BOOK OF ABSTRACTS

11TH INTERNATIONAL CONFERENCE ON HANTAVIRUSES

1-4 September 2019
Leuven, Belgium

KU LEUVEN

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Leuven, Belgium

ICH 2019, 11th International Conference on Hantaviruses, 1-4 September 2019, Leuven

is organized by the division of Clinical and Epidemiological Virology, Rega Institute, KU Leuven in collaboration with the KU Leuven Conferences and Event Office.

Book of Abstracts

Lay-out by Altera, Belgium.
Printed by Procopia, Belgium.

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ACKNOWLEDGEMENTS

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CONFERENCE VENUE

Irish College:
Janseniusstraat 1,
3000 Leuven

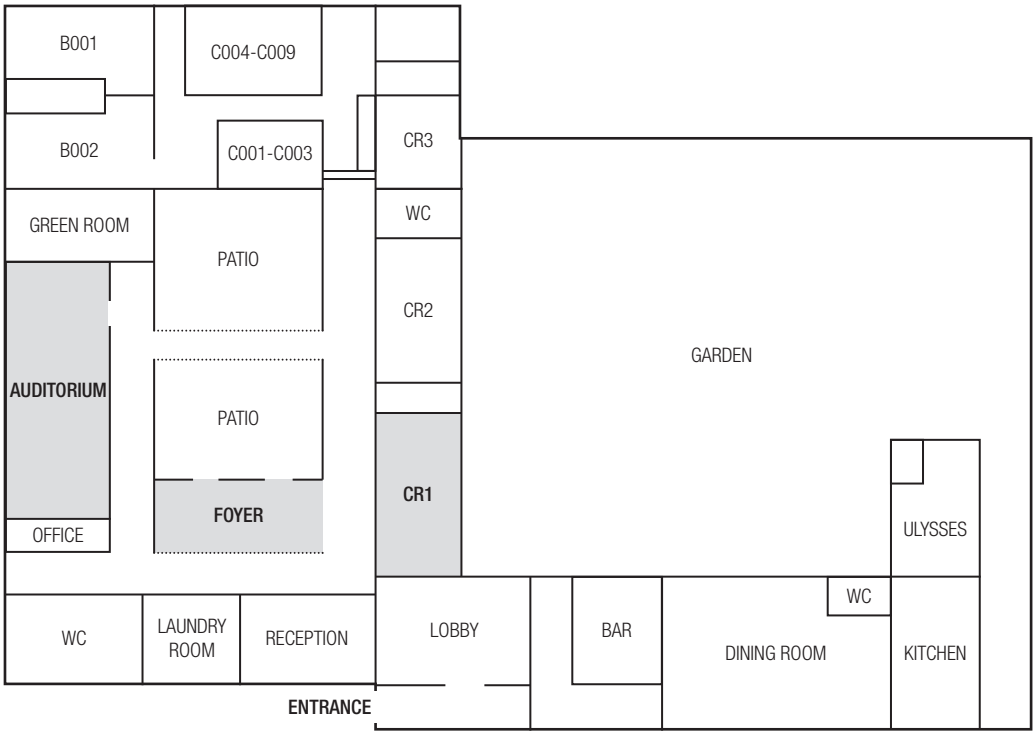
Irish College is located in the city center of Leuven on a 20 min walk from the main railway station.

Rooms:

Auditorium: conference sessions

CR1 and foyer: catering, poster and sponsor area

Floor plan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LAUNDRY ROOM
RECEPTION
LOBBY
BAR
DINING ROOM
KITCHEN
ENTRANCE

SCIENTIFIC PROGRAM

Sunday, 01/Sep/2019

04:00-05:30pm **Registration**

05:30-06:15pm **Ho Wang Lee Award Lecture**
Effect of hantaviruses on endothelial cells - Antti Vaheri.
Presentation of the awardee of the honorary Ho Wang Lee Award plaque.

06:15-07:00pm **J. Dalrymple Award Lecture**
Hantaviruses: Of mice, shrews, moles, bats and men - Boris Klempa.
Presentation of the awardee of the honorary J. Dalrymple Award plaque.

07:00-09:00pm **Welcome reception**
Location: Irish College

Monday, 02/Sep/2019

08:00-08:30am **Registration**

08:30-09:00am **Keynote 1: Welcome - Past President's Lecture**
Hantavirus vaccines: Promise and pragmatism - Connie S Schmaljohn

09:00-09:30am **Keynote 2**
Host factors that regulate virus infectivity - Jason Botten

09:30-10:00am **Ecology - Oral Talks - Part 1**
Session chairs: Herwig Leirs, co: Rainer Ulrich

09:30-09:45am Why hantavirus prevalence does not always increase with host density: modelling the role of host spatial behaviour and maternal antibodies - Herwig Leirs

09:45-10:00am Molecular phylogeny of mobatviruses (*Hantaviridae*) in Myanmar and Vietnam - Satoru Arai

10:00-10:25am **Coffee Break + Poster Viewing**

10:25-11:55am **Ecology - Oral Talks - Part 2**
Session chairs: Herwig Leirs, co: Rainer Ulrich

10:25-10:40am Development and characterization of a Sin Nombre virus transmission model in *Peromyscus maniculatus* - Bryce M Warner

10:40-10:55am Ecology and public health impact of African bat-borne hantaviruses - Sabrina Weiss

10:55-11:10am Emergence of hantavirus species as a consequence of host hybridization - Gerald Heckel

11:10-11:25am Population dynamics and Bayou virus prevalence in the marsh rice rat (*Oryzomys palustris*) following the 2010 Deepwater Horizon oil spill - Anna Perez-Umphrey

11:25-11:40am Seoul virus tropism and pathology in naturally infected feeder rats - Barry Rockx

11:40-11:55am Brno hantavirus - bat-borne hantavirus in the Czech Republic - Petra Strakova

11:55am-12:10pm **Ecology - Lightning Talks**
Session chairs: Herwig Leirs, co: Rainer Ulrich

11:55am-12:00pm	*Jemez Springs orthohantavirus* diversification in the *Sorex monticola* complex reflects host response to climatic cycling - Schuyler W. Liphard
12:00-12:05pm	Hantavirus ecology and evolution in Chile - Fernando Torrez-Perez
12:05-1:25pm	**Lunch Break + Poster Viewing**
01:25-01:55pm	**Keynote 3** The hantavirus surface: molecular insights into glycoprotein dynamics and cell entry - Nicole Tischler
01:55-02:55pm	**Pathogenisis and Immune Responses - Oral Talks - Part 1** Session chairs: Detlev Krüger, co: Alemka Markotic
01:55-02:10pm	Longitudinal transcriptome characterization of the immune response in patients infected with *Andes orthohantavirus* (ANDV) - Grazielle Esteves Ribeiro
02:10-02:25pm	Direct and indirect effects of hantaviruses on human B cells - Marina Garcia
02:25-02:40pm	Depletion of peripheral nonclassical monocytes during acute Puumala virus infection is associated with increased endothelial adhesion - Sindhu Vangeti
02:40-02:55pm	Acute *orthohantavirus* infection induces increase in serum free light chains - Jussi Matias Hepojoki
02:55-03:20pm	**Coffee Break + Poster Viewing**
03:20-04:35pm	**Pathogenisis and Immune Responses - Oral Talks - Part 2** Session chairs: Detlev Krüger, co: Alemka Markotic
03:20-03:35pm	RIG-I and MDA5 play essential and nonredundant roles in innate sensing and control of *Puumala orthohantavirus* infection - Daniel Bourquain
03:35-03:50pm	Functional consequence of atypical B cells in patients with acute hantavirus infection - Andy Dernstedt
03:50-04:05pm	Transcriptome analysis reveals modulations of immune and cell-cycle functions in monocytes of HFRS patients - Lidija Cvetko Krajinovic
04:05-04:20pm	Hantaviruses delay apoptosis in infected human endothelial cells by preventing mitochondrial membrane potential loss through up-regulation of the pro-survival factor BCL-2 - Carles Solà Riera
04:20-04:35pm	Changes in IGG glycosylation profile in HFRS patients infected with Puumala Virus - Alemka Markotic
04:35-05:00pm	**Pathogenisis and Immune Responses - Lightning Talks** Session chairs: Detlev Krüger, co: Alemka Markotic
04:35-04:40pm	Pulmonary endothelial glycocalyx degradation in *Orthohantavirus* infection - Clas Ahlm
04:40-04:45pm	Andes virus nucleocapsid protein binding to RhoGDI activates RhoA and directs endothelial cell permeability - Erich Mackow
04:45-04:50pm	Role of soluble mediators in the immune response in patients with hemorrhagic fever with renal syndrome - Misa Korva
04:50-04:55pm	Immunoregulatory responses as mediators of persistent hantavirus infections in reservoir hosts as determined with a novel reservoir host cell-isolated Puumala hantavirus - Tomas Strandin

04:55-05:00pm	*Orthohantavirus* infection triggers differentiation and subsequent polarization of human blood monocytes into macrophages - Petra Svoboda
05:00-05:05pm	**Short announcement of the Social Program** - Jan Clement/Piet Maes
06:00-07:00pm	**Guided Tour at the Beguinage with Carillon Concert** Location: Great Beguinage
07:30-09:30pm	**Belgian Beer Tasting Evening** Location: Irish College

Tuesday, 03/Sep/2019

08:30-09:00am	**Keynote 4: ISH President's Lecture** Proteinuria is an early, but rapidly evanescent diagnostic sign in probably all hantavirus infections - Jan Clement
09:00-09:30am	**Keynote 5** Immune response during human hantavirus infection: immunopathogenesis and keys to treatments - Jonas Klingström
09:30-10:00am	**Virus Phylogeny, Replication and Morphogenesis - Oral Talks - Part 1** Session chairs: Colleen Jonsson, co: Richard Yanagihara
09:30-09:45am	Cryo-EM analyses of New World orthohantaviruses - Amar Dhananjai
09:45-10:00am	Comprehensive distinct genotypes of *Seoul orthohantavirus* using multiplex PCR-based next-generation sequencing - Won-Keun Kim
10:00-10:25am	**Coffee Break + Poster Viewing**
10:25-11:55am	**Virus Phylogeny, Replication and Morphogenesis - Oral Talks - Part 2** Session chairs: Detlev Krüger, co: Alemka Markotic
10:25-10:40am	Puumala virus S-segment sequence evolution in the bank vole and functional analysis of NSs protein - Florian Binder
10:40-10:55am	Sequence analysis of L-based screening PCR product allows for molecular typing of Puumala virus - Joerg Hofmann
10:55-11:10am	*In vitro* study on interactions of pathogenic and non-pathogenic orthohantaviruses with cellular factors of human host and rodent reservoir - Giulia Gallo
11:10-11:25am	Tigray virus is a rodent-borne hantavirus which might have emerged from ancient reassortment events involving a shrew-born hantavirus - Boris Klempa
11:25-11:40am	Characterization of the field vole associated hantavirus Tatenale Virus (TATV) - Lorraine M. McElhinney
11:40-11:55am	The unconventional hantavirus square surface glycoprotein lattice resolved at high resolution by allied X-ray crystallography and electron cryo-tomography - FA Rey
11:55am-12:10pm	**Virus Phylogeny, Replication and Morphogenesis - Lightning Talks - Part 1** Session chairs: Colleen Jonsson, co: Richard Yanagihara
11:55am-12:00pm	Delineating the interplay of host factors in New World hantavirus entry into endothelial cells - Maria Eugenia Dieterle

12:00-12:05pm	Insights into Andes virus mRNA synthesis - Yaiza Fernandez-Garcia
12:05-12:10pm	Identifying the patterns and drivers of Puumala hantavirus enzootic dynamics using reservoir sampling - Lies Laenen
12:10-01:25pm	**Lunch Break + Poster Viewing**
01:25-01:40pm	**Virus Phylogeny, Replication and Morphogenesis - Lightning Talks - Part 2** Session chairs: Colleen Jonsson, co: Richard Yanagihara
01:25-01:30pm	Complete genome sequences from hantavirus pulmonary syndrome cases involved in person-to-person transmission, southwestern Argentina, 2014 - Valeria Martínez
01:30-01:35pm	Phylogeography of American rodent-borne hantaviruses - Renata Carvalho de Oliveira
01:35-01:40pm	Imprint of negative selection evidenced on hantaviral S genetic segment - Maja Stanojevic
01:40-02:10pm	**Keynote 6** Understanding hantavirus cardiopulmonary syndrome (HCPS) - Cecilia Vial
02:10-02:55pm	**Epidemiology - Oral Talks - Part 1** Session chairs: Anna Papa, co: Dexin Li
02:10-02:25pm	Molecular epidemiology of hantavirus disease in Germany - Detlev H. Kruger
02:25-02:40pm	Hantavirus surveillance of peri-domestic rodent species in Great Britain - Ellen G Murphy
02:40-02:55pm	A major outbreak of hantavirus pulmonary syndrome caused by person-to-person transmission of Andes virus in Epuyén, Southwestern Argentina - Valeria Paula Martinez
02:55-03:20pm	**Coffee Break + Poster Viewing**
03:20-04:35pm	**Epidemiology - Oral Talks - Part 2** Session chairs: Anna Papa, co: Dexin Li
03:20-03:35pm	*Seoul orthohantavirus* in captive rat populations in the Netherlands - Miriam Maas
03:35-03:50pm	Insectivore-borne hantaviruses in Asian Russia - Liudmila Yashina
03:50-04:05pm	Association of hantavirus infections and leptospirosis with the occurrence of chronic kidney disease of uncertain etiology (CKDu) among patients in a high prevalent and a low prevalent area of the North Central province of Sri Lanka - N. P. Sunil-Chandra
04:05-04:20pm	Hemorrhagic fever with renal syndrome: Current status in Russia - Evgeniy Alexander Tkachenko
04:20-04:35pm	Comparative study of Puumala virus infection in French endemic and peri-endemic areas - Sarah Madrières
04:35-05:00pm	**Epidemiology - Lightning Talks** Session chairs: Anna Papa, co: Dexin Li
04:35-04:40pm	A human Dobrava-Belgrade virus infection in France - Abbas Deeb
04:40-04:45pm	Andes hantavirus mother to child transmission and role of breastfeeding: epidemiological and virological data - Marcela Ferres

04:45-04:50pm	A small-scale epidemiologic survey and phylogenetic analysis of rodent- and shrew-borne Orthohantaviruses in Western Poland - Seung-Ho Lee
04:50-04:55pm	Castelo dos Sonhos hantavirus: an unnoted etiological agent of HPS in Brazilian Amazon - Jorlan Fernandes
04:55-05:00pm	Molecular detection of *Puumala orthohantavirus*: struggling with high nucleotide sequence variability - Rainer Ulrich
05:00-05:05pm	**Short announcement of the Social Program** – Piet Maes/Jan Clement
06:00-07:00pm	**Reception at the Historical Town Hall of Leuven** Location: Historical Leuven Town Hall
07:30-11:00pm	**Conference Dinner** Location: Faculty Club

