INTERNATIONAL HANTAVIRUS SOCIETY

IX International Conference on HFRS HPS & Hantaviruses

Beijing, China

June 5-7, 2013

Scientific Program & Abstracts

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Tuesday, June 4, 2013

13:00-21:00 **REGISTRATION**
Beijing International Convention Center (BICC)

Wednesday, June 5, 2013

08:00-09:00 Registration and set up of Posters

09:00-09:15 Welcome to the Conference: **Connie Schmaljohn (USA), President ISH**

09:15-09:30 Welcome to Beijing and Meeting Logistics: **Dexin Li and Mifang Liang (CHINA)**

09:30-12:30 **HO-WANG LEE AWARD SYMPOSIUM "EVOLUTION OF HANTAVIRUSES AND HANTAVIRUS RESEARCH"**
Connie Schmaljohn (USA), Mifang Liang (CHINA), *co-chairs*

The purpose of this award session is to both honor the contributions of the founder of hantavirus research, Professor Ho Wang Lee, and to provide the most current findings on the evolutionary relationship among hantaviruses, including newly discovered hantaviruses, throughout the world.

09:30 10:00 Keynote Speaker: **Prof. Ho-Wang Lee (KOREA)**: "Origins and Evolution of Hantavirus Research"

10:00-10:15 Introduction of Ho Wang Lee award recipient: **Prof. Ho-Wang Lee**

10:15-11:00 Award Lecture: **Jin-Won Song (KOREA)** "Hantaviruses, from Hantaan River to Imjin River"

11:00-11:30 **BREAK**

11:30-11:50 Keynote Speaker: **Dexin Li (CHINA)** "HFRS and Hantaviruses in China"

11:50-12:10 Keynote Speaker: **Jan Clement (BELGIUM)** " HFRS in the New, and HPS in the Old World: Paradi(se)gm Lost - or Regained?"

12:10-12:30 Keynote Speaker: **Luiz Tadeu Figueiredo Moraes (BRAZIL)** "Hantaviruses in South America"

12:30-14:00 **LUNCH AND POSTERS** (Presenters: please attend your poster from 1315-1345)

14:00-16:30 **JOEL M. DALRYMPLE AWARD SYMPOSIUM "A LOOK TO THE FUTURE"**
Jiro Arikawa (Japan), Jim LeDuc (USA), *co-chairs*

The purpose of this award session is to both honor the contributions of the ever forward looking Dr.

Joel M. Dalrymple and to provide state-of-the-art information on new frontiers in hantavirus research.

14:00-14:15 Introduction of the Joel M. Dalrymple Award winners and presentation of plaques

14:15-15:00 Award Lecture: **Tatjana Avšič-Županc (SLOVENIA)** "Dobrava Virus: Past, Present and Future"

15:00-15:45 Award Lecture: **Richard Yanagihara (USA)** "Hantaviruses: A Story of Rediscovery and New Beginnings"

15:45-16:00 Introduction of Chinese Sponsors: **Mifang Liang (CHINA)**

16:00-16:30 Special Lecture: Chinese Delegate

18:30-21:00 **WELCOME RECEPTION**

North Star Continental Grand Hotal

Thursday, June 6, 2013

08:30-10:30 **ECOLOGY AND EPIDEMIOLOGY-1**

Paula Padula (Argentina), Ake Lundkvist (Sweden), *co-chairs*

The purpose of this session is to introduce participants to virus-host interactions of hantaviruses and to highlight research that has revealed fundamental new information about their ecology and epidemiology.

08:30-09:00 Keynote Speaker: **Heikki Henttonen (FINLAND):** "Biome-Specific Epidemiologies of Puumala Virus"

09:00-09:30 Keynote Speaker: **Detlev Kruger (GERMANY):** "Epidemiology and Clinical Significance of Highly Pathogenic Sochi Virus"

09:30-10:30 4 oral presentations selected from submitted abstracts

- O1-1 Hemorrhagic fever with renal syndrome in Russia, 2000-2012, **Evgeniy Tkachenko**
- O1-2 Function Peculiarities of Natural Foci of Hantaan- and Amur-viral Infections on Far East Russia, **Tatyana Kushnareva**
- O1-3 Characterization of Dobrava-Belgrade virus, genotype Kurkino, infections in Germany, **Jörg Hofmann**
- O1-4 Serological Evidence of Human Hantavirus Infections in West Africa, where Hantaviruses are carried not only by Rodents and Shrews but also Bats **Peter T. Witkowski**

10:30-11:00 **BREAK**

11:00-13:00 ECOLOGY AND EPIDEMIOLOGY- 2

Hiroaki Kariwa (JAPAN), Anna Papa (GREECE), *co-chairs*

11:00-11:30 Keynote Speaker: **Yong-Zhen Zhang (CHINA)** "Phylogeny and Origins of Hantaviruses Harbored by Bats, Insectivores and Rodents"

11:30-12:00 Keynote Speaker: **Alexander Plyusnin (FINLAND)** "Evolution of Hantaviruses: Co-Speciation With a Host or Preferential Host-Switching?"

12:00-13:00 4 oral presentations selected from submitted abstracts

- O1-5 Life-Long Shedding and Non-transient Viremia of Puumala Hantavirus in Wild Bank Voles (Myodes glareolus), **Liina Voutilainen**
- O1-6 What bank vole genomics tells us about the uneven distribution of Puumala virus in Sweden, **Nathalie Charbonnel**
- O1-7 Isolation and Characterization of Hokkaido Virus, Genus Hantavirus **Hiroaki Kariwa**
- O1-8 Epidemiological and Clinical Outcome of Hantavirus Infection Associated with Two Viruses in Salta, Northern Argentina. **P. Padula**

13:00-15:00 LUNCH AND POSTERS (Presenters: please attend your poster from 1345-1445)

15:00-17:00 VIRUS PHYLOGENY, REPLICATION, AND MORPHOGENESIS

Christina Spiropoulou (USA), Rainer Ulrich (GERMANY)*, co-chairs*

The purpose of this session is to update attendees on the discovery of novel hantaviruses, or the detection hantaviruses in new environments.

15:00-15:30 Keynote Speaker: **Colleen Jonsson (USA**) "Structural Studies of Hantaan Virus"

15:30-16:00 Keynote Speaker: **Boris Klempa (SLOVAKIA)** "Complex Evolution and Epidemiology of Dobrava-Belgrade Virus: Definition of Genotypes and Their Characteristics"

16:00-17:00 4 oral presentations selected from submitted abstracts

- O2-1 Novel hantavirus strains in apodemus-mice in Estonia and Latvia, **Tarja Sironen**, Heikki Henttonen
- O2-2 Evolutive history of Sigmodontinae hantavirus in South America, **William M. Souza**
- O2-3 The N terminus of Andes virus L protein supresses mRNA and protein expression in mammalian cells, **Patrick Heinemann**
- O2-4 Autophagic Clearance of Sin Nombre Hantavirus Glycoprotein Gn Promotes Virus Replication in Cells, **Mohammad Mir**

Friday, June 7, 2013

08:30-10:30 PATHOGENESIS AND IMMUNE RESPONSES-1

Tony Schountz (USA), Jonas Klingström (SWEDEN), co-chairs

Novel animal models and new findings on the relationships of immune responses and pathogenicity or protective immune responses will be presented

08:30-09:00 Keynote Speaker: **Antti Vaheri (FINLAND)** "From Pathogenesis of Hantavirus Infections to Therapy"

09:00-09:30 Keynote Speaker: **David Safronetz (USA)** "Animal Models for Hantaviral Diseases"

09:30-10:30 4 oral presentations selected from submitted abstracts

- O3-1 A Novel Mechanism of Endothelial Cell Dysfunction in Hantavirus Pathogenesis Involves Contact Activation and Increased Liberation of Bradykinin, **Victoria Jensen**
- O3-2 Hantavirus-infection confers resistance to cytotoxic lymphocyte-mediated apoptosis, **Jonas Klingström**
- O3-3 Distinct Innate Immune Responses in Human Endothelial, Epithelial and Macrophage Cells Infected with Hantaviruses **Ok Sarah Shin**
- O3-4 Viral Load and Immune Response Dynamics in Patients with Hemorrhagic Fever with Renal Syndrome, **Misa Korva**

10:30-11:00 BREAK

11:00-13:00 PATHOGENESIS AND IMMUNE RESPONSES-2

Delia Enria (ARGENTINA), Olli Vapalahti (FINLAND), *co-chairs*

11:00-11:30 Keynote Speaker: **Erich Mackow (USA)** "Interferon, Signaling Pathway, Virulence"

11:30-12:00 Keynote Speaker: **Ellen Krautkrämer (GERMANY)** "Old World Hantaviruses: Aspects of Pathogenesis and Clinical Course"

12:00-13:00 4 oral presentations selected from submitted abstracts

- O3-5 Pronounced Th2 Gene Expression in Helper T cells from Deer Mice (Peromyscus maniculatus) Experimentally Infected with Andes Virus, **Tony Schountz**
- O3-6 Acute Hantavirus Infection Induces the Production of Galectin-3 Binding Protein (90K/Mac-2BP) , **Jussi Hepojoki**
- O3-7 HTNV-induced High Expression of CXCL10 is Mediated Through the Activation of TLR3, RIG-I and MDA5 Pathways,**Yusi Zhang**
- O3-8 Viral load and immune responses in patients with Puumala hantavirus infection, **Clas Ahlm**

13:00-14:30 **LUNCH AND POSTERS** (Presenters: please attend your poster from 1345-1415)

14:30-17:15 **DIAGNOSTICS, TREATMENTS AND CLINICAL FINDINGS -**
***Jay Hooper (USA), Dexin Li (CHINA)**, co-chairs*

The purpose of this session is to update attendees on new developments in diagnostic tests, patient symptoms, clinical observations and advances in vaccines and therapeutics

14:30-15:00 Keynote Speaker: **Alemka Markotić (CROATIA)** "Clinical and Laboratory Findings of HFRS Patients in the South-East Europe"

15:00-15:30 Keynote Speaker: **Connie Schmaljohn (USA)** "Phase 1 Clinical Studies of DNA Vaccines for HFRS Delivered by Electroporation"

15:30-17:15 7 oral presentations selected from submitted abstracts

- O4-1 Development of an Immunochromatography Strip Test for Detecting Anti-hantavirus Antibody in Rodent and Human Sera by Using an N-terminal Common Antigenic Site of Hantavirus N protein, **Jiro Arikawa**
- O4-2 Simultaneous Detection of IgG Antibodies Associated with Viral Hemorrhagic Fever by a Multiplexed Luminex-based Immunoassay, **Shuo Zhang**
- O4-3 Passive immune therapy for ANDV HPS with human plasma: an open trial, **Francisca Valdivieso**
- O4-4 A Severe Capillary Leakage Syndrome in Hantavirus Infection Treated with Bradykinin Receptor Antagonist Icatibant, **Jukka Mustonen**
- O4-5 Cardiopulmonary Involvement in Puumala Hantavirus Infection, **Johan Rasmuson**
- O4-6 Development and evaluation of a broad reacting sybr-green based quantitative real-time pcr for the detection of different hantaviruses, **Goran Bucht**
- O4-7 A Sin Nombre virus DNA vaccine delivered using the PharmaJet needle-free jet injector elicits high-titer neutralizing antibodies in rabbits and nonhuman primates, **Jay W. Hooper**

17:15-17:30 Concluding Remarks, and announcement of next meeting: President ISH

17:30 Meeting Adjourns

19:00-22:00 **CONFERENCE BANQUET**
Pangu 7 Star Hotel

Connie Schmaljohn, USA
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Anna Papa, Greece
Secretary

Jan Clement, Belgium
Vice President

Mifang Liang, China
Local Organizer

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Award Lecture

AL-1 Hantaviruses, from Hantaan River to Imjin River

Jin-Won Song

AL-2 Dobrava virus: past, present and future.

Tatjana Avšič-Županc

Institute of Microbiology and Immunology, Faculty of Medicine, Ljubljana, Slovenia

The first case of HFRS in Slovenia was laboratory confirmed in 1985. Soon, reports on the presence of two different hantaviruses responsible for human infections have been documented, as both severe and mild clinical courses of the disease were seen. In 1988, DOB virus was isolated from the lungs of a yellow-necked field mouse (Apodemus flavicollis), captured in the Dobrava village (Dolenjska region, Slovenia) where a number of cases of severe HFRS had occurred. Extensive genetic and antigenic characterization of Dobrava virus revealed that this is a unique species of the Hantavirus genus, family Bunyaviridae. By retrospective and simultaneous diagnostic analysis it was obvious that severe and even fatal HFRS cases are due to DOB virus. Among 505 HFRS cases occurred sporadically or in small epidemics so far, 132 DOBV cases have been confirmed, with 9,1% fatality rate. Based on several years of clinical, virological and immunological investigation of HFRS patients we have found: that DOBV tend to produce the most severe disease, with pronounced hemorrhages, shock, acute renal failure; patients usually require longer hospitalization, having viremia lasting on average 30 days, their viral load is on average 107 RNA copies/ml and their antibody and cytokine response kinetics, that in part is related to HLA type (HLA-B*35) reflect in disease severity and clinical outcome. Epizootiological surveys in Slovenia indicated that A. flavicollis (12.5 %) and M. glareolus (16.5 %) were most often infected with DOBV and PUUV, respectively. Later on, infection has been demonstrated in multiple rodent and shrew species. When genetic variability of DOB virus was evaluated in rodents, a closely-related strain of DOBV was found in A. agrarius (9.6 %), but was not so far molecularly detected in patients in Slovenia.

AL-3 Hantavirology: A Story of Rediscovery and New Beginnings

Richard Yanagihara

Department of Pediatrics and Tropical Medicine, Medical Microbiology and Pharmacology, John A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, United States
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The history of hantavirology has been one of rediscovery and new beginnings. More than a decade before the seminal discovery of Hantaan virus in the striped field mouse (Apodemus agrarius), Thottapalayam virus (TPMV) was isolated from the Asian house shrew (Suncus murinus). But even after this formerly unclassified virus was shown to be a hantavirus, the prevailing assumption was that TPMV represented a spillover event from a rodent host. Opportunistic studies to ascertain if small mammals having shared ancestry with rodents serve as reservoirs have been guided by

decades-old reports of hantaviral antigens in shrews and moles. Also, access to well-curated archival specimens has accelerated the acquisition of new knowledge about the spatial and temporal distribution of nonrodent-borne hantaviruses. Frozen, ethanol-fixed and RNAlater®-preserved tissues from 1,546 shrews (9 genera, 47 species), 187 moles (8 genera, 10 species) and 520 bats (26 genera and 53 species), collected in Europe, Asia, Africa and North America in 1981-2012, analyzed by RT-PCR, have yielded 25 novel hantavirus species in shrews, moles and bats. Phylogenetic analysis indicates four distinct phylogroups, with the most divergent comprising Nova virus from the European common mole and insectivorous bat-borne hantaviruses from Asia and Africa. An additional sublineage, consisting of hantavirus sequences that do not conform to their reservoir host species or geographical location, may provide new insights into hantavirus evolution. Host switching of hantaviruses has occurred between species of the same family (Soricidae and Soricidae), of different families (Soricidae and Talpidae) and of separate orders (Soricomorpha and Rodentia). The realization that newfound hantaviruses detected in shrews, moles and bats are genetically more diverse than those harbored by rodents suggests that the evolutionary history of hantaviruses is far more ancient and complex than previously conjectured. Thus, a new beginning in hantavirus research is now focused on exploring the 'inconvenient' evidence that rodents may not be the original mammalian hosts of primordial hantaviruses. Also, the once-growing complacency and indifference is being replaced by a renewed zeal to determine the ecology, evolution and pathogenic potential of still-orphan, newfound soricomorph- and chiropteran-borne hantaviruses.

Keywords: shrew; mole; bat; phylogeny;

Keynote Speak

KS-1 Origins and Evolution of Hantavirus Research

Ho-Wang Lee

KS-2 HFRS and Hantavirus in China

Dexin Li

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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 2011 in China.

From 2006 to 2011, a total of 64250 HFRS cases and 762 deaths were reported in China with the average annual incidence rate of 0.81/lakh and case fatality rate of 1.18%. Morbidity and mortality had been annually decreasing from 2006 to 2009 but had been increasing since 2010. The top 8 provinces with HFRS cases were Heilongjiang, Shanxi, Shandong, Jilin, Liaoning, Zhejiang, Hunan and Hebei, which accounted for 80.2% 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.

It is noteworthy that vaccination, along with public education, rodent control measures and social changing, have made a reduction in HFRS cases till 2009, however, cases and natural foci of HFRS has been increasing in China since 2010. It is an urgent need to define the factors, which may affect HFRS incidence and make the control measures more effective.

KS-3 HFRS in the New, and HPS in the Old World: Paradi(se)gm lost - or regained?

Jan Clement, Piet Maes, Valentijn Vergote, Lies Laenen, and Marc Van Ranst.

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Since the first (1994) description of HPS, literature inevitably mentions a distinct dichotomy between clinics in the New World (with HPS), versus the Old World (with HFRS). Although now a never questioned paradigm, the premise that genetically related viruses should generate different clinical pictures is all but obvious, since the human host's immunoreaction is considered the main pathogenic determinant, not the virus itself. Moreover, both "syndromes" are too often misnomers, cardinal symptoms being rarely complete. Should we continue to call New World infections without pulmonary involvement still "HPS", or Old World infections with transient mild proteinuria still "HFRS"? These "clinical" denominations become utterly paradoxical if we realize that 10% of South-American "pulmonary syndromes" need dialysis for acute treatment, whereas less than 5% of European "nephropathia" cases needs the same. Naming emerging viral infections after some "newly recognized" symptoms, and not after the new etiologic agent, may be attractive to the lay press, but is scientifically precarious.

Seemingly now forgotten, pioneers in American hantavirology isolated Seoul virus (SEOV) from a rat caught in Belém, Brazil, as the first (1984) hantaviral pathogen in the New World, followed by Tchoupitoulas (1985) and several other SEOVs from the USA (Girard Point, Baltimore, etc.). Moreover, the first (1993) published American account of symptomatic seroproven hantavirus were HFRS, not HPS, cases in Brazil, followed in 1994 by three USA HFRS cases of PRNT-proven SEOV. Whereas American SEOV does not cause big epidemics like in China, isolated cases go nowadays probably unrecognized or misdiagnosed, as proven by a distinct HFRS case, despite typical clinics and kidney biopsy, in a renowned American clinical series (NEJM 2007;357:1531-41).

In Europe, more than 225,000 HFRS cases, mostly induced by Puumala virus (PUUV) were registered since a first description in Sweden in 1934. More in-depth clinical experience during all these years may therefore be assumed. Thus, it should come as no surprise that in European series, "acute lung injury" was already reported with or without "acute kidney injury", before or concomitantly with the American HPS discovery. Swedish authors recently (2011) described three severe PUUV cases leading to death in refractory shock in two patients, showing on autopsy irrefutable HPS-like lung lesions, but no prominent kidney lesions.

It is now perhaps time to revise the sacro-saint paradigm of two "different" infectious diseases, and accept a simple, short name like "hantavirus disease", having the additional merit of fulfilling at least part of Koch's postulates.

KS-4 Hantaviruses in South America

Luiz Tadeu Moraes Figueiredo

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Hantavirus Pulmonary Syndrome (HPS) is a severe acute disease associated with rapid-onset respiratory failure and cardiogenic shock. In South America, the primary HPS-causing hantaviruses include Andes virus, harbored by the rodent Oligoryzomys longicaudatus in Argentina and Chile; Laguna Negra virus (LNV) harbored by Calomys laucha in Paraguay; and Rio Mamore virus (RMV) in Oligoryzomys microtis, from Bolívia. In Brazil, more than 1600 HPS cases have been reported, caused by Araraquara virus (ARAV), harbored by Necromys lasiurus, Juquitiba virus (Oligoryzomys nigripes), Anajatuba virus (Oligoryzomys fornesi), Castelo dos Sonhos virus (Oligoryzomys utiaritensis), and Laguna Negra-like virus (Calomys callidus).

Benign infections could explain the high frequencies of hantavirus seropositive individuals who never had recognizable HPS in South America. Recently, researchers from all Brazilian regions and also from Colombia, have performed serologic surveys to hantavirus using an ELISA with a recombinant nucleocapsid N protein of ARAV. In Cassia dos Coqueiros county (state of São Paulo) 4.7% of 1876 sera obtained from 1987 to 1990 were positive for anti-hantavirus antibodies, showing that those infections occurred even before the description of HPS. Other surveys were performed from 2007 to 2011, including one in the state of Santa Catarina, at the Brazil–Argentina border, where 3.5% of 350 participants had antibodies to hantavirus. In another study in the eastern side of the same state, 2.3% of 257 participants were seropositive, and reported previous severe pneumonia. In the northeastern state of Ceará, 2.8% of 72 patients with dengue-like disease had IgG and 1.4% had IgM antibodies to hantavirus. In the northern state of Amazonas, 5.7% of 1,731 participants had antibodies to hantavirus. In the midwestern state of Mato Grosso 13% of 54 participants were seropositive to hantavirus. In the Caribbean region of Colombia, 8.4% of 284 agricultural workers had IgG antibodies to hantavirus, including 2 who were also IgM-positive. These surveys show that hantavirus infections are common in all regions of Brazil, and that they also occur in Colombia.

In an epidemiological study of hantavirus reservoirs, 568 small mammals were captured from 2008 to 2009 in the countryside of the state of São Paulo, and 6.3% of them were infected by hantavirus. Infections were more frequent in the dry season and in places with degraded environment that favors abundance of N. lasiurus, Akodon sp and Calomys tener. In another study, 6 Necromys lasiurus and Akodon sp were infected by IM route with RMV. Hantavirus genome was detected in urine and feces of the rodents 4-5 days post infection (p.i.), in sera and saliva 9 days p.i., and remaining 18 days p.i. Thus, N. lasiurus and Akodon shed virus in urine, feces and saliva, posing risk for rodent and human infection.

The association of genetic polymorphisms with outcome of hantavirus infection was studied. The TNF2 allele was more frequent in HPS patients than in individuals with positive serology for hantavirus without HPS, suggesting that this allele represents a risk factor for developing HPS. On the opposite, the TGF-β high producer phenotype was more frequently found in hantavirus seropositive individuals without history of HPS, and thus seems to be associated with protection against severe disease.

A molecular evolution study based on the N gene sequence indicate that rodent-borne hantaviruses

date back ~1915 years, and reached South America from Central America. A common ancestral for hantavirus of Sigmodontinae occurred ~405 years ago. A hantavirus ancestral in Bolivia and Peru originated LNV, RMV and Jabora virus ~354 years ago. Hantaviruses were present in Brazil and Argentina ~ 341 years ago, and the divergence of Juquitiba and ARAV-Andes virus branches occurred ~306 years ago.

KS-5 Biome Specfic Epidemiogies of Puumala Hantavirus

H. Henttonen, and EDEN & EDENext teams

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Hantaviral epidemiological patterns are related to the dynamics of reservoir rodent species. Globally, rodent outbreaks due to masting of various kinds, often climate induced, usually contribute to human epidemics. Decadal changes in rodent dynamics on several continents caused by climatic oscillations like the Pacific Oscillation, El Niño, Antarctic Oscillation, and North Atlantic Oscillation have been documented. In SW USA and Chile, El Niño, Southern and Antarctic Oscillations impact rodent dynamics, often via changes in rainfall. Rodent outbreaks in SE Asia are regionally due to masting cycles of bamboos. In temperate Europe masting is the important driver of PUUV epidemics. It has been suggested that warm summers induce masting, while in snowy conditions N Europe, predation is thought be the main driver of vole fluctuations. In the large collaborative EU projects EDEN and EDENext our aim was to understand the human epidemiology of nephropathia epidemica (NE) between boreal and temperate Europe in different climatic conditions. In temperate Europe, there are suggestions that masting frequency has increased due to the warming of summers, resulting in increased frequency outbreaks of forest rodents and NE. We documented differences in the transmission dynamics of PUUV between these two biomes. Consequently, the underlying top-down or bottom-up causes of rodent fluctuations and PUUV outbreaks are different. We have further documented the role of landscape patterns (homogenous taiga vs. fragmented temperate forests) in rodent/virus dispersal, and in the presence or absence of host threshold densities for the PUUV occurrence. In addition, temperature and moisture affect the virus survival outside the host, affecting indirect transmission. Also, geographic differences in the immunogenetics of host rodents can affect their susceptibility. In addition to short-term fluctuations or outbreaks in rodent dynamics, there can be long-term trends, superimposed on the shorter "cycles", possibly due to the climatic changes, as exemplified e.g. by the national long-term monitoring of vole fluctuations in Finland. These results are essential for human risk evaluation with regard to both long-term and seasonal occurrence of PUUV in the environment. In conclusion, it is important to realize that within the same host/virus system, biome specific PUUV epidemiologies occur, which highlights the importance of geographically comparative studies in Europe, or in any host/pathogen system.

KS-6 Epidemiology and clinical significance of highly pathogenic Sochi virus

Detlev H. Kruger[1], Evgeniy A. Tkachenko[2], Vyacheslav G. Morozov[3], Yulia V. Yunicheva[4], Dmitriy L. Zavora[5], Gennadiy Malkin[2], Peter T. Witkowski[1], Boris Klempa[1,6], Tamara K. Dzagurova[2]

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A new genotype of Dobrava-Belgrade virus (DOBV), Sochi virus, was found in the Black Sea field mouse, Apodemus ponticus. This mouse is naturally occurring in the Southern European Russia and transcaucasian countries between the Black and the Caspian Sea. Recently, cell culture isolates of Sochi virus have been generated from A. ponticus and an HFRS patient with fatal outcome. At the present state of knowledge, Sochi virus seems to be the most dangerous representative of DOBV. Virus diagnostics in patients was accomplished by immunofluorescence assay, serotyping of neutralizing antibodies, and RT-PCR amplification of viral genome segments. In phylogenetic analyses we found a spatial clustering of the viral nucleotide sequences derived from patients and mice trapped at different localities of the Russian Black Sea coast region demonstrating Sochi virus as the causal pathogenic agent in humans. We currently oversee in detail the clinical courses of 52 patients with confirmed Sochi virus infection. The case fatality rate was determined to be as high as 14%. About 60% of clinical courses were defined as severe (including deaths) and nearly 40% as moderate. Four times more males than females were affected. Quite unusual for hantavirus disease, also young people became ill due to Sochi virus infection; 10% of patients were found between 7 and 15 years old and the age average of all patients was not much higher than 30 years. There is an urgent need to monitor the epidemiology of the new virus - not only because of its health-threatening character in this particular geographical area but also because of its potential ability to overcome host species barriers. Host switch of the virus to closely related host species, as A. flavicollis or A. sylvaticus, could dramatically increase its geographical spread and consequently further enhance the danger for the human population.

Keywords: Sochi virus; Dobrava-Belgrade virus; Apodemus ponticus; Transcaucasus; case fatality;

KS-7 Phylogeny and Origins of Hantaviruses Harbored by Bats, Insectivores, and Rodents

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Hantaviruses are among the most important zoonotic pathogens of humans and the subject of heightened global attention. Despite the importance of hantaviruses for public health, there is no consensus on their evolutionary history and especially the frequency of virus-host co-divergence versus cross-species virus transmission. Documenting the extent of hantavirus biodiversity, and particularly their range of mammalian hosts, is critical to resolving this issue. Here, we describe four novel hantaviruses (Huangpi virus, Lianghe virus, Longquan virus, and Yakeshi virus) sampled from bats and shrews in China, and which are distinct from other known hantaviruses. Huangpi virus was found in Pipistrellus abramus, Lianghe virus in Anourosorex squamipes, Longquan virus in Rhinolophus affinis, Rhinolophus sinicus, and Rhinolophus monoceros, and Yakeshi virus in Sorex isodon, respectively. A phylogenetic analysis of the available diversity of hantaviruses reveals the existence of four phylogroups that infect a range of mammalian hosts, as well as the occurrence of ancient reassortment events between the phylogroups. Our phylogenetic analysis also suggests that hantaviruses might have first appeared in Chiroptera (bats) or Soricomorpha (moles and shrews), before emerging in rodent species. Overall, these data indicate that bats are likely to be important natural reservoir hosts of hantaviruses. Notably, the phylogenetic histories of the viruses are not always congruent with those of their hosts, suggesting that cross-species transmission has played a major role during hantavirus evolution and at all taxonomic levels, although we also noted some evidence for virus-host co-divergence. Our genetic analyses of all known Murinae-associated hantaviruses (both established and tentative species) revealed that many of them might have originated from host-switching. Remarkably, some viruses generated from host jump including Dabieshan virus (DBSV) show >7% differences from their presumable parental/or sister species in all three encoded proteins, while other viruses have at least one protein sequence to be <7% differences from its sister virus. The estimation of the evolutionary rate and divergence time also supports the critical role of cross-species transmission in the evolution of Murinae-associated hantaviruses. The detection of positive selection suggests that genetic drift might have played an important role, and adaptation might have also been at work in the speciation of the currently known hantaviruses.

Keywords: Hantavirus; Phylogeny; Host range; Bats; Shrews;

KS-8 Evolution of Hantaviruses: Co-Speciation With A Host Or Preferential Host-Switching?

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The most recent (9th) Report of the International Committee on Taxonomy of viruses lists 23 established and 30 provisional species in the genus Hantavirus (family Bunyaviridae) [Plyusnin et al., 2012]. These virus species are harbored by altogether 51 species of rodents, shrews and moles and thus in most cases it is a relationship “one hantavirus-one host”. Such a tight bond between the two, in combination with the observed association between the whole groups of hantaviruses and (sub)families of rodents, helped to develop the widely accepted idea on a long-term co-evolution (co-speciation) of these viruses with their hosts. However, a discrepancy of divergence dates which resulted from some estimates, the outburst of newly discovered insectivore-borne hantaviruses, and

especially an accumulating evidence for host-switching events, both recent and ancient, challenged some of the earlier views on hantavirus evolution. In this talk the issue of a hantavirus-host co-phylogeny versus host-switching will be discussed in greater detail.
Keywords: hantavirus; evolution; co-speciation; host-switching;

KS-9 Structural Studies of Hantaan Virus

Colleen Jonsson

KS-10 Complex Evolution and Epidemiology of Dobrava-Belgrade Virus: Definition of Genotypes and Their Characteristics

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Dobrava-Belgrade virus (DOBV) is the most pathogenic hantavirus in Europe which causes hemorrhagic fever with renal syndrome (HFRS). The virus is hosted by mice of several species of the genus Apodemus. Molecular phylogenetic analyses showed that DOBV forms four evolutionary lineages. One of these lineages, Saaremaa virus (SAAV), is currently recognized as an independent virus species on the ICTV species list. In accordance with the four phylogenetic lineages, we propose a subdivision of the DOBV species into 4 genotypes, named Dobrava, Kurkino, Saaremaa, and Sochi according to the geographical place where the first strain of the genotype was molecularly detected. Based on the high amino acid sequence similarity and serological cross-reactivity, we are convinced that it would be more appropriate to classify these four genotypes within the Dobrava-Belgrade virus (DOBV) as a single hantavirus species. They should neither be divided into DOBV and SAAV species nor do they represent four distinct species. Interestingly, the different genotypes – despite their high genetic similarity – induce HFRS of different severity. The most severe clinical courses were observed in South-East Europe where human infections by Dobrava genotype (associated with A. flavicollis mice) occur. The case-fatality rate (CFR) of clinical cases is 10-12%. Similar CFR was observed for HFRS caused by Sochi genotype (associated with A. ponticus mice) in the Black Sea coast area of European Russia. The course of HFRS due to infection by Kurkino genotype (associated with A. agrarius mice), dominant in Central Europe and in European Russia, is mainly mild or moderate with CFR of 0.3-0.9%. Saaremaa genotype (found in A. agrarius mice on the Estonian island Saaremaa) infections seem to be subclinical. Understanding the mechanisms behind the different virulence of the DOBV genotypes could significantly advance the understanding of hantavirus pathogenesis.

