control measures.

33. **Phylogeographic diversity and reassortment of Hantaan virus in nature, the Republic of Korea**. Jeong-Ah Kim[1], Won-keun Kim[1], Jin Sun No[1], Seung-Ho Lee[1], Sook-Young Lee[1], Ji Hye Kim[1], Jeong Hoon Kho[1], Daesang Lee[2], Dong Hyun Song[2], Se Hun Gu[2], Seong Tae Jeong[2], Man-Seong Park[3], Heung Chul Kim[4], Terry A. Klein [5], Jin-Won Song[1]. [1]Department of Microbiology, College of Medicine, Korea University, Seoul, Republic of Korea, [2]Agency for Defense Development, Daejeon, Republic of Korea. [3]Department of Microbiology, College of Medicine, the Institute for Viral Diseases, Korea University, Seoul, Republic of Korea. [4]5th Medical Detachment, 168th Multifunctional Medical Battalion, 65th Medical Brigade, Unit 15247, APO AP 96205–5247, USA. [5]Force Health Protection and Preventive Medicine, 65th Medical Brigade/US Army MEDDAC-Korea, Unit 15281, APO AP 96205–528, USA. E-mail: youminlove3@hotmail.com

Objective: Gangwon and Gyeonggi provinces are highly endemic areas of Hemorrhagic Fever with Renal Syndrome (HFRS) in the Republic of Korea (ROK). However, previous studies conducted epidemiological and phylogeographic analyses of Hantaan virus (HTNV) in Gyeonggi province. The present study first demonstrated the geographic distribution and phylogenetic diversity of HTNV in Gangwon provience.

Methods: Rodents (Apodemus agrarius) were captured in the Gangwon and Gyeonggi provinces of the ROK, from 2003 to 2014. Using indirect immunofluorescence antibody (IFA) and HTNV-specific RT-PCR, a total of 5,929 A. agrarius were examined for detecting anti-HTNV IgG from sera and a partial sequence of M segment (1,982-2,327nt) from lung tissues of the seropositive rodents. Whole genome sequences of HTNV were recovered using conventional RT-PCR. Phylogenetic and Recombination/Reassortment analyses were performed using the whole sequences of HTNV tripartite genomes.

Results: Among a total of 5,929 A. agrarius, 774 (13.1%) of A. agrarius were seropositive for anti-HTNV IgG. HTNV-specific RT-PCR showed that 70.3% (544/774) A. agrarius were positive for the partial sequence of HTNV M segment. To investigate the molecular diversity of HTNV in the endemic areas, the whole genome sequences of 34 HTNV strains were completely obtained, consisting of Cheorwon, Hwacheon, and Yanggu in Gangwon province and Paju, Pocheon, and Yeoncheon in Gyeonggi province. Phylogenetic analysis showed the geographic distribution and diversity of HTNV in the ROK. Using recombination detection program (RDP), the recombination/reassortment analysis suggested a genome organization compatible with reassortment of HTNV L segment in nature.

Conclusions: The phylogeographical analysis of HTNV showed the genetic diversity and a likely natural HTNV reassortant in Gangwon and Gyeonggi provinces. These observations may provide important insights into the molecular evolution and epidemiology of hantaviruses in HFRS-prevalent areas.

34. **Genetic diversity of Artybash virus in the Laxmann's Shrew (*Sorex caecutiens*)**. Satoru Arai[1], Hae Ji Kang[2], Se Hun Gu[2], Satoshi D. Ohdachi[3], Joseph A. Cook[4], Liudmila N. Yashina[5], Keiko Tanaka-Taya[1], Sergey A. Abramov[6], Shigeru Morikawa[1], Nobuhiko Okabe[1,7], Kazunori Oishi[1], and Richard Yanagihara[2]. [1]National Institute of Infectious Diseases, Tokyo, Japan; [2]University of Hawaii at Manoa, Honolulu, HI, USA; [3]Hokkaido University, Sapporo, Japan; [4]University of New Mexico, Albuquerque, NM, USA; [5]State Research Center of Virology and Biotechnology, Koltsovo, Russia; [6]Institute of Systematics and Ecology of Animals, Novosibirsk, Russia; [7]Kawasaki City Institute for Public Health, Kanagawa, Japan. E-Mail: arais@nih.go.jp

Objective: Although based on very limited M- and L-segment sequences, Artybash virus (ARTV) was proposed previously as a unique hantavirus harbored by the Laxmann's shrew (Sorex caecutiens) in Russia.

Methods: Total RNA, extracted from RNAlater®-preserved lung tissues of 68 Laxmann's Shrew (Sorex caecutiens), captured during 2006 to 2014 in eastern Siberia, Russia, and Hokkaido, Japan, were analyzed for ARTV RNA using RT-PCR.

Results: ARTV RNA was detected in 5 of 39 (12.8%) and 1 of 29 (3.4%) Laxmann's shrews captured in Russia and Japan, respectively. All but one of the 6 ARTV-infected shrews were males. Taxonomic identity of the ARTV-infected Laxmann's shrews was confirmed by full-length cytochrome b mitochondrial DNA sequence analysis. Pair-wise alignment and comparison of partial and full-length S-, M- and L-segment sequences from these Laxmann's shrews, as well as phylogenetic analyses, using maximum likelihood and Bayesian methods, indicated that ARTV was distinct from other soricine shrew-borne hantaviruses, and from representative hantaviruses harbored by rodents, moles and bats. Analysis of the full-length nucleotide sequences of the S-, M- and L-genomic segments of ARTV strain Mukawa AH301 showed considerable divergence from representative hantaviruses: S, 22.9–47.2%; M, 20.9–48.3%; and L, 20.2–38.1%. However, the amino acid sequence variation of ARTV strains Mukawa AH301 and Amga MSB148558 was low, ranging from 4.0–6.5%. Phylogenetic analyses of the full-length coding regions, comprising 1,290-nucleotide S, 3,420-nucleotide M and 6,456-nucleotide L segments of ARTV strain Mukawa AH301, showed virtually identical topologies with ARTV, detected independently in Laxmann's shrews captured in the Altai Republic and Khabarovsk Krai of Russia.

Conclusions: Our data indicate that the hantavirus previously known as Amga virus (MGAV) represents genetic variants of ARTV. Thus, the previously proposed designation of ARTV/MGAV should be replaced by ARTV.

35. **HFRS outbreak caused by Sochi virus on the Russian Black Sea coast**. Tamara Dzagurova[1], Boris Klempa[2,3], Maria Balovneva[1], Natalia Korotina[1], Vyacheslav Morozov[4], Olga Pilikova[5], Yuliya Yunicheva[6], Viktoriya Bakhtina[7], Peter Witkowski[2], Evgeniy Tkachenko[1], Detlev Krüger[2]. [1]Chumakov Institute of Poliomyelitis and Viral Encephalitides, Moscow, Russia; [2]Institute of Virology, Charité Medical School, Berlin, Germany; [3]Institute of Virology, Biomedical Research Center, Bratislava, Slovakia; [4]Medical Company "Hepatolog" LLC, Samara, Russia; [5]Anti-Plague Station, Novorossiysk, Russia; [6]Anti-Plague Department, Sochi, Russia; [7]Infectious Disease Hospital, Krasnodar, Russia.

Objective: Sporadic HFRS cases associated with Sochi genotype of Dobrava-Belgrade virus (DOBV) are registered on the Russian Black Sea Coast annually. The case fatality index was estimated to be 15 %. Here we report on a local HFRS outbreak in Gelendzhik, situated on the Black Sea coast 250 kilometers north from the city of Sochi.

Methods: Clinical and epidemiological analysis; IgM, IgG antibody identification by ELISA and IFA with DOBV and PUUV antigens; hantavirus antigen detection by "HANTAGNOST" ELISA; L segment-targeting RT-PCRand nucleotide sequence analysis; cytochrome b gene and D-loop analysis for molecular host identification..

Results: Four men, 23, 31, 36 and 50 years old, fell ill between 16th and 24th of October, 2013. All of them were temporarily residing in the region as seasonal construction workers and lived in an uncomfortable, mice-infested wooden house, on the outskirts of the city. The patients were hospitalized with a suspected diagnosis of "HFRS". The diagnosis was confirmed one day after admission owing to anti-DOBV IgM detection by ELISA and IFA. A severe form of HFRS developed in all patients. Clinical manifestations were characterized by uremic intoxication and a moderate hemorrhagic syndrome including scleral hemorrhages and micro/macro hematuria. The 50-year-old man died on day 7 after onset (third day of hospitalisation, second day of artificial lung ventilation) as a result of renal, lung, and cardiovascular failure (max. blood creatinine - 310.7 μmol/L, min. platelet count - 3 x109/L). Post-mortem findings included multiple internal hemorrhages, pleurorrhea, lung and brain edema. Hantavirus antigen was detected in lung, brain, kidney, liver, pancreas, spleen, heart, and lymph nodes of the patient. Infection by Sochi virus was verified by RT-PCR detection of Sochi virus partial L segment sequences in these biosamples. Six small wood mice and 4 Caucasian mice were captured in the vicinity of the house. Antibodies, antigen, and RNA of Sochi virus were found in 2 Caucasian mice, whose species classification has been confirmed by mitochondrial DNA analyses.

Conclusions: The high virulence of Sochi virus was confirmed in this local outbreak. Sochi virus infects multiple human organs, what leads to severe clinical course of the disease.

36. **PUUV active endemic areas patterns in Russia: Results of 40-years monitoring**. Alla Bernshtein[1], Natalia Apekina[1], Marina Ostanina[2], Elena Mutnykh[1], Evgeniy Tkachenko[1], Irina Gavrilovskaya[3]. [1]Chumakov Institute of Poliomyelitis and Viral Encephalitis, Moscow, Russia; [2]Udmurtia Republic Centre for Hygiene and Epidemiology, Izhevsk, Russia; 3Department of Molecular Genetics and Microbiology SUNY at Stony Brook, Stony Brook, USA.

Obiective: A long-term investigation of PUUV active endemic areas with optimal bank vole (*Myodes glareolus*) habitats and annually high HFRS incidence have been conducted in purpose to reveal epizootical and epidemiological regularity.

Methods: Zoological, epizootological and epidemiological monitoring was performed by standard methods in linden- spruce forests of the Cis-ural region (56051N, 53013E).Criteria of acute rodent infection were the viral antigen detection in the lungs, which correlated with actively virus transmitting.

Results: In extensive predominantly linden forests bank vole populations reach a high density without a subsequent deep depressions (30-230 per 1 ha). In addition, each of 2-4, more often 3 years there is early reproduction of voles after a heavy crop of linden (14 of 40 years). Epizootic process has a pronounced cyclical nature on this background: 1) The increase and peak: 1 year with a maximum in late summer, 12-20 infected voles per 1 ha; 2) Decrease: 1 year with a minimum in fall, 1 infected vole on 1 ha; 3) Low activity: 0-2 years, 1-4 infected voles per 1 ha. All 14 peaks were triggered by the voles early reproduction, accompanied by increased mobility, contacts, and of infection of intensively maturing animals, including the youngs. HFRS outbreaks arise only in the epizooty peak years. Voles density rises above the threshold level, 45-60 per 1 ha in different periods (n-25), in only 56% led to outbreaks. The sharp decreases of the epizootic and epidemic activity always occurred in the years after the peaks, regardless of voles density, as the result of young animals low infection. Since 1985 the incidence increased more than 3.5 times, in parallel with the increase in density of bank vole. However, the pattern of endemic areas has not changed in 40 years of observation.

Conclusions: In bank vole optimum range epizootic and epidemic cycles in PUUV endemic areas not regulated by the dynamics of rodents population density. The growth of endemic areas activity is initiated by early voles reproduction. The sharp decline in activity may be due to qualitative changes in the virus and/or virus host population in the peak phase of the epizootic.