Wednesday, 04/Sep/2019

09:00-09:30am	**Keynote 7** Genetic diversity, geographic distribution and evolutionary history of bat-borne Loanviruses and Mobatviruses - Richard Yanagihara
09:30-10:00am	**Keynote 8** Unraveling and inhibiting the cell entry mechanisms of hantaviruses - Kartik Chandran
10:00-10:25am	**Coffee Break + Poster Viewing**
10:25am-12:10pm	**Vaccines, Therapeutics and Prevention - Oral Talks** Session chairs: Connie Schmaljohn, co: Mifang Liang
10:25-10:40am	Macropinocytosis contributes to hantavirus entry into human airway epithelial cells - Giulia Torriani
10:40-10:55am	Anti-Hantaan virus human IgG produced in transchromosomic bovines has potent neutralizing activity and is protective in animal models - Jay W Hooper
10:55-11:10am	Recombinant human monoclonal antibody therapy against Andes hantavirus infection - Maria Ines Barria
11:10-11:25am	Vesicular stomatitis virus-based vaccines provide cross-protection against Andes and Sin Nombre virus - Derek R Stein
11:25-11:40am	Monoclonal antibodies against the Andes virus surface glycoprotein GnGc - Danny Noack
11:40-11:55am	*In vitro* enzymatic analysis reveals small molecule inhibitors of the hantavirus endonuclease - Erich Mackow
11:55am-12:10pm	Neutralizing antibodies against hantaviruses derived from human survivors - Eva Mittler
12:10-01:30pm	**Lunch Break + Poster Viewing**
01:30-01:55pm	**Vaccines, Therapeutics and Prevention - Lightning Talks** Session chairs: Connie Schmaljohn, co: Mifang Liang

01:30-01:35pm	TIM-1 contributes to entry of HTNV in human airway epithelial cells - Jennifer Mayor
01:35-01:40pm	Alpaca polyclonal IgG antibodies demonstrate protection against lethal Andes virus hamster infections - Patrycja Magdalena Sroga
01:40-01:45pm	Evaluation of adjuvants efficiency in the HFRS vaccine - Svetlana Kurashova
01:45-01:50pm	Pre-clinical studies of inactivated combined HFRS vaccine - Tamara Dzagurova
01:50-01:55pm	Chloroquine, an anti-malaria drug as effective prevention for hantavirus infections - Valentijn Vergote
01:55-02:55pm	**Clinical Aspects and Diagnosis - Oral Talks - Part 1** Session chairs: Jan Clement, co: Clas Ahlm
01:55-02:10pm	Sequential assessment of clinical and laboratory parameters in patients with hemorrhagic fever with renal syndrome - Emil Pal
02:10-02:25pm	A comprehensive and comparative study of SISPA-, RNA-access, and multiplex PCR-based next-generation sequencing for *Hantaan orthohantavirus* in *Apodemus agrarius* lung tissues - Jin Sun No
02:25-02:40pm	Positive urine glucose predicts the overall severity of acute Puumala hantavirus infection - Jukka Mustonen
02:40-02:55pm	Development and validation of a European orthohantavirus microneutralization test - Tabitha Hoornweg
02:55-03:20pm	**Coffee Break + Poster Viewing**
03:20-04:05pm	**Clinical Aspects and Diagnosis - Oral Talks - Part 2** Session chairs: Jan Clement, co: Clas Ahlm
03:20-03:35pm	Increased secretion of urinary kidney injury molecule-1 in patients with hemorrhagic fever with renal syndrome caused by Puumala virus - Ivan-Christian Kurolt
03:35-03:50pm	Fatal case of Puumala virus infection and attempt to treatment with icatibant - Johan Rasmuson
03:50-04:05pm	Hantavirus or leptospirosis? A case report from the United Kingdom - Martin Edward Winstanley
04:05-04:35pm	**Clinical Aspects and Diagnosis - Lightning Talks** Session chairs: Jan Clement, co: Clas Ahlm
04:05-04:10pm	Hantavirus infections and Crimean-Congo hemorrhagic fever in Bulgaria - Iva Christova
04:10-04:15pm	Development of a pan-hantavirus enrichment protocol for next generation sequencing - Shannon LM Whitmer
04:15-04:20pm	Molecular diagnosis of hemorrhagic fever with renal syndrome - Misa Korva
04:20-04:25pm	Case report: severe Seoul hantavirus infection during peripartum period - Galina Kompanets
04:25-04:30pm	Next-generation approaches for full-length sequencing *Prospect Hill orthohantavirus* - Mariah Katherine Taylor
04:30-04:50pm	**Wrap-Up and Closing Remarks**

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ABSTRACTS

Awards

- **Ho Wang Lee Award Lecture: Effect of hantaviruses on endothelial cells**

Antti Vaheri

Department of Virology, Medicum, University of Helsinki

Endothelial cells and monocyte/macrophages are generally considered to be the principal target cells of hantaviruses. We have shown that in monocyte/macrophages interferon can induce tissue plasminogen activator (tPA) and in human endothelial cells when PUUV-infected we have seen interferon-mediated induction of tPA. PAI-1, the primary inhibitor of tPA was not induced. tPA, in the concurrent absence of neutralizing PAI-1, correlated with hemorrhages in PUUV-HFRS. Analogously, the highly elevated levels of PAI-1 could also explain the absence of hemorrhages in severe HCPS, which is in striking contrast to severe HFRS cases. Vascular leakage of endothelial cells (increased capillary permeability), not cell lysis or apoptosis, is another key element in the pathobiology of hantavirus disease. The capillary leakage seems to be largely mediated by bradykinin (BK), a potent vasodilator, generated locally by endothelial cells from HMW kininogen by the kallikrein-kinin proteolytic system. BK receptor 2 can be blocked by icatibant and has been successfully used in severe cases of PUUV infection with capillary leakage and shock. Moreover, we have described fatal PUUV cases with capillary leakage, fibrinolysis and complement activation. Interestingly, it has been reported that (i) the soluble terminal complement activation product (SC5b-9) can increase the permeability of endothelial cells through release of BK and the effect is reduced by icatibant and that (ii) BK stimulates dose-dependently release of tPA from human endothelium. Thrombocytopenia and altered coagulation are key elements in the pathobiology of all hantavirus disease, both HFRS and HCPS. The mechanism of thrombocytopenia remains elusive but we expect that altered platelet-endothelial cell interaction is involved. We have detected and studied a number of clinical and laboratory parameters such as creatinine, leukocytosis, CRP, von Willebrand factor antigen, fibrinogen, decreased fibronectin, regulatory T-cell (Treg) response and indoleamine 2,3-dioxygenase (IDO) activity, circulating cell-free DNA, histones and neutrophil elastase (presumably from neutrophil extracellular traps, NETs), complement activation and the recently found biomarkers: resistin (an adipocytokine), YKL-40 glycoprotein, Tie1, and galectin-3-binding protein (90K/Mac-2 binding glycoprotein). Albuminuria, hematuria and glucosuria predict the severity of acute kidney injury (AKI). The severity of thrombocytopenia we found to be associated with biomarkers reflecting degree of inflammation (such as IL-6, TNF-alpha, pentraxin-3, soluble urokinase-type plasminogen activator receptor, suPAR) and variables reflecting capillary leakage, but not with the severity of AKI in PUUV disease. Interestingly, however, glucosuria predicts thrombocytopenia.

- **J. Dalrymple Award Lecture: Hantaviruses: Of mice, shrews, moles, bats and men**

Boris Klempa[1,2]

1: Biomedical Research Center of the Slovak Academy of Sciences, Institute of Virology, Bratislava, Slovak Republic; 2: Institute of Virology, Charité, Universitätsmedizin Berlin, Germany

The story of hantaviruses has started 43 years ago when Hantaan virus was isolated. Perhaps, it is no coincidence that I was born in 1976, too. I feel very privileged that I could contribute to the story of hantaviruses. This privilege was provided to me by Detlev Krüger who gave me the opportunity to study and later work in his laboratory at the Charité Medical School in Berlin, Germany, by all the colleagues, especially the heart and soul of the laboratory, Brita Auste, and by the international partners who made our studies possible.

First, we focused on the diversity of Dobrava-Belgrade virus (DOBV) as the most virulent hantavirus in Europe. We showed that the virus is rather exceptionally hosted by several species of the *Apodemus* mice and forms host-specific lineages which we nowadays recognize as DOBV genotypes. We also generated several virus isolates including reassortants, characterized their receptor usage and innate immunity modulation, and, probably most important, showed that the genotypes induce disease of varying severity.

By a combination of good luck and perseverance, we were then able to identify and isolate the first autochthonous African hantavirus, associated with *Hylomyscus simus* mice trapped in Guinea. The prerequisite for this discovery was the development of the pan-hanta PCR assay. This assay turned out to be an instrumental tool not only for the identification of the second African hantavirus, the shrew-borne Tanganya virus but also for our following discoveries such as the bat-borne Magboi and Makokou viruses or the mole-borne Wandlitz (Bruges) virus. It is also widely used by the colleagues worldwide and one can conclude that the assay played a substantial role in uncovering the parallel universe of the non-rodent-borne hantaviruses. Phylogenetic analyses of these, for decades overlooked, viruses indicate that not rodents but members of the mammalian superorder Laurasiatheria (such as shrews, moles, and bats) were the hosts of the ancestral mammalian hantaviruses. Moreover, recent progress in the next-generation sequencing technologies helping to recover full genome sequences generates accumulating evidence that ancient reassortment events contributed to the currently recognized hantavirus diversity.

Although several important chapters have been added during the last decades, it is obvious that the story of hantaviruses is not over. Hantaviruses remain to be a serious health threat for humans, leading to fatal diseases. It is therefore highly desired that new chapters will be added especially in the fields of pathogenesis, prophylaxis, and treatment of hantavirus diseases.

Keynote Talks

- **Welcome - Past President's Lecture - Hantavirus vaccines: Promise and pragmatism**

Connie S Schmaljohn, Jay W. Hooper

US Army Medical Research Institute of Infectious Diseases, Frederick, MD, United States of America

We developed and clinically tested DNA vaccines for HFRS by expressing the Gn and Gc genes of Hantaan virus (HTNV) and Puumala virus (PUUV). Our initial Phase 1 clinical study demonstrated that gene gun delivery of the vaccines to skin resulted in strong but inconsistent immune responses in humans. In a second Phase 1 study we showed that intramuscular electroporation (IM-EP) delivery of the vaccines elicited robust humoral immune responses to both viruses, but that when mixed, there was interference between the two vaccines. After solving the interference issue by optimizing the gene sequences of the DNA vaccines, we conducted a Phase 2a clinical study in 120 subjects in which we compared two doses and two schedules of the mixed vaccines delivered by IM-EP. The results of those studies showed that more than 80% of recipients developed neutralizing antibodies against HTNV or PUUV and more than 70% developed neutralizing antibodies against both hantaviruses. Although these results clearly demonstrated the promise of the vaccines, we have continued to search for a more pragmatic delivery method. Toward this goal, we are currently comparing IM-EP and intradermal-EP delivery in a Phase 1 study and have recently completed another Phase 1 study using the needle-free PharmaJet Stratis® disposable syringe jet injection device to deliver the DNA vaccines intramuscularly. This device is cleared by the US FDA and several other countries for commercial use. Further, it requires no electricity, is needle-free, and has been used to deliver conventional vaccines to more than 100 thousand of people worldwide. The Stratis® Phase 1 trial consisted of three groups of nine subjects, who received the individual or mixed vaccines three times four weeks apart. We measured neutralizing antibody responses at several time points by a plaque reduction neutralization test (PRNT50) and by a pseudovirion neutralization assay (PsVNA50). Overall 25 of 27 subjects seroconverted and all of those individuals were still positive through at least Day 252. All (100%) of subjects in the HTNV or PUUV groups who received all three vaccinations seroconverted. Day 196 geometric mean titers (GMTs) for HTNV by PRNT/PsVNA were 1560/1946 and for PUUV were 537/641, indicating good correlation of the assays. The seroconversion rates and GMT of subjects receiving the combined vs the individual DNAs were generally lower, especially for the anti-HTNV response, but the seroconversion rates were still ≥78%. These encouraging results have led us to plan another Phase 2 study using the PharmaJet Stratis® delivery device. Our results demonstrate both the promise and pragmatism of DNA vaccines for HFRS.

- **Host factors that regulate virus infectivity**

Joseph Klaus[1,4], Philip Eisenhauer[1], Joanne Russo[1], Anne Mason[1], Danh Do[1], Benjamin King[1,5], Douglas Taatjes[1], Cromwell Cornillez-Ty[2], Jonathan Boyson[1], Lujian Lao[2], John Yates III[2], Bin Zhang[3], Bryan Ballif[1], Jason Botten[1]

1: University of Vermont, Burlington, VT, United States of America; 2: The Scripps Research Institute, La Jolla, CA, United States of America; 3: Cleveland Clinic, Cleveland, OH, United States of America; 4: Celgene, San Diego, CA, United States of America; 5: New York University School of Medicine, New York, NY, United States of America

Arenaviruses and New World hantaviruses cause severe and often fatal diseases in humans. Effective antivirals or FDA-approved vaccines do not exist for these pathogens and little is known regarding host proteins required for their propagation. To address this deficiency, we have focused on the discovery of key virus-host interactions that can be targeted for the development of therapeutics and vaccines. In the current study, we identified human proteins that interact with the glycoproteins (GPs) of a prototypic arenavirus and hantavirus. We discovered that the human ER-Golgi intermediate compartment 53 kDa protein (ERGIC-53), a mannose-specific lectin that functions as an intracellular cargo receptor, associates with envelope GPs encoded by pathogenic hantaviruses, arenaviruses, coronaviruses, and orthomyxoviruses, and is incorporated into virions. Importantly, ERGIC-53 is critical for the propagation of arenaviruses, coronaviruses, and filoviruses in a GP-specific manner; in the absence of ERGIC-53, although structurally intact viral particles containing the viral genome and structural proteins form, they are no longer infectious because ERGIC-53 is required for their ability to attach to host cells. We have identified a minimal domain of ERGIC-53 that is required for virion infectivity as well as a second host protein that (i) binds to this critical region of ERGIC-53 and (ii) inhibits ERGIC-53's ability to confer infectivity to viral particles. Further, we have evidence that extracellular, virion-associated-ERGIC-53 can be targeted to neutralize virion infectivity. Notably, ERGIC-53 is not essential for humans and thus may be an ideal broad-spectrum antiviral target.

- **The hantavirus surface: molecular insights into glycoprotein dynamics and cell entry**

Eduardo Bignon[1], Amelina Albornoz[1], Pablo Guardado-Calvo[2], Félix Rey[2], Nicole Tischler[1]

1: Fundación Ciencia & Vida, Chile; 2: Structural Virology Unit, Virology Department, Institut Pasteur, CNRS UMR 3569, Paris, France

The hantavirus envelope glycoproteins Gn and Gc mediate virion assembly and cell entry, with Gc inducing membrane fusion at low pH after viral uptake into endosomes. Recent X-ray structures revealed that hantavirus Gn is homologous to alphavirus E2 which belongs to positive-sense, single-stranded RNA viruses, while Gc is folded as a class II viral fusion protein, presenting specific features different from arbovirus class II fusion proteins. Although the overall arrangement of Gn and Gc on the hantavirus spikes are known, their detailed interactions are not.