Keywords: Dobrava-Belgrade virus; evolution; epidemiology; Apodemus;

KS-11 From pathogenesis of hantavirus infections to therapy

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Many types of cultured cells are susceptible to hantavirus infection and likely due to this a generalized infection in man is common. Infection of cultured cells causes very little cytopathic effects, suggesting that the disease is caused by indirect mechanisms. Host genes affect the severity of hantavirus infection in humans and the infection may have significant long-term consequences. The key elements in the pathophysiology include vascular leakage, cytokines (especially TNF-alpha and interleukin-6), cytotoxic and regulatory T cells, thrombocytopenia and complement activation. These conclusions are also based on observations on lethal HFRS cases (collaboration with Clas Ahlm and Magnus Evander, University of Umeå, Sweden; and Timo Hautala, University of Oulu, Finland) and on Puumala virus (PUUV) infected macaques. We have recently reported that a patient with severe capillary leakage syndrome caused by PUUV hantavirus infection was successfully treated with icatibant, a bradykinin receptor antagonist currently registered for the treatment of hereditary angioedema, sometimes a life-threatening condition in which vascular leakage is a key feature.

Keywords: pathogenesis; therapy; vascular leakage; icatibant; complement;

KS-12 Animal models for hantaviral diseases

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The intent of an animal model is to provide insight into the pathogenesis of disease for the purpose of developing and evaluating the effectiveness of medical countermeasures. This is especially true for pathogens, like hantaviruses, which are believed to be primarily immunopathogenic, since the underlying immune activation/suppression needs to be accurately recapitulated in order to provide the best predictive power for the use of specific therapeutics in humans. Currently, there has been no animal model described which mimics the disease manifestations associated with severe hemorrhagic fever with renal syndrome (HFRS), although lethal models based on suckling mice have been characterized. In 2001, a lethal animal model of hantavirus pulmonary syndrome (HPS) was described in which hamsters infected with Andes virus develop respiratory distress similar to human HPS. Further analysis has shown that, similar to the current dogma of HPS in humans,

infected hamsters experience signs of cardiogenic shock and tissue specific immune activation suggesting that hamsters are an appropriate model for HPS. The caveat for this model, and any hamster model, is the uncertainty regarding the effectiveness of therapeutic agentstargeting specific immune responses or improving endothelial barrier function, since cross-reactivity for many of these compounds has not been evaluated in hamsters. For this reason, a non-human primate (NHP) model for HPS or HFRS would be preferred, since in general NHPs are believed to be the gold standard surrogate model for infectious disease in humans, especially for those which are believed to be immunopathogenic.To date, efforts at developing an NHP model for HFRS or HPS have been unsuccessful. The exception is a macaque model for nephropathia epidemica which is based on infection with wild-type Puumala virus. Using a similar strategy, we have developed a NHP model for HPS which is strikingly similar to the human condition with respect to incubation period and disease progression, tissue and cellular tropism, histopathology and host responses. The utility of the novel NHP model for HPS will be compared to the previously characterized hamster model of HPS as well as the current understanding of human disease. The development of an NHP model for HPS is a milestone for the study of hantaviruspathogenesis and the evaluation of appropriate therapeutic strategies to treat and prevent this rare but frequently fatal disease. The methodologies developed in the NHP model of HPS may readily translate into a disease model for the study of HFRS.

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KS-13 ANDV Regulation of Signaling Responses in Primary Human Micro−vascular and Lymphatic Endothelial Cells

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Hantaviruses primarily infect ECs and cause HFRS and HPS. Acute pulmonary and organ edema is associated with disease and may be caused by capillary leakage of microvascular ECs (MECs) and effects on lymphatic ECs (LECs) that clear edema fluids from tissues. LECs control fluid clearance, although roles for lymphatic vessels in hantavirus disease remain undefined. We have shown that LECs and MECs are infected by hantaviruses and that signaling pathways regulating capillary and lymphatic vessel functions are altered by ANDV and HTNV infections. In contrast to TULV, VEGFR2 signaling responses induced by hypoxia and VEGF-A are uniquely altered in ANDV and HTNV infected ECs. This suggests that selective regulation of VEGFR2 responses alter normal vessel functions. However, ~70% of ANDV infected LECs resulted in the formation of giant cells, >4 times larger than normal, in response to VEGF-A suggesting that signaling responses downstream of VEGFR2 are also altered by ANDV. Giant cells are associated with mutations in TSC1 and TSC2 proteins resulting in constitutive activation of the mammalian target of rapamycin (mTOR). In fact, TSC1/2 complexes normally inhibit mTOR activation directed by hypoxia, VEGF-A and HIF1α transcriptional responses suggesting that ANDV disrupts normal VEGFR2-mTOR inhibitory responses. We found that both giant LECs and LEC permeability were sensitive to rapamycin, an mTOR inhibitor, and VEGF-C addition. VEGF-C uniquely regulates LEC responses

by activating discrete VEGFR3 receptors that form heterodimeric complexes with VEGFR2 and thereby regulate VEGF-A directed LEC responses. Our recent studies demonstrate that ANDV proteins expressed in primary human MECs and LECs alter normal cell signaling responses to hypoxia and VEGF-A, transcriptional induction and miRNA regulation. These findings suggest that ANDV proteins directly alter normal endothelial cell functions that contribute to altered capillary permeability and lymphatic vessel clearance functions. These findings suggest that ANDV uniquely alters normal LEC and lymphatic vessel functions which may contribute to edematous fluid accumulation during HPS. Moreover, the ability of VEGF-C and rapamycin to normalize LEC responses suggests a potential therapeutic approach for reducing pulmonary edema and the severity of HPS following ANDV infection by targeting functions of lymphatic vessels.

Keywords: ANDV Signaling; pathogenesis; Lymphatic endothelial cell; Signaling pathway; mTOR;

KS-14 Old World hantaviruses: aspects of pathogenesis and clinical course

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Infections cause often epithelial and endothelial dysfunction resulting in increased permeability or even organ failure. The degree of damage and mechanisms of repair play a key role in pathogenesis. One of the target organs that is affected by Old World hantaviruses is the kidney characterized by often massive proteinuria. Our studies analyzed the mechanisms that are responsible for kidney dysfunction and factors that may influence the clinical course of the infection. We examined the effects of infection on cell contacts of human renal cells in vitro and in kidney biopsy specimens obtained from patients with hantavirus infection. To identify factors that are involved in the restoration of damaged monolayers, we analyzed the number of endothelial progenitor cells (EPCs). EPCs released upon damage from the bone marrow are responsible for maintenance and repair of the endothelial barrier. Studies demonstrate that EPCs are crucial in the outcome of vascular diseases or sepsis. However, the role of EPCs in the pathogenesis of infections with hemorrhagic fever viruses is not known yet. Renal function depends on the integrity of the glomerular and tubular apparatus. Our studies demonstrate that glomerular endothelial cells, podocytes and tubular epithelial cells are susceptible for infection and that the infection disturbs the integrity of cell contacts leading to loss of barrier function. This effect was also observed in the glomeruli and tubuli of biopsies of infected patients demonstrated by the redistribution and reduction of the tight junction protein ZO-1. The analysis of factors of endothelial repair revealed an increase in the number of EPCs and levels of cytokines responsible for EPC mobilization. After the acute stage, EPC and cytokine levels decreased to baseline values. Hantavirus infection is characterized by proteinuria, decreased levels of platelets and serum albumin. The observed effects on cell contacts and levels of EPCs are directly linked to the clinical picture. The decrease in glomerular ZO-1 correlates with the decrease in levels of serum albumin. The analysis of EPC levels together with the clinical course revealed that normalization of laboratory parameters is paralleled to the mobilization of EPCs. These results provide important insights in the mechanism of hantavirus induced damage and the factors that are involved in repair.

Keywords: acute renal failure; cell-to-cell contacts; EPC; cytokines;kidney;

KS-15 Clinical and Laboratory Findings of HFRS Patients in the South-East Europe

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Two hantaviruses, Puumala virus (PUUV) and/or Dobrava virus (DOBV) cause hemorrhagic fever with renal syndrome in South-East Europe. Although some other hantaviruses, like Tula or Saarema were found among small rodents in the region, so far there is no evidence about human infections caused by some other hantaviruses. Several countries in the South-East Europe (e.g. Croatia, Bosnia and Herzegovina and Slovenia) experience both PUUV and DOBV among humans with differences in disease severity. Thrombocytopenia and increased level of CRP are the most common laboratory findings during the first week of the disease. Renal and liver impairment occurred at the beginning of the second week of the disease. Usually, DOBV is considered to cause more severe clinical pictures than PUUV, however the exceptions are not rare as well. Significantly higher proportion of DOBV-infected patients had acute renal failure, visual disturbance, severe thrombocytopenia, and elevated levels of non-segmented leukocytes, creatine, and total bilirubin. The prevalence of gastrointestinal and electrocardiography disorders also was more frequent in DOBV-infected patients. Interestingly, significantly more PUUV-infected patients had elevated systolic blood pressure on admission to the hospital. However, patients infected with PUUV or DOBV may develop oliguria or anuria as well. In the recent study, we identified the following risk factors for the development of oliguria and anuria: conjunctival hyperaemia or bleeding, diarrhoea, serum sodium of ≤133 and dipstick protein value of $p > 1.5$ g/L. Such findings may help physicians in the earlier identification of patients with a more severe form of HFRS caused by PUUV or DOBV. It would be of significant importance, especially for the severe HFRS cases, which are common in the region, to define early biomarkers of disease severity, progression and outcome. In the past, several immune parameters (e.g. soluble IL-2 receptor, IL-6, TNF-alpha, soluble IL-6 receptor etc.) were shown to correlate with clinical parameters in HFRS patients. An innovative approach to the peripheral blood mononuclear cells and urine immunomarkers and biomarkers (at the gene expression and protein level) will be presented here in correlation with other clinical and laboratory data in HFRS patients.

KS-16 Phase 1 Clinical Study of DNA Vaccines for Hantaan virus and Puumala virus delivered by Intramuscular Electroporation

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At least four distinct hantaviruses cause most cases of hemorrhagic with renal syndrome (HFRS) in Asia and Europe. We previously demonstrated in animals that DNA vaccines expressing genes encoding the envelope glycoproteins of two of these viruses, Hantaan (HTNV) and Puumala (PUUV) viruses, when given together could elicit neutralizing antibodies and provide protective immunity

against all four HFRS-causing hantaviruses. We also showed that although the vaccines could be delivered at the same time, they could not be mixed, due to interference resulting in greatly reduced responses to HTNV. We evaluated these vaccines alone and in combination in a Phase 1 clinical study using gene gun for delivery. We showed that both vaccines were able to elicit high levels of neutralizing antibodies in some individuals, but that the responses were inconsistent among the volunteers. To attempt to improve the overall seroconversion rate with these vaccines, we are conducting a second Phase 1 study, in which we delivered the vaccines using Ichor Medical System's intramuscular electroporation device (Tri-Grid™). The study includes 3 randomized groups of 9 subjects, each of whom received three vaccinations at days 0, 28, and 56 with 2 mg of DNA/ 1 mL of the HTNV vaccine, the PUUV vaccine, or a mixture of both vaccines. The study initiated in January of 2012, with final dosing in July 2012. No serious adverse events have been observed to date. Preliminary analysis of blinded serum samples indicate that neutralizing antibodies were elicited against both HTNV and PUUV. Issues relating to potential interference of the mixed vaccines, as well as assessment of seroconversion rates await unblinding of the study. Meanwhile, we have continued to investigate possible solutions for the interference observed in animals, and have generated a modified HTNV plasmid, which appears to be unaffected when mixed with the PUUV DNA vaccine. Consequently, we have begun planning for a third Phase 1 Study in which we will compare intramuscular and intradermal electroporation of this modified HTNV vaccine delivered alone or mixed with the PUUV vaccine.

Keywords: DNA vaccine; Phase 1

Oral Presentation

Topic 1 ECOLOGY AND EPIDEMIOLOGY

O1-1 Hemorrhagic fever with renal syndrome in Russia, 2000-2012

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A total of 92,350 HFRS cases were registered from 2000 to 2012 from 55 out of 83 administrative regions of Russia involving 90,850 cases from the European part (from 46 out of 58 administrative regions), and 1,500 cases from the Asian Russia (from 11 out of 24 regions). Thus, 98.3% of total number of HFRS cases were registered in the European, and 1.7% only in the Asian (mainly in Far-Eastern regions) Russia. Annual average morbidity rate is 6.5 in European regions and 1.9 per 100,000 population - in Far-Eastern regions. On the whole, in Russia it is 4.9. Children under the age of 14 years represented approximately 2.6% of the cases on the whole, in Russia. The distribution of HFRS in Russia was found to be scattered throughout the country. However, different geographical regions are distinguished by the morbidity rates due to HFRS very considerably. The most high rate of annual HFRS incidence occur in the South East of the European Russia. Practically more then 80% of all HFRS cases in Russia are registered on this territory with annual average morbidity rates of 20 per 100,000 population. Results of examination of small mammals showed that practically each landscape zone has natural foci with different degrees of hantavirus activity. More or less polyhostality of HV was observed in all the vegetative zones. The vast majority of rodents and insectivore species as well as other mammals and birds orders harboring hantavirus are probably ancillary hosts. Alongside with this the epidemiological significance of certain rodents is established now in different regions of Russia. HFRS cases in Far-Eastern Russia is etiologically conditioned mainly by HTNV and AMUV and less by SEOV. The principal source of these viruses are: A. agrarius, A. peninsulae and R.norvegicus. 98% of HFRS cases in European regions are caused by PUUV associated with bank vole, Myodes glareolus - natural reservoir and vector of the virus. Kurkino genotype of Dobrava/Belgrad virus (DOBV) associated with A. agrarius is the causative agent of HFRS sporadic cases and outbreaks in Central European regions as well as A. ponticus-borne DOB virus (Sochi genotype) is causative agent of HFRS cases registered in Sochi region, southern part of Russia. Comparative analyses of clinical data of HFRS cases out of different endemic foci using a common scheme of severity criteria indicated that all viruses caused HFRS in Russia may produce three clinical forms of disease: mild, moderate and severe. The rate of detection of severe forms as well as case-fatality rate are significantly higher for Sochi genotype of DOBV and HTNV than for PUUV, SEOV and Kurkino genotype of DOBV.

Keywords: HFRS; Hantavirus;

O1-2 Function Peculiarities of Natural Foci of Hantaan- and Amur- viral Infections on Far East Russia

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Objective: Seasonal and multi-annual dynamics of epizootic and epidemic processes were studies on endemic areas of HTNV (genovariant FE) and AMRV on Primorye Territory of Far East Russia for comparison of function of two different HFRS natural foci. Eastern subspecies of the field mouse (Apodemus agrarius) and Korean field mouse (Apodemus peninsulae) are the main hosts of HTNV and AMRV correspondingly. Methods: Complex monitoring was carried out in the forest-steppe anthropogenic landscape that is favorable for A. agrarius and in the cedar-oak forests in the optimum of A. peninsulae distributions (2001-2012). Trapping index estimated number of rodents on 100 trap-nights. Criteria of acute infection were detection the viral antigen/RNA in the lungs and organs of secretion/excretion and low avidity antibody in the blood of rodents. Basic criterion of epizootic activity was the trapping index of infected rodents with acute infection. Cases of HFRS diseases were summed in every epidemic season (from April to March). Results: We found principal differences between epizootic and epidemic processes in HTNV (FE) and AMRV natural foci that are directly depended on biotope distributions, behavior and the dynamics of specific sexual-age structure populations of reservoir hosts of pathogenic hantaviruses. Epizootic cycle duration (years) in populations of A. agrarius and A. peninsulae was 2-4 and 3-6 correspondingly. Peak of seasonal epizootic activity was observed in populations of A. agrarius in September-November and A. peninsulae in May-July. Trapping index of infected rodents with acute infection in phase of high activity of epizootic process was in populations of A. agrarius and A. peninsulae more 2 and 5 correspondingly. This index in phase of low activity of epizootic process was in populations of A. agrarius less 0,1 and A. peninsulae less 0,3. This index in phase of activity increase of epizootic process was in populations of A. agrarius more 0,6 and A. peninsulae more 1,5. In the HTNV (FE) and AMRV natural foci activation of epidemic process was observed over 3-4 and 3-6 years and seasonal prevalence of HFRS cases manifested in September-November and May-June correspondingly. Conclusions: Annual variations of epidemic activity of different HFRS natural foci directly correlated with numbers of acute infection carriers in A. agrarius and A. peninsulae populations. Outbreaks of HFRS on Primorye Territory were observed at the same year of high epizootic activity in Hantaan and Amur natural foci.

Keywords: hantavirus; epizootic process; HFRS natural foci; rodent-host; epidemic process;

O1-3 Characterization of Dobrava-Belgrade virus, genotype Kurkino, infections in Germany

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Dobrava-Belgrade virus (DOBV) is a distinct species within the genus Hantavirus and causes hemorrhagic fever with renal syndrome (HFRS) in Europe. The natural rodent hosts of DOBV are Apodemus mice species whereas humans are "dead-end hosts". According to their molecular phylogeny and natural Apodemus host species, DOBV can be subdivided into at least 3 genotypes pathogenic for humans; (i) Kurkino (formerly called DOBV-Aa) hosted by the striped field mouse, A. agrarius, (ii) Dobrava (DOBV-Af) by the yellow-necked mouse, A. flavicollis, and (iii) Sochi (DOBV-Ap) by the Black Sea field mouse, A. ponticus. Sporadic cases of clinically apparent DOBV infections are annually reported to the Robert Koch-Institute as the responsible Federal agency in Germany and anecdotic cases of mild and even severe clinical courses in DOBV-infected patients have been described. So far, comprehensive studies on molecular and serological diagnostics, epidemiology, and clinics of DOBV-Kurkino infections exist only from European Russia. Therefore, we have analyzed hantavirus infections from Germany to clarify the amount of DOBV infections and to determine the genotype. During 2007-2012, the German National Consultation Laboratory (NCL) for hantavirus infections has received serum samples from 570 patients living in the natural habitat of A. agrarius in North and North-East Germany. The specimens were tested for the presence of DOBV- and PUUV- specific antibodies in in-house EIAs and serological confirmation assays. Altogether, 86/570 (15.1%) patients with DOBV infections could be identified, including 60 males (age mean 43.0, range 13.9-77.8), 17 females (age mean 46.0, range 20.3-60.4) and 9 of unknown age and gender. Attempts to specify the DOBV genotype included neutralization assays (FRNT),, based on representatives of the different DOBV genotypes, as well as analyses of nucleotide sequences obtained from patient-derived viral RNAs. The analysis of neutralizing antibodies by FRNT failed to discriminate the genotypes in sera both from the acute and convalescent phase, as demonstrated with twelve well-characterized patient samples. Viral RNAs were obtained from 4 samples, and sequences of partial L- and M- segments revealed the appearance of genotype DOBV-Kurkino as human pathogen in Germany.
Keywords: Dobrava-Belgrade virus; Kurkino; human infections;

O1-4 Serological Evidence of Human Hantavirus Infections in West Africa, where Hantaviruses are carried not only by Rodents and Shrews but also Bats

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After discovering the first African hantaviruses - Sangassou virus in a rodent and Tanganya virus in a shrew - we extended our molecular screening to bats. Bats are known to harbor a broad variety of emerging pathogens. Their ability to fly and the forming of colonies in many different habitats enable an efficient maintenance, evolution, and spread of pathogens. A new hantavirus sequence was found in a hairy slit-faced bat (Nycteris hispida) from Sierra Leone. The virus was tentatively named Magboi virus (MGBV). Phylogenetic analyses show a close relationship to shrew- and mole-associated hantaviruses as well as to Mouyassué virus recently found in a banana pipistrelle (Neoromicia nanus). Following virus detection in these different animals we performed serological studies to investigate the occurrence of human hantavirus infections in West and South Africa, using a battery of screening, confirmatory, and typing assays. While the seroprevalence in the Western Cape region of South Africa is rather low (1.4%), we detected hantavirus-specific antibodies in up to 3.9% of Ivorian and Guinean populations in West Africa. A study in a group of Guinean patients with fever of unknown origin showed a 3.7-fold higher seroprevalence compared to the general population as well as occurrence of neutralising hantavirus antibodies. Our data let us conclude that hantavirus infections may be an unrecognised medical problem at least in parts of West Africa but possibly beyond. Ongoing deforestation and exploitation of the Guinean Forest Block, covering the coastal regions between Guinea and Ghana, may exacerbate the problem and increase the public health relevance of hantavirus infections in this area, by facilitating extensive contact between humans and hantavirus hosts.
Keywords: Hantavirus; Africa; Seroprevalence; Bats; Public Health;

O1-5 Life-Long Shedding and Non-transient Viremia of Puumala Hantavirus in Wild Bank Voles (Myodes glareolus)

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Temporal patterns of hantavirus shedding and viremia in rodent hosts have been studied in numerous laboratory experiments. On the basis of these studies, hantavirus shedding through saliva, urine and feces is believed to occur more intensively during the first weeks of infection, after which virus in only shed periodically in low quantities. In most studies, only a brief viremia was reported. However,

under the limited resources provided in natural environment, rodents are subjected to trade-offs between reproductive effort, longevity and immune response. These trade-offs may translate to enhanced or prolonged replication of pathogens. Thus far, temporal patterns of hantavirus shedding and viremia have rarely been studied in natural settings. Here we present the dynamics of Puumala hantavirus (PUUV) shedding and viremia in naturally infected, wild bank voles. In a monthly recapture study, we analyzed the presence and relative quantity of PUUV RNA in saliva, feces, urine and blood of 18 bank voles from 2 months before to 8 months after PUUV seroconversion. The proportion of animals shedding PUUV RNA in saliva, urine, and feces peaked during the first month after seroconversion, but continued throughout the study period with only a slight decline. The quantity of shed PUUV was constant in PUUV RNA positive excretions over time. More than 50% of animals showed viremia until 4 months from seroconversion, but both the probability of viremia and the virus load declined by time. Overall, wild bank voles showed notable individual variation in patterns of hantavirus shedding and viremia. Our results contradict the current view of hantavirus shedding to decline after an acute phase after hantavirus infection and a short viremic period. We suggest the long periods of shedding as a means of hantaviruses to disperse in patchy habitats where local host and/or virus populations face temporary extinctions and to survive over population bottlenecks in local populations. These findings also suggest that a revision of the present epidemiological models predicting the risk for human hantaviral epidemics may be necessary.

Keywords: Puumala virus; shedding; temporal patterns; bank vole; viremia;

O1-6 What Bank Vole Genomics Tells Us About The Uneven Distribution Of Puumala Virus In Sweden

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Nephropathia epidemica (NE) is a mild form of hemorrhagic fever with renal syndrome (HFRS) caused by the hantavirus Puumala (PUUV). In Sweden, almost all human cases are notified in the four northernmost counties, whereas the bank vole Myodes glareolus, which is the reservoir of PUUV, is common all over the country. Determining the causes underlying this heterogeneity is of main importance to better understand and prevent the risks of NE emergence throughout Sweden and, ultimately, Europe. Besides some climatic and ecological hypotheses, we proposed that geographic variability of bank vole immune responses to PUUV infection could shape differences in PUUV prevalence, and consequently NE incidence. We tested this hypothesis by performing a genome scan study of six bank vole populations sampled along a North/South transect in Sweden, including PUUV endemic and non endemic areas. The high throughput sequencing of RAD (Restriction-site Associated DNA) markers was applied, as it allowed rapid SNP (Single Nucleotide Polymorpshim) discovery and, in parallel, high-throughput genotyping of populations for thousands of SNPs, even when genomic resources are not available. We obtained 340 millions of sequences (100 bp). Among them, we isolated about 100.300 RAD-tags (DNA sequences that flank the restriction sites) that are reliable for the analysis of bank vole genomic adaptation. Population

genomic analyses were conducted to disentangle SNPs influenced by neutral processes only (genetic drift, mutation, migration) from those also influenced by selection, especially mediated by PUUV. Loci showing unusually high or low levels of genetic differentiation were assumed to be subject to natural selection, and consequently, to belong to genome regions involved, directly or indirectly, in M. glareolus / PUUV interactions. In the future, the genotyping of these SNPs for M. glareolus populations distributed all over Europe will help determining geographic areas where bank voles exhibit the same genomic signatures of selection, and therefore where the risks of PUUV emergence are important.

Keywords: genomics; immunity; selection; distribution; nephropathia epidemica;

O1-7 Isolation and Characterization of Hokkaido Virus, Genus Hantavirus

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Hantaviruses belong to the Bunyaviridae family and cause two severe human illnesses, hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). More than 30 viruses have been reported in the genus Hantavirus and are carried by the specific rodent host. We established new cell line, designated as “MRK101”, derived from a kidney of gray red-backed vole (Myodes rufocanus bedfordiae), the natural host of Hokkaido virus (HOKV). Using this cell line, we isolated HOKV Kitahiyama128/2008 strain. To characterize the newly isolated HOKV in more detail, we analyzed the viral growth of HOKV in VeroE6 and MRK101cells. In addition we determined the full-length sequences of S, M, and L segments of the virus. To observe the growth of HOKV, the HOKV Kitahiyama128/2008 strain was inoculated to MRK101 and VeroE6 cells. Culture fluids and infected cells were collected at 1, 3, 5, 7, 10, and 14 dpi. The collected fluids were subjected to focus-forming assays, and the presence of viral N protein in infected cells was evaluated by Western blotting. To determine the full-length sequences of S, M, and L segments of the newly isolated HOKV strain, each segment of the HOKV was amplified using specific primers and then sequenced directly. In MRK101 cells, the expression of HOKV N protein was confirmed at 14 dpi. The level of progeny virus increased gradually until 14 dpi. In contrast, no HOKV propagation was observed in VeroE6 cells; neither expression of N protein nor infectious virus was detected. The S segment sequence of the isolated HOKV was almost identical to previously reported HOKVs (97–98.7% nt and 99.8–100% aa identity). HOKV was the most closely related to PUUV (Identities in S segnemt, 81.9–83.8% nt and 94.7–95.8% aa; M segment, 78.1–79.9% nt and 90.6–91.6% aa; L segment, 79.7–80.7% nt and 95.3–95.6% aa) which is the etiological agent of HFRS. Phylogenetic analysis based on the nucleotide sequence of the coding region of the S, M, and L segments supported the close relationship between HOKV and PUUV. This is the first report of the hantavirus which propagates in a cell line that originated from the natural host but not VeroE6 cells. At present, the reason for the inability of HOKV propagation in VeroE6 cells is not known, but analysis of this phenomenon would provide us important information such as the host specificity of hantavirus.

Keywords: Hantavirus; Hokkaido virus; Puumala virus; Isolation; Propagation;

O1-8 Epidemiological and Clinical Outcome of Hantavirus Infection Associated with Two Viruses in Salta, Northern Argentina

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A broad spectrum of clinical manifestations of Hantavirus Pulmonary Syndrome (HPS) was reported in America, ranging from asymptomatic forms to classical picture of HPS. In Argentina, Andes virus (ANDV) is the major causative agent of HPS. In 2010, an HPS outbreak occurred in Northern Argentina Oran Salta Province, and two clusters were identified, one of them had unusually mild clinical symptoms and asymptomatic infection, supporting the idea that a less pathogenic hantavirus could be circulating in that area.

The objectives of this study were to asses if different clinical features and outcome are related to the infection with different genotypes of hantavirus, and investigate the rodent species implicated in hantavirus transmission in this endemic part of the country.

The first cluster (Cluster A) included 4 HPS cases with severe disease, 2 of them died.

The second one, a familiar cluster (Cluster B), included 5 persons, only 2 required three hospitalization days, but intensive care and intubation were not required. The severity of cases was reported as mild and evolution of the disease was favourable for the 2 cases. Asymptomatic hantavirus infection was identified in 3 persons. Hospitalized patients and the 3 contacts, have specific IgM and IgG against N-AND virus. The medical records of 6 hospitalized patients are analyzed for this study.

Small mammals trapping were carried out near these events. Results showed that 2 of 34 (5.9%) rodents (identified in the field as Calomys sp. and O. chacoensis) were hantavirus antibody positive and the RNA from lungs were amplified. Also cyt b gene (1,143 nt) from the rodents was sequenced. The mitochondrial analysis sequence identifies the two rodent hosts as C. fecundus and O. Chacoensis.

The viral sequence analysis of 950 nt from S segment showed the highest degree of identity with Laguna Negra (LANV) (C. fecundus), and with ANDV-Oran (O. Chacoensis).

The highest viral identity of Cluster A was observed with the strain found in O. chacoensis. The viral sequence analysis of cluster B showed that the 3 human sequences (2 patients and one asymptomatic contact) and viral RNA from C. fecundus were closely related.

Evidence that LANV is responsible for mild and subclinical disease in this region is presented and C. fecundus as a rodent host of LANV associated with HPS in Argentina.