37. **Presence of Hantaan virus RNA from anti-Hantaan virus IgG seronegative rodents in a highly endemic area, the Republic of Korea.** Jin Sun No[1], Won-keun Kim[1], Jeong-Ah Kim[1], Seung-Ho Lee[1], Sook-Young Lee[1], Ji Hye Kim[1], Jeong Hoon Kho[1], Daesang Lee[2], Dong Hyun Song[2], Se Hun Gu[2], Seong Tae Jeong[2], Heung-Chul Kim[3], Terry A. Klein[4], Jin-Won Song[1]. [1]Department of Microbiology, College of Medicine, Korea University, Seoul 136-705, Republic of Korea. [2]The 5th R&D Institute, Agency of Defense Development, Yuseong P.O. Box 35, Daejeon 305-152, Republic of Korea. [3]5th Medical Detachment, 168th Multifunctional Medical Battalion, 65th Medical Brigade, Unit 15247, APO AP 96205-5247, USA. [4]Public Health Command District-Korea (Provisional), 65th Medical Brigade, Unit 15281, APO AP 96205-5281, USA.

E-mail: dybono@korea.ac.kr

Objective: Hantaan virus (HTNV), the family *Bunyaviridae*, causes hemorrhagic fever with renal syndrome (HFRS) in human. Recently, the viral RNA of Hokkaido virus (HOKV), a genus of Hantavirus, was detected in anti-PUUV seronegative grey red-backed voles (Myodes refocanus). The outbreak of HPS occurred in the USA showed high percentage of hantavirus RNA positivity from seronegative rodents. Despite of majority of epidemiologic studies in seropositive rodents for hantavirus-specific immunoglobulin, the discovery of hantavirus RNA in seronegative hosts leads to an investigation of the presence of HTNV RNA in the rodents captured in endemic areas.

Methods: Apodemus agrarius, natural reservoir of HTNV, was captured in Gyeonggi and Gangwon provinces from 2013 to 2014. Indirect immunofluorescent antibody (IFA) test was performed in 240 A. agrarius and total RNA was extracted from lung tissues and analyzed by HTNV M segment-specific RT-PCR. RT-qPCR was performed targeting the HTNV S segment in the lung, liver, kidney, and spleen of rodents.

Results: A total of 186 (77.5%) A. agrarius were negative for anti-HTNV IgG antibodies. HTNV RNA was detected in seven (3.8%) of the anti-HTNV IgG seronegative rodents collected in Gyeonggi province. In anti-HTNV IgG seronegative and HTNV RNA positive (IFA-PCR+) rodents, the Ct value was in the range of 27–36. Anti-HTNV IgG seropositive and HTNV RNA positive rodents showed lower Ct values (high levels of HTNV RNA) than the IFA-PCR+ rodents in all of tissues examined in this study.

Conclusions: The detection of HTNV RNA from the IFA-PCR+ rodents may reflect the early phase of HTNV infection since the antibody can be detected in hantavirus-infected animals at 14 days after infection. Thus, our data suggest the dynamic circulation of HTNV in highly endemic areas, providing important insights into the epidemiology and virus-host interaction of hantaviruses in nature.

38. **Inactivated purified hemorrhagic fever with renal syndrome vaccination based comprehensive intervention to prevent hemorrhagic fever with renal syndrome epidemiology effect evaluation in Weifang of Shandong Province.** Wang Zhiqiang,Zhai Wenji,Kang Dianmin. Shandong Center for Disease Control and prevention,Jinan,Shandong Province,P.R. China.

HFRS caused by Hantavirus is a natural focal disease，and has been popular in many countries in Europe and Asia. Among these countries, the number of China's HFRS patients occupies 90.1% of the number of the world's HFRS patients, and the HFRS is one of the infectious disease which is seriously endangering the people’s health. Since the discovery of the first HFRS case in China, its researchers conducted a series of epidemiological studies. They have basically defined the type of HFRS virus, the species of the host animal and the prevalent characteristics of the disease, and implemented a variety of control strategies. Among a variety of control strategies, the vaccine-based comprehensive prevention and control measures recommended by the Ministry of Health has become the preferred HFRS control measures. However, the investigation about the measures at home and abroad is mainly focused on unit or unpurified bivalent HFRS vaccine’s protective effect in the area of the short-term immunology and epidemiology; the researchers rarely study the purified bivalent inactivated HFRS vaccine’s long-term

epidemiological protective effect in the large-scale population. In addition, HFRS vaccine has been widely used in all over the world, but it is still a controversial issue about its immunization strategies. Therefore, using experimental epidemiological research methods, we select the Weifang of Shandong Province as the test area in 2012, which is the high incidence area of HFRS and the house mouse widely distributed. We adopt a vaccination-based intervention method, establish intervention groups and control groups, and observe their 3-year follow-up, in order to evaluate long-term epidemiological protective effects of purified bivalent inactivated vaccine, and to explore the immune strategy. This study has an important significance for formulating a scientific and rational vaccine strategies and policies.

The results show：

1． Compared with the period before intervention, in the intervention period, in Weifang area, HFRS annual incidence, the number of villages which have the HFRS patients, the incidence density, and so on were significantly lower than the period before the intervention. The protection rate of the entire test area was 72.94%, and the effect index is 3.69. The vaccination intervention measure for the prevention and control of Weifang HFRS has played a good result.

2. The results of cohort studies shows: the incidence density of HFRS vaccine group population is 0, but the incidence density of the control group population is 6.51/10 million. The incidence density of the vaccinated group was significantly lower than that of the control group population; the protection rate of the general population was 100%.The results confirm that the mass vaccination in the population has a good long-term epidemic protective effect.Conclusion:

1. For the prevention and control of the Shandong HFRS ,to implement a vaccination-based integrated intervention measures has played a good epidemiological protective result.2. The 3-year cohort study confirmed that the HFRS vaccine in large-scale vaccination crowd has a good long-term epidemiological protective effect.

3. The strategy, "0, 14 day" 2-pin-based immunization, six months after a needle vaccination, is suitable for the vaccination of the mass crowd.

39. **Characterisation of the endonuclease activity of Hantaan virus L polymerase.** Sylvia Rothenberger[1], Giulia Torriani[1], Stefan Kunz[1], Olivier Engler[2].[1]Institute of Microbiology, University Hospital Center and University of Lausanne, Rue du Bugnon 48, CH-1011 Lausanne, Switzerland. [2]Spiez Laboratory, CH-3700 Spiez, Switzerland. E-mail: Sylvia.Rothenberger-Aubert@chuv.ch

Objective: Replication and transcription of the viral RNA genome represent crucial steps of viral multiplication, which are highly conserved among hantaviruses and therefore represent promising drug targets for the development of effective broad-spectrum antivirals. The aim of this study is to develop a cell-based assay that could be implemented in high-throughput screening of small chemical compounds.

Methods: Negative strand RNA viruses of the *Arenaviridae*, *Bunyaviridae* and *Orthomyxoviridae* family are capable of recruiting the 5'-cap structures of host cell mRNAs for the priming of viral mRNA transcription in a process called "cap-snatching" which requires a viral endonuclease (EN) activity. We combined molecular modeling with mutagenesis and biochemical assays to characterize the EN activity of Hantaan virus L polymerase.

Results: Based on the existing high-resolution structure of La Crosse orthobunyavirus (LACV) EN domain, we modeled the active sites of the putative EN of Old World Hantaan virus (HTNV) polymerase. Our model predicts that HTNV L residues H36, E54, D97, E110 and T112 correspond to the key residues of the LACV EN active site, H34, D52, D79, D92 and K94. Using HTNV L constructs comprising the N-terminal domain, we could show that a single mutation in the putative EN catalytic site of HTNV L, such as H36, E54 or D97, efficiently rescues the expression of the construct, validating our molecular model. Co-expression of WT HTNV L EN domain with an NLuc reporter plasmid resulted in markedly reduced luciferase activity, in line with published data using ANDV L (Heinemann P et al., J Virol 87:6975-6985, 2013). We further constructed C-terminal fusion proteins of WT and D97A mutant full-length HTNV L and EN domain with NLuc. When expressed in cells, the fusion constructs containing WT L or EN domain showed markedly reduced NLuc activities when compared to the "EN-dead" D97A mutants. Furthermore, we could quantify the relative ratio of expression between wild-type and endonuclease dead L.

Conclusions: Our study reveals remarkable conservation of the viral endonuclease activity among hantaviruses and paves the way for the identification of novel synthetic inhibitors of hantavirus endonuclease that will be evaluated as anti-viral drugs.

40. **Clinical assessment of a bivalent DNA vaccine for hemorrhagic fever with renal syndrome caused by hantavirus infections.** Connie Schmaljohn[1], Drew Hannaman[2], Kristopher Paolino[3], Jay W. Hooper[1]. [1]US Army Medical Research Institute of Infectious Diseases, Frederick, MD, [2]Ichor Medical Systems, San Diego, CA, [3]Walter Reed Army Institute of Research, USA.

Objective: Demonstrate safety and efficacy of DNA vaccines against hemorrhagic fever with renal syndrome (HFRS).

Methods: We developed DNA vaccines for HFRS expressing the Gn and Gc genes of HTNV or PUUV. To assess the clinical safety and immunogenicity of the DNA vaccines, we conducted Phase I studies using gene gun or intramuscular electroporation (IM-EP) delivery devices and have now advanced into Phase 2 clinical testing.

Results: The gene gun study demonstrated that the HTNV and PUUV DNA vaccines delivered as separate administrations were safe and immunogenic; however, the low overall seroconversion rate (~50%) indicated the need for improved vaccine delivery. Consequently, we conducted a Phase 1 study using Ichor Medical Systems IM-EP TriGrid Delivery System. All vaccinations were administered at a 2 mg total dose and comprised HTNV DNA alone, PUUV DNA alone, or a combined vaccine (1 mg each HTNV and PUUV DNAs). Neutralizing antibody responses were detected in 5/9 or 7/9 of individuals receiving three doses of the HTNV or PUUV DNA vaccines, respectively. In the combined vaccine group, 7/9 and 3/9 subjects developed neutralizing antibodies to PUUV or HTNV, respectively, signifying potential vaccine interference. To address this, we modified the HTNV vaccine to reflect the codon bias of humans and to remove elements known to negatively impact expression or mRNA stability. We initiated a Phase 2a dose ranging study with the modified mixed DNA vaccines in July, 2014, which will conclude in December, 2016. 120 subjects have been randomized into four vaccination groups. Subjects are receiving 1 mg or 2 mg of the mixed DNA either on Days 0, 28 and 56, or on Days 0 and 56, with an optional 6-month booster. Although group assignments will remain blinded until the study conclusion, preliminary analyses of serum samples from completed subjects indicate that the modified HTNV/PUUV vaccines can consistently induce responses against both viruses.

Conclusion: The HTNV and PUUV DNA vaccines delivered by IM-EP are safe and immunogenic in humans. Preliminary evidence indicates that observed interference issues can be mitigated through vaccine refinement, permitting further development of this HFRS vaccination strategy.

41. **Advanced Development of a Candidate Combination DNA Vaccine For Hemorrhagic Fever With Renal Syndrome (HFRS) Caused by Hantaviruses Using a Cross-Cutting Delivery Technology to Accelerate Protective Immune Responses.** Amy C. Shurtleff[1,2], Genevieve Long[2], Jay Hooper1, Robert Eackles[3], Julia Donnelly[3], Dan McLain[4], Drew Hannaman[5] and Connie S. Schmaljohn[1]. [1]US Army Medical Research Institute of Infectious Diseases (USAMRIID), Fort Detrick, Maryland, USA; [2]The Geneva Foundation, Tacoma, WA, USA; [3]US Army Medical Materiel Development Activity (USAMMDA), Fort Detrick, MD, USA; [4]Walker Downey & Associates, Inc., Verona, WI, USA; [5]Ichor Medical Systems, Inc., San Diego, CA USA.