Here we present data showing that the lateral contacts between spikes are mediated by the same 2-fold contacts observed in Gc crystals at neutral pH, allowing the engineering of disulfide bonds to cross-link spikes. Disrupting the observed dimer interface affects particle assembly and overall spike stability. We further show that the spikes display a temperature-dependent dynamic behavior at neutral pH, alternating between “open” and “closed” forms. We show that the open form exposes the Gc fusion loops but is off-pathway for productive Gc-induced membrane fusion and cell entry. We also present mechanistic insights of Gc residues that favor spike disassembly and that drive membrane insertion and pore opening as well as residues promoting the stable, trimeric post-fusion structure.

Together, the structure-function insights into the dynamic hantavirus surface are crucial for the design of optimized Gn/Gc immunogens to elicit protective immune responses.

Funding: CONICYT grants FONDECYT 1181799 and AFB 170004, Infect-ERA IMI European network, program “HantaHunt”, “Integrative Biology of Emerging Infectious Diseases” Labex (Laboratoire d’Excellence) grant ANR-10-LABX-62-IBEID.

- **ISH President's Lecture – Proteinuria is an early, but rapidly evanescent diagnostic sign in probably all hantavirus infections**

Jan Clement

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Endothelial hyperpermeability is often described as the primordial mechanism for transient “capillary leak”, causing acute symptoms in both HFRS and HCPS. However, in all affected organs, including the kidney, this hyperpermeability involves in fact 3 successive layers, all functioning normally as leaking barriers: endothelium, basement membrane, and epithelium. Concomitant breaching of all 3 necessitates an intense “inter-talk”, of which hitherto little is known. Moreover, the renal capillary tuft is covered with an unique epithelial layer, consisting of podocytes, having a key role in the renal filtration process.

Podocytes are highly specialized renal cells with long foot processes (FP), forming a tightly interdigitating epithelial capsule. The narrow FP contact points or “slit diaphragms”, are presumably the place where most of the glomerular filtration rate (GFR) takes place. The “door” in this “filtration gate” is nephrin, synthesized by podocytes, and required for normal GFR functioning. For downmodulating nephrin, soluble urokinase-type plasminogen activator receptor (suPAR) needs interaction with αvβ3integrin, which in turn is needed for cell entry by pathogenic hantaviruses. All known experiments to diminish podocytal nephrin, e.g. infusion of suPAR, result in proteinuria, a cardinal symptom of “podocyte injury”(PI), and recognized in electron microscopy (EM) as “foot process effacement” (FPE). PI and FPE are in various (mostly emerging) infections part of acute kidney injury (AKI), a renal lesion consisting of 3 +/- concomitant symptoms: 1) ↓GFR, 2) proteinuria, and 3) microhematuria. AKI is too often considered as synonymous to ↓GFR, but in mild (not only hantaviral) infections, AKI can consist of only transient proteinuria and/or microhematuria.

In hantaviral AKI, all 3 symptoms are rapidly self-remitting, mostly within 2 weeks, without sequels, and without remaining arterial hypertension (AHT). In contrast, progressive chronic glomerulonephritis (CGN) is marked by podocyte loss, and measured by degrees of podocyturia. In CGN, proteinuria antedates podocyturia, and FPE can exceed 80% of the capillary tuft in EM. Since podocytes cannot regenerate, this is an irreversible process, ultimately leading to end-stage renal failure (ESRF). In hantaviral PI, proteinuria, but without podocyturia, likewise antedates FPE, mostly limited however to < 20%, and considered a reversible process. Consequently, each HFRS case presenting after AKI with both protracted proteinuria, ↓GFR, and AHT, should be examined for another, often pre-existent renal affection, and ESRF could hitherto never be ascribed convincingly to prior HFRS.

To date, very few studies of SNV-induced HCPS contained data documenting proteinuria and/or microhematuria. However, by far the most frequent HCPS-form is ANDV-induced, which shows, when examined, unmistakable signs of concomitant AKI, often with its 3 components, prompting even acute hemodialysis in 10% of Argentinian hantaviral “pulmonary syndromes”. In a recent NYV-induced HCPS case, full-blown AKI was likewise present (Fernando EID 2019;25:1241-3). Pet rat-transmitted SEOV-HFRS has recently (2017) been discovered in North-America, and one wild rat-transmitted Texan case was even fatal, due to a HCPS-like complication (Roig CID 2012; 54: 91-4).

In all these instances, presumptive diagnosis of a hantaviral infection can preliminarily be confirmed by demonstrating thrombocytopenia and both proteinuria and microhematuria, by simple, but preferentially daily urine dipstick examination.

- **Immune response during human hantavirus infection: immunopathogenesis and keys to treatments**

Jonas Klingström

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Two related hyperinflammatory syndromes are distinguished following hantavirus-infection of humans; hemorrhagic fever with renal syndrome (HFRS), mainly in Eurasia, and hantavirus pulmonary syndrome (HPS) in the Americas. Fatality rates can be high; up to 10% for HFRS and around 35-40% for HPS. Novel treatment strategies are clearly needed for the most severe cases of hantavirus infection: as of now, no specific curative treatment or FDA-approved preventive vaccine exist. Hantaviruses infect endothelial cells, but also epithelial cells, mononuclear phagocytes (MNP), follicular dendritic cells (DC) and likely other types of cells. While infection with hantaviruses is not cytopathic per se, hantaviruses manipulate several cellular functions, including apoptosis and innate immune responses, thereby affecting both infected cells and, indirectly, also uninfected bystander cells including immune cells. Human hantavirus infection leads to exceptionally strong immune responses, which include strong inflammatory responses displayed for example by increased levels of pro-inflammatory cytokines and vigorous natural killer (NK) cell, CD8 T cell, and B cell responses. Recent studies have revealed more detailed insights into the generation of innate and adaptive cell-mediated immune responses following clinical infection with PUUV and other hantaviruses. While the exact mechanisms behind HFRS/HPS pathogenesis are poorly understood, the direct effects of hantaviruses on infected cells together with subsequent indirect effects on immune cells may together trigger a “perfect storm” leading to hyper-inflammation and increased risk for fatal outcome. An interesting and important question is the role of deregulated immune responses/immunopathogenesis in fatal outcome, and what specific factors/mechanisms that are linked to severe/fatal outcome. Equally important is to better understand what parts of the immune responses that lower the risk for severe/fatal outcome, thereby protecting the infected patient. Recent observations, indicating an important role for hantavirus-mediated immune responses in driving immunopathology, may provide new insights into HFRS and HPS disease pathogenesis indicating novel possible treatment strategies. Based on similarities between inflammatory responses in severe hantavirus infections and other hyperinflammatory disease syndromes, it can be speculated that some therapeutic interventions that have been successful in the latter conditions, including aggressive immunochemotherapy and anti-IL-6R treatment, may also be applicable in severe hantavirus infections. Further, direct antiviral treatment and passive immunization, alone or in combination with HLH-2004 and anti-IL-6R treatment, will also be discussed.

- **Understanding hantavirus cardiopulmonary syndrome (HCPS)**

Eduardo Duran[1], Flavio Carrión[1], Analia Cuiza[1], Marcela Ferres[2], Jonas Klingstrom[3], Mario Calvo[4,5], Perez Jose Luis[4], Rioseco Maria Luisa[7], Raul Riquelme[7], Jerónimo Graf[8], Pablo Vial[1,8], Leonila Ferreira[6], Cecilia Vial[1]

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In Chile, ANDV infection is considered an important public health problem affecting young, previously healthy people with a very high case fatality rate. In humans, hantavirus infection occurs following exposure to the animal reservoir and causes hantavirus cardiopulmonary syndrome (HCPS). Regarding pathogenesis of HCPS, host-related immune mechanisms rather than direct viral cytopathology are postulated to be responsible for the principal manifestations of the disease, along with infection of endothelium which can disrupt tight junctions among them to cause leakage. A major role for T cell-directed immune responses in development of the cardiopulmonary stage is suggested, especially CD8+ T cells along with Natural Killer cells (NK), which also seem to have an important role in hantavirus infection.

To understand the pathophysiology of this disease, we have started to perform a detailed clinical, immunological and molecular characterization of infected ANDV patients, since hospital admission up to 2 months later. In this matter we have analyzed peripheral blood mononuclear cells subpopulation, activation and memory by flow cytometry. The study included nine healthy subjects as controls and 14 ANDV infected patients, which were sampled on days 1, 3, 5, and 60 post-hospitalization. We saw an increase on B cells, TCD8 cells and a contraction on NK cells during the acute phase. CD4 and CD8 cells are highly activated and Regarding memory the majority are effector cells. To further understand disease pathophysiology and the host response to the virus we did a Genome Wide Association Study to analyze genetic variation between patients with mild and severe clinical course of the disease. We found genetic differences between these two groups in genes participating in innate immune response and genes from the cadherin family.

- **Genetic diversity, geographic distribution and evolutionary history of bat-borne Loanviruses and Mobatviruses**

Richard Yanagihara[1], Satoru Arai[2]

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The recent discovery that shrews and moles of multiple species (order Eulipotyphla, families Soricidae and Talpidae) from Europe, Asia, Africa and/or North America harbor genetically distinct viruses belonging to the family *Hantaviridae* (order *Bunyavirales*) has prompted a further exploration of their host diversification. In analyzing more than 3,000 frozen, RNAlater®-preserved and ethanol-fixed tissues from approximately 100 bat species (order Chiroptera), representing 11 families (Emballonuridae, Hipposideridae, Megadermatidae, Miniopteridae, Molossidae, Mormoopidae, Nycteridae, Phylostomidae Pteropodidae, Rhinopholidae, and Vespertilionidae) by reverse transcription polymerase chain reaction (RT-PCR), multi-national teams of mammalogists and virologists have identified five mobatviruses and five loanviruses to date in bat species belonging to the suborder Yinpterochiroptera (families Hipposideridae, Pteropodidae and Rhinolophidae) and the suborder Yangochiroptera (families Emballonuriade, Nycteridae and Vespertilionidae). Of these, four mobatviruses are from Asia (Đakrông virus in Aselliscus stoliczkanus from Vietnam; Láibīn virus in *Taphozous melanopogon* from China and Myanmar; Xuân Sơn virus in *Hipposideros pomona* and *Hipposideros cineraceus* from Vietnam, China and Myanmar; and Quezon virus in *Rousettus amplexicaudatus* from the Philippines) and one mobatvirus is from Africa (Makokou virus in *Hipposideros ruber* from Gabon). Of the five loanviruses, two are from Asia (Huángpí virus in *Pipistrellus abramus* and Lóngquán virus in *Rhinolophus affinis*, *Rhinolophus monoceros* and *Rhinolophus sinicus* from China), two from Africa (Magboi virus in *Nycteris hispida* from Sierra Leone; and Mouyassué virus in *Neoromicia nanus* from Côte d'Ivoire and in *Neoromicia capensis* from Ethiopia), and one from Europe (Brno virus in *Nyctalus noctula* from the Czech Republic). Phylogenetic analyses, based on partial and full-length S-, M- and L-genomic sequences, using maximum-likelihood and Bayesian methods, indicate that all bat-borne loanviruses and mobatviruses share a common ancestry, which is consistent with their host phylogeny. The basal position of mobatviruses suggests that bats, rather than rodents, may have served as the primordial mammalian hosts of ancestral hantaviruses. Phylogenetic trees, reconstructed for co-phylogeny mapping, using consensus topologies based on amino acid sequences of the nucleocapsid protein, Gn and Gc glycoproteins and RNA-dependent RNA-polymerase, exhibit congruent segregation of viruses within the family Hantaviridae, according to the subfamily of their reservoir hosts, with no evidence of host switching, except for three orthohantaviruses and one mobatvirus harbored by moles. Molecular identification of many more bat-borne viruses of the family Hantaviridae is expected. However, thus far, none of these newfound viruses has been isolated in cell culture and it is unclear if they cause infection or disease in humans. Future investigations must focus on myriad unanswered questions about the genetic diversity and geographic distribution, as well as the pathogenic potential, of bat-borne loanviruses and mobatviruses.

- **Unraveling and inhibiting the cell entry mechanisms of hantaviruses**

Kartik Chandran

Albert Einstein College Of Medicine, United States of America

The mechanisms by which hantaviruses enter cells are poorly understood, and few entry-related determinants of hantavirus virulence, host range, and tissue tropism have been identified. Here, I will discuss our ongoing work to elucidate host-virus interactions crucial for hantavirus entry using genetic, biochemical, and cell-biological approaches. Most significantly, we recently identified a cadherin superfamily member, protocadherin-1 (PCDH1), as a critical entry factor for New World hantaviruses and a key determinant of Andes virus virulence in the lethal Syrian hamster model. I will present our characterization of the molecular mechanism by which hantavirus Gn/Gc engages PCDH1, the cell-biological roles of this interaction, and its specific functions vis-a-vís other known and putative hantavirus entry factors. I will also present findings suggesting that Gn/Gc:PCDH1 interactions can influence viral host range. Finally, I will discuss our work to develop passive anti-hantavirus immunotherapeutics that target the Gn/Gc entry machine and its interactions with PCDH1.

Oral Presentations

ECOLOGY - ORAL TALKS

- **Why hantavirus prevalence does not always increase with host density: modelling the role of host spatial behaviour and maternal antibodies**

Jonas Reijniers[1], Katrien Tersago[2], Benny Borremans[3], Nienke Hartemink[4], Liina Voutilainen[5], Heikki Henttonen[6], Herwig Leirs[1]

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For zoonotic diseases, it is often assumed that the force of infection on humans increases with host density, yet the field data supporting this are often absent. In Europe, the most widespread hantavirus is Puumala hantavirus (PUUV), which is carried by the bank vole and causes Nephropathia Epidemica (NE) in humans. Extensive field campaigns have been carried out in Central Finland to shed light on the supposed relationship between bank vole density and PUUV prevalence and to identify other drivers for the infection dynamics. This resulted in the surprising observation that the relationship between bank vole density and PUUV prevalence is not purely monotonic on an annual basis, contrary to what previous models predicted: a higher vole density does not necessarily result in a higher infection prevalence, nor in an increased incidence of human NE cases.

Here, we propose a novel individual-based spatially-explicit model of the infection in the host population which 1° takes into account the immunity provided by maternal antibodies and 2° simulates the spatial behaviour of the voles, both processes that were not accounted for in previous models. We show that the reduced prevalence in peak years can be attributed to transient immunity; the density-dependent spatial vole behaviour plays only a minor role.