Topic 2 VIRUS PHYLOGENY, REPLICATION, AND MORPHOGENESIS

O2-1 Novel Hantavirus Strains in Apodemus-Mice in Estonia and Latvia

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Apodemus-mice in Europe host hantaviruses that are severe human pathogens. They cause haemorrhagic fever with renal syndrome (HFRS), and they have been reported from central and eastern Europe, and European part of Russia. ICTV recognizes these as two different viruses: Dobrava-Belgrade virus (DOBV) and Saaremaa virus (SAAV). Alternative nomenclature was recently proposed (Klempa, 2012) suggesting re-classification with one virus (DOBV) sub-divided into four different genotypes: Dobrava and Sochi genotypes from current DOBV strains, and Saaremaa and Kurkino from current SAAV strains. These viruses/genotypes show characteristic differences in their phylogeny, specific host reservoirs, geographical distribution, and pathogenicity for humans. Here, viral genome sequences were recovered from Apodemus-mice captured in Estonia and Latvia. Partial S, M and L segment sequences were recovered from lung tissue samples using RT-PCR. Apodemus species were identified using CytB sequences, and these sequences were further used to study the evolutionary history of Apodemus mice in Europe. The hantavirus strains form distinct evolutionary lineages according to their host species and geographical origin. However, some conflicts are apparent in the tree topologies, and the data reported here suggest reassortant origin for the prototype SAAV strain originating from the Saaremaa island in Estonia.
Keywords: Apodemus; Dobrava virus; evolution;

O2-2 Evolutive history of Sigmodontinae hantavirus in South America

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Hantaviruses (Bunyaviridae) are an emerging public health problem in South America where more than 2500 cases of pulmonary syndrome (HPS) were reported. This bioinformatics study aimed to recognize common ancestors of South American hantavirus and to understand their dispersion in the continent. Thus, 190 sequences of the complete N protein gene at S segment of hantaviruses from 35 countries, collected during 1986 thru 2010, were retrieved from GenBank. Sequences were aligned using the Clustal W program and manually edited according to their codon-reading frame,

using the BioEdit software. Identical sequences (mutants and clones) were identified and removed using the DAMBE 5.2.6 program. The evolutionary history was estimated using the Markov Chain Monte Carlo (MCMC) Bayesian approach under GTR model with Gamma-distributed rate variation (γ) using a relaxed (uncorrelated lognormal) molecular clock implemented in the program BEAST v1.7.4. The GTR+ γ substitution model was used since it was the best model obtained in jModelTest. Four independent MCMC runs, of four chains each, were run for 100 million generations. The evolutionary rate was estimated based on age of the most recent common ancestor. The tree showed high probability density (> 95%) and the convergence of parameters during MCMC runs were supported by effective sample sizes (> 200). Our obtained maximum clade credibility tree showed that rodent-borne hantaviruses are approximately 1915 years old reaching Sigmodontinae and Neotominae from American continente, 548 years ago. A common ancestral for Sigmodontinae hantaviruses was found 405 years ago. Hantaviruses probably reached South America from Central America and were presente in Bolivian and Peruvian hantaviruses 354 years ago. Laguna negra, Rio Mamore and Jabora viruses were probably originated at this time. Shortly after, 341 years ago, hantaviruses were present in Brazil and Argentina and the divergence of Juquitiba and Araraquara-Andes virus branchs occured 306 years ago. We show here that South American Sigmodontinae hantaviruses appeared recently, in the last 4 centuries, and probably, are in plain evolution and spreading.

Keywords: Phylogeny;Sifmodontinae;Evolutive history;South America;

O2-3 The N terminus of Andes virus L protein supresses mRNA and protein expression in mammalian cells

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Andes virus (ANDV) is a member of the genus Hantavirus (Family: Bunyaviridae). In humans, ANDV causes a highly lethal respiratory disease called Hantavirus Cardiopulmonary Syndrome (HCPS). Little is known about structure and function of the Hantavirus L protein. Sequence alignments revealed a putative RNA-dependent RNA polymerase domain in the center and a putative endonuclease of the PD-(D/E)-K superfamily at the N terminus. Studying hantaviral L proteins is difficult, because recombinant L protein expression in mammalian cells is very ineffective. Deletion analysis revealed that the 534 N-terminal amino acid residues determine the low-expression phenotype. Inhibition of translation due to RNA secondary structures around the start codon, rapid proteasomal degradation, and reduced half-life time were excluded. We show that ANDV L protein expression can successfully be rescued upon mutation of catalytic amino acids and further conserved residues of the putative endonuclease domain. In addition, wild-type ANDV L rather than expressible L mutants suppressed the level of L mRNA as well as co-transfected reporter mRNAs. Wild-type L protein also reduced the synthesis of cellular proteins in the high-molecular weight range. Using expressible ANDV L mutants as a tool for localization studies, we show that L protein colocalizes with ANDV N and NSs, but not with the glycoprotein subunit Gc. A fraction of L protein also colocalized with the cellular processing (P) body component DCP1a. Overall, these data suggest that ANDV L protein possesses a highly active endonuclease at the N terminus

suppressing the level of its own as well as heterologous mRNAs upon recombinant expression in mammalian cells.

Keywords: Andes virus; L protein;

O2-4 Autophagic Clearance of Sin Nombre Hantavirus Glycoprotein Gn Promotes Virus Replication in Cells

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Hantavirus glycoprotein precursor (GPC) is posttranslationally cleaved into two glycoproteins, Gn and Gc. Cells transfected with plasmids expressing either GPC or both Gn and Gc revealed that Gn is posttranslationally degraded. Treatment of cells with the autophagy inhibitors 3-methyladenine, LY-294002, or Wortmanin rescued Gn degradation, suggesting that Gn is degraded by the host autophagy machinery. Confocal microscopic imaging showed that Gn is targeted to autophagosomes for degradation by an unknown mechanism. Examination of autophagy markers LC3-I and LC3-II demonstrated that both Gn expression and Sin Nombre hantavirus (SNV) infection induce autophagy in cells. To delineate whether induction of autophagy and clearance of Gn play a role in the virus replication cycle, we downregulated autophagy genes BCLN-1 and ATG7 using small interfering RNA (siRNA) and monitored virus replication over time. These studies revealed that inhibition of host autophagy machinery inhibits Sin Nombre virus replication in cells, suggesting that autophagic clearance of Gn is required for efficient virus replication. Our studies provide mechanistic insights into viral pathogenesis and reveal that SNV exploits the host autophagy machinery to decrease the intrinsic steady-state levels of an important viral component for efficient replication in host cells.

Keywords: Hantavirus; Glycoproteins; Autophagy; Negative strand RNA virus; Bunyaviridae

Topic 3 PATHOGENESIS AND IMMUNE RESPONSES

O3-1 A Novel Mechanism of Endothelial Cell Dysfunction in Hantavirus Pathogenesis Involves Contact Activation and Increased Liberation of Bradykinin

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Hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS) are diseases caused by hantavirus and characterized by vascular leakage due to unknown alterations of the endothelium barrier. Current hypotheses have focused on the influx of immune cells and release of cytokines or vascular endothelial growth factor (VEGF) as mechanisms for pathogenesis. Here we report, an alternative and novel mechanism for hantavirus-induced vascular leakage. We investigated if hantavirus-infected endothelial cells caused any abnormalities in the activation of the kallikrein-kinin system (i.e., contact activation, instrinsic pathway). When the purified plasma proteins, Factor XII (FXII), plasma kallikrein (PK), and high molecular weight kininogen (HK) were exposed to hantavirus-infected endothelial cells, we detected in increase in cleavage of HK and increased enzymatic activity of FXII and PK when compared to mock-infected controls. This cleavage resulted in increased levels of bradykinin (BK) within the supernatants of infected cells. BK is a potent inducer of inflammation and most notably, vascular leakage. Measuring cell permeability in real-time using electric cell-substrate impedance sensing (ECIS), we identified dramatic increases in endothelial cell permeability after activation of the kallikrein-kinin system and release of BK. Furthermore, the alterations in permeability could be blocked using inhibitors that directly block BK binding, the activity of FXII or the activity of PK.

O3-2 Hantavirus-infection confers resistance to cytotoxic lymphocyte-mediated apoptosis

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Rodent-born hantaviruses cause two severe emerging diseases with high case-fatality rates in humans; hemorrhagic fever with renal syndrome (HFRS) in Eurasia and hantavirus cardio-pulmonary syndrome (HCPS; also called hantavirus pulmonary syndrome (HPS)) in the Americas.

A hallmark of HFRS/HCPS is increased vascular permeability. While endothelial cells are the main targets for hantaviruses, infection per se is not lytic. Patients suffering from HFRS and HCPS show remarkable strong cytotoxic lymphocyte responses including high numbers of activated NK cells and antigen-specific CD8 T cells. Hence, it has been suggested that cytotoxic lymphocyte-mediated killing of hantavirus-infected endothelial cells might contribute to HFRS/HCPS-pathogenesis.
We report that hantaviruses protect infected endothelial cells from being killed by NK cells. Further, we also show that hantaviruses inhibit apoptosis in general. Interestingly, the nucleocapsid protein was shown to inhibit the enzymatic functions of both granzyme B and caspase 3, two enzymes crucial for cytotoxic lymphocyte-mediated killing of virus-infected cells, and to inhibit staurosporine-induced apoptosis.
These findings may explain why infected endothelial cells in hantavirus-infected patients are not destroyed by the strong cytotoxic lymphocyte response and argues against a role for cytotoxic lymphocyte-mediated killing of virus-infected endothelial cells in causing HFRS/HCPS.

O3-3 Distinct Innate Immune Responses in Human Endothelial, Epithelial and Macrophage Cells Infected with Hantaviruses

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Although hantaviruses have been previously considered as rodent-borne pathogens, recent studies demonstrate genetically distinct hantaviruses in evolutionarily distant non-rodent reservoirs, including shrews, moles and bats. The immunological responses to these newfound hantaviruses in humans are unknown. We compared the innate immune responses to Imjin virus (MJNV) and Thottapalayam virus (TPMV), two shrew-borne hantaviruses, with that toward two rodent-borne hantaviruses, pathogenic Hantann virus (HTNV) and nonpathogenic Prospect Hill virus (PHV). Infection of human macrophages and endothelial cells with either HTNV or MJNV triggered productive viral replication and up-regulation of anti-viral responsive gene expression from day 1 to day 3 postinfection, compared with PHV and TPMV. Furthermore, HTNV, MJNV and TPMV infection led to prolonged increased production of pro-inflammatory cytokines from days 3 to 7 postinfection. By contrast, PHV infection failed to induce pro-inflammatory responses. To further characterize the mechanisms of hantavirus-induced modulation of host cellular immunity, we examined host cellular microRNA (miRNA) expression signatures of human endothelial cells, epithelial and macrophage cells in response to HTNV, MJNV, TPMV and PHV infection. Recently, miRNAs have emerged as a class of essential regulators for host immune response genes during pathogenesis. We identified several miRNAs associated with the pathogenicity of the hantaviruses, with expression patterns inversely correlated with that of predicted gene targets. Pathway analyses confirmed that these targets were associated with aberrant Wnt, integrin signaling, and inflammatory responses. This study suggests that differential innate immune responses following hantavirus

infection may be mediated in part by cellular miRNAs through dysregulation of genes critical to the inflammatory process.

Keywords: Innate Immunity; Shrew-borne hantaviruses; microRNA expression; endothelial cells; macrophages;

O3-4 Viral Load and Immune Response Dynamics in Patients with Hemorrhagic Fever with Renal Syndrome

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Hantavirus pathogenesis is likely to be a complex multifactorial process that includes contributions from immune responses, platelet dysfunction and the deregulation of endothelial cell barrier functions. For HFRS a correlation between higher viral load levels and the severity of the disease has been reported. We presume that the wide spectrum of disease observed among HFRS patients might be related to differing immune responses and viral load kinetics. To test this hypothesis we analyzed sequential blood samples from 29 HFRS patients hospitalized in Slovenia. Although DOBV and PUUV-infected patients both had IgM at the time of hospital admission, there was a difference in the subsequent viral loads with DOBV-infected patients having much higher levels than PUUV-infected patients (107 vs. 105 RNA copies/ml). Measuring viral RNA in sequential patient samples revealed that viremia is longer, than previously believed, with DOBV or PUUV-infected patients having viremia lasting on average 30 days or 16 days, respectively. Even more all patients entered the convalescent phase before they were discharged from the hospital, but most of them had still detectable viral loads. In our study, elevated levels of IL-10 and TNF-α, INF-γ were detected in all of patients regardless of the causative agent. But, in DOBV-infected patients the change in cytokine secretion level appeared around day 20 post infection, while in PUUV-infected patients the change was earlier. Above that, we did observe a significant correlation between the viral load dynamics and secretion of IL-10, in which an increase in viral load led to higher production of IL-10. IL-10 displays a potent ability to suppress the antigen-presentation capacity of antigen presenting cells and stimulates antibody production, which might explain the effect on viral load. Consistent with earlier data, we observed a decrease in most measured cytokines in PUUV-infected patients during the late phase of acute disease. In contrast, in DOBV-infected patients showed increases in measured cytokines during the late phase of disease. One possible explanation for this is that in DOBV-infected patients in the late phase of acute disease there is insufficient induction of immunosuppressive mechanisms, which leads to a more severe disease form of HFRS.

Keywords: serial samples; viral load; cytokines; humoral response; dynamic;

O3-5 Pronounced Th2 Gene Expression in Helper T cells from Deer Mice (Peromyscus maniculatus) Experimentally Infected with Andes Virus

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Deer mice (Peromyscus maniculatus) are the principal reservoir hosts of Sin Nombre virus (SNV), which causes the great majority of hantavirus cardiopulmonary syndrome (HCPS) cases in North America. Infection of rodent reservoirs results in life-long persistence without signs of disease or tissue pathology. Andes virus (ANDV), which causes the great majority of HCPS cases in South America, is naturally hosted by the long-tailed pygmy rice rat (Oligoryzomys longicaudatus) and is thought to similarly cause persistent infection without conspicuous disease in its host. We developed a bioinformatics approach for designing real-time PCR arrays using the unannotated deer mouse genome to examine the expression of immune genes of deer mice infected with either SNV or ANDV. While deer mice remain persistently infected with SNV without disease, deer mice infected with ANDV clear the virus but also without disease. ANDV-infected deer mice seroconverted by day 14 while most SNV-infected deer mice had not. Lymph node cell recall proliferation to viral nucleocapsid antigen was similar in SNV or ANDV infected deer mice. We determined that many of the same genes were expressed in each infection but that levels of expression were substantially higher for some genes in ANDV-infected deer mice. While Th1, Treg and Th17 gene expression profiles were similar in SNV or ANDV-infected deer mice, Th2 gene expression was substantially elevated in deer mice infected with ANDV. These results suggest an early, quantitative effect in antibody responses may be responsible for ANDV clearance from deer mice.
Keywords: Andes virus; Sin Nombre virus; deer mice; reservoir; immune response

O3-6 Acute Hantavirus Infection Induces the Production of Galectin-3 Binding Protein (90K/Mac-2BP)

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Hantaviruses are a global health concern with thousands of casualties annually. The innate and cellular immune responses contribute to the pathogenesis of hantavirus infection; however, the underlying mechanisms are not yet clearly defined. Herein we report that acute hantavirus infection induces the production of galectin-3 binding protein (LGALS3BP, hereafter referred to as 90K/Mac-

2BP). We initially observed that hantaviruses (Tula and Puumala) propagated in Vero E6 cells co-purify with 90K/Mac-2BP in density gradient ultracentrifugation. 90K/Mac-2BP contains a scavenger receptor cysteine-rich (SRCR) domain that is typically linked to the recognition of foreign antigens. Using a panel of monoclonal anti-90K/Mac-2BP antibodies we confirmed that there is, indeed, an interaction between 90K/Mac-2BP and hantaviruses. Geimonen et al. (in PNAS 2002; 99(21):13837-42) observed using a DNA array that 90K/Mac-2BP, among various other genes, is upregulated at mRNA level in human umbilical vein endothelial cells (HUVEC) as a result of hantavirus infection. We observed that Vero E6 cells constitutively produce and secrete 90K/Mac-2BP while HUVECs produce detectable levels of 90K/Mac-2BP only when infected with hantaviruses. Increased concentrations of 90K/Mac-2BP have previously been reported in the serum of patients with chronic viral infection (human immunodeficiency, and hepatitis B and C viruses) or with cancer. As a next step we analysed the 90K/Mac-2BP levels in serum samples collected from Cynomolgus macaques experimentally infected with Puumala virus (see Klingström et al. in J Virol. 2002;76(1):444-9 and in Antivir Ther. 2008;13(1):125-33 for reference). We observed that the level of 90K/Mac-2BP is upregulated in the acute phase of infection together with various acute phase proteins and cytokines. Finally, we analysed plasma samples of 61 patients with acute, serologically confirmed, PUUV infection for 90K/Mac-2BP level. The 90K/Mac-2BP level was found to be higher in acute as compared to convalescent phase, and the increase was correlated with complement activation and with various clinical parameters reflecting the severity of acute hantavirus infection. Our findings suggest that 90K/Mac-2BP has a potential role in the defence against acute virus infection possibly via recognition of non-self and activation of the innate immune response.

Keywords: 90K; Galectin-3 binding protein; Mac-2BP; innate immunity; complement;

O3-7 HTNV-induced High Expression of CXCL10 is Mediated Through the Activation of TLR3, RIG-I and MDA5 Pathways

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Hantaan virus (HTNV) is a major agent causing hemorrhagic fever with renal syndrome (HFRS), a high-mortality-rate disease threatening about 100,000 people around the world yearly. However, the pathogenesis of HFRS remains to be elucidated. It may involve the abundant production of proinflammatory cytokines and uncontrolled inflammatory responses. CXCL10, a member of CXC chemokine family, also called interferon gamma (IFN-γ)-inducible protein 10 (IP-10). We have found that CXCL10 expresses highly in the HFRS-patients' sera and the increased CXCL10 is positively correlated with the severity of HFRS. However, the molecular mechanisms of HTNV infection inducing CXCL10 expression are still elusive. To clarify the regulation mechanism of CXCL10, the HTNV-infected human umbilical vein endothelial cells (HUVEC) model has been used. The results indicated that the dsRNA, produced in the process of HTNV replication, rather than the nucleocapsid protein and envelope glycoproteins of HTNV, could activate TLR3, RIG-I,

and MDA5 pathway, which facilitate the translocation of IRF7 and NF-κB. Afterwards, the two transcription factors bind directly to the CXCL10 promoter region and promote the transcription and expression of CXCL10. Furthermore, we found that the expression of CXCR3, the receptor of CXCL10, increased on the surface of monocytes at the early stage of HFRS. It suggested that CXCL10 may recruit monocytes at the onset of the HFRS, which have played an important role in the cytokine storm and the pathogenesis of HFRS. However, further studies are still needed to reveal the function of CXCL10 in the pathogenesis of HFRS. In conclusion, we report that the increased CXCL10 level is associated with the state of the HFRS. The activation of innate immunity induced by HTNV replication play an important role in the regulation of CXCL10 expression and the abundant CXCL10 may be involved in the pathogenesis of HFRS.

Keywords: Hantaan virus; HFRS; CXCL10; Innate immunity

O3-8 Viral Load and Immune Responses in Patients with Puumala Hantavirus Infectiion

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Background The clinical picture and severity of hantavirus infection varies depending on the particular virus involved. The pathogenesis of human hantavirus infections are complex and not fully understood. It likely involves both virus and host-mediated mechanisms. The immune responses, including cytokines, cytotoxic NK- and T cells and antibodies might have an important role. The aim of the present study was to investigate the viral load and humoral immune response in patients with Puumala hantavirus infection. Methods: In total, 105 patients with verified Puumala hantavirus infection were included in this prospective study. Blood samples were collected and clinical symptoms were studied from the acute onset of disease to the convalescent phase. The kinetics of laboratory parameters, viral load and the humoral (IgA, IgM and IgG) response were investigated. Results: The patients had classical symptoms of acute hemorrhagic fever with renal syndrome (HFRS), 88% of the patients had signs of renal impairment. 1/3 of the patients had low platelets and clinical signs of milder bleeding manifestations. Fifteen of the 105 patients (14%) were classified as having a moderate/severe illness. A correlation was observed between severe illness and low platelets ($p<0001$), a high creatinine ($p=0.01$) and a high white blood cell count (WBC) ($p=0.001$). A high WBC was also associated with high creatinine values. In a majority of patients viremia was detected for up to 9 days after symptom debut. The viral load was not associated with severe illness, neither with a high creatinine level. A low Puumala virus specific IgG response was significantly associated with severe disease ($p=0.023$). Conclusions: During Puumala hantavirus infection, levels of viremia seems not affect outcome. Notably, low levels of specific IgG in patients was associated to a more severe clinical outcome in these patients. A high white blood cell count is linked both to severe disease and to renal impairment. We have previously showed that a there is

marked increase of activated CD8 T cells and NK-cells during the acute phase of infection, which could contribute to the severity of disease (Björkström et al., JEM 2011; Lindgren et al., J Virol 2011). Hence, the level of host defence including neutralizing antibodies and cellular immune responses may play important roles in the pathogenesi

Keywords: hantavirus; Puumala virus; Immune response; viral load; immunoglobulins;

Topic 4 DIAGNOSTICS, TREATMENTS AND CLINICAL FINDINGS

O4-1 Development of an Immunochromatography Strip Test for Detecting Anti-hantavirus Antibody in Rodent and Human Sera by Using an N-terminal Common Antigenic Site of Hantavirus N protein

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Objectives: Hantaviruses are causative agents of two rodent-borne zoonoses, hemorrhagic fever with renal syndrome (HFRS) and nephropathia epidemica (NE) in the Old World and hantavirus pulmonary syndrome (HPS) in the New World. This study is aimed to develop immunochromatographic (ICG) test for rapid, simple and specific diagnoses of hantavirus infection among rodent and human sera. Methods:The N-terminal 103 amino acids (aa) of Hantaan virus (HTNV), Puumala virus (PUUV) and Andes virus (ANDV) nucleocapsid (N) protein were expressed in E. coli as representative antigens of three groups (HFRS, NE and HPS—causing viruses) of hantavirus. The recombinant N antigens were used as for antigens in an immunochromatography strip (ICG) test to detect anti-hantavirus IgG antibody. Rabbit anti rat IgG labeled colloidal gold (Wine red chemicals) and Protein A labeled colloidal gold (EY Laboratories) were used to detect rodent and human IgG, respectively. Serum specimens were examined at 1:75 dilution. For human serum, five different types of ICG test strips were developed, one antigen line on one strip (HTNV, PUUV and ANDV strip), 3 antigen lines on one strip and mixed antigen line on one strip.. Results: A total of 340 rat sera which consisted of 19 of experimentally infected laboratory rat sera, 38 of naturally infected sera and 283 of uninfected laboratory rat and urban rat sera were examined. ICG test detected antibody as same level as that of ELISA. The sensitivity and specificity of ICG compared to ELISA and/or IFA were 100% and 99.8%, respectively. A total of 168 sera, including sera from HFRS patients (21 acute-phase and 35 convalescent-phase patients), NE patients (29 acute and 36 convalescent patients), HPS patients (12 acute and 16 convalescent patients), and healthy people (25) as negative controls, were used to evaluate the ICG test. All of the ICG test strips showed high sensitivities to acute (79.3 to 100%) and convalescent (97.2 to 100%) sera to homologous antigen. The intensities of the ICG test line were strongest to homologous sera. Whole bloods of human and rodent, instead of serum, were also applicable to ICG test. Conclusion: These results indicated that the ICG test with the three representative antigens is an effective serodiagnostic tool for screening and typing of HFRS and HPS hantavirus infection in human.
Keywords: ICG; Multiplex; typing; Recombinant; rapid;

O4-2 Simultaneous Detection of IgG Antibodies Associated with Viral Hemorrhagic Fever by a Multiplexed Luminex-based Immunoassay

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Humans are facing the more and more serious threat posed by viral hemorrhagic fevers (VHFs), which are mainly caused by four distinct families of enveloped, single-stranded RNA viruses. With the emergence of new viruses, advanced diagnostic methods are urgently needed for identification of VHFs. Based on Luminex xMAP technology, a rapid, sensitive, multi-pathogen and high-throughput method which could simultaneously detect VHF virus-specific IgG antibodies was developed. Recombinant antigens of nine VHF viruses including Hantaan virus, Seoul virus, Puumala virus, Andes virus, Sin Nombre virus (SNV), Crimean-Congo hemorrhagic fever virus (CCHFV), Rift Valley fever virus (RFV), Severe fever with thrombocytopenia syndrome bunyavirus (SFTSV) and dengue virus (DENV) were produced and purified from prokaryotic expression system and the coupling amount was then optimized. Cross-reactions among antigens and their rabbit immune sera were evaluated. Optimal patients' sera dilution was determined based on serial dilutions of representative clinical samples and serum samples collected from 62 laboratory confirmed hemorrhagic fever with renal syndrome (HFRS) patients, 43 confirmed SFTS patients, 88 healthy donors were analyzed. Results showed that the purity and concentration of nine detection antigens met the standards of diagnostic study. Evaluation of this new method with clinical serum samples showed 98.4% diagnostic sensitivity for HFRS, 86% for SFTS detection and the overall specificity was above 98.9%. The multiplexed luminex-based immunoassay has firstly been established in our study, which provides a potentially reliable diagnostic tool for IgG antibody detection of VHFs.

Keywords: Viral hemorrhagic fever; Luminex xMAP; Diagnosis;

O4-3 Passive immune therapy for ANDV HPS with human plasma: an open trial

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Background: In Chile, Andes (ANDV) hantavirus is the sole etiological agent of HPS causing a mean of 55 annual cases with lethality rate of 32%. No specific treatment is available and low

incidence difficult performance of clinical trials. An inverse correlation between neutralizing antibody (NAb) titers at hospital admission and disease severity has been reported. In the Syrian hamster model, early administration of plasma with ANDV NAb prevents death. In Argentine hemorrhagic fever treatment with human immune plasma decreased lethality from 20% to 1%. We designed a compassionate open trial to evaluate safety and efficacy of immune plasma from HPS survivors as a treatment strategy for this disease. Methods: We performed plasmapheresis to donors survivors to ANDV infection and stored plasma in individual bags at -80 ℃. We measured NAb titers through focus reduction neutralization test and defined units as the reciprocal of the highest dilution with 80% focus reduction. We established 10 study sites along Chile. We enrolled cases with suspected/confirmed HPS after informed consent, and infused ABO compatible immune plasma to a dose of 5,000U of NAbs. HPS was confirmed through IgM serology or RT-PCR. We followed cases for six months. Main outcome was lethality and comparators were unenrolled cases in Chile in the same period; unenrolled cases in the study sites between January 2005 and March 2012 and HPS cases admitted to a methylprednisolone clinical trial. Results: We performed 44 plasmapheresis and established 3 regional immune plasma banks. Between January 1, 2008 and March 12, 2012 we enrolled and treated 32 cases, 29 (15 males and 14 females; median age=33) with confirmed hantavirus. The lethality rate of confirmed enrollees was 4/29 (13,8%) vs. 63/199 (31,7%) in non enrollees in the same period in Chile; p=0.052 and OR=0.35(IC=0.12-1.03). The lethality rate of non enrollees admitted to the study centers between 2005 and 2012 was 18/66 (27.3%) and in the methylprednisolone study was 20/60 (33%) which is not statistically different. We detected no serious adverse events associated to plasma infusion. Conclusions: Human ANDV immune plasma infusion is a safe intervention for SPH. We observed a clinically significant difference in lethality, although it did not reach statistical significance. We cannot discard biases of an open trial.

Keywords: HPS; therapeutics; ANDV; Neutralizing antibodies;

O4-4 A Severe Capillary Leakage Syndrome in Hantavirus Infection Treated with Bradykinin Receptor Antagonist Icatibant

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Case report. In March 2011 a 37-year old Finnish male experienced high fever and headache. On hospital admission six days later he was in a state of shock with acute respiratory and kidney failure. Laboratory analyses showed haemoconcentration, leukocytosis, thrombocytopenia, proteinuria and haematuria. Radiologic imaging revealed bilateral pulmonary infiltration and pleural effusion and abundant amount of fluid in the peritoneal cavity. A recent Puumala virus infection was diagnosed. The intensive care treatment included sedation, intubation, mechanical ventilation and a continuous renal replacement therapy. A massive capillary leakage syndrome was observed. The patient

received fluid replacement, vasoactive drugs, inhaled nitric oxide, corticosteroids and ceftriaxone. His physical condition became extremely critical. Then one subcutaneous dose (30 mg) of icatibant was administered. During the next day a stabilization of the course of the disease took place, followed by gradual improvement. The total hospital care lasted for four weeks. One month later the patient was in good health and all laboratory parameters had normalized. Discussion. The clinical course of the present patient seemed inevitably lethal. Currently there is no known effective therapy for hantavirus infections. Several drugs that influence the vascular permeability are in clinical trials. Hantaviruses infect endothelial cells and cause dramatic changes in barrier and other functions of the endothelium without disrupting it. Cytotoxic CD8+ T cells, cytokines and complement activation could also trigger capillary leakage. Bradykinin promotes vasodilatation and increases vascular permeability in several pathophysiological states. Therefore icatibant was administered. Icatibant is a synthetic polypeptide that acts as a selective and competitive antagonist of the bradykinin type 2 receptor. At present it is indicated for the treatment of acute episodes of hereditary angioedema in adults. It is well tolerated and most patients need only a single 30 mg dose as treatment. Conclusion. This is the first report about icatibant use in the treatment of hantavirus infection. The drug should be tested in a larger numbers of patients with hantavirus infections, especially in those characterized by high mortality.

Keywords: Puumala; icatibant; bradykinin; hantavirus;

O4-5 Cardiopulmonary Involvement in Puumala Hantavirus Infection

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Objectives: Hantaviruses in Eurasia cause human disease which is mainly characterized by coagulopathy and renal failure. However, cardiopulmonary involvement is also reported to be a common feature. We wanted to study cardiopulmonary involvement in Swedish Puumala hantavirus (PUUV) infection. Methods: Twenty-seven hospitalized patients (mean age 56 years, range 19-82 years) with verified PUUV infection were included in a prospective study. Patients were subjected to echocardiography, 24h Holter-EKG with heart rate variability (HRV) analysis, chest CT, cardiac markers and lung function. Twenty-five age and sex-matched volunteers acted as controls for echocardiography data. Results: During the acute phase, compared to controls, patients had slightly reduced left ventricular ejection fraction ($p<0.01$), together with prolonged left ventricular relaxation time ($p<0.05$), increased pulmonary artery systolic pressure ($p<0.01$) and pulmonary arterial resistance ($p<0.05$), along with shortened pulmonary artery acceleration time ($p<0.05$). After 3 months most parameters were returning towards normal. Holter-EKG revealed abnormal results in 68% and 42% of the patients in the acute phase and at follow-up, respectively. Rhythm disturbance and reduced HRV indicating autonomic dysfunction were common. Chest CT showed pleural effusion in 38% and pulmonary edema in 21%. There was an elevation of cardiac NT-

ProBNP in all but one patient while Troponin T was slightly elevated in 8 patients, suggesting ventricular dysfunction and in some patients a discrete myocardial injury. Lung function tests revealed subnormal results for gas diffusion capacity in most patients, improving after 3 months ($p<0.001$) but were still subnormal in 40 % of the patients. Conclusions: Majority of patients with PUUV infection experience affection of both cardiac and pulmonary function, that are normally found in hantavirus cardiopulmonary syndrome in the Americas. Our results demonstrate vascular leakage in the lungs leading to pleural effusion and edema that most likely is responsible for an increased vascular resistance in the pulmonary vasculature with secondary right heart distress together with decreased pulmonary function. We can also demonstrate slightly impaired left heart function and that cardiac arrhythmias and autonomic dysfunction are very common in Swedish hantavirus infected patients.