Objective: Hantaviruses are known to cause thousands of cases of hemorrhagic fever with renal syndrome (HFRS) each year. At least four hantaviruses are known to cause HFRS: Hantaan (HTNV) and Seoul (SEOV) viruses in Asia; and, Puumala (PUUV) and Dobrava (DOBV) viruses in Europe and Scandinavia. There are no FDA-licensed vaccines for hantaviruses causing HFRS. Our work will transition a lead candidate DNA vaccine for HFRS into the advanced development pipeline toward licensure and stockpiling for use in civilians and military personnel.

Methods: The vaccine proposed is a combination of two DNA plasmids expressing the envelope glycoprotein genes of HTNV and PUUV. This combination has been shown to elicit neutralizing antibodies against all four HFRS causing hantaviruses in both animals and humans. Previous Phase 1 human clinical studies have achieved a suboptimal seroconversion rate, perhaps due to the need for improved DNA vaccine delivery technology. The current project utilizes Ichor Medical Systems' TriGrid™ Delivery System (TDS) EP device, and aims to compare DNA vaccine delivery by intramuscular (IM-EP) or intradermal (IM-EP) methods, hypothesizing that delivery to skin may be optimal because it is a highly immunologically active site.

Results and Conclusions: This multi-year contract has completed GMP manufacturing of HTNV and PUUV DNA vaccines using established master cell banks of HTNV and PUUV codon-optimized DNA sequences. We produced vaccine quantities sufficient for use in toxicology studies through a planned Phase 1 trial. These GMP product lots are currently being evaluated for stability and potency using established assays for release. A pre-Investigational New Drug (IND) meeting has been held with FDA to finalize non-clinical and clinical plans. A non-clinical safety and toxicology study in rabbits will begin in March 2016, in support of the pending IND submission. Ultimately, we are planning a randomized, comparative Phase 1 clinical study to assess the safety, tolerability, and immunogenicity of the vaccine candidate administered by electroporation. Subjects (total of 60) will receive either the monovalent HTNV or PUUV DNA vaccine candidate, a 1:1 mixture of the two candidates (HTNV-PUUV) or phosphate buffered saline (PBS) control by IM- or ID-EP using the TDS-EP device technology.

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42. **Rodent and shrew-borne hantaviruses in Germany.** Petra Strakova[1,2], Stephan Drewes[1], Maysaa Dafalla[1], Sabrina Schmidt[1], Ulrike M. Rosenfeld[1], Mathias Schlegel[1,3], Hanan Sheikh Ali[1], Rainer G. Ulrich[1]. [1]Friedrich-Loeffler-Institut, Institute for Novel and Emerging Infectious Diseases, Greifswald - Insel Riems, Germany. [2]Institute of Vertebrate Biology v.v.i., Academy of Sciences, and Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic. [3]Seramun Diagnostica GmbH, 15754 Heidesee, Germany.

Objective: Central Europe is an important endemic region for hantaviruses. Puumala virus (PUUV), Dobrava-Belgrade virus (DOBV), Tula virus (TULV) and recently described hantaviruses associated with moles (Nova virus) and shrews (Seewis virus – SWSV, Asikkala virus and Boginia virus) are present in different parts of central Europe. In Germany, a small mammal monitoring of reservoir hosts for DOBV, PUUV, TULV and SWSV is ongoing.

Methods: Small mammal trapping and dissection followed standard protocols of the network „Rodent borne pathogens“. During 2004-2014, a total of 660 bank voles, 654 common voles, 249 field voles, 24 water voles, 482 striped field mice and 355 shrews of three Sorex spec. were collected in different regions of Germany. For virus RNA detection in lung tissue, S segment specific RT-PCRs for PUUV/TULV/SWSV and a L segment RT-PCR for DOBV were used. Detection of DOBV-, PUUV- and TULV-specific antibodies were based on in-house ELISAs using recombinant nucleocapsid proteins of these viruses.

Results: DOBV-specific antibodies and RNA were detected in 25 and 5 of 482 striped field mice. The RT-PCR products showed the highest sequence similarity to DOBV genotype Kurkino. The TULV RNA and/or antibody prevalence was higher in common voles (174/654), than in field voles (23/249) and water voles (3/26). A bank vole monitoring showed PUUV-infected animals at seven of eight sites during the outbreak year 2012 (117/499 PUUV-ELISA, 84/499 PUUV/RT-PCR). In 2013, out of 161 bank voles, 2 and 5 bank voles were PUUV-ELISA and PUUV-RT-PCR positive, respectively. SWSV-RNA was only detected in three common shrews.

Conclusions: The study confirmed the presence of all four hantaviruses in Germany, but with different distribution patterns. DOBV was detected at several foci in eastern and nothern Germany. TULV investigations confirmed the common vole as its reservoir, with spillover to other vole species. Similarly, the common shrew represents the reservoir of SWSV, with rare spillover to pygmy shrews. PUUV was found to be inhomogenously distributed in a highly endemic region. The overall prevalence of DOBV and TULV were not as high as reported for PUUV in its host.

43. **PUUV Active Endemic Areas Patterns in Russia: Results of 40-years Monitoring.** Bernshtein A[1], Apekina N[1], Ostanina M[1], Mutnykh E[1], Tkachenko E[1], Gavrilovskaya I[2]. [1]Chumakov Institute of Poliomyelitis and Viral Encephalitis, Moscow, Russia; Udmurtia Republic Centre for Hygiene and Epidemiology, Izhevsk, Russia; [2]Department of Molecular Genetics and Microbiology SUNY at Stony Brook, Stony Brook, New York. USA.

Objective: Investigation of HFRS endemic areas in the forest-steppe landscape of Central Russia.

Methods: Zoological, epizootological and epidemiological monitoring was performed by standard methods in 2006-2014 years. Antibodies were detected by IFA and ELISA (IgM). Rodent infection criterion was antibodies and/or viral antigen detection in the lungs by IFA and ELISA.

Results: PUUV and DOBV (Kurkino genotype) endemic areas were identified everywhere, reservoir hosts field mouse (Apodemus agrarius) and bank voles (Myodes glareolus), respectively. DOBV incidence have been sporadic. Since 1978 year only three Kurkino associated HFRS outbreaks were recorded only in wintertime. The cause of these outbreaks were a sharp increase in abundance infected in of field mice (more than 30 specimen per 100 trap-nights) number and its migration to the settlements. About 92% of the mice were Kurkino virus infected. More than 90% of patients were rural residents which were infected when caring for domestic livestock. PUUV sporadic cases were registered in all seasons and years and, as usual there were urban residents which infected when visiting the forest. In this area, the PUUV foci are tied to small fragmented areas of forest and the incidence is sporadic even in the years with increased abundance and infection of the bank vole. Therefore, HFRS outbreaks associated with PUUV, characteristic for the optimum endemic areas bank vole area, do not happen here. In areas where both field mouse and bank vole live in close proximity, combined foci formed, which is typical only for this territory. When the population of reservoir hosts of DOBV and PUUV had low abundance and infection rate, small wood mouse (Apodemus uralensis) was dominating, but it had almost no infection. And HFRS cases in this period were rare.

Conclusions: The principal epidemiological differences of DOBV and PUUV infections are closely related to their reservoir hosts population patterns. DOBV cause sporadic cases too, except of rare epidemic seasons when HFRS outbreaks caused by Kurkino virus have taken place in winter time. Located on the mosaic forest landscape of European Russia PUUV endemic areas were characterized by sporadic HFRS incidence, which is typical only for this territory.

44. **Antiviral activity of arbidol hydrochloride in hantaan virus infection in vitro and in vivo.** Nian Ma, ZhanQiu Yang. State Key Laboratory of Virology/ Institute of Medical Virology, School of Medicine of Wuhan University, Wuhan 430071, P.R of China.

Background: Hantaviruses infect their reservoir hosts and humans, but the infection only causes disease in humans. In Asia and Europe, the hantaviruses usually cause haemorrhagic fever with renal syndrome (HFRS), yet no effective prophylactic vaccines are available. Arbidol Hydrochloride is an antiviral drug that from the former Soviet union pharmaceutical chemistry center research, which is often used for treatment of respiratory infection.

45. **Use of sequence independent single primer amplification next generation sequencing (SISPA NGS) for whole genome sequencing of Hantaan virus.** Dong Hyun Song[1], Won-keun Kim[2], Daesang Lee[1], Se Hun Gu[1], Jeong-Ah Kim[2], Seung-Ho Lee[2], Jin Sun No[2], Jin-Won Song[2], Seong Tae Jeong[1]. [1]The 5th R&D Institute, Agency for Defense Development, Yuseong P.O.Box 35, Daejeon, Republic of Korea 305-152. 2Department of Microbiology, College of Medicine, Korea University, Seoul, Republic of Korea**.** Email: wkkim1061@korea.ac.kr

Objective: Hantavirus, a family of *Bunyaviridae*, is a single-stranded, negative-sense RNA virus containing tripartite genomes. Hantavirus infection causes hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS) with mortality rates ranging from 1% to 36%. The outbreak or endemic infection of hantavirus is a critical threat for world public health due to the lack of effective prophylactic and therapeutic strategies. Additionally, according to the Center for Disease Control and Prevention, hantavirus is a Category C bioterrorism agent, suggesting that it is a possible biological agent and a threat to national security given its ease of production, rapid transmission, and undefined pathogenesis. Recently, the development of next generation sequencing (NGS) provided a potential tool enabling acquisition of whole viral genome sequences for the advancement of diagnostics and vaccines. Here, NGS applied to acquire the genomic sequence of Hantaan virus (HTNV) from isolates and lung tissues of natural hosts, Apodemus agrarius.

Methods: A. agrarius was captured in HFRS prevalent areas. The rodent was determined for HTNV positivity by indirect immunofluorescence assay and RT-PCR. Using total RNA from HTNV-positive rodent samples, library preparation was performed using TruSeq Nano DNA LT sample preparation kit. The genetic sequence of HTNV was recovered by MiSeq. To validate the obtained sequences, phylogenetic clustering with HTNV from the corresponding sites was described by maximum likelihood and Bayesian method.

Results: Using SISPA NGS, HTNV M and S segments were completely sequenced whereas the L segment was almost recovered except for a specific region between 1,500 and 2,100 nt. Pairwise alignment and comparison of the S-, M-, and L-segment coding sequences of HTNV isolates obtained by SISPA NGS showed high sequence similarities and phylogenetically well-supported clades with HTNV strains collected from the corresponding region and completely sequenced by RT-PCR.

Conclusions: SISPA NGS will be a robust tool for the whole genome sequencing and genomic characterization of hantaviruses in natural reservoirs, as well as HFRS and HPS patients.

46. **Hantavirus screening of peri-domestic rodents in the United Kingdom.** Ellen G. Murphy[1], Nicola J. Williams[2], Julian Chantrey[3], Malcolm Bennett[4] and Lorraine M. McElhinney[5]. [1]NIHR Health Protection Research Unit in Emerging Zoonotic Infections, NCZR, Leahurst Campus, School of Veterinary Science, Chester High Road, Neston, CH64 7TE, UK. [2]Zoonotic Infections of People, Pigs and Poultry Group, Department of Epidemiology and Population Health Institute of Infection and Global Health, Leahurst Campus, University of Liverpool, Neston, CH64 7TE, UK. [3]Institute of Integrative Biology, University of Liverpool, Biosciences Building, Crown Street, Liverpool, L69 7ZB, UK. [4]Faculty of Medicine & Health Sciences, Veterinary Clinical Building, Sutton Bonington Campus, Sutton Bonington, Leicestershire, LE12 5RD, UK. [5]Wildlife Zoonoses and Vector Borne Disease Research Group, Animal, Plant & Health Agency, Weybridge, Surrey, KT15 3NB, UK. Email: Lorraine.McElhinney@apha.gsi.gov.uk

Objective: To gain a clear understanding of the presence and diversity of Hantaviruses in the United Kingdom.