The potential applications of the model are not limited to the prediction of PUUV prevalence and NE incidence in Europe, as it can be easily adapted to model other rodent-borne diseases, either with indirect or direct transmission.

- **Molecular phylogeny of mobatviruses (*Hantaviridae*) in Myanmar and Vietnam**

Satoru Arai[1], Fuka Kikuchi[1,2], Keita Aoki[1,3], Saw Bawm[4], Nguyễn Trường Sơn[5,6], Kyaw San Lin[7], Vương Tân Tú[5,6], Gohta Kinoshita[8], Dai Fukui[9], Hoàng Trung Thành[10], Kimiyuki Tsuchiya (deceased)[11], Keiko Tanaka-Taya[1], Yasuhiro Yoshikawa[12], Shigeru Morikawa[1,12], Kazunori Oishi[1], Richard Yanagihara[13]

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Context: Discovery of highly divergent lineages of viruses belonging to the family Hantaviridae in shrews, moles and bats of multiple species raises the possibility that non-rodent hosts may have played a significant role in the evolutionary history of hantaviruses. To investigate this prospect, a multi-national collaborative study was undertaken.

Methods: Total RNA, extracted from RNAlater®-preserved lung tissues of 498 bats, representing six families (Emballonuridae, Hipposideridae, Megadermatidae, Pteropodidae, Rhinolophidae and Vespertilionidae), 21 genera and 76 species, captured in Myanmar and Vietnam during 2012–2016, was analyzed for hantavirus RNA by reverse transcription polymerase chain reaction.

Results: Hantavirus RNAs were detected in two of 15 black-bearded tomb bats (*Taphozous melanopogon*) and in two of 26 Pomona roundleaf bats (*Hipposideros pomona*) in the Sagaing Region and Nay Pyi Taw Union Territory of Myanmar, and in one of two Stoliczka's Asian trident bats (*Aselliscus stoliczkanus*) in Quảng Trị province and in three of 12 ashy leaf-nosed bats (*Hipposideros cineraceus*) in Quảng Trị, Phú Thọ, and Vĩnh Phúc provinces of Vietnam. Pairwise alignment and comparison of the full-length S-, M- and L-genomic segments revealed three genetically distinct mobatviruses: Đakrông virus in *Aselliscus stoliczkanus*, Láibīn virus in *Taphozous melanopogon*, and Xuân Sơn virus in *Hipposideros pomona* and *Hipposideros cineraceus*. Phylogenetic analyses, generated by maximum likelihood and Bayesian methods, showed that mobatviruses shared a common ancestry, and their basal position suggested that ancestral bats might have served as the early mammalian hosts of primordial hantaviruses. In addition, phylogeographic analysis showed clustering of Láibīn virus strains from China and Myanmar, but not of Xuân Sơn virus strains from China and Vietnam.

Discussion: These findings confirm that the black-bearded tomb bat is the natural reservoir of Láibīn virus and that at least two species of Hipposideros bats can host Xuân Sơn virus. Similar examples of host sharing exist for two loanviruses: Lóngquán virus in Rhinolophus affinis, Rhinolophus monoceros and Rhinolophus sinicus from China; and Mouyassué virus in Neoromicia nanus from Côte d'Ivoire and in Neoromicia capensis from Ethiopia. Future studies, particularly of bats in the family Hipposideridae distributed across Africa, Eurasia and Australia, are expected to unveil many more mobatviruses.

- **Development and characterization of a Sin Nombre virus transmission model in *Peromyscus maniculatus***

Bryce M Warner[1], Derek R Stein[2], Bryan D Griffin[2], Kevin Tierney[2], Anders Leung[2], Angela Sloan[2], Darwyn Kobasa[1,2], Guillaume Poliquin[2], Gary P Kobinger[3,4], David Safronetz[1,2]

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Context: In North America, Sin Nombre virus (SNV) is the main cause of hantavirus cardiopulmonary syndrome (HCPS), a severe respiratory disease with a fatality rate of 35–40%. SNV is a zoonotic pathogen carried by deer mice (*Peromyscus maniculatus*), and few studies have been performed examining its transmission in deer mouse populations. Previous studies examining SNV transmission either have shown very low transmission rates, or did not seek to determine the main mechanisms of transmission.

Objectives: We sought to determine the main mechanism(s) of SNV transmission between deer mice. We also wanted to establish an experimental model of SNV transmission to further examine factors that contribute to SNV replication, shedding, and transmission in deer mouse populations.

Methods: We housed SNV-infected deer mice with naïve, uninfected deer mice and monitored the number of transmission events through seroconversion or the presence of SNV RNA. Alternatively, we housed uninfected deer mice in cages that had previously housed SNV-infected deer mice and were left uncleaned and unchanged to determine if exposure to contaminated caging leads to SNV infection.

Results: We examined the transmission of SNV and found that direct contact between deer mice is the main driver of SNV transmission rather than exposure to contaminated excreta/secreta, thought to be the main mechanism of transmission of the virus to humans. Interestingly, exposure to contaminated caging did not result in infection of uninfected deer mice, highlighting the likely importance of direct contact for SNV transmission. Furthermore, we have used this approach to show that increases in heat shock responses, thermogenesis, or testosterone levels in SNV-infected deer mice do not increase the replication, shedding, or rate of transmission.

Conclusion: Here we describe a reliable model of direct experimental SNV transmission in deer mice, the natural rodent reservoir for the virus. This model has also been valuable in assessing the protective efficacy of vaccine platforms in protecting deer mice from contracting SNV. The use of this consistent experimental transmission system will have important implications for further examining SNV transmission and in developing strategies for the prevention of SNV infection in deer mouse populations.

- **Ecology and public health impact of African bat-borne hantaviruses**

Sabrina Weiss[1], Leonce Kouadio[2,3], Sophie Köndgen[1], Lwitiho E. Sudi[4], Chacha Mangu[4], Boris Klempa[1,5], Fabian H. Leendertz[2], Detlev H. Kruger[1], Sebastien Calvignac-Spencer[2]

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Most hantavirus research has understandably focused on the group of rodent-borne hantaviruses which contains all currently recognized human pathogens of the family *Hantaviridae*. Consequently, ecology and evolution of these viruses are relatively well understood. Only in recent years bats have been increasingly recognised as hantavirus reservoir hosts. However, most of these viruses were identified in one or few individuals in single studies, leaving open the questions of stable bat/hantavirus associations and their possible impact on public health.

Aiming at answering these questions, we trapped bats in different regions in Côte d'Ivoire (West Africa) and Tanzania (East Africa) and screened organs by PCR for the presence of hantaviruses. Additionally, serum samples from humans living in the same regions were collected and tested for IgG antibodies against antigens derived from rodent- and shrew-borne hantavirus species representing three phylogroups. Moreover, using hybrid in-solution capture and next-generation sequencing, we generated the complete nucleocapsid protein coding sequence of one bat-associated virus from Côte d'Ivoire, which allowed for expression of recombinant protein for use in serological studies.

Molecular investigations of bat organs revealed infection of different bat species with distinct hantaviruses. We show that two closely related pipistelle bat species (*Neoromicia nanus* and *N. tenuipinnis*) in Côte d'Ivoire were enzootically infected with two distinct hantaviruses (Mouyassue and Ponan virus) and that a 20% virus prevalence in these animals was stable across five years. In Tanzania, six Angolan free-tailed bats (*Mops condylurus*) were infected with a yet unknown hantavirus, tentatively named Kiwira virus. All infected animals were captured within or in immediate vicinity of human settlements and viral sequences were detected in all tested organs. We further show that human sera from people living in the same areas showed hantavirus-specific IgG antibodies.

We provide evidence that different bat species adapted to human habitats are reservoir hosts of hantaviruses and that humans are exposed to these animals and their viruses.

- **Emergence of hantavirus species as a consequence of host hybridization**

Gerald Heckel

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The number of recognized hantavirus taxa has increased markedly over the last decade but it remains typically unknown how functionally divergent species arise. This study focuses on the relevance of host ecology and evolution for evolutionary divergence in hantaviruses and their establishment as functionally divergent species. Tula hantavirus (TULV) has a very large distribution range in Europe and Asia that is substructured into multiple deep phylogenetic clades. Some of these clades appear geographically associated with the range of parapatric evolutionary lineages in the common vole (*Microtus arvalis*), the reservoir host of TULV in Europe. Our fine-scale genetic analyses of a natural hybrid zone between two common vole lineages revealed an extremely tight spatial association between host and TULV taxa in the open landscape. Remarkably, hybridization and local gene flow between common vole lineages was abundant but there was no evidence of mixing, reassortment or recombination between major TULV clades at ecologically relevant spatial scales consistent with an advanced stage of functional divergence. Significant shifts between geographic clines indicate that very few dominant alleles in the genome of the host constrain the distribution of TULV clades. Parapatric TULV genomes differed by up to 22% with a single consistent signal of positive selection in the N-terminal region of the viral envelope glycoprotein gene. Importantly, the deeper phylogenomic relationships among TULV clades strongly suggest that the divergence process commenced only after the common vole lineages established secondary contact in the hybrid zone. Thus the mixing of pre-diverged host genomes likely provided the “ecological” substrate for adaptive divergence in TULV that has resulted in functional isolation currently exceeding the state of speciation in the rodent host.

- **Population dynamics and Bayou virus prevalence in the marsh rice rat (*Oryzomys palustris*) following the 2010 Deepwater Horizon oil spill**

Anna Perez-Umphrey[1], Colleen Jonsson[2], Philip Stouffer[1], Sabrina Taylor[1]

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In 2010, the Deepwater Horizon oil rig blew out, severely oiling coastal saltmarshes of the U.S. Gulf of Mexico. This included habitat occupied by the marsh rice rat (Oryzomys palustris; hereafter, rice rat), a semi-aquatic rodent and endemic host of Bayou virus (BAYV), which produces hantavirus pulmonary syndrome in humans. Oil exposure and subsequent habitat disturbance have had well-documented impacts on wildlife, including both short- and long-term population dynamics, fitness, and immunosuppressive effects. This presents a unique opportunity to investigate how a significant ecological disturbance can influence the population dynamics of a host species, and whether there is a relationship to hantavirus prevalence. Using mark-recapture data from 2013-2017, we estimated abundance and apparent survival at oiled (n=4) and unoiled (n=3) sites in coastal saltmarshes of Louisiana. Blood sera was collected from a subset of rice rats (n=192) sacrificed at the end of each field season and screened for anti-BAYV antibodies using immunofluorescent assays to determine seroprevalence. Site-level rice rat density (27.0 ± 2.5 rice rats/ha) varied among years but not between oiled and unoiled treatments. Apparent survival was treatment-dependent in the best-supported Cormack-Jolly-Seber model in Program MARK. Survival was lower for all rice rats on oiled sites ($X^2=6.65$, $p=0.01$) and also lower for males ($X^2=15.07$, $p<0.01$) on both treatment types. Anti-BAYV antibodies were detected in rice rats at 2 of 4 oiled sites, and 1 of 3 unoiled sites, however seroprevalence did not differ between treatment types ($X^2<0.01$, $p=0.97$). Males were significantly more likely to be infected at both oiled and unoiled sites ($X^2=4.73$, $p=0.03$). While seroprevalence did not differ between oiling treatments, reduced male survival at oiled sites merits further exploration. Future screening of blood samples collected from live rice rats throughout the field season will help elucidate any relationship between oiling, survival, and seroprevalence. Anticipated sequencing results from the rice rat immunome will allow us to determine population genetic structure, immune gene diversity, and identify any genetic variants associated with serostatus.

- **Seoul virus tropism and pathology in naturally infected feeder rats**

Barry Rockx

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Seoul virus (SEOV) is an emerging zoonotic virus that can cause haemorrhagic fever with renal syndrome (HFRS) in humans, but results in a persistent but asymptomatic infection in rats. SEOV is associated with rats as a reservoir and is found worldwide. Human cases of SEOV virus infection have most recently been reported in the USA, United Kingdom, France and the Netherlands and were primarily associated with contact with pet rats and feeder rats. Little is known about the cell tropism of SEOV in its reservoir and most available data stems from experimental infection studies in which rats were inoculated via a route which does not recapitulate virus transmission in nature. The objective here is to identify SEOV cell tropism in key target organs following natural infection of a cohort of feeder rats associated with a human case of SEOV infection in the Netherlands.

The rats in this study were obtained from a feeder rat breeding farm and had been housed in open boxes. All adult rats tested positive for orthohantavirus antibodies. A selection of 20 seropositive rats was tested for the presence of SEOV RNA by rRT-PCR, in different biological samples. Results of the rRT-PCR show that the lung is the preferred organ for the detection of SEOV in rats: all animals that showed positive rRT-PCR results, had positive results for the lungs. The lungs and conductive airways including tracheas showed no evident inflammation nor SEOV-IHC positive epithelial cells. Most tissues showed extensive granular cytoplasmic positive IHC staining of especially interstitial endothelial cells of microvasculature, and seldom of endothelial cells lining larger blood vessels. Interestingly, a consistent finding in the liver was mild hypercellularity within the hepatic sinusoids mostly comprised of polymorphonuclear leukocytes (PMN). The number of PMNs present within the hepatic parenchyma and sinusoids were significantly higher in SEOV infected animals compared to non-infected. In conclusion, these data show that persistent SEOV infection of the liver in rats results in mild inflammation.

- **Brno hantavirus - bat-borne hantavirus in the Czech Republic**

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Here we present a continuous study on bat-borne hantaviruses in the Czech Republic. Since the first detection of bat-borne hantavirus in Europe, named *Brno hantavirus* (currently proposed as Brno loanvirus species in ICTV), in two animals of common noctule bats (*Nyctalus noctula*) collected in 2012 and 2013 in the Czech Republic (Strakova et al. 2017); more than 200 bats of 15 species, collected in 2013-2018 mostly from South Moravia in the Czech Republic, were investigated. The sample collection contained bats that died from natural causes or by accident. They were stored in -80 °C till dissection. Kidney and liver samples were used for RNA isolation and were screened by nested RT-PCR targeting small conserved region of L segment (Klempa et al. 2006). Hantavirus RNA was found in one noctule bat collected again in Brno city (sharing similarity 93-97% with already published sequences of Brno hantavirus n.7/2012 and n.11/2013, respectively), and surprisingly in one lesser horseshoe bat (Rhinolophus hipposideros) from cave located in Moravian Karst (sharing similarity 92-99% with n.7/2012 and n.11/2013, respectively). Moravian Karst is a protected nature reserve to the north of Brno. So far, we confirm the presence of bat-borne hantavirus in noctule and horsehoe bats. Presence of Brno hantavirus in new bat species could indicate a spill-over event (when working with hypothesis one virus - one host in rodent-borne hantaviruses). However, both species have fundamentally different roosting, foraging and even migration strategies. Therefore, we would need more data about prevalence of Brno hantavirus in local bat species, about its pathogenesis and ecology.