Keywords: Hantavirus; Pulmonary function; Cardiac function; Puumala; HFRS;

O4-6 Development and Evaluation of a Broad Reacting Sybr-Green based Quantitative Real-Time PCR for the Detection of Different Hantaviruses

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Hantaviruses are endemic in most parts of the world and cause hundreds of thousand human cases of hemorrhagic fever with renal syndrome (HFRS) and hantavirus cardiopulmonary syndrome annually throughout Eurasia and the Americas. They are zoonotic viruses, most commonly transmitted to humans by aerosolized rodent excreta, and new hantaviruses are frequently discovered in previously unknown reservoir species and geographic areas. Consequently, there is a need to improve hantavirus diagnostics. This paper describes the design and evaluation of a rapid and robust quantitative real-time PCR (QRT-PCR) assay able to detect a wide range of hantaviruses. Primers with the potential to detect different hantaviruses were designed from conserved regions of different hantavirus (L)arge segments, as identified from multiple sequence alignments. By using SYBR-green-based QRT-PCR 100-1000 target molecules of in vitro produced RNA and less than 100 copies of hantavirus RNA from different hantavirus clades and regions of the world were detected. When using the assay on clinical samples from patients with acute HFRS, Puumala hantavirus (PUUV) RNA was confirmed in all previously positive samples. Notably, the broad reacting L-segment QRT-PCR also detected viral RNA in HFRS patient samples, previously negative by a QRT-PCR targeting the (S)mall segment of PUUV. This novel assay provides a powerful tool for diagnosis of hantaviruses from different clades and regions and may also be useful in surveys with the purpose of finding new hantaviruses in rodent or insectivore species.

Keywords: Hantavirus; Broad-reacting; quantitative real-time PCR (QRT-PCR); SYBR-green;

O4-7 A Sin Nombre virus DNA vaccine delivered using the PharmaJet needle-free jet injector elicits high-titer neutralizing antibodies in rabbits and nonhuman primates

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Sin Nombre virus (SNV) is a rodent-borne hantavirus, family Bunyaviridae. When this virus infects humans, it can cause hantavirus pulmonary syndrome (HPS). There have been ~587 cases of HPS in the Unites States, >200 of them fatal. There are no vaccines or specific drugs to prevent or treat HPS. Recently, we constructed a SNV full-length M gene-based DNA vaccine capable of eliciting high-titer neutralizing antibodies in animals. Here, we tested whether this DNA vaccine could be delivered effectively using PharmaJet's needle-free jet injection technology. Both the PharmaJet intramuscular (IM) and intradermal (ID) devices are FDA 510(k)-cleared for commercial use in humans. First, we tested the SNV DNA vaccine for immunogenicity in rabbits. The vaccine at a dose of 0.4 mg was administered to rabbits 3x at 1-month intervals using the IM device or the ID device. Sera were collected and evaluated for the presence of neutralizing antibodies by a classical plaque reduction neutralization test, or by a pseudovirion neutralization assay. The vaccine elicited neutralizing antibodies in 8 of 8 rabbits after a single vaccination. SNV neutralizing antibody titers as measured by both assays were high (titer >1000) after a second vaccination. Sera from the vaccinated rabbits were tested for the capacity to protect hamsters in a SNV infection model and an ANDV lethal disease model. Passive transfer of the anti-SNV antibodies protected hamsters against SNV infection, but not against lethal disease caused by ANDV. Next, we tested whether the PharmaJet devices could effectively deliver the SNV DNA vaccine to nonhuman primates (NHPs). NHP were vaccinated 3x at 1-month intervals using either the IM or ID device (2x 0.5 mg of DNA per vaccination). All six of the NHPs developed neutralizing antibody responses. The IM device appeared to be more efficient at delivery in NHPs because all animals were positive after a single vaccination whereas 2 of 3 NHPs vaccinated with the ID device required a second vaccination before high levels of neutralizing antibodies were produced. Demonstrating that a hantavirus DNA vaccine can be effectively delivered to rabbits and NHPs using this needle-free jet injection technology is important because these devices are relatively inexpensive, practical, and already used commercially for the delivery of conventional vaccines around the world. Future studies will be aimed at testing whether PharmaJet technology can effectively deliver hantavirus DNA vaccines to humans.

Keywords: hantavirus; Sin Nombre virus; DNA vaccine; neutralizing antibodies; passive transfer

Poster Sessions

Topic 1 ECOLOGY AND EPIDEMIOLOGY

P1-1 Socioeconomic and environmental factors affecting spatial risk of hantavirus infection in state of São Paulo, Brazil – a case control study

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Objectives: The aim of this study was to estimate the spatial distribution of the risk of hantavirus in municipalities of the state of São Paulo, Brazil, from 1993 to 2008, considering the influence of environmental and socio-demographic characteristics.
Methods: We conducted a spatial case-control population-based study, in which cases of hantavirus (N = 136) were reported by the Epidemiological Surveillance System and controls (N = 900) were randomly selected in proportion to the population of each municipality. Besides the geographical coordinates (X, Y) of cases and controls following variables were included: socioeconomic , demographic, land cover, climatic and topographic, with spatial resolution of approximately 1 km2 (Worldclim (http://www.worldclim.org/) ; vegetation index (EVI) summarized by the MODIS satellite (resolution of 500 m) (NASA-MODIS).
Results: Maps were drawn to visualize the spatial risk interpolated surface in the region. A generalized linear model (two-dimensional spline involving the X and Y coordinates) were adjusted to data, generating maps with and without environmental covariates.
Conclusion: The spatial risk of hantavirus infection in São Paulo remains partially even with the inclusion of socioeconomic and environmental indicators. Counties with the highest rates of urbanization and the lowest GDP per capita had the highest risk, suggesting that towns and cities with the lowest percentage of residents in the field, but with less revenue per capita were the most affected. However, the effects of these variables cannot be considered in isolation, without information about the local context of human exposure, interaction between rodents and susceptible populations, housing, leisure and working conditions associated with the transmission of the disease.
Keywords: hantavirus; spatial case control study; risk; environmental factors; socioeconomic factors

P1-2 Hantaan virus seroprevalence of people in several regions of Jilin province of China, from 2007 to 2012

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Hemorrhagic fever with renal syndrome (HFRS) has been a serious health problem in Asia,

especially in China. Jilin province has been one of the most HFRS-prevalent region in China. Totally 5,787 sera were collected at five counties, Dunhua, Helong, Longjing, Tumen, and Yanji in in Jilin from 2007 to 2012. The titers of immunoglobulin G (IgG) antibody to Hantaan virus (HTNV) were assayed by immunofluorescence assays (IFA). Proportions of positive samples according to gender, age, residential area, occupation and vaccination history were analyzed. Average seroprevalence was 45.0% (2,606/5,787). Males (45.1%, 1,093/2,424) and females (45.0%, 1,513/3,362) showed almost same antibody prevalence. A peak seropositive rate was shown at people in their fifties (50.8%). Farmers were largest population and showed HTNV IgG prevalence 47.4% (2,368/5,000). Among five counties, peoples in Helong showed highest prevalence of anti-HTNV (55.8%, 279/500). 126 among 206 people (61.2%), who were previously innoculated with Hantaan virus vaccine, have antibodies to HTNV. This is a report on the study on seroprevalences of HTNV in healthy population in five provinces in Jilin province, northeast China. Our data show higher overall HTNV seropositive rates of people in this study compared with that in other studies. Vaccination program can cause the high HTNV seroprevalence of people in the study areas.

Keywords: Hantaan virus; seroprevalence; China; Jilin;

P1-3 Antibody levels to hantavirus in inhabitants of Brazil and Colombia determined by an ELISA using the N recombinant protein of Araraquara virus

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Hantaviruses are an emerging public health problem in Brazil where 1600 cases of pulmonary syndrome (HPS) were reported with a 40% case fatality rate. Recently, a recombinant nucleocapsid (rN) protein of Araraquara virus (ARAV), a brazilian hantavirus, was obtained in Escherichia coli. ARAV rN has been evaluated as antigen for antibody detection in Brazil and Argentina showing it is a suitable antigen for diagnosis of hantavirus infections. ARAV rN has been shared with laboratories from different regions of Brazil and also from Colombia allowing perform epidemiologic studies in regions where hantavirus infection is completely unknown: - In the south, at Santa Catarina state, in the western border Brazil - Argentina, in 2009, 3.5% of the studied population (340) had antibodies to hantavirus. In the eastern side of Santa Catarina, IgG antibodies

to hantavirus were found in 2.3% of 257 participants and all 9 seropositives reported a previous severe pneumonia. - In the Southeast, at Cassia dos Coqueiros county, Sao Paulo state, in a retrospective study, 89 of 1,876 participants (4.7%) that donated blood from 1987 to 1990 had antibodies to hantavirus, showing that these infections occurred completely unrecognized, even before HPS description in the Americas. - In the northeast, in 2008, from 72 dengue suspected cases at an urban area of Ceara state, 2 (2.8%) had IgG antibodies and 1 (1.4%) had IgM antibodies to hantavirus. - In the north, at Atalaia do Norte, Careiro Castanho, Itacoatiara and Labrea conties, Amazonas state, it was performed an study from 2007 thru 2009 where 10 individuals had antibodies to hantavirus among 1,731 participants. - In the Center-West, Mato Grosso state, in 2010, IgG antibodies to hantavirus were detected in 7 (13%) of the 54 participants. There was an association of seropositivity to hantavirus within the participants born in the south of Brazil. - In Caribbean region of Colombia, in 2009, 24 of 284 agricultural workers (8.4%) had IgG antibodies to hantavirus including 2 cases that were also IgM positive. We show here that the development of ARAV rN has been useful for epidemiological studies of hantavirus in South America. We also show that hantavirus infection is common in all regions of Brazil because 2.3% to 13% of the participants of our epidemiologic studies had antibodies to hantavirus and it also occurred in C

Keywords: Hantavirus; Brazil; Colombia; N antigen of Araraquara virus;

P1-4 Hantavirus epidemiological studies in Brazilian Sigmodontinae rodents

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In Brazil 1600 cases of cardiopulmonary syndrome (HCPS) were reported with a 40% case fatality rate. Seven hantavirus genotypes were reported: Juquitiba (related to Olygorizomys nigripes), Araraquara (Necromys lasiurus), Castelo dos sonhos (Olygorizomys moojeni), Laguna Negra-like (Calomy callosus), Anajatuba (Olygorizomys fornesi), Rio Mearim (Holochilus sciueus) and Jabora (Akodon montensis). Hantavirus infection was diagnosed by ELISA using as antigen a recombinant protein from Araraquara virus (ARAV) nucleocapsid, conventional RT-PCR, and a SYBR Green real-time RT-PCR. Here we show results of studies on hantavirus epidemiology in wild rodents: - 51 rodents were captured in rural areas of Rio de Janeiro state from 2004 - 2005, and 4 of them (Olygoryzomys nigripes) had hantavirus antibodies. It was showed, for the first time, hantavirus infections in Rio de Janeiro. - 174 rodents were captured in the northeast of Sao Paulo state, 2003 - 2004 and 9 (5.7%) had antibodies to hantavirus including Necromys lasiurus, Akodon sp and Oligoryzomys sp. Amplicons of RT-PCR from 5 N. lasiurus were sequenced presenting ARAV in all specimens. Thus, it was evidenced that N. lasiurus might be the ARAV natural reservoir. - In the University of Sao Paulo campus in Ribeirao Preto 10 N. lasiurus were captured in 2009 and 3 of them were infected by hantavirus. The campus community was warned on these rodents and the risk of HCPS. - Among 568 small mammals captured in the northeastern region of Sao Paulo state 36

rodents (6.3%) were infected by hantavirus during 2008-2009, as part of a study on environmental change and hantavirus infection among rodents. Hantavirus infection in wild rodents seems to have a seasonal preference (7.2%) in the dry season (April to September), and in places with degraded environment (7.7%). Environmental degradation and seasonality seems to be favoring abundance of opportunistic rodents species such as N. lasiurus, Akodon sp, and Calomys tener, and then were considered as risk factors for hantavirus infection. Therefore, loss of diversity and environmental degradation increased hantavirus infection among wild rodents. - 6 Necromys lasiurus and Akodon sp were infected by intramuscular route with Rio Mamore (RMV) hantavirus. The virus genome was detected in the urine and feces of these rodents 4-5 days after infection a.i., and in serum and saliva.

Keywords: Hantavirus; Epidemiological; Sigmodontinae; Brazil;

P1-5 Hantaviruses in the United Kingdom

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Hantaviruses are a group of emerging rodent-borne viruses which are distributed across the globe. Several distinct strains are known to circulate; those confirmed to cause human disease within Europe are Dobrava (DOBV), Hantaan (HTNV), Puumala (PUUV) and Saaremaa (SAAV) viruses. Seoul virus is considered to be a global hantavirus, although human cases outside of Asia are extremely rare. In the United Kingdom there has been growing evidence for human and animal exposure to hantaviruses since the 1970's; demonstrated by the detection of specific antibodies and classic HFRS type disease. However, until recently, no direct evidence for any hantavirus has ever been reported in the UK. The presentation will cover the work undertaken by the Health Protection Agency to firmly prove the existence of at least one hantavirus in the UK, a Seoul hantavirus designated strain Humber. Recent clinical cases, virus findings and the wider public health implications will be discussed.

Keywords: UK; Seoul; Hantavirus; Rattus norvegicus;

P1-6 Hemorrhagic Fever with Renal Syndrome in the Primorye Region of Russia: Epidemiological and Epizootological Characteristics of two Outbreaks

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Hantavirus infection is endemic in far-eastern regions of Russia. In the Primorye Region there are three pathogenic hantaviruses associated with hemorrhagic fever with renal syndrome (HFRS) – Hantaan, Amur and Seoul with natural hosts Apodemus agrarius, A. peninsulae and Rattus norvegicus, subsequently. Approximately 60 HFRS cases are reported in rural and urban foci of hantavirus infection annually with average incidence 3.0 cases per 100,000 persons and mortality

up to 12%. Large local outbreaks of HFRS were reported in our region in 1991 (30 patients) and in 1997 (17 patients), during last decade the total 11 outbreak were registered mainly in natural foci of infection. In this paper we describe two outbreaks (2009-2010) associated with different hantaviruses Amur and Hantaan that were observed in different landscapes – forest and steppe-forest areas where A. agrarius and A. peninsulae are dominated. First outbreak was registered among four employees of National Park "Zov Tigra" from May 31, 2009 to June 06, 2009. All patients used firewood stored in the place contaminated by rodent's feces for burning in stove. HFRS was confirmed by typical clinical course and serologically. There was increased number of infected A. peninsulae in this area and rate of infection was up to 4.5 rodents per 100 trap days. Notably most of infected rodent have markers of acute hantavirus infection, i.e. RNA in organs of excretion and low-avidity specific antibodies. Molecular analysis of RNA isolated from patient's blood and serological testing confirmed that Amur virus was the etiological agent in all cases. Second outbreak was registered at June 14-27, 2010 in the lowlands near Khanka Lake and involved eight cases. All patients were young man (20 - 26 years old) who worked in similar conditions of increased dust forming and lived in tents. Two patients have severe HFRS and one of them died. Among trapped in this locality rodents about 60% A. agrarius were young animals with acute hantavirus infection. Also hantaviral RNA was detected in samples of soil collected near the patient's housing. Hantaan-infection was confirmed by serological typing in all examined cases. In conclusion, outbreak of hantavirus infection are associated with active epozootic processes in populations of natural hosts and usually involved people grouped by similar conditions of work.

Keywords: hantavirus; outbreak; Hantaan; Amur;

P1-7 Molecular epidemiology of Puumala Virus in Belgium

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Puumala virus (PUUV) is the sole causative agent of HFRS in Belgium, with a high incidence in the southern part and a low occurrence in the northern part of the country. It is generally believed all HFRS cases have their origin in the southern part of the country. This study aims at analysing the genetic characteristics of PUUV isolated out of Belgian HFRS patients and bank voles. Serum samples from HFRS patients with PUUV-specific IgM-antibodies, collected from 2008 to 2012 were used. In addition, PUUV was isolated from bank vole lung tissue samples. Subsequently, the whole S segment sequences were obtained and maximum likelihood and Bayesian methods by using Paup* and Mr. Bayes software for phylogenetic reconstruction implemented. Twenty-five million generations with samples drawn every 50,000 generations were analysed. TRACER v1.5 was used to determine whether the Bayesian analysis reached appropriate convergence. All sequences recovered from patient samples and bank vole isolates were closely related and formed one monophyletic clade. Within this clade 2 distinct groups could be identified representing viruses

isolated from respectively northern and southern Belgium. Between both groups an average nucleotide and amino acid similarity of 91.8% and 98.5% was detected respectively. Most of the changes in the nucleotide sequences were silent mutations. The group with sequences originating from the northern part of Belgium exhibited a high degree of similarity (nt: 93.7-94.2%, aa: 98.4-98.6% similarity) with PUUV BE/Turnhout/1/1985, previously isolated in the same area. The sequences isolated in the South were most related to PUUV BE/Montbliart/1/1986 (nt: 95.0-99.8%, aa: 99.3-100% similarity) and PUUV BE/Montbliart/2/1986 (nt: 95.0-99.5%, aa: 99.1-99.8% similarity), which originate from Montbliart, a municipality in the South of Belgium. Our phylogenetic analysis of the whole S segment sequences recognized PUUV as the causative agent of HFRS in both parts of Belgium. Our data suggest that hantavirus infections can be contracted in both parts and confirms the geographical clustering of PUUV in Belgium. The Belgian isolates establish a genetic lineage distinct from other known PUUV lineages from Europe and Russia. Within the Belgian lineage two distinct genetic groups were observed, which might suggest a separate spatial evolutionary process for both clusters.

Keywords: Puumala virus; phylogeny; epidemiology;

P1-8 Serological Differentiation of HFRS Cases in Urban Focus of Hantavirus Infection

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The annual registration of hemorrhagic fever with renal syndrome (HFRS) in Primorski Territory is conditioned by long and active foci of hantavirus infection. Seoul virus (SEOV) is the main cause of diseases in urban focus of Primorski Territory. However some part of cases (about 34.4%) registered in urban focus amongst persons staying for 2-3 weeks before disease onset in natural foci, was associated with Hantaan (HNTV) virus. Mozaic spreading of hantaviruses in HFRS natural foci dictates need for determination of their ethiological role in epidemic process in different areas of Primorski Territory. Earlier, it was shown that hemagglutination-inhibition test is helpful not only for serodiagnostic of HFRS, but also for typing of hantavirus strain caused disease. We studied samples of blood, taken from HFRS patients from Vladivostok city in dynamics, in which the presence of specific antibodies was revealed by immunofluorescence method. Titer of antihemagglutination antibody was considered as highest dilution of the blood, which caused the delay agglutination of geese red cells. Consideration for stating etiologic diagnosis HFRS was 4-times difference in titer of antihemagglutination antibody to antigen of homologous and heterologous virus. The results of our studies have shown that antihemagglutination antibody in HFRS patients appeared for 4-6 days from diseases onset, their titer increased for 8-15 days, reached the maximum to 21 days and remained on high level before 30 days whereupon slowly decreased. Serologic typing of blood sample from HFRS patients was studies with the help of hemagglutination-inhibition test using antigen of HNTV and SEOV. As a result, the studies of sample blood from 49 HFRS patients from Vladivostok (n=64) revealed that in 28 cases of the disease (57.1%) difference in antibody titer confirmed the etiologic role of the SEOV, in 13 patients

(26.5%) - HNTV. The number of the samples with low titer of antihemagglutination antibodies and small differences in titer to different antigens was 8 (16.3%). Thereby, serotyping of HFRS cases of urban patients in hemagglutination-inhibition test at early periods of the disease enables to get clear information on role of determined serotypes of hantaviruses, namely Hantaan and Seoul in epidemic process.

Keywords: hantavirus; hemorrhagic fever with renal syndrome ;urban focus ;

P1-9 Time variant vegetation variables and their impact on the spatio-temporal patterns of HFRS in Belgium

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The incidence of HFRS is the output of a system with various processes interacting with one another at different points and scales in time and space. The components of the system are factors affecting the ecology of the host species and the pathogen as well as the physical, biological and socio-economic environment affecting the exposure of humans to the pathogen. The dynamics, dimension and overall condition of vegetative systems stand out as major determinants of the functioning of that system. The strong connection between disease incidence and vegetation features suggests that periodic observation of the earth surface from space has considerable potential to benefit spatio-temporal epidemiology. One of the major goals of earth observation from space-borne remote sensing (RS) is the assessment and monitoring of vegetation-related phenomena across the temporal and spatial dimension. Other important indicators of vegetation activity can be derived from conventional meteorological datasets. In this domain the notion of Growing Degree Days (GDD) is perhaps the most extensively used parameter that can be associated to timing and length of vegetation-related phenomena. GDD is derived from air temperature and indicates the cumulative effect of heat units that are needed to trigger and maintain various biological processes. In this study we conjugate remotely sensed and meteorological data on a temporally and spatially explicit manner as potential indicators of phenomena driving HFRS disease risk. In particular the study focused on Belgium and covers the period 2003-2010. Two major objectives were envisaged: (i) To assess the extent to which vegetation-related RS products and GDD can explain the temporal variability in HFRS risk: most studies using RS data for epidemiology focus on the derivation of risk maps and not much in exploring the potentials of RS temporal resolution. (ii) To identify indicators of annual vegetation conditions that are suited for modeling HFRS. These indicators were derived from time series of RS and meteorological data. Our results highlight the importance of considering indicators of vegetation dynamics when modeling the spatio-temporal patterns of HFRS. Moreover, it was found that the combination of meteorological data and parameters obtained from RS signals gives better results than using exclusively either of the two data sources.

Keywords: HFRS; remote sensing; growing degree days; spatio-temporal epidemiology;

P1-10 Detection of Hantavirus Circulation in Rattus rattus from Mayotte Island, Indian Ocean

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Following the exceptional Chikungunya virus (CHIKV) epidemics in the Indian Ocean Islands in 2005-06, an extensive capture of wild and domestic vertebrates was performed to evaluate their role in the CHIKV circulation. More than 3500 animals including primates, bats, rodents, and insectivores were sampled (blood, serum, sometimes organs) during 2006 and 2007 campaigns in La Réunion and Mayotte Islands. Although an extremely low sero-prevalence against CHIKV was observed, these campaigns have constituted a rich collection of samples to be used for searching traces of other pathogens and possibly virus discovery. We tested for the presence of Arenavirus and Hantavirus in samples from rodents and insectivores. Ribonucleic acids present in the serum of 539 rodents (Rattus rattus, Rattus norvegicus, Mus musculus) and insectivores (Suncus murinus, Tenrec ecaudatus) captured in 2006-7 in La Réunion island (379) and in 2007 in Mayotte island (160) were extracted, pooled by groups of 5, and then screened by nested-RT-PCR for Hantaviruses (Klempa et al, 2007, EID 13: 520-2 ; Kang et al, 2009, Virology 388: 8-14) and Arenaviruses (Vieth et al, 2007, Trans R Soc Trop Med 101: 1253-64). For each positive pool, samples were re-screened individually and then sequenced. None of the captured samples from La Reunion Island was found positive for either Arenavirus nor for Hantavirus. In Mayotte Island, no sample was positive for Arenavirus. However 29 among 160 (i.e. 18%) serums from Rattus rattus were tested positive for Hantavirus. The sequence of 246 nucleotides within the polymerase L coding region showed a limited genetic diversity among the samples but a conservation of the amino acid sequence. The phylogenetic analysis indicated that these new hantaviruses clustered closer but clearly distinct from the Hantaviruses Serang virus and Jurong virus. A phylogeographic analysis suggests that several variants seem to have been largely spread and mixed across the Mayotte Island. This work invites to a better surveillance of new Hantaviruses in rodents of Indian Ocean islands and to assess the risk of transmission of Haemorrhagic Fever with Renal Syndrome (HFRS) to the population.
Keywords: Mayotte Island;Rattus rattus;Indian Ocean;L gene;Phylogeography;

P1-11 Presence of three human pathogenic hantaviruses Puumala, Dobrava and Saaremaa in Austria

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In Austria, so far, it is confirmed that Puumala virus is endemic and is causing about 30 cases of

nephropathia epidemica per year. The most relevant Puumala-endemic regions in Austria are in the southern states Styria, Carinthia and Burgenland (southern part) and in a region bordering Germany in Upper Austria. About 90% of Puumala-cases are found in the Austrian state Styria. In 2012 a Puumala-outbreak occurred with 264 laboratory confirmed cases. The last epidemics were observed in 2004 and 2007 with 72 and 78 documented Puumala-cases respectively. In 2011 to 2013 in addition to Puumala-cases the first autochthonous Dobrava- as well as Saaremaa-cases have been detected in Austria, two hantaviruses that are known to cause hemorrhagic fever with renal syndrome (HFRS) in Europe. Up to 2011 only imported Dobrava virus infections have been detected in Austria. In 2011 to 2013 four autochthonous Dobrava virus infections have been diagnosed in Austria. The infections were confirmed by the detection of Hantaan/Dobrava/Seoul group specific IgM and IgG (recomLine Bunyavirus IgG/IgM, Mikrogen, Neuried, Germany) and of viral nucleic acid in serum using a real time PCR. In three cases it was possible to characterize the virus by sequence analysis as Dobrava. Additional, Dobrava virus was also found in its natural host Apodemus flavicollis captured at the place of residence of one patient. In 2012 the first patient with a Saaremaa virus infection was diagnosed in Austria. The infection was confirmed by the presence of Hantaan/Dobrava/Seoul group specific IgM and IgG (recomLine Bunyavirus IgG/IgM, Mikrogen, Neuried, Germany) and the detection of viral nucleic acid in serum that was characterized by sequence analysis as Saaremaa virus. Additional, Saaremaa virus was detected in its natural host Apodemus agrarius captured at the patients place of residence. The epidemiology of Puumala, Dobrava and Saaremaa virus infections in Austria is presented. The phylogenetic analyses based on partial S and M segment sequences of Puumala, Dobrava and Saaremaa virus strains from human cases as well as from rodents are shown. The data confirm that the three human pathogenic hantaviruses Puumala, Dobrava and Saaremaa, which are found in Europa, are circulating in Austria. This is proven by the demonstration and characterization of all three viruses in human cases as well as in the related natural rodent hosts.

Keywords: HFRS; hantavirus; Puumala virus; Dobrava virus; Saaremaa virus;

P1-12 Study of Spatio-temporal Dynamics of Hantavirus and Host Rat in small area based on GIS

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Spatial heterogeneity of HFRS epidemic focus has the spatial criterion dependence. So the study of spatio-temporal dynamic of epidemics of HFRS should pay attention to spatial criterion. At present, there are many studies revealed spatio-temporal character of HFRS, including large spatial scale, middle spatial scale, Meso-micro scale, small scale. But spatio-temporal dynamics of HFRS virus and host in small area hasn't expounded by micro scale. The study on spatio-temporal dynamics of HFRS epidemics in small area has a great significance for working out practical prevention and control measures suited to the time and local conditions. For this reason, this study combines spatial epidemiology, community ecology, molecular biology, molecular genetics and spatial information statistics, based GIS.Longitudinal surveillance of host rats carried out in Jvnan County from Feb. 2006 to Jan 2007, further expounding the relationship of spatio-temporal dynamic of HFRS various

and host rats in small area. Results: 1.Ecological characteristics of host rats Ecological environment influences community structure of host rat in experimental district. Rattus norvegicus and Mus musculus are major species, either in time niche or spatial niche. The ecological characteristics of host rat in experimental district as follow: ○1The community structure of host mice is different in experimental districts.○2The season distribution of community structure is different. ○3Both time niche breadth and spatial niche breadth of Rattus norvegicus and Mus musculus are very high. Both time niche overlap index and spatial niche overlap index of Rattus norvegicus and Mus musculus are highest.○4 Shannon-Weiner S indiex ,Niche Overlap index and ecological status of host mice in two experimental districts are difference due to natural condition and geographical factor.○5Food niche overlap index and breed niche overlap index between species are high. 2. The distribution character of host rat in small area The frist major specie is Mus musculus, second major specie is Rattus norvegicus in experimental districts. (1) Rat densities of seven villages in first experimental district are basically same. The constitutions of rat species in seven villages have different characteristics. The constitutions of rat species are basically similar in Xilandun village, Donglandun village,Qnliuhe village and Dashanqi

Keywords: small area; host rat; HV; spatio-temporal dynamics;

P1-13 Regression analysis of hemorrhagic fever with renal syndrome（HFRS）incidence and rodents infected hantavirus in epidemic area of Shaanxi Province，China.

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Shaanxi Province is in areas of high incidence of hemorrhagic fever with renal syndrome (HFRS), it is located in the eastern part of Northwest China, there are about 36 million people in this area. To clarify the relationship between HFRS incidence, density of rodents and the Rate of mice infected hantavirus in the past 29 years. 7186 mice was captured by night clamp method, hantavirus antigen was detected in 6928 mice lung tissue with direct fluorescence antibody test(DFA), specific hantavirus IgG antibody was detected by ELISA in 1040 mice serum, HFRS cases information came from National infectious disease reporting system.

Comprehensive data showed that the number of cumulative HFRS cases was about a hundred thousand (100,000) during 29 years from 1984 to 2012, the average annual incidence rate was about 9.94/100000. Apodemus and Rattus norvegicus mainly carried hantavirus in infected areas, the average annual rat lung Hantavirus positive rate was 4.99%(346/6928), The main virus type was Hantaan virus. There was no correlation between HFRS incidence and the density of rodents, but it showed a Statistically significant correlation between annual HFRS incidence and Hantaan virus infected rate in mice(P=0.002), the correlation coefficient was 0.52, in addition ,it derived a linear regression equation (Y case = 2089.63 +32417.21 X virus positive rate) from HFRS case number and Hantaan virus antigen positive rate in mice every year. Oterwise, specific IgG positive rate could be better reflected the active status of hantaan virus in rat in Shaanxi Infected areas (R Shaanxi =

0.52, P = 0.00).