Method: Rodents of various species, including brown rats (Rattus norvegicus), bank voles (Myodes glareolus), field voles (Microtus agrestis) and house mice (Mus musculus) were trapped from October 2014 to November 2015. Rodents were trapped at 26 peri-domestic locations from around mainland UK ranging from rural/agricultural settings such as pig farms, dairy farms and woodlands to urban dwellings such as residential properties and ports. Trapped rodents were humanly euthanised and tissue samples were taken at post mortem examination. Kidney and lung tissue from all rodents will be screened for Hantavirus RNA using a pan-hantavirus RT-PCR.

In addition, the 2nd UK Hantavirus workshop will be held on the 11 -12th February in Liverpool where academia, public health and veterinary health organisations will meet to discuss the current strategies for Hantavirus surveillance and research in the UK.

Results: Preliminary results from Hantavirus screening of UK rodents and the recommendations from the workshop will be presented.

Conclusions: From these results we will be better informed as to what Hantavirus species (Seoul, or other new novel species) are circulating in rodent populations in the UK and be able to determine if there is a significant risk to public health.

I would like to acknowledge the Emerging and Zoonotic Infections Heath Protection Research Unit (HPRU) at the University of Liverpool for supporting this work.

47. **Sin Nombre hantavirus nucleocapsid protein exhibits a metal-dependent DNA-specific endonucleolytic activity.** Elisabeth Möncke-Buchner, Michal Szczepek*, Marcel Bokelmann, Patrick Heinemann, Martin J. Raftery, Detlev H. Krüger, and Monika Reuter. Institute of Medical Virology, Helmut-Ruska-Haus, and *Institute of Medical Physics and Biophysics, Charité Medical School, Berlin, Germany. Email: detlev.kruger@charite.de

Background. Hantaviral nucleocapsid protein (N) represents a prime example of a multifunctional viral protein. It is the most abundantly produced viral protein in hantavirus-infected cells, is the major virus immunogen, and has key roles in viral replication, genome encapsidation and specific enhancement of viral translation. The N proteins of some segmented (-)ss RNA viruses can exert DNA- and/or RNA-degrading activities, although for members of *Bunyaviridae* an N-associated nuclease activity has only been shown in the genus nairovirus.

Methods. N protein of Sin Nombre virus (SNV-N) was expressed as an N-terminal GST fusion protein and was purified under native conditions. GST tag was removed during the purification procedure. Purified N protein was tested on various DNA and RNA substrates for nucleic acid-degrading activity. Analysis of N primary sequence by structure-based protein fold prediction revealed a putative active site of the endonuclease. Two of the crucial amino acids therein were replaced by site-directed mutagenesis.

Results. We show that SNV-N encodes a DNA-specific metal-dependent endonuclease activity that acts on both single- and double-stranded DNA, whereas single- and double-stranded RNA proved to be resistant to SNV-N mediated cleavage. Two Asp residues in positions 88 and 103 of SNV-N show sequence similarity with the active site in the bacterial Type II restriction endonuclease HindIII where residues Asp93 and Asp108 [as part of the canonical PD-(E/D)xK catalytic motif] play a role in binding and coordination of metal ions essential for HindIII DNA cleavage. Replacing Asp88 and Asp103 by alanine led to an SNV-N protein abrogated for endonuclease activity.

Conclusion. The purified nucleocapsid protein of Sin Nombre virus possesses a DNA-specific metal-dependent endonuclease activity. One might speculate that this nuclease can counteract the sensing of cytoplasmic DNA which induces innate IFN-responses, the formation of DNA/RNA hybrids which interfere with virus replication, or the accumulation of ssDNA in the cytosol which trigger IFN production. The fact that Asp88 and Asp103 are highly conserved in all hantaviruses known so far implies that the DNA-specific metal-dependent endonuclease activity is a novel common feature of the multifunctional hantaviral nucleoprotein.

48. **Seewis Virus in Eurasian Common Shrews (*Sorex araneus*) in Southwestern Poland.** Seung-Ho Lee[1], Ewa Gajda[2], Won-keun Kim[1], Joanna Hildebrand[2], Richard Yanagihara[3], Jin-Won Song[1]. 1Department of Microbiology, College of Medicine, Korea University, Seoul, Republic of Korea; [2]Department of Parasitology, Uniwersytet Wrocławski, Wrocław, Poland; [3] Department of Pediatrics, John A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, Hawaii, USA. E-mail: leeds1104@gmail.com

Background: Earlier studies have documented the existence of Tula virus (TULV) in common voles (Microtus arvalis) in central Poland by virus isolation. Also, in southeastern Poland, Puumala virus (PUUV) and Dobrava virus (DOBV) RNAs have been detected in bank voles (Myodes glareolus) and yellow-necked mice (Apodemus flavicollis) by real-time PCR, and cases of hemorrhagic fever with renal syndrome have been confirmed. Recently, we reported the co-circulation of Seewis virus (SWSV) in the Eurasian common shrew (Sorex araneus), Boginia virus in the Eurasian water shrew (Neomys fodiens) and Nova virus in the European mole (Talpa europaea) in central Poland. The objective of this pilot study was to ascertain the existence of shrew- and rodent-borne hantaviruses in southwestern and northern Poland.

Methods: RNAlater®-preserved lung or spleen tissues from 25 yellow-necked mice, 13 bank voles and 10 Eurasian common shrews, captured between 2009 and 2014 in northern Poland (Gdańsk in Pomeranian province) and southwestern Poland (Osobowice and Milicz in Lower Silesia province), were analyzed for hantavirus RNA by nested RT-PCR, using primers designed to detect the L, M and S segments of DOBV, PUUV and SWSV.

Results: PUUV and DOBV RNAs were not detected in tissues of bank voles and yellow-necked mice, respectively. However, of the 10 Eurasian common shrews, SWSV RNA was found in three of five males and in none of five females. Phylogenetic analyses, based on partial L- and S-segment sequences, using the maximum-likelihood method, showed that SWSV from southwestern Poland segregated along geographic-specific lineages.

Conclusions: Disappointingly, hantavirus RNA was not detected in rodents, but this may have been due to the low prevalence of infection. By contrast, despite the small sample size, the previously reported high prevalence of SWSV infection in Eurasian common shrews in other geographic regions was borne out in

southwestern Poland. Also, the clear preponderance of infected male Eurasian common shrews resembled the gender-specific prevalence of hantavirus infection in other species of shrews and in rodents. Efforts are now underway to analyze tissues from additional rodents and shrews from southwestern Poland, as well as to isolate SWSV.

49. **Rodent-Borne Viruses Antibodies in Health Professionals Who Handle Wild Animals in Brazil.** Jorlan Fernandes[1], Renata Carvalho de Oliveira[1], Alexandro Guterres[1], Raphael Gomes[1], Monique Queiroz Lima[1], Felipe Moliterno[1], Márcio Neves Bóia[1], Silvana Levis[2], Elba Regina Sampaio de Lemos[1]. [1]Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, Brazil; [2]Nacional Institute of Human Viral Diseases, Pergamino, Argentina. **E-mail:** jorlan@ioc.fiocruz.br

Objective: Health professionals who handle wild animals are considered to be highly exposed to rodent-borne diseases during their daily job. In this study, we aim to access the occupational risk of these professionals to hantaviruses and arenaviruses.

Methods: Participants of the VI Annual Meeting of the Brazilian Mastozoology Society were invited to fill a self-administered questionnaire and to donate a small volume of venous blood. The questionnaire included questions about previous exposure wild and laboratory animals, time and type of exposure, use of personal protective equipment and any previous occurrence of a severe febrile illness. Serum samples were tested for immunoglobulin G against Araraquara hantavirus (ARAV) and Junín mammarenavirus (JUNV), by using ELISA.

Results: We conduct a serological survey in 341 health professionals. Sixty one (20.8%) informed that they did not use any personal protective equipment (PPE). For the ones who always use PPE, gloves were unique PPE used during handling animals. Antibodies against ARAV were detected in 1 (0.2%) participant from São Paulo state, a highly endemic area for hantavirus pulmonary syndrome in Brazil. In the questionnaire, he reported to work with wild rodents in the same state for four years. The participant also referred to a febrile illness characterized by high fever and headache. Antibodies against JUNV were detected in 2 (0.3%) of the 341 persons in the study. One participant who worked with wild animals in the Atlantic rainforest and a second who have worked in more than six Brazilians states for approximately 8 years. All the reactive participants report the use of gloves as the only PPE at work.

Conclusion: Although the low use of PPE, in this serological survey on animal handlers, it was observed a low prevalence of antibodies against rodent-borne viruses. This results show that besides the high exposure to wild life animals, these health professionals have low risk of infection by rodent viruses.

50. **Seroprevalence of hantavirus in bats from the Atlantic Forest, Brazil.** Renata Carvalho de Oliveira[1], Alexandro Guterres[1], Jorlan Fernandes[1], Pedro Cordeiro-Estrela[2], Roberto Leonan Morim Novaes[3], Emmanuel Messias Vilar[2], Ricardo Moratelli[3], Elba Regina Sampaio de Lemos[1]. [1]Oswaldo Cruz Institute, IOC-FIOCRUZ, Rio de Janeiro, Brazil. [2]Laboratory of mammals, DSE, CCEN, UFPB, João Pessoa, PB, Brasil. [3]Fiocruz Mata Atlântica, CFMA-FIOCRUZ, Rio de Janeiro, Brazil. E-mail: reoliveira@ioc.fiocruz.br

Objective: Sera from bats (order Chiroptera) were screened for the presence of antibodies against Andes recombinant nucleoprotein (rNP-ANDV), to investigate the prevalence of hantaviruses in bats collected in the Brazilian Atlantic Forest biome. Although novel hantaviruses have been identified in bat species in Africa and Asia, but little is known on hantaviruses in Neotropical bats.

Methods: We analyzed 212 bat serum samples by using indirect enzyme-linked immunosorbent assay (ELISA) for detection of IgG antibodies to rNP-ANDV. A titer of >1:400 was considered positive. Bats were collected in four different localities spanning ~2000 km line and covering tropical and subtropical climates in the states of Rio de Janeiro, Bahia and Santa Catarina. Fieldworks were conducted from December 2013 to May 2015, in distinct expeditions, integrated with two Brazilian biodiversity research programs.

Results: Andes-specific antibodies were detected in 11 bats, which represents an overall seroprevalence of 5,2%. The species composition and trophic guild of positive bats was *Artibeus lituratus* (n=3, frugivorous), *Desmodus rotundus* (n=3, hematophagous), *Carollia perspicillata* (n=2, frugivorous and insectivorous), *Artibeus fimbriatus* (n=1, frugivorous), *Anoura caudifer* (n=1, nectarivorous) and *Phylostomus discolor* (n=1, omnivorous bat). All species belong to the family Phyllostomidae. Geographical location of positive bats were Bahia state (n=6, Northeast region), Rio de Janeiro state (n=4, Southeast region) and Santa Catarina state (n=1, South region).

Conclusions: In this study, we reported the presence of hantavirus antibodies in six species of Neotropical bats with different feeding habits. Curiously, recent studies have identified all novel hantaviruses only in insectivorous bat species in the Old World. Genetic and phylogenetic studies are needed to identify the infecting hantavirus lineage(s) hosted by these bats. Brazil has a great diversity of bat species (> 178 spp.) and ecosystems, making these animals a new target for investigations in the hantaviruses field, in the near future.

51. **Hantavirus Pulmonary Syndrome mimicking dengue in Rio de Janeiro, Brazil.** Renata Carvalho de Oliveira[1], João Bosco Júnior[2], Alexandro Guterres[1], Jorlan Fernandes[1], Liana Strecht Pereira[1], João Marcos Penna[2], Reynaldo de Jesus Oliveira Júnior[2], Cristina Giordano Dias[3], Stefan Vilges de Oliveira[4], Elba Regina Sampaio de Lemos[1]. [1]Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, Brazil. [2]Rio Claro Health Department, Rio de Janeiro, Brazil. [3]Rio de Janeiro State Health Department, Rio de Janeiro, Brazil. [4]Surveillance Service in Health, Ministry of Health, DF, Brazil. E-mail: reoliveira@ioc.fiocruz.br

Objective: Hantavirus Pulmonary Syndrome (HPS) diagnosis can easily be missed if not suspected. Early recognition of the disease, based on early signs and symptoms, is not easy to perform and can be confused with other prevalent diseases endemic in Brazil, such as dengue fever, leptospirosis, rickettsiosis and influenza. This case report described the first human case of HPS in Rio de Janeiro State, where the clinical picture was interpreted as dengue fever.