PATHOGENISIS AND IMMUNE RESPONSES - ORAL TALKS

• Longitudinal transcriptome characterization of the immune response in patients infected with *Andes orthohantavirus* (ANDV)

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Hantaviruses are important human pathogens that cause a severe zoonotic disease called hantavirus cardiopulmonary syndrome (HCPS). HCPS presents a fatality rate of ~30% in Chile. Clinical manifestations may present as a mild condition with moderate respiratory failure or progress quickly to a severe condition with shock that can be fatal. This progression towards HCPS is a complex multifactorial process that involves mechanisms directly induced by the virus or by the host response. However, the host response factors associated with the progression towards HCPS are still unknown. Our goal is to better understand the role of the host's immune responses in this progression towards HCPS, by longitudinally characterizing the transcriptional profile of the peripheral blood mononucleated cells (PBMCs) isolated from ANDV-infected patients. Total RNA was extracted from PBMCs isolated from peripheral blood of healthy controls and patients infected with ANDV between days 1-6 post onset of cardiopulmonary symptoms, during the acute phase of the disease, and on day 60, during the convalescent phase. Isolated RNA was sequenced at the Broad Institute (Boston, USA) using Illumina's HiSeq platform (50M reads aligned in pairs). We analyzed 12 patients and 9 healthy controls. Transcriptome analyses were performed with Multiqc, STAR, featureCounts, lima-voom, WebGestalt y tmod. Differential expression was evaluated by comparing the samples from day 0; 1-2; 3-4; 5-6 and 60 versus the healthy controls. The number of differentially expressed (DE) genes was 1072, on day 0, 1123 on day 1-2, 939 on day 3-4, 954 on day 4-5. There were no DE genes at day 60. These genes are associated with activation of immune response and control of apoptosis. Blood Transcriptional Modules (BTMs) associated with immune response were over-represented on different days in severe patients. Among the BTMs that were only over-represented on day 0, we found (1) viral sensing and immunity and (2) plasma cells and immunoglobulins. These results suggest that genes associated with the immune response is important in the pathogenesis. Future directions of this study include the validation of candidate genes to help us understand the mechanisms involved in the pathogenesis of HCPS.

- **Direct and indirect effects of hantaviruses on human B cells**

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In previous studies we observed a massive polyclonal activation of circulating B cells in Hantavirus Pulmonary Syndrome (HPS) patients and an increased risk for B cell lymphoma development in individuals that had suffered Hemorrhagic Fever with Renal Syndrome (HFRS). Thus, we hypothesized that B cells could be targets for hantavirus infection, as well as become activated by the strong inflammatory response characteristic of human hantavirus diseases.

To test these hypotheses, a human B cell line (BJAB) and subsequently purified primary B cells (pBC) from healthy blood donors were exposed to HTNV, ANDV and PUUV strains at a MOI of 1. Sampling was done until 96 hpi. To asses for infection, cells were processed for western blot and immunofluorescence detection of the nucleocapsid protein. To measure viral replication qRT-PCR was performed on RNA extracted from the cells. Supernatants were used to assess productive infection by measuring titers of progeny viruses. In addition, the activation state of B cells was measured by flow cytometry. Finally, to assess the indirect effects of hantaviruses, B cells were co-cultured with HTNV-infected endothelial cells (HUVEC and HMVEC-L) for up to 72h and activation markers were analyzed on B cells by flow cytometry at different time points.

We found that BJAB and pBC are susceptible to both HPS and HFRS causing hantaviruses, with productive infection clearly seen in BJAB. Furthermore, pBC showed increased levels of activation and proliferation markers when co-cultured directly with infected endothelial cells (HTNV-EC) or with supernatants from HTNV-EC, but not when directly exposed to the virus, suggesting that activation is mediated by soluble factors secreted by HTNV-EC. Interestingly, the atypical double negative B cells (CD27-IgD-), whose function is still not well understood, showed the highest increase of Ki-67 and BAFF-R (markers of proliferation and maturation).

In conclusion, these results indicate that human B cells constitute a newly described target for hantavirus infection as well as show an activating effect of HTNV-EC on B cells mediated by soluble secreted factors. Further studies will be of interest to define whether these cells could be reservoirs of hantaviruses and if this is involved in hantavirus-caused pathogenesis.

- **Depletion of peripheral nonclassical monocytes during acute Puumala virus infection is associated with increased endothelial adhesion**

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Context: Hantaviruses infect vascular endothelium without cytopathic effects yet induce increased vascular permeability. Previously, we showed that monocytes and dendritic cells (DCs) are significantly reduced in blood during acute Puumala virus (PUUV) infection in patients with hemorrhagic fever with renal syndrome (HFRS) while these cells simultaneously accumulate in the airways. Monocytes and DCs are innate immune cells critical in inflammation, initiation of adaptive effector immune responses and possibly contribute to the immunopathogenesis of HFRS.

Objectives: Here, we set out to decipher the mechanism behind the reduction of circulating monocytes and DCs during acute HFRS and also to understand the functional consequences of this on the immune response.

Methods: We determined (i) distribution of monocyte and DC subsets; (ii) maturation (CD86, HLA-DR) and (iii) adhesion (CD62L) marker expression; (iv) chemokine receptor expression (CCR2/4/6/7), and (v) functional responses to TLR7/8 stimulation in longitudinal peripheral blood samples from a cohort of 23 Finnish PUUV infected patients using multicolor flow cytometry. Patient plasma cytokines were measured using Luminex. In vitro, blood monocyte adhesion to vascular endothelium infected with PUUV propagated in bank vole cell lines was studied using confocal microscopy. Monocyte endothelial adhesion marker expression was assessed using Western blot.

Results: In line with our published findings, monocyte and DC subsets were significantly reduced in circulation also in the Finnish patients. Especially CD16+ nonclassical monocytes that patrol vasculature, aiding or controlling inflammation in a disease-specific context, were depleted from blood during the early stages of disease; normalizing over time to similar frequencies as observed in healthy individuals. Additionally, we observed an expansion of hematopoietic progenitors during acute HFRS. Monocytes from acute HFRS patients expressed higher levels of the endothelial adhesion marker CD62L as compared to cells from healthy controls, and produced IL-6 and TNFα in response to TLR stimulation. In vitro adhesion assays showed that monocytes are differentially activated by direct contact with PUUV-infected endothelial cells and culture supernatants respectively.

Conclusion: Our findings suggest that during acute HFRS, monocytes and DCs display increased endothelial attachment, and contribute to systemic inflammation. Activated monocytes may play an inflammatory role in causing endothelial dysfunction and vascular leakage.

- **Acute *Orthohantavirus* infection induces increase in serum free light chains**

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Hemorrhagic fever with renal syndrome (HFRS), caused by some of the Eurasian orthohantaviruses, is characterized by acute kidney injury (AKI) with accompanying proteinuria. However, the underlying mechanisms are largely unknown. We recently observed that immunoglobulin free light chains (FLCs) specific to orthohantavirus nucleocapsid protein can be detected from urine. The finding led us to study whether acute orthohantavirus infection would increase the level of FLCs in blood. Since FLCs possess nephrotoxic properties, we hypothesized that overproduction of FLCs might contribute to the AKI in HFRS. By studying serum samples of healthy donors, patients with non-specified acute infection, and patients with acute Puumala virus (PUUV) infection, we observed that only PUUV infected patients (ca. 50%) had FLC concentrations above the normal range. We then studied a panel of archival serum/plasma samples of patients hospitalized due to acute PUUV infection and observed increased FLC concentration in ca. 70% of the patients. To study the potential mechanisms behind FLC overproduction, we inoculated B cell enriched peripheral blood mononuclear cell (PBMC) fractions of healthy donors with PUUV and could demonstrate polyclonal activation resulting in production of IgM, IgA, IgG and FLCs. By applying flow cytometry to study PBMC fractions of patients hospitalized due to PUUV infection, we observed increased amounts of plasmablasts in samples collected during the acute phase. Furthermore, we could demonstrate that a subset of the plasmablasts were positive for viral antigen suggesting that PUUV infects a certain population of B cells. Taken together our finding suggest that orthohantavirus infection induces activation of B cells, possibly due to active replication, which leads to overproduction of FLC. Since overproduction of FLCs is connected to varying forms of kidney disease, we suggest the AKI in HFRS to be – at least partially – attributed to FLCs. It is plausible that abnormally high FLCs levels in serum contribute to the pathogenesis of not only HFRS but also of hantavirus cardiopulmonary syndrome.

- **RIG-I and MDA5 play essential and nonredundant roles in innate sensing and control of *Puumala orthohantavirus* infection**

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Hantaviruses are negative-sense RNA viruses which cause zoonotic diseases in humans. Virus recognition and response by the innate immune system are critical components of host defense against hantavirus infection. In order to replicate successfully in human cells, hantaviruses need to prevent early interferon (IFN) induction. RNA viruses trigger IFN upon non-self recognition by RIG-I-like receptors (RLRs), including RIG-I and melanoma differentiation antigen 5 (MDA5). Prior studies have shown the importance of RIG-I in hantavirus mediated induction of innate antiviral responses. We evaluated the individual functions of RIG-I and MDA5 in pathogen recognition and control of Puumala virus (PUUV) infection. Therefore, we analyzed IFN induction and PUUV replication in A549 cells deficient in either RIG-I, MDA5 or the central adapter molecule for RLR signaling MAVS.

We could show that PUUV infection triggers a strong IFN response starting from 48h post infection. This IFN response was completely abolished by the loss of MAVS, suggesting that innate sensing of PUUV is dependent on cytosolic RLRs in these cells. Consistent with the lack of an IFN response, PUUV replication was strongly enhanced in the A549 ΔMAVS cells in comparison to wildtype A549 cells. In contrast to the loss of MAVS, knockout of RIG-I alone only led to a delay of approx. 24h in IFN induction following PUUV infection. However, at 72h p.i. a strong IFN response could be detected in the PUUV infected A549 ΔRIG-I cells. In the cells lacking MDA5 alone, a similar but less pronounced delay of IFN induction could be observed.

We conclude that innate immune sensing of PUUV infection occurs predominantly via the cytosolic RLR-MAVS signaling pathway. Lack of RIG-I or MDA5 alone results in decreased innate immune signaling and virus control in these cells, while a knockout of the downstream signaling adapter MAVS completely abolishes the IFN response towards PUUV infection. Thus, RIG-I and MDA5 are both essential pattern recognition receptors which sense distinct dsRNA molecules that accumulate during PUUV infection. Although PUUV is able to delay the IFN response to allow successful replication, late IFN-induction via the RLR pathway still hampers viral replication.

- **Functional consequence of atypical B cells in patients with acute hantavirus infection**

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Circulating B cells in healthy individuals comprise a small fraction of CD27-IgD- atypical B cells (ABCs). These cells accumulate in patients with Systemic Lupus Erythematosus (SLE) with nephritis. In vitro studies imply that ABCs are dysfunctional or exhausted but their in vivo biological function remains poorly understood. Hantavirus infections that cause hemorrhagic fever with renal syndrome (HFRS) lead to transient kidney dysfunction in patients, as shown by increased serum creatinine levels. We hypothesized that (i) development of ABCs is associated with reduced kidney function, and (ii) that studies of HFRS could be used to assess if accumulation of ABCs is detrimental to the development of antiviral humoral immunity. Moreover, we assessed a potential mechanism for shedding of CD27.

Using longitudinal HFRS-patient blood samples stratified based on the median creatinine level, we show by flow cytometry that ABCs preferentially accumulate in circulation of patients with high serum creatinine levels. Phenotypical analysis showed that HFRS-induced ABCs had lower expression of activation markers and showed reduced capacity for antigen presentation to T cells. Moreover, we found the same expression pattern of ABC associated surface markers identified for SLE and other chronic infections, also in ABCs in acute HFRS. In addition, these cells had decreased expression of the complement regulatory protein CD55.

Since extracellular ATP can cleave membrane bound CD27 in mice, we hypothesized that also human CD27 could be cleaved. We here demonstrated that addition of extracellular ATP is effectively cleaving CD27 from the cell surface, and that the shedding is blocked when co-incubating with a specific MMP-8 inhibitor. Further, levels of soluble CD27 correlates both with kidney dysfunction and levels of ATP breakdown products in patient plasma. Finally, we found that increased kidney dysfunction in patients was correlated to longitudinal development of Gn/Gc-targetting neutralizing antibodies.

Collectively, this study demonstrates an association between reduced kidney function and accumulation of ABCs in circulation. Moreover, our data shed light on the potential impact that accumulation of circulating ABCs may result in a productive antiviral response in HFRS-patients.

- **Transcriptome analysis reveals modulations of immune and cell-cycle functions in monocytes of HFRS patients**

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In addition to endothelial cells, orthohantaviruses successfully infiltrate some cells of the immune system such as monocytes/macrophages and dendritic cells. Circulating monocytes generally play an important role in the recognition of virus infection and host innate immune response to viral infection. They may act inflammatory or anti-inflammatory depending on the influence of various factors from the microenvironment, including the type of virus infection. On the other hand, they potentially may contribute to the dissemination of the virus in the body and development of the disease. Literature data on the interaction of ortohantaviruses and innate immune cells during the infection are very scarce. Our previous studies have shown regulatory changes at the expression level of genes involved in the early immune response of peripheral blood mononuclear cells of patients with hemorrhagic fever with renal syndrome (HFRS). The objective of this study was to assess the impact of orthohantavirus infection on host innate immune target cells – human monocytes.

The study enrolled 15 HFRS patients infected with Puumala virus (PUUV) and 10 healthy controls. Primary blood monocytes were depleted from freshly isolated PBMCs during the early acute phase of HFRS. We performed mRNA deep sequencing (RNA-Seq) in order to analyze transcriptomic expression profiles in blood monocytes of HFRS patients. Differential expression was analyzed using DESeq package and DEGs were ranked by log2 fold change and corresponding p-values. The enrichment analysis was also conducted to illustrate altered monocyte cell functions.

A total of 5356 genes in monocytes during the acute HFRS were determined as significantly up-/down-regulated and defined as differentially expressed genes compared to healthy controls. Pathway enrichment analysis revealed that immune- and cell cycle-related functions were most relevant biological processes affected by the orthohantavirus infection in monocytes. The most significant over-represented pathways were MHC class I mediated antigen processing and presentation as well as interferon type I signaling. The study reveals that many functional alterations are happening in monocytes during their interaction with orthohantaviruses in peripheral circulation during the early phase of HFRS.