P1-14 First detection of Dobrava/Belgrade Hantavirus in Apodemus flavicollis in Albania. Some Data on the Distribution and Ecology of Small Mammals (Rodentia and Insectivora)

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Dobrava/Belgrade hantavirus was not previously studied in the small mammals of Albania, and positive human cases of HFRS were confirmed. A study was undertaken to investigate the presence, distribution, and to test the small mammals species in Albania for the hantavirus load.
This study on the small mammals was performed during a period of three years, in 31 out of 36 districts of Albania. A study on the ecosystem including the vegetation structure, and the presence of water sources, valleys, streamlets, and the distance from human inhabited areas, was done before setting the traps. Only snap traps were used and set at 167 stations in 120 villages. The trapping sites were near forests of pines, oaks, hazelnuts and shrubs, in the upper border of the forests line (1500-1800m altitude), in human inhabited areas, as well as in meadows, lawns, clearings, and ecotones. Genetic material was extracted from homogenized rodents' tissues and PCRs were performed for probable detection of hantavirus infection.
A total number of 325 small mammals were collected, belonging to 15 species: 12 in Rodentia and 3 in Insectivora order. Nine species belonged to the Muridae family: 6 of them in Murinae subfamily: A. flavicollis, A. sylvaticus, A. mystacinus, R. rattus, R. norvegicus and M. domesticus, and three of them in the Microtinae subfamily: M. glareolus, M. felteni and M. arvalis. Two species belonged to the Gliridae family: D. nitedula and M. avellanarius, and one to the Sciuridae family: S. vulgaris. While among insectivores, two species belonged to the Soricidae family: C. suaveolens and C. leucodon, and one species to Talpidae family: T. stankovici. The most predominant trapped species was A. flavicollis (52.75% of the rodents, and 50.15% of small mammals), followed by A. sylvaticus (21.68% of the rodents, and 20.62% of small mammals). 6 out of 60 (10.0 %) A. flavicollis trapped in 2006, and 4 out of 88 A. flavicollis (4.6%) trapped in 2007, were carrying Dobrava-Belgrade virus RNA.
The most prevalent species was A. flavicollis found mainly in high forests and ecotone, followed by A. sylvaticus, present from low to high altitudes. A. flavicollis were positive for DOBV. Positive A. flavicollis were detected in 6 districts, in altitude ranging from 250 to 1500m, in the border with F.Y.R.O.M and Greece in the north-east, east and south-east part of Albania. There is an increased risk for human HFRS in Albania.
Keywords: Small mammals; Distribution; Dobrava/Belgrade virus; HFRS risk; Albania

P1-15 First record of Myodes glareolus and Microtus arvalis in Albania as the main reservoirs of PUUMALA and TULA viruses

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Previous studies on the small mammals (Rodentia and Insectivora) in Albania have not recorded the presence of M. glareolus and M. arvalis. These species have specific habitat and vegetation structure preferences. M. glareolus has a limited vertical and horizontal distribution and can be present in altitudes of more than 1400m, meanwhile M. arvalis has a wider distribution in meadows, in flattened and sloped clearings.
The purpose of this study was to find and evaluate the presence of new habitats in high altitudes in deep rural, and subalpine areas, and to evaluate the possibility for the presence of new small mammals species.
After the identification of the new habitats in forest of high altitudes, a number of killing snap traps were set in the determined stations. As bait we used peanut butter, apple, salami, and sausage. Snap traps were set in deep forest of fir, pines and oaks, close to water sources like streamlet, small ponds, and natural water sources.
In 2007 only 3 individuals were trapped: 1 female of M. glareolus in Korce-Dardhe in altitude of 1438m, longitude of 40.52329o, latitude of 20.81400o, in a high and dense mixed forest of fir, pines, oaks; although vegetation like blackberry, sturgeon, willow, ferns and many sources and ponds of water were recorded nearby; 1 male of M. glareolus in Korce-Voskopoje in altitude of 1488m, longitude of 40.59438o, latitude of 20.59875o, in a mixed and isolated forest of pines and juniper, although vegetation like dog-rose, blackberry, no water sources were nearby; 1 female of M. felteni in Pogradec-Dardhas in altitude of 1188m, longitude of 40.84923o, latitude of 20.42157o, in a low and isolated forest, and vegetation like hazelnut, chestnut, oak, hawthorn, juniper, wild pear, wild apple, wild plum, gooseberry, maple, dog-rose, blackberry, in ecotone with sloped meadows. In 2008 only one male of M. arvalis was trapped in Korce-Dardhe station, in an altitude of 1431m, longitude of 40.52554o, latitude of 20.81532o, in a sloped clearing with very dense herbaceous vegetation, surrounded by a high forest of fir, pine, and oaks.
Our study showed that there was an optimal habitat and vegetation structure for the presence of Microtinae species. We recorded for the very first time the presence of M. glareolus and M. arvalis in high altitude habitats in the east and south-east of Albania. There exist the possibility of PUUMALA and TULA virus presence in Albania, so further studies need to be undertaken.
Keywords: First record; Myodes glareolus and Microtus arvalis; Puumala; Tula; Albania

P1-16 Co-circulation of Genetically Distinct Soricid- and Talpid-borne Hantaviruses in Poland

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A genetically distinct hantavirus, named Seewis virus (SWSV), has been identified in the Eurasian common shrew (Sorex araneus), throughout the vast distribution of its soricid reservoir. Also, SWSV has been detected in the Siberian large-toothed shrew (Sorex daphaenodon), Eurasian pygmy shrew (Sorex minutus), tundra shrew (Sorex tundrensis) and Mediterranean water shrew (Neomys anomalus), suggesting either local spillover or long-standing host-specific adaptation. The overlapping geographic ranges of the Eurasian common shrew and other soricomorph species in Poland prompted the present study on the phylogeography of SWSV. Hantavirus RNA was detected by RT-PCR in RNAlater®-preserved lung tissues from 9/38 Eurasian common shrews, 1/35 Eurasian pygmy shrews, 1/11 Mediterranean water shrews, 3/7 Eurasian water shrews (Neomys fodiens) and 18/35 European common moles (Talpa europaea), captured in southeastern and central Poland in 2010 to 2012. Analysis of the partial S-, M- and L-segment nucleotide and amino acid sequences revealed SWSV in the Eurasian common shrew and Mediterranean water shrew at the same trap site in Chmiel in southeastern Poland. In addition, a previously unrecognized hantavirus, named Boginia virus (BOGV), was identified in the Eurasian water shrew in Boginia, Huta Dłutowska and Kurowice in central Poland, where SWSV-infected Eurasian common shrews and Nova virus (NVAV)-infected European common moles were also found. Phylogenetic analyses showed that SWSV segregated along geographic-specific lineages, and BOGV was distantly related to SWSV, in keeping with the evolutionary relationship between their soricid hosts. The low prevalence of SWSV infection in the Mediterranean water shrew and Eurasian pygmy shrew suggested cross-species virus transmission. The co-circulation of three genetically distinct hantaviruses in syntopic shrews and moles in Poland parallels findings that multiple hantavirus species co-exist in their respective rodent reservoir species in the same locality. Also, the co-existence of SWSV in the Eurasian common shrew, Eurasian pygmy shrew and Mediterranean water shrew resembles the host sharing among rodent-borne hantaviruses and other soricid-borne hantaviruses, such as Jemez Spring virus, in North American shrews. Finally, definitive data confirm that the European common mole is the reservoir of NVAV.
Keywords: Seewis virus; Nova virus; Boginia virus; Poland;

P1-17 Phylogenetically Divergent Hantaviruses Harbored by Insectivorous Bats in Côte d'Ivoire and Vietnam

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Hantaviruses detected in multiple species of shrews and moles across four continents are genetically more diverse than hantaviruses harbored by rodents, suggesting that the host range of hantaviruses may be more extensive than previously imagined. In particular, mammals having shared ancestry with soricomorphs may have figured prominently in the diversification of hantaviruses. By virtue of their rich biodiversity, vast geographical distribution and demonstrated ability to host myriad viruses, bats were investigated as potential reservoirs of hantaviruses. Either frozen, ethanol-fixed or RNAlater®-preserved tissues and fecal samples from 520 bats (representing six families, 26 genera and 53 species), captured in Asia, Africa and the Americas in 1981-2012, were analyzed for hantavirus RNA by RT-PCR. Following numerous failed attempts, hantavirus RNA was detected in tissues from two of 12 banana pipistrelles (Neoromicia nanus) (family Vespertilionidae), captured during June 2011 near Mouyassué village, in Côte d'Ivoire, and from five of 45 Pomona roundleaf bats (Hipposideros pomona) (family Hipposideridae), captured during May 1997 and March 1999 in Tuyên Quang and Quang Nam, respectively, in northern and central Vietnam. The RNA-dependent RNA polymerase-encoding L segment of the newfound hantaviruses, designated Mouyassué virus (MOYV) and Xuan Son virus (XSV), exhibited nucleotide and amino acid sequence similarity of less than 70% to representative soricomorph- and rodent-associated hantaviruses. Phylogenetic analysis, using maximum likelihood and Bayesian methods, showed that MOYV and XSV formed highly divergent lineages, distant from all other hantaviruses, except Magboi virus recently detected in the hairy slit-faced bat (Nycteris hispida) (family Nycteridae) from Sierra Leone. Suboptimal primer design and imperfect cycling conditions may have been responsible for the failure to detect hantavirus RNA in other insectivorous bat species. The discovery of bat-borne hantaviruses heralds a new frontier in hantavirology. Many more hantaviruses are likely to be found in insectivorous bats throughout their vast geographic range. Intensive studies, including virus isolation attempts and high-throughput sequencing, are underway to clarify the phylogeography, ecology and pathogenic potential of these newfound hantaviruses.
Keywords: Bat; Mouyassué virus; Xuan Son virus; Cote d'Ivoire; Vietnam;

P1-18 New Facts of Amur Hantavirus Circulation in Apodemus peninsulae Populations

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Objective: Long-term studies of some biotic factors (sexual and age classes in the seasonal and yearly dynamics of population structure), affected on Amur hantavirus (AMRV) circulation in Apodemus peninsulae populations – the reservoir of etiologic agent of HFRS in the forest ecosystem in Primorye Region of Far East Russia – were performed. Methods: Complex monitoring of epizootic and epidemic processes on AMRV endemic areas were studied during 2001-2012 years.

Criteria of acute infection were detection of viral antigen/RNA in lungs and organs of secretion/excretion and/or antibody of low avidity in the blood of captured rodents. Results: Phase of low activity of epizootic process. Only two age classes acted the part in active circulation of AMRV: adults-overwinter – in spring and summer, adults-current year – in summer and autumn. Sexual activity males acted the dominant part in all seasons. The epizootological significance of breeding females was more considerable in summer than spring, autumn theirs constituent among infected mice reduced below 10 %. Phase of activity increase of epizootic process. Activization of process observed from spring to autumn and characterized by increase of number of infected mice (from 0,3 to more 3,5 individuals on 100 trap/night) under mean value of population number less 10 mice on 100 t/n. Adults-overwinter maintained active circulation of AMRV in spring, the others age classes were drawn in epizootic process in summer and autumn. Breeding females acted the significant epizootological part in all seasons, theirs constituent among infected mice in autumn was more 24%. Presence of acute infection of most infected mice (more 70-80%) confirmed of epizootic activity in A. peninsulae populations. Phase of high activity of epizootic process. All age classes acted the active part in circulation of AMRV. Constituent parts of adults-overwinter and adults-current year among infected mice were high during of all seasons. Sub-adults were drawn in epizootic process already in spring, and summer theirs constituent among infected mice reached to 23%. In active epizootic process breeding females acted the dominant part in spring (about 60%). In summer and autumn the epizootological importance of males and females became identical. Conclusion: Prevalence of breeding females among infected mice in all seasons of current year of observations for dynamic of hantavirus infection in A. peninsulae populations can be prognostic index of development of phase of high activity of epizootic process in next year in HFRS forest natural foci.

Keywords: hantavirus;epizootic process; rodent-host;HFRS natural foci;

P1-19 Genetic Resistance / Tolerance of M. Glareolus to Puumala Virus: Evidence of Selection and Epidemiological Consequences

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Several experimental studies have shown that bank voles (Myodes glareolus) exhibit some variability in their responses to Puumala virus (PUUV) infection. Differences in immune genes could mediate this variability and allow for higher resistance (capacity to eliminate PUUV) or tolerance (capacity to dampen the negative immunopathological damages due to immunity) to PUUV. We tested the hypothesis that bank vole / PUUV coevolution might have shaped selection in favour of lower inflammatory and antiviral responses against this hantavirus. Using the human medicine literature, we have selected five candidate genes that seem to be involved in the immune response against PUUV (TNF-α promoter, TLR4, TLR7, Mx2, Integrin β3). We have focused on ten populations of bank voles sampled in the French Ardennes, along a North-South transect including PUUV endemic and non-endemic areas. We have looked for signatures of selection using population genetics and genotype - phenotype association approaches. We observed a stronger

genetic differentiation than expected under neutral evolution for one polymorphic site of the TNF-α promoter (-296). Phenotype-genotype associations next revealed a significant relationship between individual genotype at this site and PUUV viral load: heterozygote A/G individuals exhibited higher viral load than homozygote ones (Anova, p=0.03). We further evaluated the expression level of TNF-α and Mx2 genes using spleen samples from the same voles. We observed a strong negative correlation between PUUV viral load and gene expression levels (TNF-α: p=0.004; Mx2: p=0.012). Using only non-infected individuals, we showed that TNF-α and Mx2 gene expression varied spatially, lower levels being observed in areas where PUUV prevalence are high (TNF-α : $F_{1,155}=3.16$, p=0.077; Mx2 : $F_{1,152} = 7.80$, p=0.006). Altogether, our researches have highlighted signatures of selection for TNF-α (promoter sequence and expression) and Mx2 (expression). These genes have antiviral properties but also induce immunological damages, what make them central for driving a balance of resistance / tolerance to PUUV. Bank voles vary in their basal ability to tolerate/resist to PUUV. In high PUUV prevalence areas, TNF-α and Mx2 expression seemed down-regulated, what suggest selection or phenotypic plasticity for higher tolerance to PUUV, at the benefit of lower immunopathological costs

Keywords: interaction; immune genes; selection; resistance/tolerance; landscape;

P1-20 Bank Vole Population Dynamics is crucial for understanding the epidemiology of Puumala Hantavirus

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The contribution of landscape genetics to the understanding of Puumala hantavirus (PUUV) epidemiological processes has already been discussed by Guivier et al. (2011). It allowed relating genetic parameters indicative of bank vole population dynamics with PUUV prevalence in a fragmented forest of Northeastern France. During year 2012, we tried to generalize those results into to new geographic areas, with contrasted landscape structures and PUUV prevalence: first in Scandinavia, Taivalkoski, Northern Finland (65 °N, 28 °E), where forest habitats are very connective and PUUV prevalence fairly high (20-50%); second in Flandres, Northwestern Belgium, where forest habitats are highly fragmented and PUVV prevalence very low (0-20%). In the three ecosystems (fragmented forests in France and Belgium, and continuous forest in Finland), genetic analyses suggest that bank voles form metapopulations, which comprise a set of populations fluctuating in size and in connection with others, some of them eventually going to local extinction and being recolonized by other populations. In the three studies, we demonstrated that the prevalence of PUUV is determined by the dynamics of these populations. The prevalence is low in small and isolated populations and high in large and connective populations. Generalized at a larger scale, our results suggest that the dynamics of the metapopulations of voles are a key factor of the persistence and the spread of PUUV, which could maintain themselves only where vole metapopulations are highly connective and experience low extinction rates. Differences in the

metapopulation dynamics of voles across European regions could thus (at least partly) explain why PUUV is restricted to a limited portion of the distribution of the bank vole.

Keywords: Landscape genetics; Molecular epidemiology; Hantavirus transmission; Fragmented landscape;

P1-21 Impact of Landscape Connectivity and Fragmentation on Puumala Virus Genetic Diversity and Structure

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Microevolutionary processes (drift, migration and selection) shaping Puumala hantavirus (PUUV) and its primary host, the bank vole, were studied in a fragmented landscape of Ardennes, France. We monitored the genetic diversity and the spatial distribution of PUUV lineages, together with the spatial genetic structure of neutral and immune genes of its primary host, the bank vole, in relation with forest fragmentation. The genetic diversity of the bank vole was weakly structured in space for both neutral and immune genes, revealing high gene flow and large population sizes of bank voles over the studied area. Vole populations in tiny fragments however evidenced signature of genetic drift and genetic isolation. On the other hand, the genetic diversity of PUUV lineages was highly structured in space and according to the fragmentation of the forest; each forest fragments harbouring one specific lineage. This result suggests high genetic drift and low gene flow between PUUV lineages occurring in different forest fragments. Thus, over the same area, the genetic diversity of the virus is mainly shaped by genetic drift and isolation, while the genetic diversity of its host is mainly shaped by gene flow and population exchanges. These contrasted patterns of microevolution have important consequences for the opportunity of coevolution between PUUV and the bank vole in fragmented landscapes.

Keywords: Landscape genetics; Molecular epidemiology; Forest fragmentation;

P1-22 Experimental adsorption of hantavirus to soil changes virulence of hantavirus.

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It is well known that rodents are natural reservoirs of hantaviruses and occasionally excrete hantavirus to environment. In the majority of cases, humans infected after direct contact with ro-dents or their excreta, which occurs mostly by inhaling virus-contaminated aerosol. This way suppose, what hantavirus must survive in environment for definite time. The aim of our study was to present the change of hantavirus virulence after adsorption and sur-viving of hantavirus on samples of soil. Strain AA-95 (genovariant Far East, virus Hantaan) used in this study was originally isolated from the lung of infected striped field mouse (A. agrarius) trapped in Spassk district of Primorye Region and adapted to cell line Vero E6. After full adsorption of hantavirus on samples of brown soil collected in forests and storing at 40C for 7 days the virus desorption from complexes formed by

virus and soil was observed after treatment by phosphate buffered saline supplemented by 5% bovine albumin. Newborn laboratory mice were inoculated by 0.01 ml of hantavirus after adsorption (titer up to 4.5 log FFU/ml) intracerebrally (experiment group). In control group newborn laboratory mice were inoculated by 0.01 ml of native hantavirus (untreated) titer up to 4.5 log FFU/ml. All animals in control group have external signs of disease (decreased physical activity, dystaxia, convulsions) on 18 day post inoculation (p.i.) and all mice died on 23 day p.i. In experimental group all animals look healthy until 40 day p.i. However, in all groups inoculation of virus induced formation of specific serum antibodies in equal titers (1:256 in indirect immunofluorescence assay (IFA)). Our results show that adsorption of hantavirus to soil changed his primary biological property – virulence for newborn laboratory mice. But such treatment didn't influence on induction of spe-cific immunity and capability to grow in cell line. Hantavirus circulation in natural host is followed by occasionally excretion of virus to environ-ment, where it may be exposed to different physicochemical and biological factors. It can be as-sumed that complex of these factors including adsorption to different soils can change some im-portant properties of hantavirus.

Keywords: hantavirus; virulence; biological property;

P1-23 Anjozorobe Virus: a New Hantavirus Associated to the Thailand Virus Phylogroup Detected in Rodents from Madagascar

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Till now, there were only serological evidence that hantaviruses were circulating in rodents and humans from Madagascar. To assess the presence of a hantavirus in the island, we sampled, from October 2008 through March 2010, in the Anjozorobe-Angavo forest corridor (70 km North from the capital city Antananarivo), 584 rodents belonging to 7 species. A hantavirus was detected from organs of the ubiquist Roof Rat (Rattus rattus) and of the endemic Major's Tufted-tailed Rat (Eliurus majori). Amazingly, alignment and comparison of the S and M Coding Domain Sequences (CDS) of this virus with S and M CDS of hantavirus taxa showed a high percentage identity value between these sequences and those from the Thailand virus (THAIV) phylogroup, including the South-East Asian Jurong, Serang and Thailand viruses (97.0 to 98.1% and 91.7 to 93.6% amino-acid identities respectively). S, M and L CDS phylogenetic analysis of this virus confirmed that Anjozorobe virus (proposed name) belongs to the THAIV virus phylogroup and should be considered as a variant of the THAIV species. THAIV being suspected to be a cause of hemorrhagic fever with renal syndrome in humans, further studies are underway in Madagascar to assess the risk of Anjozorobe virus infection in humans.

Keywords: Madagascar; Rattus rattus; Eliurus majori; Thailand virus; Hantavirus;

P1-24 Genetic analysis of Tula hantavirus in Siberia, Russia

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Background: Hantavirus Tula (TULV) is distributed in large parts of Europe and two regions of Asia: West Siberia and Kazakhstan. TULV is able to infect different Microtus species, including M. arvalis, M. agrestis, M. rossiaemeridionalis, M. gregalis, M. subterraneus, and related species Lagurus lagurus. Analysis of the European TULV sequences demonstrated a geographical clustering which was independent from the rodent reservoir species. Methods: A total of 254 rodents of subfamily Arvicolinae, i.e. 11 Microtus agrestis, 80 M. oeconomus, 151 M. gregalis, 12 Lagurus lagurus were trapped on the territory of six administrative regions of the western and eastern Siberia. Sera samples were screened by IFA for detection of hantavirus-reactive antibodies or by ELISA for presence of hantaviral antigen. Tissues from sero- and antigen-positive rodents were analyzed by RT-PCR, and taxonomic identification of host species was based on phylogenetic analysis of partial cytochrome b gene sequences. Results: Seropositive rodents were found in all tested species. Hantavirus sequences were recovered from three seropositive M. gregalis and three antigen-positive Lagurus lagurus. RNA positive animals were found in three localities of western and eastern Siberia. Sequence analysis showed host-specific affinities of TULV sequences. Two different rodent species were carriers of two different genetic lineages of TULV, Russia II and Russia III. The divergence level of the S-segment nucleotide sequences between two lineages from Siberia was high and ranged from 18-21%, amino acid divergence level was 6%. Distinct genetic lineages of TULV, associated with M. gregalis and Lagurus lagurus were demonstrated at one trapping site in western Siberia where both species lives in sympatry. We showed that geographic distribution of TULV among M. gregalis range as far east as Krasnoyarsk region of eastern Siberia. Conclusions: In this study, we demonstrated host-specific clustering of the TULV sequences from M. gregalis and Lagurus lagurus.
Keywords: hantavirus Tula; Siberia; Microtus gregalis; Lagurus lagurus;

P1-25 Phylogeography and Dynamics of Puumala Viruses in France : Comparison between Virus Circulation in Bank Voles and the Prevalence of Haemorrhagic Fever with Renal Syndrome (HFRS) in Humans

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Puumala virus (PUUV) is the only hantavirus circulating in France known to provoke a human disease, Nephropathia Epidemica (NE), an attenuated form of Haemorrhagic Fever with Renal

Syndrome (HFRS). Every year, about a hundred human cases of HFRS are confirmed in the North-Eastern France. PUUV is mainly hosted by bank voles (Myodes glareolus).
Since 2000, a survey of bank vole populations inside and outside the known human endemic area is performed in order to explore the relationships between the PUUV dynamics in Myodes glareolus and the occurrence of human NE cases. Rodent trapping with capture-mark-recapture protocol is regularly realized in endemic, peri-endemic and non-endemic areas. Rodents are taxonomically classified, sexed, and blood sampled for further PUUV serology and molecular analysis. A limited number of capture with sacrifice allows us to try virus isolation from positive lungs.
Two types of analysis have been performed. A phylo-geographical study to evaluate the diversity of PUUV in different regions of France with comparison to other European PUUV lineages. An analysis of PUUV evolution along time by surveying the same spots over 10 years.
A substantial genetic variability in the S segment has been evidenced in France (up to 15%) although the NP protein amino acid sequence is strongly conserved. Globally, a geographical clustering of the PUUV is observed in viral variants sequenced from both rodent and human samples. The circulation of PUUV is also attested in non-endemic areas although at a lower prevalence. In the endemic areas, different spots can show very different values of PUUV prevalence with no evident correlation with rodent density which vary from high to very low levels depending on the site and/or the year.
This work attests the circulation of PUUV outside the human endemic area. It outlines the necessity of a better exploration of the factors (genetics, tolerance, co-infection…) that may influence the circulation of PUUV in rodent population and its transmission from rodent to human. It will allow to better delineate new risk areas where NE could emerge in humans.
Keywords: Puumala virus; Myodes glareolus; Phylogeography; HFRS; endemic/peri-endemic;

P1-26 Hantvirus Infection in Rats of Rural and Peri-urban Habitats in Luzon, Philippines

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A fieldwork was conducted in Luzon, Philippines, in order to assess the presence of hantavirus and the seroprevalence of infection in rats that may be a risk to smallholder farmers in rural rice fields and peri-urban habitats.
Rodents were collected in October of 2010 in North Luzon, Phillipines. Trapping and processing were performed according to established safety recommendations. Species identification was done in the field. Serum samples dilutions were tested by an indirect IgG enzyme linked immunosorbent assay (ELISA), using a N recombinant protein of SEOV produced in *E. coli* and negative control (a protein extract of *E. coli*, not containing the plasmid encoding the recombinant protein). Total RNA was extracted from kidney tissues of seropositive rodents. Reverse transcription (RT) procedure and hemi-nested polymerase chain reaction (PCR) were performed to amplify a partial fragment from hantavirus genome. The partial M segment sequence of hantavirus genome was recovered using primers designed to amplify the Gn fragment of 437 nt (6 to 442 related to the SEOV Sapporo strain).

The PCR products were purified and sequenced. Multiple nucleotide and amino sequence aligments were done using MEGA 5.
IgG antibodies were detected in 11 of 166 serum samples analyzed. Two of them were juvenils (18,2%) and 7 were males (63,6 %). All seropositive rodents had low or moderate antibody titers. The viral genome was detected in 4 of 11 seropositive rats (36, 4 %), all males.
Comparison of the 418 nt generated from Gn-M segment with those of other representative SEOV showed the highest nucleotide identity (86,6%) with Serang virus strain Jurong TJK/06 from Singapore and an amino acid identity of 94%. This hantavirus strain form a distinct phylogroup with Serang, Cambodian and Thailand virus.
The results presented in this study demonstrate the presence of Serang virus strain Jurong TJK/06, in rats of Philippines. However, it was not surprising to find Jurong virus in Philippines where *R. tanezumi* is abundant. These highly similar virus strains have been already identified in different rodent hosts.

P1-27 Model-based prediction of nephropathia epidemica outbreaks in Belgium based on climatological and vegetation data

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Nephropathia epidemica (NE) is a human infection caused by *Puumala virus* (PUUV), which is naturally carried and shed by bank voles (*Myodes glareolus*).
The objective of this paper was to develop a method that allows prediction 3 months ahead of the occurrence of NE epidemics.
We use a dynamic linear regression model. In Belgium no time series of the bank voles' population dynamics were available. The population of bank voles is related to the variation of seed production of beech and oak trees. The NE occurrence in Belgium was predicted by remotely sensed phenology parameters of broad-leaved forests, together with the oak and beech seed categories and average monthly air temperature (℃).
NE outbreaks in Belgium were predicted three months ahead with a 40% MRPE.
Such a predictive modelling approach might be used as a step towards the development of new tools for the prevention of future NE outbreaks.

P1-28 Outbreak of Hemorrhagic Fever with Renal Syndrome in Gramsh District South-East of Albania, 2011

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Background: Hemorrhagic fever with renal syndrome (HFRS) is a rodent-borne zoonosis, which is caused by the viruses of the genus *Hantavirus*. HFRS has been recognized in Albania since 1985, and is circulating in many areas of the country. Gramsh district is one of the previous endemic areas of HFRS in our country. An outbreak of Hemorrhagic fever with renal syndrome took place in August 2011 in this district, in Kukur village. The aim of the study is to present epidemiological and clinical features of patients with HFRS.

Patients and Methods: Epidemiological investigation was conducted following the outbreak of hemorrhagic fever with renal syndrome. Medical records of total of five hospitalized patients were reviewed. Sera were collected during all phases of epidemiological investigation and analyzed by ELISA, Indirect Imunofluorescence and a recombinant Immunoblot test for the confirmation of positive cases. The tests were performed at the National Reference Laboratory at the Institute of Public Health in Tirana. Also, a serosurvey was conducted during August, and 122 blood samples were collected from the contacts and residents of the village.

Results: Four cases were confirmed by serology. Patients had symptoms such as fever, headache, fatigue, colic abdominal. Thrombocytopenia and proteinuria were present in all patients. Immunoblot test indicated Dobrava virus as the etiological agent of the outbreak. Hantavirus IgG antibodies prevalence resulted 7.3 % by commercial ELISA test. All patients were recovered. Previous studies investigating the small mammals’ viral load which were trapped close to the outbreak region revealed the presence of Hantavirus, Dobrava/Belgrade virus strain in *A. flavicolis* tested organs.

Conclusion: Our study showed that HFRS is still circulating in endemic areas in Albania. Further studies in all endemic areas of Albania need to be undertaken. A rapid diagnosis is important for the correct therapeutic approach.

Keywords: Outbreak, Hantavirus, HFRS, Kukur, Gramsh, Albania.

P1-29 Genetic detection of Belgrade-Dobrava virus from rodent reservoirs in Serbia

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Hantaviruses are known to be endemically present in Serbia, where Belgrade virus was first isolated from clinical specimens of infected patients. The aim of the study was to investigate presence of Dobrava-Belgrade virus in wild rodent reservoirs in two geographical regions, in central and

western Serbia.
Organ samples from wild rodents trapped in 2007 and 2009, on two sites in central and western Serbia (Ravanica and Tara region, 26 and 28 animals, respectively) were tested for presence of hantavirus genome. All animals were prescreened serologically and only those with positive finding of either hantaviral antibodies and/or antigen were tested. RNA was Trizol extracted from tissue samples from lung, kidney and liver samples. Nested protocol for RT-PCR was performed by using degenerate primers that amplify partial L segment sequence of 412nt of all known hantaviruses. All PCR products were sequenced automatically using the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, Foster City, CA). Multiple nucleotide alignments of attained sequences were prepared using the Clustal W program. Phylogenetic analysis was performed using the PHYLIP program package. Hantavirus sequences used for comparison were recovered from the GenBank.
Six animals tested positive for hantaviral genome, of which 5 sequences were identified as DOBV virus and one sequence was identified as TULV.by both BLAST analysis and according to clustering in phylogenetic tree. Four DOBV sequences were recovered from *Apodemus flavicolis* mouse tissues and one from *Glis glis*. Mean pairwise nucleotide divergence among newly characterized DOBV sequences from Serbia was 9% (range, 0.6–16%; SD 4.9), and 9.7% (range, 4.9–16.6%; SD 4.5) with the DOBV reference strain used in this study. In phylogenetic tree, newly detected strains clustered separately, according to sites of isolation.
Conclusion: Genetic data about hantaviruses circulating in Serbia are rather scarse. Here we report first detection of DOBV sequences in wild rodents at two geographical sites in central and western Serbia, indicating existence of possible epidemic foci.

P1-30 Detection of a novel hantavirus (Tatenale Virus) in the United Kingdom

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Serological studies and sporadic human cases have previously suggested the presence of hantavirus in the UK. However, until now the species of hantavirus present in UK wildlife has never been confirmed. Between September 2009 and November 2011, wild rodents consisting of brown rats (*Rattus norvegicus*) (n = 133), wood mice (*Apodemus sylvaticus*) (n = 269), house mice (*Mus musculus*) (n = 50), bank voles (*Myodes glareolus*) (n = 35) and field voles (*Microtus agrestis*) (n = 8) were live caught across north-west England (Cheshire, Liverpool and Wirral). With the exception of a single field vole, the lungs from all rodents sampled were negative for hantaviral RNA using a pan-hantavirus RT-PCR. However, partial sequences for small (S) and large (L) genome segments were recovered from the lung of the field vole and confirmed the presence of a novel hantavirus (Tatenale Virus) in the United Kingdom. Recently, public health investigations following two cases of haemorrhagic fever with renal syndrome in the UK, led to the subsequent isolation of Seoul hantavirus from wild and domestic brown rats. The prevalence and public health impact of the two hantavirus species in the UK are not yet known.