Methods. A 34-years old male patient, previously asymptomatic, presented to public hospital in the municipality of Rio Claro, state of Rio de Janeiro with a 4-day history of fever, headache, arthralgia and myalgia. He was managed with oral fluid replacement and symptomatic medication. Two days later, the patient returned, complaining of persistent fever associated with dyspnea nausea and vomiting. When admitted, his leucocyte count was 4000/mm3; platelets 52,000/mm3; hematocrit 46.0%; alanine aminotransferase 121 U/l; aspartate aminotransferase 48 U/l. Electrolytes, blood glucose and renal function were normal. Twenty-four hours after admission, the patient rapidly progressed to respiratory failure and shock. Cardiopulmonary resuscitation failed, and the patient died. A serum sample was collected and evaluated for hantavirus by serologic and PCR testing.

Results. ELISA result was positive for IgM against recombinant nucleocapsid protein (rN) of the Juquitiba virus (JUQV). Viral genome was detected by reverse transcription PCR, and the virus was identified as JUQV. Non-structural protein 1 (NS1), and IgM tests for dengue were negative. We also obtained and tested serum samples from 35 healthy residents from patient`s workplace and neighboring residences. IgG against hantavirus (rN-JUQV) was detected in four samples, with a seroprevalence rate of 11.2%.

Conclusion. The circulation of a pathogenic hantavirus emphasizes the need of additional local studies to define the risk areas of human disease and, HPS should be included as a differential diagnosis for patients with febrile illness and acute respiratory distress, even in non-endemic areas.

52. **Phylogeny and Origins of Arena, Bunya and Filoviruses: Highlighting their relationships.** Alexandro Guterres[1], Renata Carvalho de Oliveira[1], Jorlan Fernandes[1], Elba Regina Sampaio de Lemos[1], Carlos Guerra Schrago[2]. [1]Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, Brazil. [2]Federal University of Rio de Janeiro, Rio de Janeiro, Brazil. E-mail: guterres@ioc.fiocruz.br

Objective: The evolutionary history of viral genomes is a classical problem that has inspired a long series of questions and hypotheses in evolutionary biology. Recently, sequence analyses of emerging viruses evidenced that genes from arenaviruses share some homology with other virus such as bunyaviruses and filoviruses. In this study, we explore key aspects of these viruses evolution, particularly their phylogenetic relationships.

Methods: The genomic sequences of the family *Arenaviridae*, *Bunyaviridae* and *Filoviridae* used were retrieved from GenBank. Multiple sequence alignment was performed using TCOFFEE, MUSCLE and CLUSTAL, in the JALVIEW software. Phylogenetic relationships of the three major open reading frames (L protein, glycoprotein and nucleoprotein) were estimated using (a) Maximum likelihood (ML), Bayesian MCMC method available in the MrBayes and BEAST package. Because the MCC tree is automatically rooted on the assumption of a molecular clock it enables determination of which viral lineages are most likely to be basal. The basal clade estimated by the BEAST tree was used as an outgroup to root the phylogenetic trees inferred under the ML and Bayesian phylogenetic analyses.

Results: The phylogenetic inference based on L protein sequences confirmed the existence of two distinct monophyletic clades. The first clade is comprised by two well-supported phylogroup, one including the genus Hantavirus and the Filoviridae family and the second phylogroup, highly supported, comprised the genus Orthobunyavirus and Tospovirus. The second clade contained the genus Phlebovirus, occupying a basal position in the clade, followed by Nairovirus and Arenaviridae family branched in a well-supported monophyletic subcluster. The ML and BEAST trees produced highly similar topologies. The glycoproteins sequences have major size difference, according to each family. Through the evaluation of the alignments was possible to observe that the size difference impair the alignment of the glycoprotein.

Conclusions: The recent data reinforce the idea that Arena-, Bunya, and Filovirus are evolutionarily and genetically interconnected. The genus *Hantavirus* has a strong phylogenetic relationship with the family *Filoviridae* and the genus *Nairovirus* with the family *Arenaviridae*. Interestingly, the data suggest that the family *Bunyaviridae* is not monophyletic.

53. **Serological Evidence of Hantavirus Infection in an Indigenous Reserve in the State of Mato Grosso, Brazil.** Ana Cláudia Pereira Terças[1,2], Leonir Evandro Zenazokenae[1], Vagner Ferreira do Nascimento[1], Thalise Yuri Hattori[1], Ariadne Cristinne Pereira de Moura[1], Liana Strecht[2], Renata Carvalho de Oliveira[2], Mariano Martinez Espinosa[3], Marina Atanaka Santos[3], Alba Valéria Gomes de Melo Via[4] and Elba Regina Sampaio de Lemos[2]. [1]University of the State of Mato Grosso, Tangará da Serra - MT. Foundation for the State of Mato Grosso Research - FAPEMAT. [2]Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro - RJ. [3]Federal University of Mato Grosso, Cuiabá - MT. [4]Department of Health of Mato Grosso, Cuiabá - MT. E-mail:ana.claudia@unemat.br

OBJECTIVE: The purpose of this study was to identify the presence of hantavirus infection among 327 indigenous of the ethnic group Haliti-Paresí in the Utiariti Indian Reserve, middle northern of the Mato Grosso state, where 75% of Hantavirus Pulmonary Syndrome (HPS) cases have been notified since 1999.

METHODS: A seroprevalence study was conducted with the Indian population living in nine villages of Campo Novo do Parecis, Mato Grosso state. Serum samples were collected from 210 and 91 healthy Indian population in 2014 and 2015, respectively and tested to detect IgG antibodies against the recombinant N protein of hantavirus Araraquara, by ELISA.

RESULTS: Araraquara-specific antibodies were detected in 23 Indians, which represents an overall seroprevalence of 7.6%. Age varied from 8 to 79 years-old with mean age of 30.1 years. The frequency was higher in the older group and similar between man and woman. Concerning epidemiological aspects, 73.9% reported having contact with rodent indoors. Thirteen individuals have no symptoms 60 days before data collection, compared to others 10 Indians who had myalgia, fever, headache, back pain, asthenia, diarrhea, dyspnea and abdominal pain.

CONCLUSION: We report here a prevalence of 7.6%, similarly to a previous study conduct in the Enawene-Nawe ethnic group from the same state (8%). Although the prevalence observed in these Brazilian Indian populations have been less than that identified in other studies carried out in Indians in the American continent, the result reinforces the importance of the knowledge concerning the geographical distribution and prevalence of this zoonotic disease in Indian reserve areas in the Mato Grosso state.

54. **Hantavirus Seroprevalence in Gold-Mining Area in Mato Grosso, Brazil.** Ana Cláudia Pereira Terças[1,2], Elaine Cristina de Oliveira[3,4], Cor Jesus Fernandes Fontes[3], Rafael Gomes da Silva[2], Renata Carvalho de Oliveira[2], Marina Atanaka Santos[3], Elba Regina Sampaio de Lemos[2]. [1]University of the State of Mato Grosso, Tangará da Serra - MT. Foundation for the State of Mato Grosso Research - FAPEMAT, MT, Brazil. [2]Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro - RJ, Brazil. [3]Federal University of Mato Grosso, Cuiabá - MT, Brazil. [4]Department of Health of Mato Grosso, Cuiabá - MT, Brazil. E-mail:ana.claudia@unemat.br

OBJECTIVE: This study was carried out to determine the presence of antibodies anti-hantavirus in a gold-mining area in the Amazon region of Mato Grosso state in Brazil.

METHODS: This seroprevalence study was conducted in a hyper-endemic area for malaria in the municipality of Coloniza, Mato Grosso state during the period of 2012 and 2013. Blood samples were collected from people who working in the gold-mining area and sera were tested for hantavirus antibodies (IgG) by enzyme-linked immunosorbent assay (ELISA), using recombinant hantavirus N protein Araraquara (rN ARAV) as antigen.

RESULTS: Among the 112 people who working in the gold-mining area and were included in this study, 56.25% were men. The age varied from six months to 65 year-old with mean age of 29 years. Four (3,6%) of the 112 patients were found to have antibodies against ARAV; three women (16, 24 and 52 years-old) and one 31-year-old male patient. One of these reactive patients was malaria positive (Plasmodium falciparum).

CONCLUSION: This result highlights the importance of considering hantavirus pulmonary syndrome in the differential diagnosis of malaria in the Mato Grosso state where this viral zoonosis has been reported since 1999 and fatal cases of unknown etiology have been described.

55. **A non-fatal case of hantavirus cardiopulmonary syndrome imported into the UK (ex-Panama), July 2014.** Barry Atkinson[1], Lisa J. Jameson[1], Begoña A. Bovill[2], Emma J. Aarons[3] Jodie Clewlow[3], Sarah Lumley[1], Jennie Latham[1], Megan H. Jenkins[2], Alasdair P. MacGowan[2], Andrew J. Simpson[3], Javeed Ahmed[4], Timothy J. Brooks[3,5] and Roger Hewson[1,5]. [1]Research Department, Microbiology Services Division, Public Health England, Porton Down, Salisbury, United Kingdom. [2]North Bristol National Health Service Trust, Bristol, United Kingdom. [3]Rare and Imported Pathogens Laboratory, Microbiology Services Division, Public Health England, Porton Down, Salisbury, United Kingdom. [4]Public Health Laboratory Bristol, Bristol, United Kingdom. [5]National Institute for Health Research, Health Protection Research Unit in Emerging and Zoonotic Infections, Liverpool, United Kingdom. barry.atkinson@phe.go.uk

Objectives: In July 2014, a case of hantavirus cardiopulmonary syndrome (HCPS) was diagnosed in a UK resident returning from Panama. The patient had recently returned from a nine month stay in the Los Santos province of Panama with her partner who worked locally as a rice farmer. They lived in a rural area

and, in the weeks prior to her return, she had swept out a shed that had evidence of rodent infestation and handled packaged horse feed which had evidence of rodent damage.

Methods: On 13th July, while in Panama, she developed fever, myalgia, lethargy, headache and rigors. After return to the UK, symptoms progressed to include vomiting, cough, rapidly progressive dyspnoea and chest tightness. The clinical course of disease was typical for HCPS and matched well with the limited data available for human infection with Choclo hantavirus (CHOV), a species of hantavirus known to circulate in Panama and suspected of causing several cases of severe human disease in the Los Santos region of Panama during 2014. Samples were sent to the rare and Imported Pathogens Laboratory at Public Health England (Porton Down, UK) for confirmation.

Results: Serological analysis identified increasing titres of hantavirus IgG antibodies over the course of illness and hantavirus RNA was detected in serum taken seven days after onset of symptoms. Sequence analysis following a pan-hantavirus diagnostic assay confirmed the presence of Choclo hantavirus RNA. A set of RT-PCR, real-time RT-PCR and sequencing assays were designed to characterise the S segment of the CHOV genome.

Conclusion: This represents the first confirmed identification of the causative agent from the ongoing HCPS outbreak in Panama and only the third documented importation of HCPS in to Europe. Novel CHOV-specific assays designed can be used for rapid identification and characterisation of future cases.

56. **Molecular characterization of hantaviruses circulating in Serbia in five years period.** Gorana Stamenkovic[1], Valentina Cirkovic[2], Marina Siljic[2], Bojana Bozovic[1], Ana Gligic[1], Maja Stanojevic[2]. [1]Department of Genetics, Institute for biological research “S. Stankovic”, University of Belgrade, Belgrade, Serbia. [2]Department of microbiology, Faculty of Medicine, University of Belgrade, Belgrade, Serbia. [3]National Center for Arboviruses and HF viruses, Institute of Virology, Vaccines and sera - Torlak, Belgrade, Serbia. Email: gorana.stamenkovic@ibiss.bg.ac.rs.