- **Hantaviruses delay apoptosis in infected human endothelial cells by preventing mitochondrial membrane potential loss through up-regulation of the pro-survival factor BCL-2**

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Hantavirus are zoonotic RNA viruses belonging to the order Bunyavirales. Human infection with rodent-borne hantavirus can cause hemorrhagic fever with renal syndrome (HFRS) or hantavirus pulmonary syndrome (HPS), two lethal diseases for which currently there is neither specific treatment nor an FDA-approved vaccine. Hantavirus cause no symptoms in their natural hosts, which remain persistently infected and act as long-term virus reservoirs. Interestingly, hantavirus-infected patients show strong cytotoxic lymphocyte responses and hyper-inflammation, nevertheless infected endothelial cells remain mostly intact. Hantavirus have been reported to circumvent the apoptotic machinery of a cell through the inhibition of granzyme B activity and, to certain extent, that of caspase-3. Here we show that upon chemical induction of apoptosis, the cleavage of procaspase-8, -9 and -3 into their active form is hampered in infected cells. Hantavirus delay mitochondrial membrane potential loss and the release of cytochrome C from mitochondria upon staurosporine-mediated induction of apoptosis, consequently hampering the downstream signaling events leading to apoptosis execution. Moreover, we report that hantavirus induce the pro-survival factor BCL-2 in infected cells, while the expression of other anti- and pro-apoptotic BCL-2 family members remained unchanged. Silencing of BCL-2 by siRNA and direct inhibition of BCL-2 with the compound ABT-737, both resulted in apoptosis induction in STS-exposed hantavirus-infected cells. Overall, here we report a specific mechanism by which hantavirus infection protects endothelial cells from intrinsic apoptosis at the mitochondrial level by means of up-regulating the expression of the pro-survival factor BCL-2.

• Changes in IGG glycosylation profile in HFRS patients infected with Puumala virus

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Hemorrhagic fever with renal syndrome (HFRS) is caused by orthohantaviruses (HTV), which belonging to genus *Orthohantavirus. Puumala orthohantavirus* (PUUV) is a pathogenic HTV, which predominantly cause mild to moderate HFRS in Croatia and in the rest of Europe. Inflammation is one of the hallmarks of the HFRS immunopathogenesis. The aim of our study is that we analyze possible changes in the glycosylation profile of IgG in HFRS patients during the course of disease, in order to better understand its potential influence on inflammation and other immunopathogenetic mechanisms. It is the first such study conducted in HFRS patients.

Study was conducted in adult patients with confirmed HFRS, hospitalized at the University Hospital for Infectious Diseases, Zagreb, Croatia. Two sera samples (on admission-point 1 and dismission-point 2) from 68 HFRS patients, were tested by the Hydrophilic Interaction Liquid Chromatography-Ultra High Performance Liquid Chromatography (HILIC-UHPLC) to determine the glycosylation profile of IgG in the serum of all subjects. The results were compared with the glycosylation profile of 17 controls, which were matched by age and sex. We used a linear mixed model to analyze N-glycoprotein changes in time. Samples were grouped and compared at the time of admission and dismission and in comparison to controls. P values were corrected for multiple assays using the Benjamini-Hochberg method.

We found that the level of glycans without galactose (G0) and with one galactose (G1) is significantly higher and the level of glycans with two galactose (G2) and the level of glycans with sialic acid (S) is lower in point 2 than in point 1. In addition, at point 1 we detected less core fucosylated glycans (F) and more glycans with bisecting GlcNAc (B) than in point 2.

Our findings indicate that changes in the glycosylation profile of IgG, in sera of HFRS patients, may have influence on dynamic of inflammatory process during the course of disease and on antibody-dependent cell-mediated cytotoxicity (ADCC). Further analysis of clinical and other laboratory parameters and potential correlation with glycosylation changes are in process.

VIRUS PHYLOGENY, REPLICATION AND MORPHOGENESIS - ORAL TALKS

• Cryo-EM analyses of New World orthohantaviruses

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Scientific investigations of biosafety level 3 (BSL-3) agents require special containment facilities and highly trained staff. The risks posed by BSL-3 virus handling restrict their investigations, especially in structural biology as large quantities of the pathogens are required for high resolution studies. Currently cryo-electron microscopy and tomography (cryo-EM and ET) have been used to obtain 3D maps of viruses up to 3-4 ˚ A resolution. However, cryo-EM inside a BSL-3 facility incur very high costs and require an extensive and cumbersome protocol for decontamination. To bridge this techique gap, we modified the cryo-EM specific chemical crosslinking described in the Grafix method and optimized a protocol to inactivate viruses that can retain structural integrity and remain amenable to structural studies. The prototype study was carried out on the vaccine strain of Venezuelan Equine Encephalitis virus (VEEV TC-83). The cryo-EM map of the inactivated virus was obtained at a resolution of 7.5 Å, which was the highest resolution reported for a glutaraldehyde fixed virus. The same approach was used in BSL-3 conditions to obtain inactivated samples of New World Orthohantaviruses. While a round morphology may be predominant in Old World viruses, a variety of morphologies were observed in New World viruses with Black Creek Canal virus having a predominantly tubular morphology. Cryo-tomography studies were performed on the purified and inactivated hantaviruses with a target resolution of 20 Å of the virus glycoprotein spike complex by sub-volume averaging. The spikes of BCCV and ANDV were found to be similar to the 4-fold spikes observer in Old World viruses. It was observed that the RNP complex forms bends and kinks within the virus. Patches of naked membrane were observed on all Hantaviruses which may indicate that complete coating of viral surface with viral glycoproteins is not absolutely essential for assembly. The protocol was replicated successfully in multiple labs, on multiple viruses, at multiple times which indicate its robustness and flexibility. This could make many other hitherto challenging BSL-3/4 viruses amenable to cryo-EM analyses.

- **Comprehensive distinct genotypes of *Seoul orthohantavirus* using multiplex PCR-based next-generation sequencing**

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Context: *Seoul orthohantavirus* (SEOV) poses a critical and worldwide public health threat. SEOV, carried by *Rattus norvegicus* and *R. rattus*, causes hemorrhagic fever with renal syndrome (HFRS) in humans. Increases of pet rat markets and urbanization have significantly associated with clinical HFRS cases due to human-rat contacts and sharing habitats. SEOV-induced HFRS was found from pet rat farmers, owners and their family in the United States and Europe. However, the epidemiologic surveillance and phylogenetic characteristics of SEOV remain to be investigated for the SEOV outbreak preparedness and responses.

Objectives: This study aimed to investigate epidemiologic surveillance and phylogenetic characteristics of SEOV using multiplex PCR-based next-generation sequencing (NGS).

Methods: A total of six HFRS clinical specimens and 1,269 R. norvegicus were collected in the urban HFRS-endemic area, Republic of Korea (ROK), during 2000-2016. SEOV infection was confirmed and quantified by indirect immunofluorescence antibody test and quantitative real-time PCR, respectively. Multiplex PCR-based NGS was applied to recover the genomic sequences of SEOV, using specimens containing low copies of viral genomes from HFRS patient sera and rodent lung tissues. Phylogenetic trees were generated by the maximum likelihood (ML) method.

Results: The epidemiologic survey showed an enzootic infectious cycle of SEOV during a year regardless of sex, weight (age), and season. Viral loads of SEOV described a wide spread in the tissues and dynamic circulation among the rat populations. Multiplex PCR-based NGS revealed nearly whole-genome sequences of SEOV from HFRS patients and reservoir hosts in the ROK. The phylogenetic relationships of SEOV revealed the distinct genotypes of SEOV from East Asia (China, North Korea, South Korea, and Japan), Southeast Asia (Vietnam and Singapore), Western Europe (United Kingdom, France, and Belgium), and North America (United States). In addition, the phylogenies of SEOV M and S segments exhibited a possible occurrence of genetic reassortment in nature.

Conclusion: Comparative genomic analyses demonstrated the comprehensive distinct genotypes and a possible genetic exchange event of SEOV. This study provides significant insights into epidemiologic surveillance, well-defined genotypes, and genetic diversity of SEOV. The phylogeographic analysis will aid in tracking of virus genomes and mitigating the disease risk against SEOV outbreaks.

- **Puumala virus S-segment sequence evolution in the bank vole and functional analysis of NSs protein**

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Hantaviruses are usually associated to a single rodent reservoir host and do not cause visible disease in their host. Puumala virus (PUUV), which is transmitted by the bank vole (*Myodes glareolus*) is the main causative agent of human hantavirus infections in central Europe. The S-segment of vole-borne hantaviruses contains two major overlapping open reading frames (ORF) coding for the nucleocapsid (N) protein and a non-structural (NSs) protein, a putative interferon-antagonist. To investigate the role of PUUV NSs, a large scale sequencing study of PUUV-positive voles was done. Additionally recombinant NSs was generated and analyzed for its functional activity. For this purpose, blood and lung tissue samples of 851 bank voles trapped during 2010-2014 in Baden-Wuerttemberg and North Rhine-Westphalia were analyzed. Our investigations showed that 242 animals (28.4%) were positive for PUUV-specific antibodies, whereas in 193 animals (22.7%) PUUV RNA could be detected. In the hantavirus outbreak years 2010 and 2012 a higher PUUV prevalence could be detected compared to 2011/2013/2014. From spring to autumn a decrease in the RNA detection rate was observed in bank voles. Sequence analysis of PUUV revealed sequence types of the N-ORF and NSs-ORF with temporal and/or spatial variation. The NSs-ORF showed a higher number of non-synonymous mutations than the overlapping and non-overlapping N-ORF regions. Further analysis of sequence variations revealed positive selection for the NSs-ORF and a negative (purifying) selection for the non-overlapping N-ORF. Additionally, NSs could be detected after infection in vitro and showed inhibiting activity of the interferon (INF) -ß promoter. NSs protein function was assessed by mutational studies. The results suggest an influence of bank vole population dynamics on molecular PUUV evolution. The function of NSs protein was demonstrated in vitro and potentially important regions were identified.

- **Sequence analysis of L-based screening PCR product allows for molecular typing of Puumala virus**

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For molecular hantavirus typing and phylogenetic analyses, sequences of the small (S) segment encoding the nucleocapsid protein should be favoured. Based on this, we have previously shown that there is a clear spatial distinction of PUUV clusters even among neighboring regions in Germany. Due to the diversity of the S-segment, and the low viral loads even in the early phase of infection it can be challenging to amplify nucleotide sequences.

Molecular diagnostics of hantaviruses in Germany is based on a nested PCR targeting the conserved large (L) segment. Not all samples with a positive screening PCR were found to be also positive in a subsequent S-segment nested PCR, which shows a lower degree of conservation across different strains.

Therefore, we investigated the potential of an L-segment region, resulting from the screening PCR, to allow for spatial distinction of the virus origin, with the aim of facilitating future molecular epidemiological analyses. Of 93 PUUV-infected patients with positive screening PCR 60 (65%) were also positive in the PCR of the S-segment. Both phylogenetic trees resulted in well supported phylogenetic clusters corresponding to the geographical origin of the virus.

In summary, analysis of sequences directly obtained from a diagnostic screening PCR allow for PUUV typing and can be used to assess the molecular epidemiology of the virus.

- ***In vitro* study on interactions of pathogenic and non-pathogenic orthohantaviruses with cellular factors of human host and rodent reservoir**

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Orthohantaviruses are hosted by rodents and small insectivorous mammals. They are present all over the world, reflecting geographical distributions of their natural hosts. Orthohantaviruses are persistent and asymptomatic in their reservoirs, but may cause two types of pathology targeting different organs when transmitted to humans via aerosol of contaminated rodents' excreta: hemorrhagic fever with renal syndrome in Eurasia and hantavirus cardiopulmonary syndrome in the Americas. The way orthohantaviruses interact with their hosts to propagate and establish persistence or pathogenicity is not yet understood. Our objective is to compare interaction of the pathogenic Puumala virus (PUUV), causing epidemic nephropathy in Europe, and of the low or nonpathogenic Tula virus (TULV) or Prospect Hill virus (PHV) with cellular factors. We assessed the susceptibility to infection of different cell lines derived from various tissues of human or rodent origin. Only simian VeroE6 kidney cells and human HuH7 hepatocyte and THP1 monocyte cells were permissive to the three orthohantaviruses, PUUV, TULV and PHV. Other human cell lines were found either not susceptible to infection or could be infected by one virus but not the others. The specificity of PUUV for its natural host, the bank vole (Myodes glareolus), was reproduced by its capacity to productively infect bank vole derived lung or kidney cells, while TULV could not infect these cells. Similarly, the host specificity of TULV was demonstrated using immortalized kidney cells from the common vole (Microtus arvalis). Profiles of infection and virus production were investigated by virus titration in immunofluorescence and western blot assays, quantification of viral RNA by RT-qPCR, and co-localization of viral proteins with cell compartment markers. The results of these investigations highlight differential virus-host interactions according to the virus species and the cell type on which they were produced. These results are complementary to other proteomics and interactomic approaches, indicating differential interactions of viral proteins with cellular factors involved in apoptosis, inflammation and the interferon pathway. Such *in vitro* studies on the interaction of pathogenic and non pathogenic orthohantaviruses with rodent and human derived cells will help to understand the outcome of these viral infections leading to persistence or pathogenesis.

- **Tigray virus is a rodent-borne hantavirus which might have emerged from ancient reassortment events involving a shrew-born hantavirus**

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Over the last decade, more than 20 new hantaviruses were discovered in non-rodent hosts - such as shrews, moles, and bats - and revealed ancient hantavirus evolutionary origins in non-rodent hosts. Tigray virus (TIGV) was found in Ethiopia in 2012 in the Ethiopian White-footed mouse, *Stenocephalemys albipes*. Initial phylogenetic analyses unexpectedly placed the virus within the clade of shrew- and mole-borne hantaviruses. Analyses of complete sequences of all three genomic segments obtained from a single strain then confirmed its unusual phylogenetic position only in M and L segment-based analyses while the virus clustered with other Murinae-associated hantaviruses in the S segment-based trees.

The objective of this study was to verify the host association of TIGV with rodents, extend virus genomic data, and perform phylogenetic analyses clarifying the exceptional placement of TIGV in the previous study.

A total of 235 small mammals collected in Ethiopia in 2016 were analyzed. TIGV RNA was detected by the genus-specific nested RT-PCR. Nucleotide sequences from all three genomic segments were obtained using an in-solution hybridization capture approach followed by next-generation sequencing and subjected to phylogenetic analyses in a Maximum-Likelihood and Bayesian framework.

Altogether, 11 positive animals were found at three trapping sites separated from the previous detection by 715 km, representing not only S. albipes but also S. albocaudata and S. griseicauda, suggesting host associations beyond the species level and broad TIGV occurrence. Phylogenetic analyses with the extended datasets confirmed conflicting phylogenetic signals alternatively clustering the virus with rodent- or shrew-borne viruses. Moreover, the recently recognized mole-borne Bruges virus was shown to be the most closely related virus sharing the alternative positions in the S and M segment trees but not in the L segment.

Multiple detections of the virus in Stenocephalemys mice after the anecdotic initial finding clearly confirms these rodents as TIGV reservoir hosts. Our phylogenetic analyses including several TIGV strains showed that the conflicting placement of the virus in the segment-specific trees is valid for the whole species and suggest that the virus might have emerged from ancient reassortment events involving ancestral rodent- and shrew/mole-borne hantaviruses.