Topic 2 VIRUS PHYLOGENY, REPLICATION, AND MORPHOGENESIS

P2-1 Tracking hantavirus nucleocapsid protein using intracellular antibodies

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Background: Hantavirus nucleocapsid (N) protein is a multifunctional viral macromolecule involved in multiple stages of the viral replication cycle. The intracellular trafficking of N protein during virus assembly remains unclear. Methods: We used N protein-specific intracellular expressed antibodies to track the localization and distribution of Hantaan virus and Seoul virus N protein. The N protein-specific antibody single-chain variable antibody fragments (scFvs), which bind an N-terminal linear epitope (L13F3) and C-terminal conformational domain (H34), were intracellularly expressed in the endoplasmic reticulum (ER) by fusion of the SEKDEL retention signal peptide at the carboxyl terminus, and in the cytoplasm (Cyto) by deletion of the ER membrane target signal peptide. Stable Vero-E6 cell lines expressing intracellular scFvs were either infected with hantavirus or transfected with an N protein expression plasmid; virus replication and N protein intracellular localization were determined. Result: N protein co-localized with scFvs in the ER and cytoplasm with or without viral membrane glycoproteins. Hantavirus replication was inhibited in both the scFvs-ER- and scFvs-Cyto-expressing stable cell lines. Conclusion: N protein may be expressed in the ER retention signal peptide of KDEL circulating region (ER/cis-Golgi) without the assistance of G protein, and so expression of N protein in both the cytoplasm and within the ER/cis-Golgi plays an important role in virus replication.
Keywords: Hantavirus; N protein; Intracellular antibody

P2-2 Molecular Phylogeny of Bowé Virus, A Newfound Hantavirus Harbored by the Doucet's Shrew (Crocidura douceti) in Guinea

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Recent discovery of multiple soricid-borne hantaviruses in Eurasia and North America suggests the existence of additional hantaviruses in sub-Saharan Africa, where several shrew lineages have evolved and diversified. Lung or intercostal tissues, preserved in RNAlater® or ethanol, from 4 Buettikofer's shrews (Crocidura buettikoferi), 11 Doucet's shrew (Crocidura douceti), 15 large-headed shrews (Crocidura grandiceps), 6 Jouvenet's shrews (Crocidura jouvenetae), 41 West African pygmy shrews (Crocidura obscurior), 6 African giant shrews (Crocidura olivieri) and 27 Therese's shrews (Crocidura theresae), captured in southeastern Guinea during 2011-2012, were analyzed for hantavirus RNA by RT-PCR. A genetically distinct hantavirus, designated Bowé virus (BOWV), was detected in a Doucet's shrew. The full-length 1,731-nucleotide S-genomic segment

of BOWV contained a single open reading frame (ORF), encoding a 431-amino acid nucleocapsid protein (NP) (nucleotide positions, 28 to 1,320), and 27- and 408-nucleotide 3'- and 5'-noncoding regions (NCR). The hypothetical NSs ORF was absent. Employing prediction software available in the NPS@structure server, the BOWV NP secondary structure resembled that of other rodent- and soricomorph-borne hantaviruses. The complete M-genomic segment of BOWV was 3,597 nucleotides, with a predicted glycoprotein of 1,145 amino acids, starting at nucleotide position 36, and a 124-nucleotide 5'-NCR. Like other hantaviruses, the BOWV glycoprotein precursor had the highly conserved WAASA amino-acid motif (amino acid positions 652-656) and five potential N-linked glycosylation sites (four in Gn at amino acid positions 142, 355, 407 and 526; and one in Gc at position 936). The full-length 6,551-nucleotide L-genomic segment (2,158 amino acids) of BOWV exhibited six major conserved motifs (designated premotif A and motifs A, B, C, D and E). Phylogenetic analysis, using maximum-likelihood and Bayesian methods, under the GTR+I+G model of evolution, showed that BOWV shared a common ancestry with Tanganya virus and Azagny virus, two crocidurine shrew-borne hantaviruses in Guinea and Côte d'Ivoire, respectively. Whole genome analysis of many more hantaviruses from Africa will provide a better understanding about how the radiation of African shrews might have contributed to the evolutionary history and cross-species transmission of hantaviruses.

Keywords: Hantavirus; Bowé Virus; Crocidura douceti; Guinea;

P2-3 Amga Virus, A Newfound Hantavirus Harbored by the Laxmann's shrew (Sorex caecutiens) in Russia and Japan

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Recent discovery of genetically distinct hantaviruses in multiple species of soricine and crocidurine shrews (order Soricomorpha, family Soricidae, subfamily Soricinae and Crocidurinae), as well as in moles (family Talpidae, subfamily Talpinae and Scalopinae), challenges the conventional view that rodents (order Rodentia, family Muridae and Cricetidae) are the principal reservoir hosts and raises the possibility that soricomorphs may have played an important role in the evolutionary history of hantaviruses. The vast distribution of Sorex shrews in Russia and Japan, particularly the Laxmann's shrew (Sorex caecutiens), spurred a search for shrew-borne hantaviruses. Total RNA was extracted from lung tissues of 49 Laxmann's shrew from the Sakha Republic, Russia and four from Hokkaido, Japan, captured in 2006 and 2010, respectively. Hantavirus RNA was detected by RT-PCR in 16 Laxmann's shrew (14 of 38 males, or 36.8%, and 2 of 15 females, or 13.3%; P=0.11). In pair-wise alignment and comparison of the coding region of the 1,290-nucleotide S and 3,420-nucleotide M segments and the partial 4,245-nucleotide L segment, the newfound hantavirus, designated Amga virus (MGAV), was distinct from Seewis virus (SWSV) in the Eurasian common shrew (Sorex araneus) and exhibited nucleotide sequence similarities of 57.4-70.2%, 50.8-79.8% and 56.0-75.1% with representative rodent-, soricid- and talpid-borne hantaviruses. Comparison of

the very limited 82-amino acid M- and 115-amino acid L-segment products of Artybash virus (ARTV), detected in a Laxmann's shrew captured in the Altai Republic of Russia, showed 96.3% and 96.5% sequence similarity, respectively, with MGAV. Phylogenetic analyses, generated by the maximum likelihood and Bayesian methods using the GTR+I+Γ model of evolution, indicated a shared ancestry between MGAV and ARTV. Also, topologies suggested geographic-specific clustering of MGAV, similar to the phylogeography of other soricid-borne hantaviruses, such as SWSV in Eurasia. The clear distinction between MGAV and SWSV and the close genetic and phylogenetic relationship between MGAV and ARTV suggest that MGAV and ARTV represent the same virus. A designation of ARTV/MGAV is proposed for this newfound hantavirus harbored by the Laxmann's shrew.

Keywords: Newfound Hantavirus; Sorex caecutiens; Russia; Japan;

P2-4 Newfound Hantavirus in the Pomona Roundleaf Bat (Hipposideros pomona) in Vietnam

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Compelling evidence of genetically distinct hantaviruses in multiple species of shrews and moles (order Soricomorpha, family Soricidae and Talpidae) suggests that soricomorphs, rather than rodents (order Rodentia, family Muridae and Cricetidae), may be the primordial hosts. Recently, the host range of hantaviruses has been further expanded by the discovery that insectivorous bats (order Chiroptera) also serve as reservoirs. Conjecturing that Mouyassué virus (MOYV) in the banana pipistrelle (Neoromicia nanus) in Côte d'Ivoire and Magboi virus (MGBV) in the hairy split-faced bat (Nycteris hispida) in Sierra Leone are representative of a much broader distribution of bat-borne hantaviruses, lung tissues, collected in RNAlater®, from 33 bats (2 Aselliscus stoliczkanus, 4 Cynopterus sphinx, 4 Hipposideros larvatus, 5 Hipposideros pomona, 1 Hipposideros sp., 1 Murina eleryi, 1 Murina sp., 4 Myotis siligorensis, 2 Rhinolophus pearsonii, 2 Rhinolophus pusillus and 7 Rhinolophus sinicus) captured in July 2012 in the Xuan Son National Park in Vietnam, were examined for hantavirus by RT-PCR. Hantavirus RNA was detected in one of five Pomona roundleaf bats (family Hipposideridae). Analysis of a 4,271-nucleotide (1,423 amino acids) region of the L segment indicated sequence similarity of 69.6-71.5% and 75.1-78.3% at the nucleotide and amino acid levels, respectively, between the newfound hantavirus, designated Xuan Son virus (XSV), and the African chiropteran-borne hantaviruses, MOYV and MGBV. XSV sequences were identical in lung, liver, kidney and spleen. Phylogenetic analyses, using maximum-likelihood and Bayesian methods, indicated four distinct phylogroups, with XSV sharing a common ancestry with MGBV.

Very similar topologies, supported by high bootstrap (>70%) and posterior node probabilities (>0.70), were consistently derived, suggesting an ancient evolutionary origin of chiropteran- and soricomorph-borne hantaviruses, likely predating hantaviruses hosted by rodents. The Hipposideros genus is one of the most speciose of insectivorous bats, with more than 70 species. The vast geographic distribution of the Pomona roundleaf bat in Vietnam, as well as in Bangladesh, Cambodia, China, India, Laos, Malaysia, Myanmar, Nepal and Thailand, provides opportunities to ascertain the genetic diversity, phylogeography and pathogenic potential of XSV and XSV-related hantaviruses.

Keywords: Newfound Hantavirus; Hipposideros pomona; bat-borne hantaviruses; Vietnam;

P2-5 Genetic characterization of shrew-borne hantaviruses isolated in Finland

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Objectives. Hantaviruses are emerging viruses carried mostly by rodents. However, recently many novel hantaviruses have been detected also in shrews, moles and bats. Previous studies showed a high divergence in hantavirus from Sorex araneus in the 1980s in Finland. To study novel hantaviruses in Finland, shrews and moles have been collected. Methods. Viruses were previously isolated in cell culture from Sorex araneus (Seewis), Sorex minutus (Asikkala) and Neomys fodiens (Laihia). The host identification was confirmed with Cyt-b genes. Viruses were screened using RT-PCR and hantavirus-specific L-segment primers from lung tissue. Specific primers were applied also to amplify partial S and M genes. Multiple sequence alignment was performed using ClustalX 2.0 with default parameters and manually revised with BioEdit 7.0. The phylogenetic tree was constructed using neighbor-joining (NJ) method (PHYLIP) and Bayesian approach. Results. Hantavirus-specific RNA was found in S. araneus with a prevalence of 30%. All strains were variants of Seewis virus. Compared with the prototype mp70 strain, partial L segment showed 80-81% similarity and for partial S segment 84-85% and at the amino acid level 91% similarity was found. Partial M segment showed low sequence identity to other insectivore-borne hantaviruses such as Thottapalayam (49%), Cao Bang (65.5%), Asama (68%) and Imjin (46%). For the rodent-borne hantaviruses, Hantaan and Puumala, the identity is 74% and 44%. Phylogenetic analysis based on partial L segment showed these Seewis strains to form a distinct cluster, and the topology was congruent with the tree based on the S segment. Conclusions and future prospects. Highly divergent Seewis hantaviruses are circulating in Finland. Similar studies are scheduled for Asikkala and Laihia viruses. We aim to express their proteins to establish seroassays for all these shrew-borne hantaviruses and to learn whether they infect humans or wild or domestic animals, and if so with which consequences.

Keywords: Seewis Virus; Phylogeny; Shrew;

P2-6 Hantaan Virus Nucleocapsid Protein Stimulates MDM2-dependent p53 Degradation

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Nucleocapsid (N) protein of Hantaan virus (HTNV), that causes hemorrhagic fever with renal syndrome, is the most abundantly expressed protein in HTNV-infected cells. The influence of N protein to the HTNV-infected cells was not much studied yet. Recently, some evidences were reported that the N protein of HTNV inhibited apoptosis. To elucidate the regulation of apoptosis by N protein we studied p53 regulation by HTNV N protein. The amount of p53, increased by cytotoxic drugs (cisplatin, doxorubicin), was reduced when cells were infected with HTNV or over-expressed with HTNV N protein. We showed the p53 gene transcript level was not affected by N protein. When cells treated with a proteasome inhibitor (MG132) or a MDM2 antagonist (Nutlin-3), p53 was not reduced in N protein-overexpressed cells. We have finally concluded the HTNV N protein ubiquitinates and degradates p53 MDM2-dependently. Interestingly a non-pathogenic hantavirus, Prospect Hill virus, did not reduced p53 level unlike pathogenic hantaviruses. For the first time, herein, we report down-regulation of p53 through a post-transcriptional mechanism by the HTNV N protein. MDM2-dependent ubiquitination of p53 was involved in the decrease of the protein. A molecular mechanism of hemorrhagic fever with renal syndrome by HTNV is proposed with this noble function of the HTNV N protein.
Keywords: Hantaan virus; p53; Apoptosis; Ubiquitination; MDM2;

P2-7 Novel hantavirus identified in Major's pine voles (Microtus majori) in southern European Russia.

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Recently, plenty of novel hantaviruses have been discovered in “atypical” hosts such as shrews, moles, and even bats. Here we report on identification of a novel hantavirus associated with a rodent host, Major's pine vole (Microtus majori).
Altogether 34 hantavirus antigen- and PCR-positive Major's pine voles were identified in the Black Sea coast area of European Russia within the years 2008-2010. Initial sequence analysis revealed novel hantavirus sequences. Moreover, a single common vole (M. arvalis) infected with Tula virus (TULV) has been detected. Detailed sequence and phylogenetic analyses were then performed for 11 virus strains originating from voles of 8 trapping sites.

The obtained data show that Major's pine vole is a newly recognized natural hantavirus host. The newfound virus, provisionally called Adler virus (ADLV), is closely related to TULV. Amino acid differences to TULV (5.6-8.2% for nucleocapsid protein [N], 9.4-9.5% for glycoprotein precursor [GPC]) are on the border line of the current ICTV species definition criteria (7%) and below our recently proposed more strict criteria (10% for N, 12% for GPC). Sympatric occurrence of ADLV and TULV in the same region suggests that ADLV is not a geographical variant of TULV but a host-specific taxon. High intracluster sequence variability indicates long term presence of the virus in this region. The pathogenic potential of ADLV needs to be determined.

Keywords: Microtus majori; phylogeny; Tula virus; Russia

P2-8 Shrew-borne hantaviruses in the heart of Europe.

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Recent discovery of genetically distinct hantaviruses in bats and moles, as well as in multiple species of shrews contests the conventional view that rodents are the principal reservoir hosts of hantaviruses. In Central Europe, only one shrew borne hantavirus has been known until now; Seewis virus (SWSV) associated with common shrew (Sorex araneus). The objective of our study was to investigate the presence, distribution, virus-host specificity, and molecular evolution of shrew-borne hantaviruses in Central Europe. Therefore, a total of 445 shrews of the Soricinae and 27 shrews of the Crocidurinae subfamily were collected in Germany, Czech Republic, and Slovakia. Screening by genus-specific L-segment RT-PCR revealed specific amplification products in tissues of 50 out of 353 S. araneus and 6 out of 63 S. minutus. Phylogenetic analyses of L and S segment sequences showed that the detected hantavirus strains belong to two distinct clades. 53 samples clustered together with previously reported SWSV, while 3 samples from S. minutus formed a separate clade, which has been later shown to cluster with Asikkala virus (ASIV) from Finland. Our first comprehensive sequence analysis of SWSV strains from Germany, Czech Republic, and Slovakia showed its broad geographical distribution, high genetic divergence, and strong geographic

clustering. The detection of Asikkala virus indicates presence of another shrew-borne hantavirus in Central Europe of unknown pathogenic potential for humans.
Keywords: Seewis virus; Asikkala virus; Shrew-borne; Central Europe; Sorex;

P2-9 The molecular signature of patient-derived PUUV strains allows their assignment to particular outbreak regions

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Hantavirus disease (HFRS) is classified as a reportable infectious disease in Germany since 2001 with an average of about 200 cases in non-epidemic years. During the nationwide outbreaks in 2007, 2010, and 2012 elevated numbers of human cases have been reported to the national health authorities, with up to 2,800 laboratory confirmed cases registered for 2012. Although three hantaviruses, Puumala virus (PUUV), Dobrava-Belgrade virus, and Tula virus, circulate in rodents in Germany and can infect humans, most clinical cases are caused by PUUV, mainly circulating in South and Southwest Germany. Years of high bank vole densities are accompanied by increased numbers of human hantavirus infections. As the National Consultation Laboratory for Hantavirus Infections, we collected human samples via a nationwide network of hospitals and laboratories, for more detailed hantavirus diagnostics. Blood specimens tested positive for both PUUV-IgG and -IgM by ELISA or immunoblot, were screened for hantavirus RNA in a RT-PCR assay targeting a conserved region within the polymerase gene. All samples tested positive in the RT-PCR were subjected to an additional PCR targeting a 577 bp, less conserved region within the nucleocapsid gene. We collected 133 patient derived PUUV sequences and completed the dataset with 64 rodent derived sequences from Germany. Molecular phylogenetic analysis of these sequences revealed 7 different clades, comprising both human and rodent derived PUUV sequences. These clades clearly correspond to different outbreak regions. Furthermore, evidence was found for 7 additional clades comprising either human or rodent derived sequences. Phylogenetic analysis within a particular outbreak region reveals appearance of putative sub-clades with geographic distances of 20-30 km. We conclude that PUUV epidemics in Germany are not caused by countrywide spread of the same virus strain but result from multiple local outbreaks associated with increased densities and infection rates of bank voles. High molecular similarities of human- and rodent-derived PUUV sequences from the same geographic origin and their clear molecular distinction from strains of neighboring regions indicate spatial evolution of each virus clade. Moreover, the particular PUUV nucleotide sequence detected in a patient permits to trace back the infection to a specific geographical risk area.
Keywords: Puumala; Phylogeny; Germany;

P2-10 Genetic Characterization Of Seoul Hantavirus From *Rattus norvegicus* in Buenos Aires City

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Hantaviruses (genus *Hantavirus*, family *Bunyaviridae*) present a close association with their natural host. A geographical clustering of the different genetic variants can be appreciated. Seoul virus (SEOV) seems to be an exception to this common pattern due to the fact that this virus and their rodent hosts (*Rattus rattus and Rattus norvegicus*) have been found in different parts of the world (Asia, Africa, Europe and the Americas). In Asia, SEOV has been associated with milder forms of HFRS and few human cases have been reported outside Asia.
Serological evidence of SEOV infection was found in 10/81 *R. norvegicus* and in 1/20 *R. rattus* captured in the Buenos Aires port in 1983. In 1999 the first molecular characterization of SEOV in two *R.norvegicus* captured in the Buenos Aires port was communicated. In 2001, 29 *R. norvegicus* were captured in a marginal area of Buenos Aires city, finding serological evidence of infection in 9 of them.
RNA was extracted from the lung or kidney and subjected to a nested RT – PCR. A 692 bp for the S segment and a 659 bp for the M segment were obtained from one rodent captured in 1983 and from one rodent captured in 2001. Specific primers were used to sequence each segment with the ABI PRISM 3100 DNA Analyzer. Phylogenetic analysis was performed using the neighbor-joining method in order to compare the sequences of the two Argentinean SEOV strains with those circulating in different parts of the world. The fragment of the M segment of Argentine strains grouped in different clades; while 1983 strain located together with Asiatic human and rodent strains, 2001 strain located together with American strains from USA and Brazil.
One of the reasons for the lack of clinical cases in our country may be the presence of less virulent strains but the fact that one of the Argentinean strains grouped with rodent and human strains from Asia would indicate that SEOV is a potential pathogen for human beings in our country. Ongoing studies of complete sequences of the circulating strains would clarify this issue.

P2-11 Molecular evolution of Puumala hantavirus in an endemic region in Lower Saxony

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Puumala virus (PUUV) is the predominant hantavirus species in Germany causing mild to moderate cases of haemorrhagic fever with renal syndrome (HFRS). Lower Saxony is an endemic region for PUUV infections in Germany. Since the introduction of the German Infection Prevention Act in 2001 the number of hantavirus cases in Lower Saxony was recorded annually and the highest incidence of human PUUV infections was found in the district of Osnabrück. In this district, rodent monitoring was performed since 2005 and revealed a continuing and high prevalence of PUUV-specific antibodies in the bank vole (*Myodes glareolus*) populations. During the hantavirus outbreak in 2010, a seroprevalence of 93-100% was demonstrated in the bank vole populations from four trapping sites. RT-PCR investigations targeting the S-, M- and L-segment confirmed the high prevalence in the bank vole populations during this outbreak. Phylogenetic analyses of the partial S-, M- and L-segment sequences revealed a novel PUUV lineage clearly separated from PUUV lineages in other parts of Germany including those from neighbouring regions in Lower Saxony and North Rhine Westphalia. The first almost complete genome sequence of this PUUV lineage from Osnabrück confirmed these initial phylogenetic analyses. In conclusion, the investigations demonstrated a continuing PUUV prevalence in a highly endemic region. Future investigations will have to prove whether there are associations between changes in bank vole populations, the prevalence of PUUV infections in these populations and the frequency of human infections in these regions.

Topic 3 PATHOGENESIS AND IMMUNE RESPONSES

P3-1 New Aspects of Immunopathogenesis of Hemocoagulation Disorders in Hemorrhagic Fever with Renal Syndrome (Hfrs)

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Objectives: The hemorrhagic syndrome is one of the leading syndrome of HFRS, the severity of which often determines the outcome of disease. However, the genesis of it's main manifestations and immune mechanisms of it's regulation are poorly understood today.
Materials and methods: A comprehensive clinical and laboratory examination of 60 patients with serologically confirmed HFRS, taking place in infectious hospitals of Primorsky region (Russia) in 2010-2012 has been done. Plasma levels of activated partial thromboplastin time (APTT), prothrombin time (PT), prothrombin index (PI), fibrinogen, concentration of soluble fibrin-monomer complexes (SFMC), fibrinase, spontaneous lysis, XII-a-dependent lysis, ethanol test, the rate of aggregation with a universal inducer of aggregation (UIA) and complete blood cell count were measured. Levels of IL-1b, IL-6, IL-6sR, IL-17, TNFα, TNFαRII and TGF- b1 were measured in the serum samples with commercial ELISA tests.
Results: A significant increase IL-17 ($p<0,05$) in serum of patients with HFRS, which correlates with the level of platelets in peripheral blood ($rs = -0,805$, $p<0,001$) are revealed. Levels of TNFα and IL-6 were also significantly increased ($p<0,05$), but did not correlate with indicators of blood coagulation and with platelets count. Also found a slight increase ($p>0,05$) in levels of IL-1b, IL-6sR, TNFαRII and TGF- b1,which also did not correlate with the level of platelets in peripheral blood and indicators of hemostasiogram. Also observed a significant increase the concentration of soluble fibrin-monomer complexes (SFMC) in the serum of patients, which had the other unchanged indicators of the hemostatic system. Other indicators f blood coagulation were in the normal range, and also did not correlate with platelet count and level of serum cytokines.
Conclusions: Revealed a strong inverse correlation between the level of IL-17 and platelet count may suggest about the role of autoimmune mechanisms in the development of thrombocytopenia in HFRS. Observed significant increase the concentration of SFMC, which indicates the activation of blood coagulation in HFRS .
Keywords: immunopathogenesis; hemocoagulation disorders; hemorrhagic fever with renal syndrome; cytokines; platelet

P3-2 Pathohistological Features of Balkan Endemic Nephropathy and Hantavirus Infections

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The endemic nephropathy has been the subject of many epidemiological studies since it was first described. However, the etiology of Balkan endemic nephropathy (BEN) has remained unknown more than five decades after its original description. The occurrence of two distinct diseases in overlapping geographical areas is rarely seen. However, hemorrhagic fever with renal syndrome (HFRS, hantavirus infection) has been reported in Balkan Peninsula more than 50 years ago, along with the occurrence of endemic nephropathy.

Both diseases have the same morphological features during the early stage (renal vasculitis, interstitial lesions). Since the first registered case of HFRS in the former Yugoslavia in 1952, sporadic cases have been constantly present and occasionally major or minor epidemics with mortality rate from 1-16%. The analysis of autopsy speciments from HFRS patients in Balkan Peninsula shows that hantavirus serotype Belgrade was present more frequently as an infectious agent. The most prominent pathohistological finding in the initial stage of the disease is a dilatation of blood vessels in the corticomedullary zone, while renal interstitial haemorrhage is detectable in in the later stadium. In addition, a frequent finding is the presence of hyaline granules in epithelial cells of tubules, asociated with the degeneration and various degree of necrosis that also includes tubular basal membrane. Glomerular changes are minimal and usually are present as unequal thickenings of the glomerular basal membrane and capsule. Cases of HFRS and BEN, registered in Serbia, have rather similar pathological and immunopathological findings during the late stage of the disease, too. Electron microscopic analysis in patients with BEN revealed the presence of virus-like particles within cytoplasmic vesicles in renal cells. The fact that both BEN and viral diseases have similar slowly progressive clinical course has raised the question about the viral etyology of endemic nephropathy. However, no virus has been detected so far in the kidney tissue from these patients. Since the development of modern molecular detection methods enables the identification of new infectious agents, the biopsy and autopsy materials of these patients should be analysed for the presence of the existing and newly-discovered infective agents within multidisciplinary retrospective-prospective studies using the molecular genetic techniques.

The similarity of morphological changes and clinical features among patients who suffer from HFSR and BEN along with the fact that both diseases occur in the same specific geographical areas indicate the need for further multidisciplinary studies regarding a possible causal relationship between HFRS and BEN.

Keywords: hantavirus; endemic nephropathy; pathohistology; multidisciplinary approach

P3-3 The influence of Hantavirus on the cells of innate immunity

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The immune function of neutrophils and macrophages the cellular elements of innate immunity at infectious diseases, mainly, associates with phagocytoses and production of cytokines, including nitric oxide and oxygen radicals. We study the morphofunctional characteristic of these cells in

hemorrhagic fever with renal syndrome infections. The strain of PM-T79-95 Hantavirus (HV) selected from lung suspension of infected mice Apodemus agrarius, adapted previously to Vero E6 cells, was used. In experiments we used supernatant liquid of cells culture, included not less 5 infectious units on cells. The activity of enzymes ATPase, 5′ nucleotidase, succinate- (SDG), lactate dehydrogenases (LDG) and myeloperoxidase (MPO) were determined in infected cells by Hayhoe and Quaglino's methods with our updating. For determination of apoptosis cells the method of staining by Hoechst 333258 was used. It is established that after 15 minutes of contact with the HV 55,5 % macrophages and 36,2 % neutrophils becoming antigen-positive cells. The cytoplasm of cells displayed a specific diffuse fluorescence, after incubation of the infected culture for 60 min, the fluorescence was observed of both the plasma membrane and perinuclear area of cells, and this fluorescence did not change with time. The plasma membrane of cells is known to transform spatially during chemotaxis, and this directly depends on activities of its enzymes 5′ nucleotidase and ATPase. We found that an increase of the number of antigen-positive cells was associated with a decrease in the activity of 5'-nucleotidase and ATPase of macrophages and neutrophils, indicating stimulation of cells. The activity of the oxygen-dependent metabolism of phagocytes, in particular, the function of the hexose monophosphate shunt was assessed histochemically. We found an oxygen-dependent stimulation of macrophages and neutrophils infected by HV. Also it is established that the virus was able to induce apoptosis of neutrophils. On this indicated a moderate increase in the activity SDG and the presence in HV-infected neutrophils tendency to anaerobic energy production path, as evidenced by LDG activity increase. Thus, our results showed the increased metabolic activity of macrophage and neutrophils in process of HV infection, which suggests the important role of these cells of innate immunity in the pathogenesis of infections.

Keywords: neutrophils; enzymes; virus; monocytes/macrophages; ultrastructure;

P3-4 The cells of monocytes system infected by Hantavirus

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In the some studies was established that monocytes/macrophages are represent one of the major targets cell for human immunodeficiency virus, enterovirus, filovirus and other viruses. However the role monocytes/macrophages in protection, as well as in the pathogenesis of hantavirus infection, is not clear. We study the functional characteristic of these cells in hemorrhagic fever with renal syndrome infections. The strain 635 of Hantavirus (HV) selected from lung suspension of infected mice Apodemus agrarius, adapted previously to Vero E6 cells, was used. In experiments we used supernatant liquid of cells culture, included not less 5 infectious units on cells. The activity of enzymes ATPase, 5′ nucleotidase, lactate dehydrogenases (LDG) and cytochrome oxidases were determined in infected cells by Hayhoe and Quaglino's methods with our updating. It is established that after 60 minutes of contact with the HV 42,3 % monocytes in the absence of maturity time, 36,2 % monocytes with pre incubations time 18 h and 26,4 macrophages (pre incubations time 72 h) becoming antigen-positive cells. Thus, the susceptibility of monocyte-derived cells for HV

infection decreased when their achievement of final stage of differentiation. Found that regardless of the differentiation of monocyte activation of ATPase and 5′ nucleotidase occurred in the later stages of infection. The activation of LDH in undifferentiated monocytes reaches maximum value at 24 h, and in differentiated monocytes at 48 h post HV infection. At the same time the activity cytochrome oxidases in undifferentiated monocytes was defined as the maximum value after 5 h, and in differentiated monocytes at 48 h after infection. Determined that the infected by HV undifferentiated monocytes did not cause nitrite production throughout the period of observation, whereas in differentiated monocytes the amount of nitrite was increased in the initial period post infection, decreasing by the end of the observation period. Thus, our results showed that the monocyte-derived cells were susceptible to HV infection and activation of enzymes in undifferentiated monocytes occurred in the early stages of infection, whereas the active effect of the virus on the production of metabolites nitric oxide were found in contact with differentiated monocytes.

Keywords: virus infection; enzymes; monocytes/macrophages; ultrastructure;

P3-5 Infection of Endothelial and Mesothelial Cells with Andes Virus and NK Response

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In the early phase of the infection pathogenic hantaviruses escape induction of innate antiviral responses, specifically innate interferon system. The rol of NK cells in hantavirus infection remains to be investigated. Lung microvascular endothelium is considered to be the primary site for hantavirus replication.

To determine whether two human pulmonary cell types support Andes virus (ANDV) replication and contribute to the earliest induction of the antiviral response, human microvascular pulmonary endothelial cell line HMEC-1 and cultured primary human mesothelial cells were mock infected or infected with ANDV.

Infection of cells was confirmed by immunofluorescence and cell morphology was analyzed by confocal microscopy. Expression of cell surface markers related to NK cell activation was analyzed by flow cytometry. Viral RNA, infectious particles and cytokine and chemokine responses were quantified in culture media of both cell types. Infected cells were identified by the characteristically intra-cytoplasmatic pattern of staining for viral nucleoprotein (NP).

The infection caused morphological changes in HMEC-1 and mesothelial cells. By day 4 post infection (p.i.) more than 20% of cells were positive for NP staining in HMEC-1 infected at a multiplicity of infection of 0.1. At the same day viral RNA in culture media increased drastically to reach 2, 5 x 109 S segment molecules per ml. The kinetics of infection in HMEC-1 was similar to that seen in Vero E6 cells, although in mesothelial cells the titer reached up to 4 days p.i. was lower. Expression of IL-1 and IL-8 and major histocompatibility complex class I (MHC-I), ICAM and CD80/CD86 surface markers were evaluated in each cell type. ANDV infection in HMEC-1and mesothelial cells significantly increased MHC-1 expression in cell surface and IL-8 secretion.

However, ANDV seems to be unable to alter the pattern induced by some added inflammatory mediators such as TNF and IFN-γ.
NK cells were co-cultured with ANDV-infected or mock-infected HMEC-1 cells to study the direct effect of this interaction in the activation of NK cells. Preliminary data suggested that there were no evident differences in responses between infected and non infected co-cultures.
HMEC-1 and mesothelial cells supported ANDV replication and both of them could serve as a model to study distinct roles in modulation of the immune system during hantavirus infection.