Objective: Hemorrhagic fever with renal syndrome (HFRS) is known to be present in Serbia since mid 20th century, whereas first hantavirus isolates from Balkan peninsula originate from human cases in Serbia dating from the eighties of the last century. Although known to be endemically present, molecular genetic studies of hantaviruses in Serbia, up to now, have been very scarce. The purpose of the present investigation was to determine the genetic diversity of obtained viral isolates in Serbia during the period 2007–2011.

Methods: Blood samples of HFRS patients previously determined to be positive for anti-hantaviral antibodies by indirect immunofluorescence reaction and by ELISA test were used in the study. Immune positive sera were analysed by nested RT-PCR protocols using degenerate primers that amplify partial L and S segment following by direct nucleotide sequenced. Phylogenetic analysis was performed on all newly acquired sequences. Sequence alignments, for both partial L and S segments, were accomplished by using MEGA 5.1 software package. Sequences aligned by CLUSTAL W were examined with jModeltest 2.1.7 software to determine the best fit nucleotide substitution model. Phylogenetic analysis was performed using PAUP version 4.0b and PHYML 3.0 software packages using the maximum likelihood (ML) method with 500 bootstrap replicates.

Results: During the studied period 2007-2011, 113 human sera were reported seropositive to anti-hantaviral antibodies by the National reference laboratory for viral hemorrhagic fevers and ARBO viruses. Of these, 64 sera were accessible for PCR analyses. Of the total number of tested sera, 18/64 were found RT-PCR positive for the L segment and 11/64 for the S segment. Nucleotide sequences of 346 bp fragment of L segment differ by 0.3% to 7.4% and for 494 bp of S segment differences were from 0.6% to 4.1%. Phylogenetic analysis of the obtained hantavirus L and M genome segment identified all the recovered hantavirus genomes as DOBV.

Conclusions: Our results confirm DOBV as the main causative agent of HFRS in Serbia.

57. **Human hantavirus infections in the Czech Republic.** Hana Zelená[1], Jakub Mrázek[2]. [1]Institute of Public Health, Ostrava, Czech Republic. [2]University of Defence, Hradec Králové, Czech Republic.

Objectives: Identification of probable natural focuses of hantaviruses in the Czech Republic based on occurence of human hantavirus infections and their determination using sequencing analysis.

Methods: Patients suspected of hantavirus infection were serologically examined using the ELISA IgG and IgM assay (Euroimmun). Positive samples were further tested with Immunoblot assay (Euroimmun, Euroline Anti-Hanta Profile 1). RT-PCR home made assay with primers focused on L segment was used for molecular diagnostics. Both L and S segments were sequenced in positive samples.

Results: Between 2009-2015 acute hantavirus infection was serologically diagnosed in 29 patients. PCR was positive in 28 of them. Consecutive sequencing revealed in 14 patients infection caused by Dobrava/Belgrade virus - genotyp Dobrava, in 12 patients Dobrava/Belgrade virus– genotyp Kurkino, in 1 patient Tula virus and in the last one the sequencing was not successful.

Conclusions: It was found out that human hantavirus infections in the Czech Republic are predominantly caused by Dobrava/Belgrade virus, both genotypes Dobrava and Kurkino. Tula virus was found as causative agent in one case.

58. **Ultrastructural changes in gastric mucosa in hemorrhagic fever with renal syndrome.** Evseyev AN, Plekhova NG, Evseyeva AA. Far Eastern State Medical University, Khabarovsk. Central Research Laboratory, Pacific Medical University, Vladivostok.

Objective. Morphological assessment of changes in gastric mucosa (GM) at an acute stage of HFRS and relationship between the changes and the antigen availability in different cells.

Materials. Gastric biopsy samples of eleven HFRS patients were taken for gastroduodenal disorders in 6 to 14 days from the onset of clinical manifestation of HFRS.

Methods. Immunofluorescence and confocal laser scanning microscopy and immunoelectron microscopy of biopsy samples were used. Gastric biopsy samples were studied with electron microscope JEM-100S (JEOL, Japan).

Results. Gastroscopy of HFRS patients showed punctate hemorrhage, acute erosions, and ulcers in gastric body, cardia and antrum. With light microscopy, degenerative changes and necroses of superficial epithelial structures and focal stromal hemorrhage were detected. Confocal microscopy revealed specific coarse-granular luminescence in GM capillary epithelium and endothelium, which was indicative of Hantavirus antigen. Electron microscopy of gastric biopsy samples revealed degenerative changes in mucocytes. Positive response to Hantavirus antigen was observed in macrophage type cells. Mucocyte and macrophage cytoplasm presented with solid viroplasts, double-layer membrane structures as well as laminar structures. Cytoplasm of chief and parietal cells also presented with solid viroplasts, double-layer membrane structures, laminar and tubular structures, increased number of lysosomes and autophagosomes.

Conclusion. Our research has shown that GM at the acute stage of HFRS presents with prevalence of alterative ultrastructural changes in cells combined with signs of focal hyperplasia of gastric superficial foveolar epithelium and incipient shift of epithelial differentiation towards mucocyte, secondary to the profound epithelial proliferation combined with poor differentiation of highly specialized cells (chief and parietal cells) were revealed. Ultrastructural virus-specific inclusions in the cells were found and dependence thereof on the adaptive rearrangement of gastric mucosa was established.

59. **Evolutionary history of Puumala Virus : analyse and comparison of different ancestral states inference methods.** Guillaume Castel[1,2], François Chevenet[3,2] and Olivier Gascuel[4,2]. [1]INRA, UMR 1062 CBGP, F-34988 Montferrier-sur-Lez, France. [2]Institut de Biologie Computationnelle (IBC), 34095 Montpellier, FRANCE. [3]UMR MIVEGEC, IRD, Montpellier, France. [4]Centre de Bioinformatique, Biostatistique et Biologie Intégrative (C3BI), Institut Pasteur, Paris, France.

Understanding the phylogeography of pathogenic viruses (their origins, evolutionary history, and factors shaping their actual spatial distributions) is particularly important on the epidemiological point of view to predict their spread and to improve assessment of emergence risks. Last years, phylogeography has expanded rapidly triggered by important methodological advances but the challenge is still to test different evolutionary scenarios and to make correct inferences of the mechanisms of spread of these pathogens based on the shape of the phylogenies obtained from collections of sequences. Puumala hantavirus (PUUV) is found across Europe where it is one of the main viruses responsible for clinical hantavirus infections in Europe. To date, eight PUUV lineages have been described in Eurasia. PUUV is transmitted by the bank vole, Myodes glareolus which is widespread in Europe. Evolution of all hantaviruses is strongly linked to phylogeography (Bennett 2014). As for other hantaviruses, the evolutionary history of PUUV is quite controversial and has mosltly been studied at the regional level, most often from small data sets (Asikainen et al 2000, Sironen et al 2001, Plyusnina et al 2001) of partials sequences. Recently, the phylogeography of the genus Hantavirus have been revisited with the last phylogeographical methods (Bennett 2014). In this study we focus on the dynamics and the evolutionary history of viral populations of PUUV at the European level at the light of the recent acquisition of new sequences of complete segments of PUUV and by using the most recent methods in phylogeny and in ancestral states reconstruction. We are using PAMELA, a new software (in development), to objectively analyse the results of a Bayesian ancestral states inference method (BEAST) and to compare it with the results of Maximum Likelihood and/or Maximum Parcimony methods implemented in PAMELA. The explanatory potential will be improved by combining these different approaches with different resolution power and should help to design an overall analytical framework focused on biologically-relevant scenarios. This study underline some uncertainty in the evolutionary history of PUUV which could be a starting point for future sampling in neglected areas.

60. **Complete genome, phylogeny and diversity of Puumala hantavirus isolates circulating in France.** Guillaume Castel[1], Mathilde Couteaudier[2], Elodie Monchatre-Leroy[3], Frank Sauvage[4], Franck Boué[3], Jean-Baptiste Pons[4], Adélaïde Dubois[5], Séverine Murri[5], Angelina Plyusnina[6], Jean-François Cosson[7], Dominique Pontier[4], Nathalie Charbonnel[1], Alexander Plyusnin[6], Philippe Marianneau[5] & Noël Tordo[8]. [1]INRA - UMR 1062 CBGP, F-34988 Montferrier-sur-Lez, France. [2]INRA - UR1282, Biologie Virus Aviaire, 37380 Nouzilly, France. [3]ANSES - Laboratoire de la rage et de la faune sauvage, Domaine de Pixérécourt - CS 40009 - 54220 Malzéville, France. [4]CNRS - Université Lyon 1, Laboratoire de Biométrie et Biologie Evolutive (UMR5558), F-69622 Villeurbanne, France & LabEx ECOFECT Ecoevolutionary Dynamics of Infectious Diseases, Lyon, France. [5]ANSES - Laboratoire de Lyon, Unité Virologie, 31 avenue Tony Garnier 69007, Lyon, France. [6]Department of Virology, University of Helsinki, Finland. [7]INRA - Bipar, 23 Av. Général de Gaulle, Maisons-Alfort, France. 8Antiviral Strategies Unit, Institut Pasteur, 25 rue du Dr. Roux, 75724 - Paris - France.

Puumala hantavirus (PUUV) is hosted by the bank vole, *Myodes glareolus*, and causes Nephropathia epidemica (NE), a mild form of haemorrhagic fever with renal syndrome (HFRS). Although HFRS is prevalent throughout Europe, clear variations in the incidence of reported human cases are observed at the regional scales. If the presence of infected rodents is necessary for human contamination, strict correlation between the number of human cases and number of infected rodents is not so clear. Thus, in France high PUUV seroprevalence and viral load in bank vole population have yet been observed in areas with no reported human cases (peri-endemic and non-endemic areas). In order to clearly establish the current circulation of PUUV in France and to investigate the genetic diversity of its isolates, trapping was done in several regions between 2009 and 2014 (Ardennes, Jura, Alsace, Orléans, Troyes). Bank voles were tested for IgG ELISA serology and seropositive animals were then subjected to sequence analysis of the complete S segment (Alsace, Troyes, Orléans, Jura) or of the complete genome (Ardennes, Jura, Orléans) of the infecting PUUV. Phylogenetic analyses revealed that French PUUV isolates globally belong to the Central European lineage. However, isolates from Ardennes are clearly distinct from those of the other French regions and there is a strong spatial structure between the different regions. Sequence analyses revealed significant differences at the genetic level and specific amino acid signatures along the N protein that discriminates regional variants. Detailed phylogeographic analyses are performed to precisely estimate the dynamics and the evolutionary history of viral populations of PUUV at the European level and notably to reconstruct the precise origins of the French lineages.

61. **Epidemiology and Emergence of Hantaviruses in Georgia.** Giorgi Babuadze, Nana Mamuchishvili, Giorgi Chakhunashvili, David Tsereteli, Tamar Chikviladze, Marina Chubinidze. National Center for Disease Control and Public Health, Tbilisi, Georgia.

Objective: Determine the prevalence of hantavirus infections in Georgia

Methods: Following the first report of a suspected lethal hantavirus infection in Western Georgia in 2009, the National Center for Disease Control and Public Health conducted an epidemiological investigation. A case definition for hantavirus infections was developed, which was based on the U.S. Centers for Disease Control and Prevention case definition. A standard questionnaire to find an epidemiological link to exposure or source of infection was used. Specific anti-hantaviral IgM and IgG ELISA was performed for laboratory confirmation.