- **Characterization of the field vole associated hantavirus Tatenale Virus (TATV)**

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The recently identified hantavirus, Tatenale virus (TATV), has been detected in a number of field voles (*Microtus agrestis*) at several sites in England and Wales. Partial sequence data previously suggested TATV to be a new orthohantavirus species within the Arvicolinae associated members of the family *Hantaviridae* (group 3a). Whilst efforts to isolate TATV in vitro continue, we now report the genome sequence of TATV. RNA was extracted from lung and kidney samples from one field vole (V23) and lung from another filed vole (V16), representing the organs with the highest viral load, as determined by PCR. Host genomic DNA (gDNA) and ribosomal RNA (rRNA) was depleted, and ds cDNA synthesised. The DNA was sequenced using an Illumina MiSeq. A total of 95% of all reads were identified as host-specific and removed. De novo alignments of the remaining sequences failed to result in hantavirus-specific contigs, therefore genomic sequences of the most closely related group 3a hantaviruses (Tula and Khabarovsk) were used to map the remaining reads. A hybrid TATV sequence was constructed combining the TATV-specific reads obtained from all three samples. The hybrid sequence was used to realign reads and build up the TATV genome. Gaps were closed using a combination of manually extending trimmed reads, using the PRICE (paired read iterative contig extension) tool and PCR followed by Sanger sequencing. Mapping of the final genomic sequence back to the original read data confirmed that some regions of the genome had no representative reads, despite the large amount of read data obtained. The low abundance of the virus in these clinical specimens proved a bioinformatics challenge and required PCR data to complete the genomic sequence. The final segments were 1,994 (S), 3,501 (M) and 6,446 (L) nucleotides long. Nucleic acid identity of the segments compared to the other group 3a genomes was 60.4-64.8% for S, 65.1-72.9% for M and 74.0-76.0% for L. This is comparable to the overall intra-3a group identity reinforcing TATV as a new orthohantavirus species.

- **The unconventional hantavirus square surface glycoprotein lattice resolved at high resolution by allied X-ray crystallography and electron cryo-tomography**

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Hantaviruses are responsible for important zoonoses around the world: a severe hantavirus cardiopulmonary syndrome is caused by several New World hantaviruses, and a hantavirus hemorrhagic fever with renal syndrome by several Old World hantaviruses. Such episodes account for more than 100,000 cases yearly, and have high lethality. In spite of their medical importance, the organization of the hantavirus particles is not known in detail. All medically relevant hantaviruses are very closely related, with the envelope glycoproteins Gn and Gc sharing more than 60 % and 70% amino acid sequence identity, respectively. Structural data obtained for any of hantavirus therefore will also shine light on all of them. Here we report the X-ray structures of the Gn/Gc ectodomain of two New-World hantaviruses: Andes virus (ANDV) and Maporal virus (MAPV), to 2.6 and 3.2 Å resolution, respectively. We further docked the resulting atomic model into a 12 Å resolution three-dimensional reconstruction of Tula hantavirus (an Old-World hantavirus). The Gn/Gc heterodimers of either MAPV or ANDV Gn/Gc completely fill the projecting square spikes of the Tula virus particle, showing that the spikes interact laterally via Gc:Gc contacts, and that each spike is made by Gn:Gn intra-spike interactions. The structures also showed that the overall organization of the Gn/Gc heterodimer has many features in common with the alphavirus p62/E1 heterodimer, which forms trimeric spikes instead. Our resulting pseudo-atomic model for the hantavirus particle allowed the mapping of all currently available data on the epitopes of hantavirus-neutralizing antibodies. It also allowed to understand how Gn engages the fusion loop of Gc, which is a class II membrane fusion protein evolutionary related with the alphavirus and flavivirus fusion proteins, and to dissect the low pH induced conformational change of Gc to drive fusion with endosomal membranes.

EPIDEMIOLOGY - ORAL TALKS

• Molecular epidemiology of hantavirus disease in Germany

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In Germany, hantavirus disease is notifiable by law since 2001; altogether 12,383 clinical cases have been registered between 2001 and 2018. The 3 largest outbreaks happened in 2010, 2012, and 2017 with 2,000 – 3,000 registered patients each. About 97% of virus-typed cases were caused by Puumala virus (PUUV). These *Myodes glareolus*-associated infections usually occurred in the West and South of the country. The minority of cases was due to infections by Dobrava-Belgrade virus (DOBV), genotype Kurkino. The natural host of DOBV-Kurkino is *Apodemus agrarius* (with some virus spillover to *A. flavicollis* animals) which can only be found in the North-East of the country; consequently, DOBV-Kurkino infections are restricted to this region. In 6 cases hantavirus infection might have contributed to the death of the patients.

PUUV and DOBV-Kurkino infections are not only different in their geographical but also temporal occurrence; the incidence peak for PUUV cases occurred in early summer but DOBV-Kurkino case number peaked in early winter. Whereas PUUV is responsible for large outbreaks in Germany, the number of clinical DOBV-Kurkino cases seems to be relatively constant over time and on a lower level.

From 202 PUUV-infected patients, sequence analysis of parts of the S segment and phylogeographic analysis allowed the construction of a detailed “molecular registry” of PUUV strains. The strains fell into 7 main clusters representing the various geographical outbreak regions and even allowed a further breakdown to smaller regions, i.e., counties and their segmentations. Virus sequences from voles trapped in the respective areas completely corresponded to the human-derived sequences. Moreover, we amplified and analyzed DOBV-Kurkino L-segment sequences from 10 patients which indicated a similar spatial clustering and accordance with sequences from local A. agrarius. Our “molecular registry” allows the allocation of the concrete geographical place of infection of a patient.

- **Hantavirus surveillance of peri-domestic rodent species in Great Britain**

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Context: Human cases of Seoul virus (SEOV) associated haemorrhagic fever with renal syndrome (SEOV-HFRS) have been reported in Great Britain (GB) since 1977 and SEOV RNA has been detected in GB wild brown rats (*Rattus norvegicus*). Novel hantavirus, Tatenale virus (TATV), was discovered in GB in 2013 in a field vole (*Microtus agrestis*). The prevalence and distribution of both of these viruses in wild rodents is unknown.

Objective: To increase the understanding of the prevalence, diversity and epidemiology of Hantaviruses in the GB.

Method: Rodents of various species, including brown rats (n=68), field voles (n=23) and bank voles (*Myodes glareolus*, n=56) were trapped from October 2014 to January 2016 from a variety of peri-domestic sites including pig farms, commercial premises and rural dwellings. Tissue samples (kidney and lung) were taken at post mortem examination and RNA extracted. These rodents were screened for hantavirus RNA using a published pan-hantavirus RT-PCR assay.

Results: SEOV RNA was detected in 19 per cent (13/68, 95% CI 11 to 30) of rats and all sequences fell within SEOV lineage 9. Twelve sequences were highly similar to each other and to the previously reported GB Humber strain of SEOV (98%). One rat SEOV sequence was more distant. The SEOV prevalence in rats from pig farms was significantly greater (p=0.047) than other sites sampled. Tatenale virus (TATV) RNA was detected in 7/23 (30.4%, 95% CI, 11.6-49.2%) of field voles from one location in North Wales. No Puumala virus (PUUV) RNA was detected in this study. No significant sex or age differences were observed among positive and negative rats or voles.

Conclusions: The results from this study suggest that SEOV could be widespread in wild rats in GB and therefore pose a potential risk to public health. The zoonotic potential and, therefore the public health risk, of TATV are unknown.

- **A major outbreak of hantavirus pulmonary syndrome caused by person-to-person transmission of Andes virus in Epuyén, Southwestern Argentina**

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Hantavirus infection occurs after the inhalation of aerosolized viral particles produced by infected rodents. Andes virus (ANDV) was firstly characterized in 1995 from a patient with hantavirus pulmonary syndrome (HPS) in southwestern Argentina; one year later person-to person transmission was suspected, after an outbreak that led to the infection of 16 people. The objective of this work was to describe the major outbreak of person-to-person transmission to date, that began in a rural town, Epuyén, during the spring/summer season 2018-2019, and the adopted measures that helped to control viral dispersion.

For this purpose all suspected cases were laboratory-confirmed at the National Reference Laboratory. The epidemiological information related to each case was analysed. Incubation periods were estimated considering time elapsed from most probable exposure event since the beginning of fever. Blood samples were collected and RNA were subjected to next generation sequencing and nucleotide analysis to confirm person-to-person transmission. Seroprevalence studies in human and rodent populations were performed to detect previous infections with ANDV. An isolation program for high risk contacts together with laboratory follow up for early viral detection were implemented.

A total of 33 cases with epidemiological link to a previous HPS case were reported. ANDV, variant South, was characterized. The most probable incubation period ranged from 14 to 37 days. The level of nucleotide variation observed between full-genome sequences was minimal (nucleotide identity: 99.9% - 100%) confirming person-to-person transmission. The isolation of contacts together with their close clinical and laboratory follow up were successful for the early detection of cases and for the limitation of viral transmission in late stages of the outbreak.

We described the largest chain of transmission of ANDV spreading from person-to-person. The outbreak lasted 97 days and implicated 12 deaths. Conversely to the outbreak occurred in 1996, strict control measures were necessary to limit viral spreading avoiding more infected cases. Since 1995 to 2017 the percentage of epidemiologically linked cases was 7.5% in the southwestern region of the country. After this outbreak, that percentage increased to 22%. This number is probably underestimated.

- ***Seoul orthohantavirus* in captive rat populations in the Netherlands**

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In 2016, the first patient was diagnosed in the Netherlands with *Seoul orthohantavirus* (SEOV), and in 2017, three additional cases were diagnosed. All patients had been in contact with either feeder rats or pet rats. These rats were tested using serology and RT-qPCR and were also positive for SEOV. During the investigations, signals about the exchange of rats, both nationally and internationally, suggested that the boundaries between trade of feeder rats and pet rats are less strict then was assumed. This raised the question about the spread of SEOV in the various rat populations in the Netherlands and the consequent public health risk. We therefore performed a study to assess this spread.

We approached commercial (feeder) rat breeding farms, ratteries (non-commercial breeders of pet rats) and individual rat owners who did not breed or only did so on a small scale. Participating farms, ratteries and owners were requested to send in dead rats and to fill in a questionnaire with questions regarding the housing, contacts with other rats, exchange with other farms, ratteries and owners and hygiene measures. Rats were tested for SEOV by serology and RT-qPCR, using lung tissue. A virus neutralization test was performed on seropositive samples that were RT-qPCR negative.

Nine commercial farms participated, of which six were actual breeding farms, and three were trading farms. Of each, 10 rats were tested. Of two breeding farms, rats were positive for SEOV (5/10 and 6/10 positive). Of the eight participating ratteries, five to ten rats were collected per rattery. Also two shelters sent in six and nine rats. Of one rattery, two out of eight rats were SEOV positive. 29 Rats of private owners were tested, of which one was SEOV positive. The questionnaires gave a general view of a very dynamic business, with links between breeding farms, the feeder and pet rats, and countries in Eastern Europe. In conclusion, we found SEOV in various types of captive rat populations in the Netherland, but not on a very large scale. Results will be used for public health communication to risk groups.

- **Insectivore-borne hantaviruses in Asian Russia**

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Insectivores are the new emerging reservoir of hantaviruses. Here, we present the results of investigation of its genetic diversity and geographic distribution in Asian Russia. Lung tissues from 455 insectivores, captured during 2007-2009, 2011, 2015-2017 in 30 localities in Western, Eastern Siberia and Far East of Russia, were analyzed for hantavirus RNA using RT-PCR. Viral RNA was detected in 73 samples.

Phylogenetic analyses of partial- and full-length S, M, and L segment sequences from these shrews and moles indicated that seven distinct insectivore-borne hantaviruses are endemic in Asian Russia. This report demonstrates the impressive distribution of Seewis (SWSV) virus among phylogenetically related *Sorex* species and Artybash (ARTV) virus in the Laxmann's shrews (*Sorex caecutiens*). Kenkeme virus harbored by the flat-skulled shrew (*S. roboratus*) was detected in geographically distant localities of Western Siberia and far-eastern Russia. Yakeshi virus was detected among taiga shrews (*S. isodon*) in the Far East of Russia and long-clowed shrews (*S. unguiculatus*) from Sakhalin Island.

We describe two novel hantaviruses Altai (ALTV) and Lena (LENV), which shows distant relation to other Sorex-borne hantaviruses, suggesting that both have emerged from cross-species transmission. ALTV harbored by the common shrew (*S. araneus*) and LENV harbored by the Laxmann`s shrew (*S. caecutiens*), are also the hosts of SWSV and ARTV, respectively. A novel hantavirus, named Academ virus, was detected in the Siberian mole (*Talpa altaica*) from Western Siberia.

- **Association of hantavirus infections and leptospirosis with the occurrence of chronic kidney disease of uncertain etiology (CKDu) among patients in a high prevalent and a low prevalent area of the North Central province of Sri Lanka**

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Chronic kidney disease of uncertain etiology (CKDu) has become a major disease burden, affecting the farming community of Sri Lanka and the exact etiology, which could be multifactorial is not yet established. Although it has been hypothesized that the hantavirus infection and leptospirosis as possible causes for CKDu in Sri Lanka, to date, the association of hantaviruses, leptospira or both pathogens for the occurrence of CKDu is not sufficiently explained. This study is aimed to determine the association of past exposure to hantaviruses and leptospirosis among CKDu patients and healthy individuals under similar living conditions and lifestyles.

A total of 180 known CKDu patients {albumin-to-creatinine ratio (ACR) ≥ 30 mg/g urine} living in a high CKDu prevalent areas of Anuradhapura district of the North Central Province of Sri Lanka was compared with a group of 49 healthy (ACR) < 30 mg/g urine), age and sex matched individuals who were blood relatives of CKDu patients (control-1) and another group of 48 healthy (ACR) < 30 mg/g urine), age and sex matched individuals (control-2) from a low CKDu prevalent area of the same district.

50/179 (28%) CKDu patients, 16/49 (32.6%) of the healthy blood relatives living in the same area and 7/48 (14.6%) healthy individuals living in a low CKDu prevalent area were found positive for IgG antibodies to either Puumala or Hantaan or both strains. However, only 14/179 (7.8%) CKDu patients, 3/49 (6.1%) control 1 group individuals and 3/48 (6.2%) control 2 group had IgG antibodies to leptospira respectively.

Results show a clear difference in sero-prevalence to hantavirus infections in CKDu patients from a high CKDu prevalent area compared to healthy individuals from a low CKDu prevalent area. In contrast, detection of higher but similar sero-prevalence rates to hantaviruses in CKDu patients and their healthy blood relatives in high CKDu prevalent area indicates that there is no association of hantavirus infection with the CKDu. It may also be possible that healthy blood relatives who are seropositive to hantaviruses may develop CKDu subsequently which require further follow up. However, IgG antibody positivity to Leptospira has shown no association between CKDu, control 1 and 2 groups.