P3-6 Regulation of endothelial cell functions by hantaviruses

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Hantaviruses are zoonotic viruses carried by rodents and insectivores. Hantavirus pathogenesis in humans has thus far only been associated with rodent-borne viruses which can be divided to Arvicolinae-, Murinae- and Sigmonontinae-borne viruses with increasing disease severity. Induced leakage of microcapillaries is the known 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 and thus alternative explanations for hantavirus pathogenesis need to be sought after. Earlier studies have suggested that hantaviruses might differ in their ability to induce innate immunity responses in human endothelial cells. The apathogenic Arvicolinae-borne Tula and Prospect Hill viruses (TULV and PHV) show more rapid induction of cellular antiviral state as compared to pathogenic hantaviruses. In this study we infected primary human dermal microvascular endothelial cells (HDMECs) with pathogenic, Arvicolinae-borne, Puumala virus (PUUV) and the above mentioned TULV and PHV. We compared the results to those obtained with widely used cell model of endothelial hantavirus infection, human umbilical vein endothelial cells (HUVECs). Our results show that induction of innate immunity by PUUV is delayed as compared to PHV and TULV in both cell types. More dramatically, in contrast to TULV and PHV, PUUV was capable of replicating even in the presence of an induced antiviral state in HDMECs. However, this phenomenon is possibly not related to virus pathogenesis since the Murinae-borne Hantaan virus (HTNV) is also not capable replicating in antivirally activated HDMECs. The hantavirus-induced innate immunity involved upregulation of at least IFN-beta, MxA, RIG-I and complement factor B, the last being a known component of the alternative complement pathway. Interestingly, complement activation is known to correlate with Puumala virus pathogenesis. It is thus conceivable that hantavirus infection of endothelial cells could promote complement activation and subsequent vascular leakage through upregulation of complement factor B.

Keywords: hantavirus; endothelial cells; innate immunity; complement; vascular permeability;

P3-7 Influence of genetic markers on hantavirus disease and development of pulmonary syndrome in Brazil

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Hantaviruses are an emerging public health problem in Brazil where 1600 cases of pulmonary syndrome (HPS) were reported with a 40% case fatality rate. We inves- tigated the association of the TNF2 allele, polymorphisms in TGF-β, IL-10, IL-6 and IFN-γ cytokines genes, human leukocyte antigens (HLA) and human platelet alloantigen (HPA) genes with the outcome of hantavirus infection in patients from the northeast of the state of Sao Paulo where Araraquara virus (ARAV) is the causative of HPS. The participants of the study were divided into 3 groups including: 26 HPS patients (6 of them were fatal cases); 96 individuals with IgG antibodies to the N protein of ARAV, but no previous history of HPS and different control groups depending on the genetic marker. Blood samples were collected in tubes containing EDTA (BD Vacutainer, USA), and the genomic DNA was isolated using a salting-out procedure. The TNF genotype was determined using Cytokine Genotyping Tray kit (One Lambda Inc., Canoga Park, CA, USA) and HLA-A, -B, -Cw and –DRB1 alleles and alleles of TGF-β (codons 10, 25), IL-10 promoter (-1082, -819, and -592), IL-6 (–174) and IFN-γ (-874) cytokines genes were determined by SSP-PCR using commercially available kits (Micro SSP HLA DNA Typing and Cytokine Genotyping Tray; One Lambda, CA, USA). Differences among gene frequencies detected in HPS group, seropositive group and control group were analyzed by Fisher's exact test with corrections. It was found that the TNF2 allele was more frequent in HPS patients than in individuals with positive serology for hantavirus but without a history of HPS illness, suggesting that the TNF2 allele could represent a risk factor for developing HPS. On the opposite, the TGF-β high producer phenotype was more frequently found in individuals with hantavirus infection. This phenotype, more than represent a risk factor for hantavirus infection, seems to be protective against most severe form of HPS, because it is associated with thrombocytopenia less intense than other phenotypes of TGF-β. No significant influence of HLA alleles, HPA, IL-6, IFN-γ and IL-10 genotypes was associated to hantavirus disease.
Keywords: Araraquara virus; Cytokines genes; HLA; Genetic markers;

P3-8 Corticosteroids Do Not Prevent Pathogenesis in the Syrian Hamster Model of Hantavirus Pulmonary Syndrome

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Andes virus (ANDV) is associated with a lethal vascular leak syndrome in humans termed hantavirus pulmonary syndrome (HPS). The mechanism for the massive vascular leakage associated with HPS is poorly understood, however immune dysregulation is often suggested as a possible

cause. A small number of studies have suggested that treatment of human HPS with corticosteroid may provide some protection from lethal disease arguing that limiting inflammation and immune responses may prevent pathogenesis. This has never been formally tested in an adult lethal disease animal model of HPS. In hamsters, ANDV causes a respiratory distress syndrome closely resembling human HPS. To determine whether corticosteroid treatment would prevent lethal disease, hamsters were challenged with ANDV and then were treated with corticosteroids at various times after challenge. Neither dexamethasone nor methylprednisolone prevented disease in hamsters following a high dose (2000 pfu) intramuscular ANDV challenge when steroids were administered in a dose-escalating manner, or when steroids were front-loaded and then administered in a dose-deescalating manner. Similar results were also obtained when steroid dosage and timing of administration were altered following a low dose (80 pfu) challenge. In all cases, corticosteroid treatments did not prevent pathogenesis or prolong disease despite evidence of reduced adaptive immune responses in animals receiving corticosteroids as measured by flow cytometry. These data indicate that the immune responses suppressed by corticosteroids are not necessary for HPS pathogenesis in the hamster model of HPS and suggest that corticosteroid treatment alone is insufficient to prevent HPS.

Keywords: Pathogenesis; HPS; syrian hamster model; corticosteroid; immune response;

P3-9 Sangassou virus, the first hantavirus isolate from Africa, displays biological properties distinct from those of other Murinae-associated hantaviruses.

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The first indigenous African hantavirus, Sangassou virus (SANGV) was isolated from an African wood mouse (Hylomyscus simus), trapped in a forest habitat in Guinea, West Africa. Here, we report on the characterization of the genetic and functional properties of the virus. The complete genome of SANGV was determined and showed typical hantavirus organization. The small (S), medium (M), and large (L) genome segments containing genes encoding nucleocapsid protein, two envelope glycoproteins, and viral polymerase were found to be 1,746, 3,650, and 6,531 nucleotides long, respectively. The exact 5' and 3' termini for all three segments of the SANGV genome were determined and were predicted to form the panhandle structures typical of bunyaviruses. Phylogenetic analyses of all three segment sequences confirmed SANGV as a Murinae-associated hantavirus most closely related to the European Dobrava-Belgrade virus. We showed, however, that SANGV uses β(1) integrin rather than β(3) integrin and decay-accelerating factor (DAF)/CD55 as an entry receptor. In addition, we demonstrated a strong induction of type III lambda interferon (IFN-λ) expression in type I IFN-deficient Vero E6 cells by SANGV. These properties are unique within Murinae-associated hantaviruses and make the virus useful in comparative studies focusing on hantavirus pathogenesis.

Keywords: Sangassou virus; receptor; interferon lambda; genome; phylogeny;

P3-10 Mutating Specific Residues in the NY-1 Virus Gn Cytoplasmic Tail Abolishes Interferon Regulation and Defines a Hantavirus Virulence Determinant

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Pathogenic hantaviruses (NY-1V, ANDV and HTNV) and nonpathogenic TULV regulate early cellular IFN responses and replicate successfully in human endothelial cells. Expressing M segment encoded GnGc proteins or the Gn protein cytoplasmic tail (Gn-T) from these viruses blocks RIG-I and TBK1 directed transcription from interferon stimulated response elements (ISREs) and IFNβ promoters (>90%), but not transcription directed by constitutively active interferon regulatory factor-3 (IRF-3). In contrast, expressing the PHV Gn-T had no effect on TBK1 induced transcriptional responses. Analysis of NY-1V Gn-T truncations demonstrated that the C-terminal 42 residues of Gn inhibit RIG-I, MAVS or TBK1 directed IFN induction 70-90%. Recent analysis of NY-1V Gn-T protein functions demonstrated that the protein binds to TRAF3, a signaling protein which forms complexes with TBK1 required for IFN induction. Further truncations of the NY-1V Gn-T demonstrated that residues 1-14 from the Gn C-terminus were dispensable for TBK1 regulation. Further, Gn-T binding to TRAF3 was directed by residues 14-42 amino acids from the C-terminus and constructs expressing this domain regulated TBK1 induced IFN responses. Mutations in 14 individual residues of the NY-1V Gn-T were evaluated for their ability to inhibit IFN transcriptional responses. We found that only mutations of the tyrosine 21 residues from the Gn C-terminus (Y21A) prevented the Gn-T from inhibiting TBK1 directed ISRE or IFN responses. Interestingly, the Gn-T-Y21A mutant was still capable of binding to TRAF3. These findings suggest that TRAF3 binding is at a discrete site within the Gn-T. This data indicates that Gn-T-Y21A mutations prevent TBK1 regulation via a TRAF3 independent mechanism that may instead result from GnGc directed TBK1 degradation. These findings define a NY-1V residue required for Gn-T mediated IFN regulation and suggest the potential for modifying this virulence determinant as a means of attenuating NY-1V.

Keywords: Interferon; Signaling pathway; virulence determinant; GnGc Protein; Mutagenesis;

P3-11 Levels of vascular endothelial growth factor in Dobrava/Belgrade virus infections

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OBJECTIVES: Dobrava/Belgrade virus (DOBV) causes to humans hemorrhagic fever with renal syndrome (HFRS). The main pathophysiologic finding is increased vascular permeability. Vascular endothelial growth factor (VEGF) is one of the most potent vascular permeability agents. Aim of the study was to evaluate the VEGF levels according to the day of illness, and the clinical course in laboratory confirmed DOBV infections. METHODS: The case-controlled study included 40 serum samples from 26 Greek HFRS patients (22 males), 17-70 years old (median 35), in whom laboratory

diagnosis was set by serological and/or molecular methods. The earliest available sample was taken on the 5th day of the disease, and the latest on the 70th day. The samples were divided into 4 groups, according to the day of illness, A: 5-7 days, B: 8-14 days, C: 15-21, D: 22-70 days. A control group of apparently healthy individuals was included. VEGF levels were estimated using a commercial ELISA kit (Human VEGF-A Platinum ELISA, Bender MedSystems GmbH Vienna, Austria). According to the manufacturers, the sensitivity of VEGF detection is >7.9 pg/mL. Mann-Whitney U-test or Kruskal-Wallis test was used to evaluate the difference between groups. The p value less than 0.05 was considered significant. RESULTS: Serum VEGF levels ranged from 0 to 2058.57 pg/ml (mean 648.68, SD 579.09) and were significantly increased compared to the control group (mean 78.20, SD 34.7) (p=0.006). Highest VEGF levels (mean 970.33 pg/ml, SD 642.6) were observed during the 2nd week of the disease. The respective values in the 1st and 3rd week were 355.67 pg/ml (SD 374.3), and 635.81 pg/ml (SD 484.5). Levels decreased further after the 4th week (143.93 pg/ml, SD 78.7). It was observed that the highest VEGF values were seen when the renal failure was already established and that the peak of VEGF in patients with severe disease was seen later than in those with milder clinical course.CONCLUSION: Significant higher VEGF levels are observed in HFRS patients after the 1st week of illness, and especially in the severe cases, the VEGF levels are even higher during the convalescence, suggesting that VEGF plays a role in the repair mechanism by contributing to the vascular repair of the damaged tissues.
Keywords: Dobrava/Belgrade; VEGF;

P3-12 RNA interference screen for human membrane trafficking genes associated with Andes virus infection

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Andes virus (ANDV) causes hantavirus pulmonary syndrome (HPS), and is the only hantavirus known to cause human-to-human transmission. To date, no effective vaccines, immunotherapeutics, or antivirals exist for HPS treatment. Overall, very little is known about the interactions between ANDV and host proteins during infection. In this study, we performed a small interfering RNA (siRNA) screen in primary human microvascular endothelial cells (HMVEC) to identify host factors involved in membrane trafficking processes important for ANDV infection, including endocytosis, vesicular transport, cytoskeletal formation, and exocytosis. The inhibitory effects of these siRNAs were verified by analyzing host and viral protein expression. We identified several cell migration/adhesion, endocytosis, and exocytosis genes and cellular pathways that play significant roles in ANDV infection. Furthermore, using human immunodeficiency virus (HIV)-based pseudoparticles containing ANDV Gn/Gc proteins, we narrowed down host factors involved mainly in the viral entry process. We also verified the importance of these cellular factors in the ANDV life cycle in various cell lines. The results of our study will lead to a better understanding of ANDV/host cell interactions, and may aid in future development of antiviral therapies against ANDV infection.
Keywords: Andes virus; HPS; siRNA library; Virus entry; HMVEC;

P3-13 Establishing a Human Immune System (HIS) Mouse Model to Study Hantavirus Infection

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Hantavirus-associated pathogenesis is characterized by increased vascular permeability and a drastic decrease in platelet counts. Interestingly, hantaviruses do not cause any direct cytopathic effect in vitro suggesting that an over-stimulated human immune response is responsible for both the elimination of the virus and the symptoms observed in vivo. We have previously reported that infection of human hematopoietic cells with pathogenic hantaviruses does not cause reduction in cell survival or alteration of cell differentiation; however, it leads to strong upregulation of HLA class I molecules, the recognition structures for antiviral cytotoxic T cells (CTLs). This supports the hypothesis that pathogenic hantaviruses cause damage by inducing unusually strong CTL responses. A major obstacle in hantavirus research is the lack of an animal model that allows insight into hantavirus-associated immunopathogenesis in humans. In order to solve this problem we generated human immune system (HIS)-engrafted mice. For this purpose newborn pups of non-obese diabetic/severe combined immunodeficiency interleukin-2 receptor γ-chain knockout (NOD-scid IL2rγnull) mice were injected with CD34+ human hematopoietic stem cells. At the age of 12 weeks reconstitution with human immune cells was confirmed. Thereafter, HIS mice were infected with Haantan virus (HTNV) by different infection routes and viral dosages. Currently, we are analyzing this mouse model of hantavirus infection.
Keywords: hantavirus; immunopathogenesis; animal model; humanized mouse;

P3-14 Contribution of Hantaan Virus-Specific $CD4^+$ T Cells to Anti-Viral T-Cell Response in Humans

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Background: Hantaan virus (HTNV), the prototype of genus Hantavirus, could cause the lethal hemorrhagic fever with renal syndrome in humans. However, the contribution of $CD4^+$ T cells to the host response against HTNV infection has not been clear.
Methodology/Principal Findings: We found the ratio of the $CD4^+$/$CD8^+$T cell percentage was reverse at acute phase of the patients compared with healthy control. The reverse ratio was negatively correlated with the level of plasma viral load ($P<0.05$, r=-0.33), the days of the disease course ($P<0.05$, r=-0.39) and the frequency of dialysis of the patients ($P<0.05$, r=-0.50). Therefore we mapped the $CD4^+$ T-cell epitopes with IFN-γ ELISPOT assays, using 351 overlapping peptides spanning the entire structure protein of HTNV in a large cohort of HTNV-infected individuals. A total of 65 novel 15-mer $CD4^+$ T-cell epitopes of the HTNV were identified, among which 10 peptides were dominant target epitopes. Moreover, the HTNV-nucleoprotein-specific $CD4^+$T-cell

response was stronger that that specific to HTNV-glycoprotein (P<0.01). Both the magnitude and breadth of specific $CD4^+$T-cell responses were inversely associated with the level of plasma viral load(P<0.001 for magnitude, P<0.05 for breadth) at acute phase of the disease. The response in patients with severe/critical course showed narrower antigenic repertoire and much weaker responses than that in mild/moderate group (P<0.05). Using intracellular cytokine staining we found that the epitopes-specific $CD4^+$ T cells displayed multiple-cytokine patterns including IFN-γ, TNF-α, IL-2 and IL-4, meanwhile the CD107a, granzyme B and perforin. The frequency of $CD4^+$IL-2^+T cells positively associated with the frequency of $CD8^+$IFN-γ^+T cells at the acute phase of each patients(P<0.05, r=0.50), suggesting the HTNV-specific $CD4^+$T cell may help the effect function of $CD8^+$T cell through the secreting of IL-2. In addition, the frequency of $CD4^+$IFN-γ^+granzyme B^+T cells inversely associated with the level of plasma viral load(P<0.01, r=-0.65) and positively correlated with the frequency of $CD8^+$IFN-γ^+granzyme B^+T cells(P<0.01, r=0.68), suggesting the probable cytotoxic ability of $CD4^+$T cells against HTNV infection. Furthermore, the HTNV specific-$CD4^+$T cells in milder cases showed a terminal differentiation effector phenotype ($CD27^-$ $CD28^-CCR7^-CD45RA^-CD127^{high}CD57^{low}PD\text{-}1^{low}$). The proliferation assay showed that there was an impaired proliferative capacity of HTNV-specific $CD4^+$T cells in severe/critical patients compared with those with milder outcome(P<0.05). *Conclusions:* Taken together, these results indicate that the effective HTNV-specific $CD4^+$ T-cell response could contribute against the HTNV infection, probably through the $CD8^+$T cell response and the cytotoxic effect.

Key words: Hantaan virus, $CD4^+$ T cell response, epitope, virus control

P3-15 Characterization of Natural Killer Cell Phenotype during HFRS

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Aim: To investigate the phenotypic characterization of NK cells from the patients with HFRS, and the function of NK cells in the pathogenesis of HFRS.

Methods: Flow cytometric analysis was used to detect the NK cell subsets and the phenotype of NK cells. Then several receptors were detected in HTNV infected HUVEC.

Results: The three subsets of NK cell (CD56bright, CD56dim, CD56neg) did not show significant difference in the five phases of HFRS; neither did several activated and inhibited receptors. The expression of HLA-ABC, ICAM-1, VCAM-1, E-Selectin, P-Selectin was up-regulated in HTNV infected HUVEC.

Conclusion: NK cells maybe not involved in the pathogenesis of HFRS, and during the acute phase of HFRS, NK cells maybe inhibited by the up-regulated MHC-I molecules.

P3-16 Involvement of Akt/NF-κB Pathways in High Level Expression of Cytokines/Chemokines, Adhesion molecules induced by Hantaan Virus Infection

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Objective: Hantaan virus (HTNV), major serotype of the genus *Hantavirus*, causes a severe form

of HFRS. NF-κB represents a family of eukaryotic transcription factors involved in the early pathogen response, which plays an important role in promoting inflammation and viral gene expression. It is well known that different DNA and RNA viruses infection can trigger NF-κB activation via different signaling mechanisms. However, little is known regarding the signaling pathway utilized by HTNV and the role of the nuclear factor in the infection process.

Methods and Results:1. HTNV infection can up-regulate the expression of IL-6, IL-8, RANTES, ICAM-1, VCAM-1, E-selectin, P-selectin, but the expression of TNF-α and IL-1β does not have significant difference before and after HTNV infection. 2. Cardamomin, NF-κB inhibitor, can reduce Akt Activation in HTNV infected HUVEC and inhibit nuclear translocation of NF-κB by HTNV. 3. Cardamomin inhibits the expression of IL-6, RANTES, ICAM-1 and VCAM-1, but not IL-8.

Conclusion: HTNV replication was independent of the ability of the virus to activate NF-κB in HUVEC. The selective induction of the cytokines/chemokines and adhesion molecules may provide a clue for the involvement of the specific immune response in the pathogenesis of HFRS, and the blockade of Akt/ NF-κB pathways maybe reduce the excessive inflammatory reaction. The Akt/ NF-κB pathways maybe involved in the expression of these molecules in HTNV infected HUVEC, and cardamomin may be a potential beneficial agent for therapy of HFRS.

Keywords： HTNV; Akt; NF-κB; ICAM-1; VCAM-1; IL-6; IL-8; RANTES;

Topic 4 DIAGNOSTICS, TREATMENTS AND CLINICAL FINDINGS

P4-1 Sodium Dodecyl Sulphate (SDS)/EDTA-Pretreated Chromatography Paper Strips for Sampling Hantavirus Positive Rodents in the Field

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Simple and standardized sampling techniques are essential tools during rodent trapping field campaigns as field conditions may limit handling and transportation of the collected specimens. In this study we describe the potential of SDS/EDTA-pretreated chromatography paper strips for the collection, storage, and transportation of hantavirus-positive urine samples for subsequent genetic analysis. In order to inactivate the hantaviruses and other potential microorganisms upon contact with the strips, the latter were pretreated with SDS, a surfactant with protein denaturising ability. This can allow safe transportation of the strips without extensive biohazard precautions. To protect the viral RNA from degradation by ribonucleases the chromatography strips were additionally treated with EDTA and Tris-HCl. EDTA chelates magnesium ions, a necessary cofactor for most nucleases. The weak organic base Tris-HCl ensures the proper action of the chelating agent in binding the divalent cations. With this method rodent or insectivore urine samples can be collected and stored in a biosafe and convenient way. The use of the SDS/EDTA paper strips was validated by applying serial tenfold dilutions of the quantified Puumala virus (PUUV) strain BE/Montbliart/1/1986 to the pretreated strips. The strips were stored at different environmental conditions thereafter (room temperature, 4 °C, and -20 °C). At week 1, 4, 8 and 12 the presence of PUUV on the strips was detected by quantitative PCR. Our results show that PUUV RNA can remain stable for at least 12 weeks. With all temperatures tested the concentration of PUUV RNA remained stable. For the strips stored at room temperature however, the concentration dropped considerably after 12 weeks and viral RNA was not detectable in the lowest dilutions tested. For longer storage periods we advise to store the strips at lower temperatures. Additionally, the strips were tested during a bank vole trapping campaign. Urine was collected from PUUV IgG-positive bank voles. The strips were air-dried, kept at ambient temperature during the field campaign and stored at -20 °C upon arrival in the lab. PUUV RNA could be successfully extracted from all strips. We conclude that the use of SDS/EDTA-pretreated chromatography paper strips for retrieval and subsequent analysis of hantavirus RNA from urine specimens is a feasible method for sample collection.
Keywords: Puumala virus; chromatograhy paper strips; SDS/EDTA; sampling;

P4-2 A rapid method for hantavirus titration

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Human infections through Andes virus (ANDV) are common during summer months in southern Chile, where they currently cause 30 % of fatality in infected patients. It has been reported that this particular species can be transmitted from person to person, probably through saliva or other corporal fluids. In cell culture, hantaviruses produce non-lytic infections and yet, techniques like focus reduction assays (FRA) to detect infectious virus are limited due to their low sensitivity, reproducibility and long incubation times (7 days). In order to improve ANDV detection and quantification, we have established a novel, rapid and highly quantitative method which involves the detection of ANDV infected cells by flow cytometry. To this end, the expression of the N protein during viral infection was measured using monoclonal antibodies which were previously developed and characterized in our laboratory. The N protein has been reported to be expressed at early times points post-infection and to be the most abundant viral protein during infection of cells. To develop this assay, the time course of N protein expression versus virus release was analyzed in Vero E6 cells. The expression of N was detected as early as 6 hrs post-infection whilst virus release was detected only 24-48 hrs post-infection. This window of time was used to detect virus during its first round of infection and allowed us to reduce the time of this assay to two days. Titers determined by flow cytometry correlated well with titers obtained by FRA. Furthermore, viral titers fall to zero when viral stocks were pretreated with heat, confirming that the detected N protein was not derived from input virus. Further, the assay was applied to quantify inhibition of virus cell entry by the use of drugs that prevent acidification of endosomes. Further, infection of cells was not detected when the non-permissive HeLa cells were used. Taken as a whole, the novel quantitative hantavirus titration assay offers several advantages over FRA in terms of time, reproducibility and sensitivity. Among its many applications, it will be useful to titer virus stocks, to detect infectious virus in human samples and to screen for antiviral drugs.

Keywords: ANDV; Flow cytometry; Detection; Hantavirus; titration;

P4-3 Evaluation of a line immunoassay using recombinant hantavirus antigens for detection of Dobrava/Belgrade virus

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The main hantavirus circulating in Greece is Dobrava/Belgrade virus (DOBV), causing to humans severe hemorrhagic fever with renal syndrome (HFRS). Aim of the present study was to evaluate the performance of a commercial line immunoassay for the detection of IgM and IgG DOBV antibodies in Greek HFRS patients.

Material and Methods: Seventy serum samples collected through 1995 to 2012 were tested; 64 were obtained from confirmed and 6 from suspected HFRS cases. DOBV associated with *Apodemus flavicollis* has been previously detected by RT-nested PCR and sequencing in 42 of the 64 samples. The samples were analyzed for hantavirus IgM and IgG antibodies using the recomLine HantaPlus IgG, IgM line immunoassay (Mikrogen, Neuried, Germany) which includes recombinant antigens for DOBV, Hantaan virus (HTNV), Seoul virus (SEOV), Puumala virus (PUUV), and Sin Nombre virus (SNV). The assay detects the serotype specific N-terminal part of the virus nucleocapsid

antigen. Sandfly fever virus antigen is also included, but the testing for phleboviruses is out of the scope of the present study.
Results: The line immunoassay reliably detected IgM and IgG hantavirus antibodies in all 64 samples (100%) from the confirmed HFRS cases: DOBV antibodies was detected in 62 samples, while antibodies against Puumala virus (PUUV) were detected in 2 samples. Differentiation between groups DOBV-HTNV-SEOV and PUUV-SNV was successful in all samples. Cross-reactivity was seen mainly between DOBV and HTNV in the IgM assay and in less extend in the IgG. Among the 42 PCR-confirmed DOBV samples, 26 were clearly characterized as DOBV by the immunoassay; a cross-reactivity with HTNV was observed in the rest 16 samples.
Conclusion: The recomLine HantaPlus IgG, IgM line immunoassay, which is well established in laboratories across Europe, is a helpful tool for the laboratory diagnosis of DOBV infections.

P4-4 Detection of Puumala virus in bronchoalveolar lavage during “hantavirus pulmonary syndrome” in a Belgian NE case

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INTRODUCTION: In Europe, Puumala hantavirus (PUUV) commonly causes “nephropathia epidemica” (NE), but a primary hantavirus pulmonary syndrome (HPS)-like presentation is rare.
CASE REPORT: A 42 year-old female was admitted into intensive care unit (ICU) after four days of progressive dyspnea with fever, visual disturbance and myalgiae. Blood pressure on day 5 was 100/70 mm Hg, heart rate 101/min; temperature 39,5 ℃ and oxygen saturation 92% under 15 L O2/min. Bibasal lung crackles were noted. CRP level was 18 mg/dl, platelets 84,000/µl, leukocytes 7,460/µl, Sodium 130 mEq/l, LDH 1815, ASAT 61, and ALAT 51 UI/l. Creatinine and urea were normal. Chest radiograph showed bilateral infiltrates. Echocardiography revealed normal left ventricular function. Antibiotics IV were started for suspected community-acquired pneumonia (CAP). Due to worsening of respiratory failure, non-invasive ventilation with high oxygen levels was initiated. Chlamydia, legionella and mycoplasma pneumoniae serologies were negative, as well as pneumococal and legionella urinary antigens. At day 7, bronchoscopy with BAL was performed. Respiratory mucosa appeared slightly inflammatory and BAL fluid macroscopically gray. Microscopic examination revealed numerous macrophages with multiple slightly greenish homogenous cytoplasmic inclusions, surrounded by a fine clear halo. These last features were compatible with a «viral» infection. Lymphocyte subtyping revealed 81% CD8 and 17% CD4 T-cells. BAL fluid analyses were negative for bacteria, mycoses, mycobacterium and viruses. Only at day 10, renal failure with oliguria appeared, suggesting a possible NE, subsequently characterized by high urinary levels of protein (12.8 g/l) and sodium (68 mEq/l). A preserved acute serum sample

appeared positive for PUUV in IgM EIA (ratio 14) and RT-PCR. Sequencing confirmed a PUUV belonging to the same clade of four other PUUV geographically close lineages of the Belgian Ardennes.

Intermittent haemodialysis was required. The patient was discharged from ICU on day 15 without renal assistance, and from hospital on day 24. Later immunostaining of the BAL macrophage inclusions was positive with specific anti-PUUV antibodies.

DISCUSSION: Although a NE diagnosis remains difficult before renal involvement, the combination of a severe CAP, elevated LDH, hyponatremia and thrombocytopenia must alert for a possible HPS, especially in endemic areas. To date, PUUV in the lung could only be demonstrated at autopsy. Revelation of these particular greenish intracytoplasmic inclusions in BAL macrophages, proved by immunostaining to be PUUV, was of great importance for confirmation of a hantaviral lung involvement. Such finding can strengthen clinical suspicion of HPS and supports BAL usefulness in severe CAP of unknown etiology.

P4-5 Development of a SYBR Green Ⅰ based One-step Real-time PCR Assay for Detection and Quantification of Hantaan Virus

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Hantaan virus (HTNV) is a major zoonotic pathogen belonging to Hantavirus and causing hemorrhagic fever with renal syndrome (HFRS) in Asia, especially in China. The diagnosis of HFRS is mainly dependented on clinical manifestation and detection of IgM and IgG antibodies agasinst HTNV. In order to get the accurate viral load of HTNV, the development and evaluation of a SYBR Green Ⅰ based on one-step real-time PCR assay was established. The standard curve was generated as mean Ct versus log dilution of known virus titer over a 10-log range from 10^7 to 1 PFU/ml. The sensitvity and specificity of the assay was invetstigated, and 100-fold more sensitive when compared with conventional RT-PCR and there was no cross-reaction was observed with other viruses (HBV, HCV, and HIV). The viral RNA load from serum of clinical HRFS patients was also investigated with the assay. This sensitive and specificity assay has been proved to be useful for detection of HTNV both in labrotary and in clinical.

Keywords: Hantaan virus; real-time PCR; hemorrhagic fever with renal syndrome

P4-6 Lung and heart lesion in patients with HFRS

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Hemorrhagic fever with renal syndrome (HFRS) is an actual problem for the Republic of Bashkortostan, where circulating Puumala hantavirus. The main syndromes are including fever, renal and haemorrhagic. In addition to kidney damage in some patients with moderate to severe forms of the disease are observing changes in the lungs and heart. Compliance with bed rest limits

the use of instrumental methods of diagnosis, and therefore especially important to determine organospetsificum serum biomarkers.

We studied in the blood of patients with HFRS in dynamics following indicators: alveomutsin (n = 96), brain natriuretic propeptide NT-proBNP (n = 88) and troponin I (n = 24), depending on the severity of the disease, function of the lung , kidney and heart . Contents alveomutsin HFRS patients was depended on the severity of the disease and the period; was higher in patients with lung damage ($p < 0.01$) and correlated with oxygen saturation (SpO2) in moderate ($r = -0,61$; $p = 0.001$) and severe ($r = -0,55$; $p = 0.02$) forms of the disease. NT-proBNP was higher than the control value for moderate to severe forms of the disease in the initial period and correlated with systolic blood pressure ($r = -0,63$; $p=0.001$ and $r = -0,46$; $p = 0.011$, respectively), even more increased in the oliguric period and correlated with serum creatinine ($r = 0,64$; $p = 0.001$ and $r = 0,72$; $p = 0.002$, respectively). In 24 patients with electrocardiographic changes studied troponin I. It was exceeded the normal value in 2 patients with deep negative T waves in the ECG and tachycardia in oliguric period. These clinical and laboratory symptoms were regarded by us as a manifestation of myocarditis. Thus, for HFRS caused by serotype Puumala, characterizing by lesions of the heart and lungs. To confirm this, along with the instrumental methods can be used serum biomarkers.