Results: The first patient (lethal case) was referred to the ambulatory hospital in May, 2009 with a 1-month history of fever, abdominal pain and cough and symptomatic self-treatment. An initial diagnosis of bilateral pneumonia was made. The patient died on the same day of admission. Clinical samples were not available. The household included 9 members (including the lethal case) (3 males, 6 females) ranging in age from 6 years to 70 years. All of them were determined to have had exposure to rodents. In 2012, 6 hantavirus cases were registered: 1 case in the Guria region, 1 case in the capital city Tbilisi, and 4 cases in a family in the village Perevi, in NW Georgia. In the family cases, the mother and 1 child were hospitalized due to the severe pulmonary symptoms, while the father and the other child developed mild symptoms. All 4 patients were hantavirus IgM positive. All 4 patients recovered after anti-viral therapy. In 2009-2015 a total of 27 hantavirus laboratory confirmed cases were registered in different parts of Georgia including 4 lethal cases. One of the lethal cases was a citizen of Denmark who had been traveling in Georgia for 10 days before becoming ill. According to the PCR results performed in a Danish hospital, the patient sample was positive for the hantavirus, Dobrava virus.

Conclusion: Since 2009, hantavirus cases have increased in Georgia, which is likely associated with improved surveillance. The emergence of hantaviruses indicates a need for continued epidemiological investigations as well as studies of the ecology of the viruses in wild rodents.

62. **Development of minireplicon systems for Tula and Dobrava hantaviruses.** Kirill Nemirov[1], Alexander Plyusnin[2], Åke Lundkvist[3], and Noël Tordo[1]. [1]Antiviral Strategies Unit, Virology Department, Institut Pasteur, Paris, France. [2]Department of Virology, University of Helsinki, Haartman Institute, Helsinki, Finland. [3]Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden.

Hantaviruses (genus *Hantavirus*, family *Bunyaviridae*) are rodent-borne viruses that cause two severe and often fatal human zoonoses: hemorrhagic fever with renal syndrome (HFRS), and hantavirus cardiopulmonary syndrome (HCPS). For many years research on these pathogens was hampered by the lack of a reverse genetics system (RG). Such systems, developed for other RNA viruses, may be

extremely useful for dissecting the basis of viral pathogenesis, generation of live-attenuated vaccines, and testing of antivirals. Although several reports claiming the development of minireplicons (MR) for hantaviruses were published, these MRs appeared to have very limited capacity and have not been developed for practical use. We are currently exploring different approaches in order to develop functional MR and rescue systems for non-pathogenic Tula hantavirus and highly pathogenic Dobrava hantavirus. Current progress of the work as well as specific issues related to adaptation of RG approaches to hantaviruses will be presented.

63. **Extracorporeal life support for severe hantavirus cardiopulmonary syndrome.** Tomicic V., Graf J., Umaña A., Abarca J., López R., Howard M., Castillo R., Cisterna S., Vial P. Departamento de Paciente Crítico, Clínica Alemana de Santiago, Santiago, Chile.

INTRODUCTION. Hantavirus Cardiopulmonary Syndrome (HCPS) has a mortality rate between 30-40%. A combination of refractory shock and severe acute respiratory distress leads to death in severe cases. Extracorporeal life support (ECLS) supports circulation and gas exchange while patients recover. The largest case series reports a survival rate of 60% in 51 HCPS patients supported with ECLS (1994-2010, New Mexico, US).

METHODS. We report 8 cases of severe HCPS supported with ECLS between 2001 and 2015 in a single center in Chile. ECLS connection criteria were: Cardiac index < 2.5 L/min/m2 under maximal vasoactive support and lactate > 4 mmol/L or PaO2/FiO2 ratio < 60.

RESULTS. Two patients died, one due to refractory shock and the other due to sepsis. One patient developed severe ischemia of the lower limb with large muscle mass loss; subsequently all patients have received direct perfusion of the limb under ischemic threat ever since. Two patients had large femoral hematomas, one of them with severe superinfection. Two patients required cholecystectomy due to acalculous cholecystitis. One patient developed recurrent hepatic bleeding and hemoperitoneum that required hepatic arterial embolization.The survival rate was 75%. All survivors returned to their prior activities after extensive rehabilitation.

CONCLUSIONS. ECLS allowed rescue of 75% of severe HCPS patients. Severe HCPS patients treated with ECLS have a high complication rate that protracts their hospital stay and rehabilitation process.

64. **Hantavirus infection confers resistance to Natural Killer cell-mediated killing and activates Natural killer cells through IL-15/IL15Rα expression.** Shawon Gupta[1,2], Carles Sola Riera[2], Monika Braun[2], Nicole Tischler[3], Malin Stoltz[1], Karin Sundström[1], Niklas Björkström[2], Hans-Gustaf Ljunggren[2] and Jonas Klingström[2]. [1]Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, 171 77 Stockholm, Sweden. [2]Center for Infectious Medicine, Department of Medicine, Karolinska Institutet, Karolinska University Hospital Huddinge, 141 86 Stockholm, Sweden. [3]Fundación Ciencia & Vida, 778 0272 Santiago, Chile.

Objective: Endothelial cells are the main targets for hantaviruses and vascular permeability is a hallmark of HFRS/HCPS. An intriguing observation in patients with HFRS/HCPS is that the virus infection leads to strong activation of cytotoxic lymphocytes, CD8 T cells and Natural Killer (NK) cells, but no obvious destruction of infected endothelial cells. Here, we provide a possible explanation for this dichotomy.

Methods: Primary NK cell were isolated and used to kill target cells *in vitro*. NK cells were co-incubated with hantavirus-infected cells and the NK cell activity was measured.

Results: Hantavirus-infected endothelial cells are protected from NK cell-mediated killing. This protection was attributed to inhibition of granzyme B and caspase 3 by the viral nucleocapsid protein. Next, we sought to explain the strong NK cell activation. Hantavirus-infected cells were shown to strongly activate NK cells in a cell-cell contact-dependent way, this was due to virus-induced IL-15 and IL-15Rα on infected cells and the response was blocked with anti-IL-15 antibodies. A consequence of this IL-15-dependent NK cell response was that it led to killing of uninfected cells despite expression of normal levels of HLA class I. Our findings provide a tentative explanation for the hantavirus-mediated block of cytotoxic granule-mediated killing, and hence the protection of infected cells from cytotoxic lymphocytes.

Conclusion: Taken together, hantavirus-infection protected cells from NK cell-mediated killing and hantaviruses activated NK cells and these NK cells killed uninfected cells Thus, our data add further insights into mechanisms behind the immunopathogenesis of hantavirus infections in humans.

65. **Spatial diffusion of Nova virus, a divergent hantavirus harbored by the European mole, in Belgium.** Lies Laenen[1], Simon Dellicour[2], Valentijn Vergote[1], Inne Nauwelaers[1], Sarah De Coster[1], Ina Verbeeck[1], Marc Van Ranst[1], Philipphe Lemey[2], Piet Maes[1]. [1]KU Leuven - University of Leuven, Department of Microbiology and Immunology, Laboratory of Clinical Virology, Rega Institute for Medical Research, Leuven, Belgium. [2]KU Leuven - University of Leuven, Department of Microbiology and Immunology, Laboratory of Evolutionary and Computational Virology, Rega Institute for Medical Research, Leuven, Belgium

The last decade, genetically distant hantaviruses have been discovered in *Soricidae*, *Talpidae* and *Chiroptera.* While these insectivore-borne hantaviruses provided new evolutionary insights, little is known about their possible pathogenicity or the relationship with their natural hosts. Mole-borne hantaviruses

have played a substantial role in hantavirus evolution, with several hantaviruses jumping host species boundaries. In this study we characterized Nova virus (NVAV), a hantavirus detected in the European mole (*Talpa europaea*) in 2008, in order to better resolve its origins, dispersal and host relationship. Phylogenetic analysis of the complete Nova virus genome revealed a close relationship with bat-borne hantaviruses for all 3 segments, indicating its involvement in cross-species transmission events. Screening of the renal tissue of 479 moles resulted in a Nova virus positivity rate of 53.2%. Positive samples covered the entire sampling area, indicating a widespread distribution and high transmission efficiency of NVAV. To further investigate Nova virus dispersal into the European mole population, the complete sequence of the S segment of 100 samples, evenly distributed across Belgium, has been determined. A continuous Bayesian phylogeographic reconstruction demonstrated the correlation between geographical location and dispersal time. Our dataset could be split up into 2 distinct clades, separating samples from East and West Belgium. Additionally, the influence of landscape heterogeneity on Nova virus diffusion in the mole population was investigated. Our results indicate that main waterways do not act as an environmental resistance factor, slowing down virus dispersal. While more research is needed to elucidate its possible pathogenicity, it appears that Nova virus is well adapted to its mole host.

66. **Cytokine and chemokine kinetics and their potential as biomarkers in patients with Puumala virus infection.** Petra Svoboda*[1], Lidija Cvetko Krajinović*[1], Petra Čikeš[1], Antea Topić[1], Martina Bosnar[2], Vesna Eraković Haber[2], Davor Jugović[1] and Alemka Markotić[1]. [1]University Hospital for Infectious Diseases “Dr. Fran Mihaljević”, Zagreb, Croatia; *equal contributions; e-mail: psvoboda@bfm.hr. [2]Fidelta Ltd, Zagreb, Croatia

Objective: Hemorrhagic fever with renal syndrome (HFRS) is a viral disease caused by hantaviruses, however, it is primarily considered as an immune-mediated disease. Several studies have shown deregulated pro- and anti-inflammatory cytokine/chemokine production during the acute phase of hantavirus infection in sera of HFRS patients. Monocytes/macrophages are important immune cells since they are producing different cytokines/chemokines and are considered one of the target cells for hantaviruses. The aim of our study was to analyze the monocytes/macrophages related cytokines/ chemokines profile in sera of HFRS patients infected with Puumala virus (PUUV) according to the severity of their clinical picture. Also, looking at the correlation with some clinical parameters we would like to determine possible cytokines/chemokines as the useful biomarkers for the HFRS prognosis.
Methods: The study included 34 HFRS patients with a serologically confirmed PUUV diagnosis, hospitalized in 2014 in Zagreb, Croatia. Patients' serum samples were taken in two acute time points – upon arrival at the hospital and before discharge. The level of PUUV viral load was detected. For analysis of cytokine and chemokine levels Luminex technology of (multiplexed) immunoassay with magnetic beads was used for 16 analytes, namely: IL-1β, IL-1RA, IL-12(p70), IL-15, IL-18, IL-23, IL-27, MIF, CCL2, CCL3, CCL4, CCL5, CCL7, CCL20, CXCL12a, TGF-β1.
Results: The results showed in general, suppression of the early immune response to PUUV i.e. some pro-inflammatory cytokines were down-regulated or absent (IL-1β, IL-12(p70), IL-23). CCL4, IL-1RA, IL-18 and MIF were elevated in both phases compared to the controls, while CCL5 was downregulated in the first and upregulated in the second phase. In opposite, low levels of IL-15 were recorded in the first serum and not in the second serum sample. Some differences in the measured cytokines/chemokines were detected in patients with mild in comparation to the patients with moderate clinical picture and correlation with several clinical laboratory parameters were observed as well.
Conclusions: Some differences in the levels of monocytes/macrophages related cytokines/chemokines in HFRS patients with mild or moderate clinical pictures can be useful biomarkers for the prognosis of the HFRS clinical picture.

67. **Hantavirus and Mammarenavirus infection in a single rodent host in in the area influenced by construction of Hydroelectric Power Plant in Brazil.** Jorlan Fernandes, Renata Carvalho de Oliveira, Alexandro Guterres, Thayssa Alves Coelho, Cibele Bonvicino, Paulo Sérgio D’Ándrea, Elba Regina Sampaio de Lemos. Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, Brazil.