Keywords: Puumala hantavirus; alveomutsin; NT-proBNP; troponin I

P4-7 The Spleen is Enlarged during Acute Puumala Hantavirus Infection and The Enlargement does not Associate with the Platelet Count

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Background: Puumala hantavirus (PUUV) infection is associated with thrombocytopenia and mild bleedings. We aimed to evaluate if the spleen is enlarged during acute PUUV infection, and to determine its possible association with thrombocytopenia.

Methods: Abdominal magnetic resonance imaging (MRI) was performed in 20 consecutive hospitalized patients with acute serologically confirmed PUUV infection. MRI study was repeated 5-8 months later, at recovery. The maximum length of spleen in the acute phase and at the recovery was measured and the change was compared with the laboratory findings reflecting the clinical course of the disease.

Results: In all patients, the length of the spleen was increased in the acute phase compared with the recovery phase (median 129 vs. 111 mm, $p<0.001$). There was no association between the change in the length of the spleen and the minimum platelet count ($r=0.171$, $p=0.472$). The change correlated with the maximum C-reactive protein value ($r=0.513$, $p=0.021$). Interestingly, the association between the change in the length of the spleen and the other variable measuring inflammation, the maximum leukocyte count, was negative ($r=-0.471$, $p=0.036$). No association was found between the change and the maximum serum creatinine concentration and the length of the hospital stay ($r=-0.306$, $p=0.189$ and $r=0.034$, $p=0.887$, respectively).

Conclusions: The spleen, measured by MRI, is enlarged in the acute phase of PUUV infection. The

change does not associate with the main clinical findings of acute PUUV infection, namely thrombocytopenia and acute kidney injury. The negative association between the change in the length of the spleen and the acute-phase maximum leukocyte count may reflect the abnormal pooling of leukocytes in the enlarged spleen.

Keywords: hantavirus; platelet; spleen; MRI; enlargement

P4-8 Renal and Pulmonary Syndrome Caused by Tula Virus in Immunocompromised Host? First Molecular Evidence

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Authors refer the first molecular evidence of Tula virus as an etiologic agent of renal and pulmonary syndrome in an immunocompromised patient.14-years old boy from rural area of the Czech Republic treated for acute lymphoblastic leucaemia (ALL) was admitted to the hospital for febrile respiratory infect complicated with respiratory distress and desaturation due to severe bronchopneumonia. In the course of the hospitalisation he developed renal failure with oliguria and thrombocytopenia. The relapse of ALL was excluded by the bone marrow evaluation. Acute hantavirus infection was confirmed using both serologic and molecular methods. Sequencing of the PCR products revealed that the causative agent was Tula virus. This case shows the real evidence for previously suspected human pathogenicity of Tula virus. In addition to PUUV and DOBV this is the third Central European hantavirus pathogenic for humans.

Keywords: Tula virus; Hantavirus; Czech Republic; Acute Lymphoblastic Leucaemia;

P4-9 Plasma Levels of Soluble Urokinase-type Plasminogen Activator Receptor Associate with the Clinical Severity of Acute Puumala Hantavirus Infection

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Background: Urokinase-type plasminogen activator receptor (uPAR) is released during infection and inflammation, and it promotes the migration and adhesion of leukocytes by binding to β-integrins. Pathogenic hantaviruses use β3-integrins to enter endothelial cells and inhibit β3-integrin directed cell migration. Previous studies indicate that a high plasma concentration of the soluble form of the receptor (suPAR) predicts disease severity in various infections, including sepsis. Recent work also suggests that circulating suPAR can activate podocyte β3-integrins in the glomeruli, causing foot process effacement and proteinuria. We aimed to evaluate if plasma suPAR levels associate with the clinical severity of Puumala hantavirus (PUUV) infection. Methods: Plasma suPAR levels were measured in 97 patients (63 males, 34 females, aged from 22 to 77 years) with acute serologically confirmed PUUV infection using a commercial enzyme-linked immunosorbent

assay (ELISA). Levels were measured twice during the acute phase (on day 1 and 2 after admission to the hospital) and once during the convalescent phase (median 13 days after discharge from the hospital). The maximum value of suPAR in the acute phase was compared with laboratory findings reflecting the clinical severity of the disease. Results: Plasma suPAR values in the acute phase were significantly higher than control values taken after hospitalization (median 8.7 vs. 4.7 ng/ml, $p<0.001$). Maximum plasma suPAR levels correlated positively with maximum blood leukocyte count ($r = 0.475$, $p<0.001$), maximum plasma creatinine concentration ($r = 0.378$, $p<0.001$), change in weight during the hospital stay ($r=0.406$, $p<0.001$) and the length of hospital stay ($r = 0.326$, $p=0.001$), and inversely with minimum blood platelet count ($r = -0.325$, $p=0.001$) and minimum hematocrit ($r = -0.369$, $p<0.001$). Conclusions: Plasma suPAR values are markedly increased in the acute phase of PUUV infection and correspond with the levels previously reported in patients with sepsis. Plasma suPAR levels associate with several variables indicative of severe disease. It is possible that an increase in suPAR activates β3-integrin in PUUV infection. Circulating suPAR thus might be involved in the pathogenesis of PUUV infection.

Keywords: suPAR; severity; Puumala; hantavirus;

P4-10 Effect of Smoking on Risk and Clinical Severity of Puumala Hantavirus Infection

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Objectives: There are two previous reports of smoking as a risk factor to Puumala hantavirus (PUUV) infection. Smoking has local and systemic adverse effects on the immune system and might have impact on outcome of infections. The aim of this study was to evaluate the prevalence of smoking in patients with acute PUUV infection and the possible influence of smoking on the clinical severity of PUUV infection. Methods: The questionnaire was sent to 494 patients, who had been treated in Tampere University Hospital with serologically confirmed PUUV infection during the years 1982 to 2012. Patients were asked if they smoked at the time of acute PUUV infection. Results: Out of all patients 357 (72 %) participated the survey (M257/F100, mean age 41.3 ±12.2 years). Altogether 51 % of responders answered that they smoked at the time they caught PUUV infection. Fifty-seven percent of males and 36 % of females were smokers. During concurrent years, smoking has decreased from 33 % to 22 % among Finnish males of the same age and among females it has varied between 14 to 20 %. Maximum blood leukocyte count measured during the hospital stay was significantly higher among smokers than in non-smokers (median 10.8 vs. 8.9 x10E9/l, $p<0.001$). Smokers had also higher maximum plasma creatinine level than non-smokers (median 273 vs. 184 µmol/l, $p<0.001$). There were no differences between the smokers and the non-smokers in maximum or minimum hematocrit value, maximum C-reactive protein level, minimum platelet count, or in the duration of hospitalization, age, blood pressure or body mass index. Conclusions: Hospital-treated

patients with PUUV infection smoke clearly more often than the average population in Finland. Certain clinical manifestations are more severe in smokers than in non-smokers. Pathogenetic mechanisms need further studies.

Keywords: smoking; Puumala; hantavirus; risk factor;

P4-11 Sochi Virus as a Highly Pathogenic and Life-Threatening Agent

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Sochi virus, a novel genetic variant of Dobrava-Belgrade virus, was isolated in cell culture from a patient suffering from a fulminant lethal course of hemorrhagic fever with renal syndrome (HFRS).The patient presented with shock and combined kidney and lung failure. Sochi virus is transmitted to humans from its host reservoir Apodemus ponticus. In virus neutralization assays, the Sochi virus isolated from the HFRS patient (Sochi/hu strain) and another Sochi virus isolate from A. ponticus (Sochi/Ap strain), were found to be neutralized to identical extents by sera of HFRS convalescents infected by Sochi virus. The complete nucleotide sequences of the S, M, and L segments demonstrated a strong similarity between Sochi/hu and Sochi/Ap strains.
In total, 56 HFRS patients with serologically proven Sochi virus infection have been recognized in the south of the Krasnodar region (Black See area), with available clinical data for 52 of them. The clinical courses were found as severe for 31 patients, moderate for 20 patients and mild for only 1 patient. Eight fatal cases were registered which led us calculate a case fatality index of 8/56 = 14.3 %. In one case death occurred on the 4th day of fever, prior to the provision of medical care, all other patients died during hospitalization. The main causes of death were renal, lung, and cardio-vascular failure, with findings as DIC syndrome, pulmonary and cerebral edema, endotoxic shock. Based on the severity and case fatality of disease, Sochi virus is the most dangerous pathogen in comparison with other hantaviruses causing HFRS on the territory of Russia.

Keywords: HFRS; Sochi;

P4-12 Atypical Presentation of Hantavirus Infection without Pulmonary Edema in Andes Virus Endemic Area.

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Hantaviruses are rodent-borne zoonotic agents that cause hantavirus pulmonary syndrome (HPS) in the Americas. In Southern Argentina, HPS was recognized in 1995 and Andes virus (ANDV) was

characterized as the etiologic agent, being the only genotype identified in this region. Although clinical spectrum of ANDV infections is not completely known, not fulfilling HPS definition cases are exceptional. We present the case of a previously healthy 43-year-old man, initially seen as an outpatient with a 4 days history of fever (40 ℃), headache, dizziness and asthenia. The patient was a rural resident in Southern region and had peridomestic activities with risk exposure to rodents. Clinical examination disclosed fever (39 ℃) and a cardiac systolic murmur (diagnosed in his teens). Laboratory values on day 9 after onset of symptoms showed: hematocrit 43%; WBC 7800 /mm3; neutrophils 83%; limphocytes 15%, atypical lymphocytes; platelets 76000 / mm3; LDH 1019 U/l (NV <320 U/l), ESR 55 mm and cholesterol 100 mg/dl. AST, ALT, BUN, alkaline phosphatase, creatinine, Na, K, bilirubin, KPTT, prothrombin time, total protein, albumin and urinalysis were normal. He continued with fever and headache and, on day 13, he referred blurred vision and was hospitalized. Physical findings included a temperature of 38.5 ℃, hepatomegaly and small cervical, axilar and inguinal lymph nodes. The admitting presumptive diagnoses were meningoencephalitis, infectious endocarditis, HIV acute infection and mononucleosis. Ophthalmologic exam disclosed acute reduction of visual acuity (20/200). Chest roentgenography and abdominal ultrasond were normal. Echocardiogram showed thickness of mitral valve. Computed tomography of the brain showed signs of incipient cerebral edema. Electroencephalogram and CSF analysis were normal. Abnormalities in laboratory were: platelets 106000 mm3; AST 63 UI/l (normal value 22 UI/l) and 81 UI/l (normal value <22 UI/l). During hospitalization, fever descended, visual acuity improved, and platelets returned to normal values. He was discharged 5 days after. In day 22 AST raised to 122 UI/l and ALT to 142 UI/l. Blood, CSF and urine cultures were negative. Serologic tests for Treponema pallidum, Brucella, Chlamydia, HIV, HAV, HBV, HCV, CMV, Epstein Barr virus, influenza A and B, adenovirus were negative. Because of undeterminant cause of this illness, hantavirus infection was investigated: IgM an IgG against recombinant ANDV nucleocapsid were positive confirming acute infection. Conclusions: ANDV can produce a wider spectrum of clinical disease than initially was thought. In this patient, the unusual presentation postponed the diagnosis suspicion.

Keywords: atypical presentation; Andes virus; Argentina;

P4-13 Clinical Characteristics of Hantavirus Infections in Southern Argentina

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Clinical Characteristics of Hantavirus Infections in Southern Argentina Andes virus (ANDV) is the only genotype characterized in hantavirus infections cases in Southern Argentina. During an outbreak in 1996 person-to-person transmission was confirmed. Family clusters are a common presentation associated with interhuman transmission. To describe the clinical characteristics of ANDV infections we analysed 94 confirmed cases acquired in the region and/or with molecular evidence of ANDV infection. The mean age was 31.5 years (+15.2; 1-70) and 63% were males. Of

the 94 patients, 62% were sporadic, 21% belong to 9 family clusters and 17% to the 1996 ´outbreak. Hantavirus Pulmonary Syndrome (HPS) was the clinical presentation in 90/94 cases. Common symptoms were fever (98%), dysnea (69%), myalgia (67%), headache (64%), cough (63%), vomiting (50%), abdominal pain (28%), arthralgia (17%) and diarrhea (13%). Physical findings were tachypnea (95%), tachycardia, (94%), hypotension (91%), hyperthermia (80%), conjunctive injection (27%), pharyngeal congestion (23%), hemorrhages (20%); facial flushing (15%) and pethechia (15%). During the cardiopulmonary phase, 69% patients required inotropics and 63% respiratory mechanical ventilation (RMV). Three patients required hemodialysis . Creatinine values (mean 20.7 mg/l +17.0; 7.0-110) were higher than 25 mg/l in 25% cases. BUN was increased in 83% cases (mean 0.76 g/l +0.4; 0.31-2.0). AST and ALT were elevated in 93% of cases rising 5 to 10 times in 34%; CK was >5 times above normal in 20%. Case-fatality rate with RMV requirement was 74%. Most patients (97%) died during the first 10 days of illness (mean 6.8 days +0.7; 3-33) and 2 days after admission (mean 1.9 days +4.4; 0-28). Hemorrhages were a clinical predictor of death; laboratory abnormalities associated to mortality were PaO2/FIO2 at admission, maximal increases in hematocrit and in creatinine values, and minimal platelet count. One of the 4 patients that did not fulfill HPS case-definition had fever, meningism and elevated transaminases; the other 3 were children with subclinical infection (1) or febrile syndrome (2). The lethality rate was 46% but differences were observed according to the presentation: In sporadic cases this rate was 40%. In the clusters, 9/10 (90%) index case-patients died while mortality in secondary cases varied from 29% in family clusters to 53% in the 1996 ´outbreak. Conclusions: ANDV infections are characterized by a high lethality rate with more renal, hemorrhagic, hepatic and muscular involvement than described for Sin Nombre virus. In clusters presentations, index cases are almost always fatal with clinical severity descending in secondary cases.

Keywords: Andes virus; Argentina; clinical characteristics;

P4-14 A Case of Hantavirus Nephropathy presenting with a “HUS-like” Syndrome

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Introduction: Hantavirus nephropathy is a zoonotic viral infection with possible lethal consequences. In Europe, infections are mainly caused by the Puumala (PUUV) strain with generally a good prognosis. Case description: A 58-year-old Belgian farmer was referred for plasmapheresis because of suspected diagnosis of TTP-HUS. The patient was admitted with a fever of 39 ℃, shivers, arthralgia and gastro-intestinal complaints of nausea, epigastric pain and anorexia. In the first days he developed a productive cough. Further anamnesis revealed recent contact with rodents, more specifically the removal of a bank vole nest 3 weeks prior to admission. He had acute renal failure (ARF) (plasma creatinin 1,89mg/dl), proteinuria (14g/L), thrombocytopenia (20.10E9/L) and haemoconcentration with a mildly elevated schistocytosis. Elevated levels of D-dimers, haptoglobin,

CRP, LDH and a left shift in the leukocytosis were noted. Diagnostic immunoblasts were present in as well the peripheral blood as the bone marrow. Arterial sampling showed mild hypoxaemia and hypocapnia. Mild coagulopathy with prolonged APTT, decreased levels of protein C and protein S, next to positive levels of lupus anticoagulans and initially a positive Coombs were puzzling. Because of the tentative diagnosis of hantavirus nephropathy, we opted for a conservative approach and the patient fully recovered within 6 weeks. By that time the results of PUUV IgM were strongly positive while the IgG were on the verge of positivity. RT-PCR and sequencing proved to be positive for PUUV, originating from the Turnhout region, North of Belgium. Discussion: This case demonstrates the clinical variability progressing from a flu-like syndrome to ARF with thrombocytopenia. However, this patient was referred with a suspected diagnosis of TTP-HUS, though without arguments for severe hemolysis. Tests showed signs of endothelial dysfunction and leakage, next to a diagnostic hematologic triad and nefrotic range proteinuria. Also remarkable was a mild coagulopathy with a prolonged APTT, a positive lupus anticoagulans in the early fase and low levels of protein C and S. Thorough history taking is necessary since prior contact with bank voles was practically diagnostic. Misdiagnosis was avoided, so was unnecessary treatment with immunodepressants or plasmapheresis, allowing correct serology, leading to positive IgM.

Keywords: Hantavirus; Nephropathy; HUS;

P4-15 Hantavirus pulmonary syndrome: prognostic factors for death in Brazil

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Hantavirus pulmonary syndrome (HPS) was described for the first time in Brazil in 1993 and has occurred endemically throughout the country. This study analysed clinical and laboratory aspects as well as death-related factors for HPS cases in Brazil from 1993 to 2006. The investigation comprised a descriptive and exploratory study of the history of cases as well as an analytical retrospective cohort survey to identify prognostic factors for death due to HPS. A total of 855 Brazilian HPS cases were assessed. The majority of cases occurred during spring (33.5%) and winter (27.6%), mainly among young male adults working in rural areas. The global case fatality rate was 39.3%. The mean interval between the onset of symptoms and hospitalisation was 4 days and that between hospitalisation and death was 1 day. In the multiple regression analysis, adult respiratory distress syndrome and mechanical respiratory support were associated with risk of death; when these two variables were excluded from the model, dyspnoea and haemoconcentration were associated with a higher risk of death.

Keywords: Hantavirus; Hantavirus Pulmonary Syndrome; Epidemiology; Prognostic factors; Brazil;

P4-16 The optimal timing of CRRT for critical patients with hemorrhagic fever with renal syndrome

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Background: High mortality in critical patients with HFRS is probably associated with the severity of ARF. The aim of this study is to explore the optimal timing of CRRT starting in critical patients with HFRS.

Methods: A retrospective analysis was performed on 59 critical patients, treated with CRRT or without hemodialysis in our Center, from January 2008 to August 2012. According to the RIFLE criteria, all the patients were divided into risk, injury and failure stage. Loss and end stage were not found. Each stage was further divided into CRRT group and control group. We compared the progress rate of renal function and mortality between CRRT group and control group of each stage. Furthermore, Univariate analyses were performed to determine the potential risk factors for mortality before the deterioration of renal function.

Results: 59 critical patients met the inclusion criteria, of whom 27 patients (45.8%) died. The median age was 52 years (range 16-70) and 47 (79.7%) were males. The median hospital day in survivors was 26 days (Range 7-93). Renal recovery, defined as independence from hemodialysis at discharge, was documented for 27/32 (84.4%) of the survivors. The progress rate of renal function was 10.0% in CRRT group and 33.3% in control group at risk stage (P=0.021). The progress rate was 16.7% and 50.0% in CRRT group and control group separately at injury stage（P=0.039). No significant difference was found at failure stage (60.0% in CRRT group vs. 80.0% in control group, P=0.180). The mortality showed no significant difference between CRRT group and control group at risk stage (P=0.070) and injury stage (P=0.508), yet significant difference was found at failure stage (P=0.007). Furthermore, the mortality showed no significant difference among the three stage in CRRT group (P＞0.05). The potential risk factors for mortality included the rate of mechanical ventilation (P=0.004), plasma lactic acid（≥4mmol/L）at hypotensive phase (P=0.041).

Conclusions: Starting CRRT at risk and injury stage in critical patients with HFRS might be the optimal timing to improve the renal function. Compared with the patients without hemodialysis, starting CRRT at failure stage might decrease the mortality. Starting CRRT at the early stage of ARF on the basis of supportive and symptomatic treatment actively would decrease mortality more efficiently.

P4-17 Manufactured post-exposure prophylactics for hantavirus pulmonary syndrome

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Andes virus (ANDV) is the predominant cause of hantavirus pulmonary syndrome (HPS) in South America and the only hantavirus known to be transmitted person-to-person. There are no vaccines,

prophylactics, or therapeutics to prevent or treat this highly pathogenic disease (case-fatality 35-40%). Infection of Syrian hamsters with ANDV results in a disease that closely mimics human HPS in incubation time, symptoms of respiratory distress, and disease pathology. Here, we report on the feasibility of two post exposure prophylaxis strategies in the ANDV/hamster lethal disease model. First, we evaluated a natural product, human polyclonal antibody, obtained as fresh frozen plasma (FFP) from a HPS survivor. Second, we used DNA vaccine technology to manufacture a polyclonal immunoglobulin-based product that could be purified from the eggs of vaccinated ducks. The natural "despeciation" of the avian IgY (i.e., Fc removed) results in an immunoglobulin predicted to be minimally reactogenic in humans. Administration of ≥5,000 neutralizing antibody units (NAU)/kg of FFP-protected hamsters from lethal disease when given up to 8 days after intranasal ANDV challenge. IgY/IgYΔFc antibodies purified from the eggs of DNA-vaccinated ducks effectively neutralized ANDV in vitro as measured by plaque reduction neutralization tests (PRNT). Administration of 12,000 NAU/kg of duck egg-derived IgY/IgYΔFc protected hamsters when administered up to 8 days after intranasal challenge or 5 days after intramuscular challenge. These experiments demonstrate that convalescent FFP shows promise as a postexposure HPS prophylactic. Moreover, these data demonstrate the feasibility of using DNA vaccine technology coupled with the duck/egg system to manufacture a product that could supplement or replace FFP. The DNA vaccine-duck/egg system can be scaled as needed and obviates the necessity of using limited blood products obtained from a small number of HPS survivors. We also provide an update to alternative treatment routes for administration of polyclonal antibodies and evaluate an alternative avian species as an IgYΔFc source. Ultimately, our goal is to develop a capability to use DNA vaccine technology to manufacture polyclonal antibodies for use as post-exposure prophylactics, and possibly therapeutics, to prevent and treat lethal viral diseases such as HPS.

Keywords: Andes virus; HPS; polyclonal antibodies; avian; IgY

P4-18 DNA vaccine encoding Hantavirus glycoprotein N-terminal, targeted to the major histocompatibility complex II compartment by lysosome-associated membrane protein, significantly elicits both specific humoral and cellular immune responses and induces immune protection against Hantavirus challenge in Balb/c mice

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Endogenous antigen encoded by conventional naked DNA was processed by antigen-presenting cells (APCs) and only presented to CD8+ T cell through forming peptide/MHCI (major histocompatibility complex type I molecules) complex. Whereas, antigen presentation by MHCII molecules and activation of CD4+ helper T cells are critical for the generation of effective immue response and long-term immune memory. Our previous studies showed DNA vaccine encoding

SARS Coronavirus nucleocapsid (CoV-N), even epitope-based SARS CoV-N70-122 DNA vaccine with the lysosome-associated membrane protein (LAMP) as a chimera can not only elicit both humoral and cellular immune responses, but also promote a long-lasting T-cell memory response. Here, LAMP was as a key molecule trafficking DNA vaccine-encoding target protein to the MHC II compartment.

Hantaan virus (HTNV), an old world Hantavirus, is mainly distributed in Asia. Haemorrhage fever with renal sydrome (HFRS) caused by HTNV is one of three Hantavirus-causing diseases (EN, HFRS and HPS). The fatality is among 3%-15%. In the past two years, sporatic cases of HPS obviously increased with a high fatality in the North America. There is no effective treatment to Hantavirus infection yet. Hence, vaccination prophylaxis is considered preferable solution against HV. Currently, inacitve HTNV vaccines are only applied in Asia, mainly China, Japan and South Korea. A continued reduction in HFRS was obseved while these vaccines distributed widely. However, short term of immunogenicity and low efficacy are disadvantages of inactive vaccine. development of a more effective vaccine of HTNV is of great need. Hantavirus glycoprotein N-terminal, named Gn, was reported that could induce neutralizing antibody production although with a low serum titer as natural infection. No study yet evaluates immune efficiency elicited by Gn as a vaccine. Therefore, we constructed DNA vaccine with HTNV Gn targeted to the MIIC by trafficking molecule LAMP.

In this study, we have investigated the cellular and humoral immune responses in ex vivo and immune protection in vivo of immunized mice by DNA vaccine encoding HTNV glycoprotein N-terminal (Gn). The cDNA encoded HTNV Gn was acquired by PCR from a full-length HTNV M segment. First of all, effective expression of the proteins encoded by the DNA plasmid constructs in transfected 293 cells was confirmed by fluorescence microscopy for proteins containing the enhanced green fluorescent protein (EGFP) tag, as well as by western blot. And then, Four groups of Balb/c mice were immunized with four different plasmids: ① pVAX1 vector with the Food and Drug Administration (FDA) document as a negative control. ② DNA encoding unmodified Gn as an endogenous antigen (pVAX-Gn); ③ DNA encoding Gn as a LAMP chimera targeted to the MIIC (pVAX-LAMP/Gn); ④ DNA encoding LAMP alone as a control (pVAX-LAMP) of pVAX-LAMP/Gn group. We tested specific antibody production in serum two weeks after each immunization by ELISA for humoral response evaluation. As a major indicator of induction of functional antibodies most likely conferring protective immunity, neutralizing activities against HTNV were evaluated by the cell microculture neutralization test. At the same time, we measured the peptide-specific IFN-γ secretion by ELISpot and specific CTL cytotoxicity against P815 cells which were loaded with Gn overlaping peptide-pool by LDH assay to evaluate cellular immune response. After Balb/c mice were immunized three times, animal protection experiment in vivo was also performed with Hantavirus challenging, and virus load in different tissues was detected by Sandwich ELISA and qRT-PCR.

The results demonstrated that the immune responses differed markedly. Twice immunizations with pVAX-LAMP/Gn were sufficient to generate strong antibody response (P/N>2.1 at dilution rate 1:1000). In contract, pVAX-Gn need to be administrated more than three (P/N>2.1 at dilution rate 1:100). No significant HTNV Gn-specific antibody was detected in mice vaccinated with pVAX-LAMP or pVAX. ELISpot analyses indicated that pVAX-LAMP/Gn elicited much greater IFN-γ response than pVAX-Gn. Whereas, pVAX-LAMP and pVAX induced very low levels, respectively. The cell microculture neutralization test suggested that immunization with pVAX-L-G could induce

neutralizing antibodies in mice against HTNV. The neutralizing titer of mice immunized with pVAX-LAMP/Gn was the highest among all the different groups (p<0.05). The neutralizing antibodies of mice immunized with pVAX-Gn could also be detectable. The sera from the pVAX or pVAX-LAMP control showed no neutralizing activity. For nonradioactive cytotoxicity assay, P815 cells were loaded with 83 overlaping peptides-pool of Gn as target cells. Among the experimental groups, splenocytes from mice immunized with the pVAX-LAMP/Gn showed higher specific cytotoxicity than that from mice immunized with the pPVAX-Gn at E/T ratios of 20:1, 10:1 and 5:1 (p<0.05). In contrast, no cytotoxicity against peptides loaded P815 cells in control mice immunized with pPVAX-LAMP or pVAX was detectable at each E/T ratios. Moreover, we investigated the protection efficacy in vivo after three times administration with naked DNA plasmids and carried out HTNV challenge. As no available animal model existed for HTNV challenge, which reflects the disease manifestations of severe HFRS, we made an observation of the Balb/c mice infected with HTNV 76-118 strain and established an evaluation system about animals infected with HTNV 76-118 before the animal protection experiment. We ran a set of programs (post-immunization viral intramuscular injection method) to infected the Balb/c mice with HTNV 76-118. Four groups of Balb/c mice were exposured to HTNV 76-118 at day 14 after third immunizations. And then, experimental mice were sacrificed and brain, heart, liver, spleen, lung and kidney were exteriorized after three days. Evaluation of viral load was performed by sandwich ELISA and qRT-PCR, and significant differences among the groups were observed. That is, the HTNV specific antigen could be detected in the brains and livers of the Balb/c mice immunized with pVAX-LAMP or pVAX, but undetected in that of all mice immunized with pVAX-LAMP/Gn or pVAX-Gn (p<0.05) .

In summary, HTNV Gn usually cannot induce a strong immune response. However, Gn showed a strong immunogenicity to elicit both humoral and cellular responses with LAMP as a chimera. And results of protection assay in vivo indicated that the immune response established was HTNV specific and protective. These findings not only demonstrate that the LAMP as a trafficking molecule can introduce Gn to MHCII presenting pathway and significantly enhance HTNV specific immune response, but also suggest that the pVAX-LAMP/Gn as a DNA vaccine has potential application on clinic for HFRS immunoprophylaxis.

Keywords: HTNV; Gn; LAMP; DNA vaccine; ELISpot; Cytotoxicity; Animal protection; HFRS

P4-19 A Retrospective Study on Continuous Renal Replacement Therapy versus Intermittent Hemodialysis in Critical Patients with Hemorrhagic Fever with Renal Syndrome

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Objective: The optimal pattern of blood purification therapy for critical patients with HFRS is still a pending problem until now. The aim of this study is to explore the efficacy of continuous renal replacement therapy (CRRT) versus intermittent hemodialysis (IHD) in critical patients with HFRS. Methods: A retrospective analysis was performed on different severity of patients with HFRS in our Center, between January 2008 and August 2012. All the patients treated with CRRT or IHD were divided into severe type and critical type. We compared the mortality between CRRT group and

IHD group, the mortality and dialysis rate of each type between CRRT and IHD separately. Furthermore, Univariate analysis and x^2 test were used to determine the potential influential factors between the two groups.

Results: 147 patients met the inclusion criteria, including 78 severe type and 69 critical type, and were treated with 65 (43.5%) CRRT and 82 (57.5%) IHD. 44(67.7%) patients in CRRT group survived while 79(96.3%) patients survived in IHD group ($P<0.001$). All the severe patients survived no matter using CRRT or IHD, while in critical patients the fatality was 18.8% in IHD and 39.6% in CRRT (P=0.124). The CRRT dialysis rate was 18.4% in severe patients and 76.8% in critical patients separately ($P < 0.001$). In addition, the critical patients had more organs dysfunctioned compared with the severe patients ($P<0.001$). Patients in CRRT group were more severe, as manifested by longer refractory shock($P < 0.001$), longer hospital day in survivors （P=0.002）, higher WBC ($P<0.001$)，higher HGB (P=0.043)，higher plasma lactic acid (P=0.030), lower ALB($P<0.001$) and higher mechanical ventilation rate ($P<0.001$) during the acute stage compared with the IHD group. Furthermore, other complications such as heart failure, ARDS, coagulopathy, central nervous system dysfunction and secondary infection in CRRT group were more frequent than the IHD group($P<0.05$).

Conclusions: CRRT may be a better choice and suitable to be used in the treatment of critical patients compared with IHD. CRRT is found to improve hemodynamic stability with a better fluid balance and control of biochemical status during the hypotensive phase and oliguria stage. These may contribute to going through the acute stage steadily and improving the survival rate, particularly for critical patients with HFRS.