Objective: Deforestation and habitat destruction are one of the main causes of the emergence or re-emergence of infectious diseases. Particularly, the construction of hydroelectric power plants may cause a diverse array of problems to local people and biodiversity, including forest fragmentation, biodiversity loss and disease emergence. In this study, we investigated Hantavirus and Mammarenavirus infection on wild rodents collected in the influence area of a small hydroelectric power plant (SHPP) in Midwestern Brazil.
Methods: During an eco-epidemiological study performed during the implementation of the SHPP Mambaí, in the Municipality of Cassilândia, Mato Grosso do Sul, in 2008, 142 rodents were captured. The genomic RNA was extracted from liver tissue samples following RT-PCR using as target the nucleoprotein and glycoprotein precursor, from hantavirus and mammarenavirus, respectively. Additionally, we conducted the genetic characterization of the amplified DNA obtained from positive rodents. **Results**: Two rodents of the species Oligoryzomys matogrossae were positive for hantavirus and one of this were also positive for mammarenavirus. Sequences obtained from the two rodents showed high similarity with Juquitiba virus (JUQV) a highly pathogenic hantavirus from endemic HPS areas in South America. The JUQV strains obtained from O. matogrossae samples, together with the all the sequences of other JUQV strains, form a well-supported clade (branch support = 1), with nucleotide diversity ranging from 0.3%–

12.7%. **Conclusion**: Infection of the same rodent by two rodent-borne viruses at the same time is a rare event. Here we found a single rodent infected with JUQV and an unidentified arenavirus in a highly-degraded area. Our results suggests that the negative effect of the SPPH Mambaí in the environment could be responsible for the infection of this two virus in the same rodent host, probably cause for an increase in the encounters of rodents species. More studies should be conducted in these areas in order to better understand the frequency of this event, and its implications on the dynamic and maintenance of the enzootic cycles.

68. **Development of minireplicon systems for Tula and Dobrava hantaviruses.** Kirill Nemirov[1], Alexander Plyusnin[2], Åke Lundkvist[3], and Noël Tordo[1]. [1]Antiviral Strategies Unit, Virology Department, Institut Pasteur, Paris, France, [2] Department of Virology, University of Helsinki, Haartman Institute, Helsinki, Finland, [3] Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden.

Hantaviruses (genus Hantavirus, family *Bunyaviridae*) are rodent-borne viruses that cause two severe and often fatal human zoonoses: hemorrhagic fever with renal syndrome (HFRS), and hantavirus cardiopulmonary syndrome (HCPS). For many years research on these pathogens was hampered by the lack of a reverse genetics system (RG). Such systems, developed for other RNA viruses, may be extremely useful for dissecting the basis of viral pathogenesis, generation of live-attenuated vaccines, and testing of antivirals. Although several reports claiming the development of minireplicons (MR) for hantaviruses were published, these MRs appeared to have very limited capacity and have not been developed for practical use. We are currently exploring different approaches in order to develop functional MR and rescue systems for non-pathogenic Tula hantavirus and highly pathogenic Dobrava hantavirus. Current progress of the work as well as specific issues related to adaptation of RG approaches to hantaviruses will be presented.

69. **Sequence variability of Puumala virus strain cg1820.** Szemiel, A.M., Vatipally, S., Wilkie, G., and Elliott, R.M. MRC - University of Glasgow Centre for Virus Research, 464 Bearsden Road, Glasgow, G61 1QH, Scotland, UK

Puumala hantavirus (PUUV), from the Bunyaviridae family, causes a relatively mild form of hemorrhagic fever with renal syndrome. It is the most common rodent-borne pathogen in Europe. The natural host of PUUV is the bank vole, Myodes glareolus. The hantavirus genome consists of three single stranded RNA segments of negative sense polarity: the L segment, which codes for the RNA-dependent RNA polymerase, the M segment, where glycoproteins Gn and Gc are encoded, and the S segment, which encodes N protein, and sometimes contains an overlapping NSs ORF. Open reading frames within each segment are flanked by untranslated regions (UTRs). As the UTRs are considered to be conserved within each genus, most publications use consensus oligonucleotide primers for amplification and Sanger sequencing of all the segments. In this study we have demonstrated by RACE analysis that the PUUV UTRs are different to those reported in the databases. We have also used virion RNA from two independently prepared stocks of PUUV cg1820 strain for deep sequencing. The resulting reads were mapped to our reference genome of PUUV cg1820. Deep sequencing analysis showed that our stocks are a mixture of substrains. Although there was no variability in the M segment, we have observed variability in the L and S segments. In approximately 40% of reads that mapped to L segment we observed an A521V polymorphism. We also found variability in the S segment 3' UTR at the position 20. Additionally in over 20% of reads there was E55K change in the N ORF, and W41stop polymorphism in the NSs ORF, which were caused by one point change in the S segment. The observed variability within the segments could be the obstacle in solving hantavirus reverse genetics. This study is the first deep sequencing analysis of the full-length genome of PUUV.

Attendees

First name	Last name	Email
Stephan	Aberle	virologie@meduniwien.ac.at
Clas	Ahlm	clas.ahlm@umu.se
Linda	Allen	linda.j.allen@ttu.edu
Satoru	Arai	arais@nih.go.jp
Jiro	Arikawa	j_arika@med.hokudai.ac.jp
Tatjana	Avsic Zupanc	tatjana.avsic@mf.uni-lj.si
George	Babuadze	gbabuadze@ncdc.ge
Catherine	Badger	catherine.v.badger.civ@mail.mil
Faezzah	Baharom	faezzah.baharom@ki.se
Xuefan	Bai	xfbai2011@163.com
Daniel	Bourquain	bourquaind@rki.de
Gavin	Braunstein	gavin.m.braunstein.civ@mail.mil
Rebecca	Brocato	rebecca.l.brocato.ctr@mail.mil
Guillaume	Castel	Nathalie.Charbonnel@supagro.inra.fr
Nathalie	Charbonnel	Nathalie.Charbonnel@supagro.inra.fr
Jan	Clement	jan.clement.dr@telenet.be
Lidija	Cvetko Krajinovic	lcvetko.krajinovic@gmail.com
Adelaide	Dubois	Nathalie.Charbonnel@supagro.inra.fr
Lesley	Dupuy	lesley.c.dupuy.ctr@mail.mil
Gillian	Eastwood	geastwoo@utk.edu
Fredrik	Elgh	fredrik.elgh@umu.se
Myriam	Ermonval	myriam.ermonval@pasteur.fr
Jorlan	Fernandes	jorlan.fernandes.j@gmail.com
Claudia	Filippone	cfilippone@pasteur.mg
Maxime	Galan	Nathalie.Charbonnel@supagro.inra.fr
Marina	Garcia	marinagarcia.ar@hotmail.com
Irina	Gavrilovskaya	irina.gavrilovskaya@stonybrook.edu
Felix	Geeraedts	f.geeraedts@labmicta.nl
Elena	Gorbunova	elena.gorbunova@stonybrook.edu
Ann	Hawkinson	ann.hawkinson@unco.edu
Heikki	Henttonen	Heikki.Henttonen@luke.fi
Roger	Hewson	roger.hewson@phe.gov.uk
Joerg	Hofmann	joerg.hofmann@charite.de
Jay	Hooper	jay.w.hooper.civ@mail.mil
Rommel	Iheozor-Ejiofor	rommel.iheozor-ejiofor@helsinki.fi
Rohit	Jangra	rohit.jangra@einstein.yu.edu
Colleen	Jonsson	cjonsson@utk.edu
Hiroaki	Kariwa	kariwa@vetmed.hokudai.ac.jp
Jeong-Ah	Kim	youminlove3@hotmail.com
Won-keun	Kim	wkkim1061@korea.ac.kr
Boris	Klempa	boris.klempa@savba.sk
Jonas	Klingström	jonas.klingstrom@ki.se
Misa	Korva	misa.korva@mf.uni-lj.si
Detlev H.	Krüger	detlev.kruger@charite.de
Ivan-Christian	Kurolt	ikurolt@bfm.hr
Lies	Laenen	lies.laenen@uzleuven.be
Jason	Lanman	jlanman@purdue.edu
James	Le Duc	jwleduc@utmb.edu
Seung-Ho	Lee	leeds1104@gmail.com

First name	Last name	Email
Dexin	Li	lidx@chinacdc.cn
Mifang	Liang	mifangl@hotmail.com
Schuyler	Liphardt	liphardt@unm.edu
Chaofeng	Ma	mark7447@xiancdc.cn
Erich	Mackow	erich.mackow@stonybrook.edu
Piet	Maes	pmaes3@uzleuven.be
Kimia	Maleki	kimia.maleki@ki.se
Joshua	Marceau	joshua.marceau@nih.gov
Philippe	Marianneau	philippe.marianneau@anses.fr
Alemka	Markotic	alemka.markotic@gmail.com
Lorraine	McElhinney	lorraine.mcelhinney@apha.gsi.gov.uk
Annabel	Meade	annabel.offer@ttu.edu
Greg	Mertz	gmertz@salud.unm.edu
Ellen	Murphy	hlemurph@student.liverpool.ac.uk
Jukka	Mustonen	jukka.mustonen@uta.fi
Nana	Nana Jules	africcare@gmail.com
Jin Sun	No	dybono@korea.ac.kr
Renata	Oliveira	reoliveira@ioc.fiocruz.br
Gustavo	Palacios	travel@genevausa.org
Anna	Papa-Konidari	annap.med@gmail.com
Richard	Peluso	r.peluso@euroimmun.us
Anna	Perez-Umphrey	Aperezumphrey@gmail.com
Casey	Perley	casey.c.perley.ctr@mail.mil
Douglas	Petty	pett7237@bears.unco.edu
Sandra	Quackenbush	sandra.quackenbush@colostate.edu
Martin	Raftery	martin.raftery@charite.de
Johan	Rasmuson	johan.rasmuson@umu.se
Jean-Marc	Reynes	jean-marc.reynes@pasteur.fr
Charlotte	Robin	charlotte.robin@liverpool.ac.uk
Barry	Rockx	barry.rockx@rivm.nl
Sylvia	Rothenberger	Sylvia.Rothenberger-Aubert@chuv.ch
Joel	Rovnak	joel.rovnak@colostate.edu
Kati	Ryynanen	kati.ryynanen@reagena.com
David	Safronetz	david.safronetz@phac-aspc.gc.ca
Matthias	Schade	matthiasschade.de@googlemail.com
Connie	Schmaljohn	connie.s.schmaljohn.civ@mail.mil
Günther	Schönrich	guenther.schoenrich@charite.de
Tyler	Sherman	tsherman11@gmail.com
Amy	Shurtleff	amy.c.shurtleff.ctr@mail.mil
Matthew	Simons	matthew.simons@stonybrook.edu
Megan	Slough	slough@mail.einstein.yu.edu
Anna	Smed-Sorensen	anna.smed.sorensen@ki.se
Darci	Smith	darci.r.smith.ctr@mail.mil
Carles	Sola riera	carles.sola.riera@ki.se
DongHyun	Song	swpia@kaist.ac.kr
Jin-Won	Song	jwsong@korea.ac.kr
Christina	Spiropoulou	css8@cdc.gov
Krystin	Steelman	krystin.steelman@ttu.edu
Petra	Strakova	strakova.p@centrum.cz
Tomas	Strandin	tomas.strandin@helsinki.fi

First name	Last name	Email
Petra	Svoboda	psvoboda@bfm.hr
Agnieszka	Szemiel	agnieszka.szemiel@glasgow.ac.uk
Nicole	Tischler	ntischler@cienciavida.org
Fernando	Torres-Perez	fernando.torres@pucv.cl
Giulia	Torriani	giulia.torriani@chuv.ch
Katerina	Tsergouli	ktsergouli@gmail.com
Antti	Vaheri	antti.vaheri@helsinki.fi
Leonardo	Valdivieso-Torres	lvaldivi@utk.edu
Cecilia	Vial	mcvial@udd.cl
Bryce	Warner	warnerb@myumanitoba.ca
Evan	Williams	ewilli99@utk.edu
Peter	Witkowski	peter.witkowski@charite.de
Richard	Yanagihara	ryanagih@hawaii.edu
Liudmila	Yashina	yashina@vector.nsc.ru
Kumiko	Yoshimatsu-Morimatsu	yosimatu@med.hokudai.ac.jp
Hana	Zelena	hana.zelena@zuova.cz