- **Hemorrhagic fever with renal syndrome: Current status in Russia**

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In Russia, more than 137 thousand cases of hemorrhagic fever with renal syndrome (HFRS) were reported between 2000 and 2018; they occurred in 68 out of 85 administrative regions of the country. Most cases, 98.4% of the total number, were registered in European Russia and only 1.6% in Asian Russia. Approximately 80% of patients were males. The majority of HFRS patients was 20-50 years old, while only 2.4 % were children up to the age of 14 years. The average fatality rate was 0.4%. Six hantaviruses, causing HFRS of different clinical severity, were recognized as pathogens in Russia; Hantaan, Amur, Seoul, Puumala, and two subtypes (Kurkino and Sochi) of Dobrava-Belgrade virus with the principal hosts *Apodemus agrarius mantchuricus, Apodemus peninsulae, Rattus norvegicus, Myodes glareolus, Apodemus agrarius*, and *Apodemus ponticus*, respectively.

HFRS represents a serious medical problem in Russia, especially in the European part of the country where the case numbers are by far the highest in Europe. There are strong regional differences in HFRS incidence rates across the country reflecting spatial differences in the distribution and endemic infection level of small mammals as virus reservoirs and, moreover, differences in pathogenicity between the hantaviruses.

This unique combination of various causative agents together with high number of human clinical cases enables the accumulation of highly valuable epidemiological data and allows an understanding of the epidemiological and clinical differences between the HFRS forms.

- **Comparative study of Puumala virus infection in french endemic and peri-endemic areas**

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In Europe, nephropathia epidemica (NE), a mild form of haemorrhagic fever with renal syndrome (HFSR), is caused by Puumala virus (PUUV). The distribution of NE cases is heterogeneous and endemic (circulation of PUUV in bank vole populations and high number of NE cases), peri-endemic (circulation of PUUV in bank vole populations but rare NE cases) and non-endemic (no detection of PUUV in bank vole populations and no NE case) areas are reported.

To better understand these epidemiological differences, it is important to characterize bank vole-PUUV interactions and their geographic variability. We therefore performed crossed experimental infections of wild bank voles captured in French endemic (Ardennes) and peri-endemic (Loiret) areas, with two French PUUV strains, recently isolated and genetically characterize from the same areas.

PUUV fitness was evaluated using serological and qRT-PCR data. Results were compared between PUUV strains and bank vole populations. We found that the serological response and the distribution of PUUV in lungs and excretory organs seem to be more important in bank voles infected by the endemic PUUV strain, whatever their population of origin. This result suggests that the Hargnies-PUUV strain, isolated in a French PUUV endemic area (Ardennes), has a different capacity of replication inside host, than the Vouzon-PUUV strain that circulates in the peri-endemic area (Loiret). It could be interesting to perform comparative genomics on these PUUV strains to identify the mechanisms underlying these differences.

VACCINES, THERAPEUTICS AND PREVENTION - ORAL TALKS

• Macropinocytosis contributes to hantavirus entry into human airway epithelial cells

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Context: Hantaviruses represent an important group of emerging pathogenic viruses in many regions of the world and are an important paradigm for zoonotic airborne transmission. The lack of a licensed vaccine and the limited therapeutic options at hand make the development of novel anti-viral drugs an urgent need. Host cell attachment and entry are the first and most fundamental steps of virus infection and represent major barriers for zoonotic transmission.

Objectives: During host cell invasion, viruses critically depend on cellular factors, including receptors, co-receptors, and regulatory proteins of endocytosis. Here, we investigated cellular factors involved in entry of the pathogenic Old- and New World hantaviruses Hantaan virus (HTNV) and Andes virus (ANDV) into human respiratory epithelial cells.

Methods: To identify cellular factors involved in hantavirus entry into human airway epithelial cells we combined an unbiased small molecule screen with functional studies testing specific inhibitors of endocytosis using a biocontained recombinant Vesicular stomatitis virus pseudotype platform.

Results: Screening of a kinase inhibitor library covering major cell signaling pathways revealed differential requirement for host cellular factors for HTNV and ANDV entry and provided first hints for an involvement of macropinocytosis. Examination of a selected panel of well-defined inhibitors of endocytosis confirmed that both HTNV and ANDV enter human respiratory epithelial cells via a pathway that critically depends on sodium proton exchangers and actin, hallmarks of macropinocytosis. However, HTNV and ADNV differed in their individual requirements for regulatory factors of macropinocytosis, indicating important virus-specific differences.

Conclusion: Our results support the hypothesis that HTNV and ANDV have evolved to recognize distinct sets of receptors and/or co-receptors on human respiratory epithelial cells, perhaps due to divergent virus evolution. Differential receptor/co-receptor engagement by the two viruses may induce distinct signaling that could explain the diverse dependence on cellular regulatory factors. Our study further identifies specific small molecule inhibitors that may help to develop novel antiviral strategies against these emerging pathogens.

- **Anti-Hantaan virus human IgG produced in transchromosomic bovines has potent neutralizing activity and is protective in animal models**

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Efforts to develop vaccines targeting hantaviruses have invariably focused on the production of neutralizing antibodies as a predictor of vaccine potency and a likely surrogate of vaccine protective efficacy. Here, we have explored the possibility of using a novel technology to produce anti-Hantaan virus (HTNV) neutralizing antibodies for use as a pre or post-exposure prophylactic. The technology involves hyperimmunization of transchomosomic (Tc) bovines engineered to express human polyclonal IgG antibodies. The vaccine used in this study is a HTNV DNA vaccine encoding Gn and Gc. Tc bovine were either hyperimmunized with 1.2 mg/vaccination of lipid nanoparticle (LNP)-formulated DNA, or 12 mg of DNA plus an adjacently administered SAB proprietary adjuvant. The LNP-formulated vaccine yielded approximately 5-fold higher neutralizing antibody titers despite using 10-fold less DNA. Human IgG was purified from the plasma collected from the animal vaccinated with the LNP-formulated DNA. Purified IgG at 50.48 mg/mL, termed SAB-159, had anti-HTNV neutralizing antibody titers >100,000 as measured by pseudovirion neutralization assay (PsVNA) or plaque reduction neutralization test (PRNT). As little as 4 microliters of this material was sufficient to protect Syrian hamsters against infection (EC50 < 2 mg/kg) in passive transfer experiments. Bioavailability studies in hamsters indicated that neutralizing antibody titers >100 were still present in sera four weeks after administration of 10 mg/kg of SAB-159 (half life= 11.3 days). A HTNV PsVNA50 titer =103.1 was calculated to predict an estimated probability of protection of 80% with a lower and upper 95% fiducial limit of 73.2 and 167.3, respectively. Each unit log10 increase in pre-challenge HTNV PsVNA50 titer was associated with an increase in odds of protection against HTNV of 83.0 times (p>0.0001). Efficacy of SAB-159 to protect in nonhuman primates was also evaluated using a marmoset infection model. A single subcutaneous injection of 12.32 mg/kg SAB-159, but not a negative control antibody, one day prior to HTNV challenge substantially limited HTNV infection. Together these data demonstrate a proof-of-concept that the Tc bovine-based manufacturing platform can be used to produce exceedingly potent anti-HTNV neutralizing antibodies that are polyclonal, human, and protective in animal models.

- **Recombinant human monoclonal antibody therapy against Andes hantavirus infection**

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Context: Hantavirus cardiopulmonary syndrome (HCPS) caused by Andes hantavirus (ANDV) is a severe disease that can progress to hypotension, pulmonary failure, and cardiac shock, which results in around 36% case-fatality rate in humans. Currently, there is no specific treatment or vaccine; however, several studies have indicated that generation of neutralizing antibody responses correlates with survival of HCPS.

Objectives: Evaluate the potential of human monoclonal Abs as post exposure therapy at different stages of infection using the Syriam hamster HCPS model.

Methods: Recombinant immunoglobulin G antibodies isolated from a high neutralizer responder previously infected with ANDV were cloned, produced and characterized. For the in vivo protection experiment, Syriam hamsters were challenged with 200 focus-forming units (FFUs) of ANDV and administrated intraperitoneally with isotype control (50mg/kg) or a combination of two mAbs (25mg/kg JL16 + 25mg/kg MIB22). Hamsters were monitored daily for signs of disease, respiratory rates and weight changes were also recorded. Survivors were euthanized and sera and lung were collected for analysis.

Results: Two mAbs, JL16 and MIB22, recognized ANDV-GP and neutralized ANDV using pseudoviral particles and full replicative virus. In addition, our data suggest that both mAbs bind different regions on the ANDV-GP and that mAb JL16 posses a higher relative affinity than MIB22. We examined the post-exposure efficacy of the mAbs in a Syrian hamster model of ANDV-induced HCPS, and combination of both mAbs protected 100% of animals from a lethal challenge even when the Ab cocktail was administrated 5 days post-infection. Interestingly, the combination of mAbs protected 50% of animals even when Ab cocktail was administrated at day 8-10 post infection.

Conclusion: These data suggest that combination therapy of JL16 and MIB22 mAbs could be an effective post-exposure treatment for patient infected with ANDV.

- **Vesicular stomatitis virus-based vaccines provide cross-protection against Andes and Sin Nombre virus**

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Context: Andes virus (ANDV) and Sin Nombre virus (SNV) are the main causative agents responsible for hantavirus cardiopulmonary syndrome (HCPS) in the Americas. HCPS is a severe respiratory disease with a high fatality rate for which there are no approved therapeutics or vaccines available. Some vaccine approaches for HCPS have been tested in preclinical models, but none have been tested in infectious models for their ability to protect against multiple species of HCPS causing viruses.

Objectives: We sought to determine if recombinant vesicular stomatitis virus-based vaccines for Andes and Sin Nombre viruses could provide cross-protection in infectious challenge models.

Methods: Both rVSVΔG/ANDVGPC or rVSVΔG/SNVGPC vaccines were assessed for viral growth kinetics in vitro and subsequently used to vaccinate Syrian hamsters. Vaccinated animals were assessed for cross-reactive IgG antibodies by ELISA and neutralization assay. In addition animals were subjected to a homologous or heterologous challenge with ANDV or Hamster-adapted SNV (HA-SNV) viruses, respectively.

Results: We show that while both rVSVΔG/ANDVGPC and rVSVΔG/SNVGPC display attenuated growth as compared to wild type VSV, each vaccine is able to induce cross-reactive IgG responses. Both vaccines protected against both homologous and heterologous challenge with ANDV and HA-SNV and prevented HCPS in a lethal ANDV challenge model.

Conclusions: This study provides evidence that the development of a single vaccine against an HCPS-causing hantaviruses could provide protection against multiple HCPS-causing agents.

- **Monoclonal antibodies against the Andes virus surface glycoprotein GnGc**

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Hantaviruses cause a number of severe diseases in humans, the most severe of which is Hantavirus Cardiopulmonary Syndrome (HCPS). Although HCPS cases are relatively rare (approximately 300 cases recorded annually in the Americas), HCPS has a recorded case fatality rate of up to 50%. Moreover, HCPS viruses remain pathogens of major concern due to their potential for misuse as biological weapons, aerosol transmission route, and widespread availability in wild hosts. Nevertheless, no specific licensed treatments or vaccination strategies exist so far. Here, we describe our ongoing efforts to generate and characterize protective and neutralizing novel monoclonal antibodies against Andes virus (ANDV), a major causative agent for HCPS in South America. These antibodies are directed against the glycoprotein complex (Gn and Gc) of ANDV. Several monoclonal antibodies isolated from immunized mice were highly neutralizing, as determined by plaque reduction neutralization assays against a recombinant vesicular stomatitis virus expressing the ANDV glycoproteins (VSV-ANDV). To characterize antibody-virus interactions and elaborate on currently limited knowledge of hantavirus glycoproteins, VSV-ANDV escape mutant viruses were subsequently generated and analyzed via deep sequencing, cross-neutralization assays, and enzyme-linked immune sorbent assays (ELISAs). These monoclonal antibodies will drive vaccine development towards key epitopes associated with neutralization and protection, and could themselves have major implications as therapeutics, and analytic or diagnostic tools.

- ***In vitro* enzymatic analysis reveals small molecule inhibitors of the hantavirus endonuclease**

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Hantaviruses (HVs) are negative stranded RNA viruses with a tri-partite segmented genome. Similar to influenza virus (IAV), the HV polymerase (Pol) contains an N-terminal endonuclease (Endo) domain that cleaves caps from cellular mRNAs to prime viral mRNA transcription. In contrast to IAV, HVs accomplish this in the cytoplasm with a robust endonuclease that blocks Pol expression. Mutations in the Endo domain permit HV Pol expression by reducing endonuclease function and purified expressed Endo domains permit the evaluation of potential Endo inhibitors. The recent success of IAV inhibitors that target endonuclease function suggested the potential for novel HV Endo inhibitors as therapeutic targets. We recently purified Andes virus (ANDV) Endo domain mutants and N proteins and respectively demonstrated their cleavage activity and interactions with RNAs in vitro. RNA degradation, protection and FRET based assays we have developed permit us to assess endonuclease degradation and potential Endo inhibitors. We recently identified compounds that block HV endonuclease activity in vitro with nM IC50s which also reduce ANDV titers. These lead compounds serve as scaffolds for in silico analysis of Endo domain interactions and iterative inhibitor development. These findings permit us to mechanistically examine Pol, Endo and N protein interactions that direct HV RNA transcription and have the potential to reveal a therapeutic means of reducing HV replication.

- **Neutralizing antibodies against hantaviruses derived from human survivors**

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Hantaviruses are the causative agents of two severe human diseases - hemorrhagic fever with renal syndrome (HFRS) in the Old World, and hantavirus cardiopulmonary syndrome (HCPS) in the New World, respectively. Although no FDA-approved vaccines or specific treatments are currently available to prevent or treat infections caused by these agents, previous findings suggest that human infection and disease may be amenable to treatment with antibody-based therapeutics and prophylactics. Further, the human antibody response has been shown to be a protective correlate of HFRS caused by the Old World hantavirus, Puumala virus (PUUV). Here, we sought to characterize the human antibody response to natural PUUV infection and to identify fully human monoclonal antibodies (mAbs) with potential therapeutic value. Accordingly, we report on the isolation of a panel of approximately 200 glycoprotein-specific mAbs from PUUV convalescent patients by viral antigen-based sorting and high-throughput mAb cloning and production in yeast. MAbs were screened for antigen binding affinity and antiviral neutralizing activity to identify approximately 50 mAbs with exceptional PUUV neutralization potency. The binding epitopes of PUUV neutralizers were defined by analyses of binding competition and viral escape, and mAbs were down-selected further on the basis of their neutralization breadth against other virulent hantaviruses. Currently, we are probing the mechanism of neutralization of the most promising candidate mAbs and plan to evaluate their efficacy in animal models of hantavirus challenge.

CLINICAL ASPECTS AND DIAGNOSIS - ORAL TALKS

• Sequential assessment of clinical and laboratory parameters in patients with hemorrhagic fever with renal syndrome

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Background: Information on the sequential appearance, duration, and magnitude of clinical and laboratory parameters in haemorrhagic fever with renal syndrome (HFRS) is limited. In Slovenia, several hantaviruses have been confirmed in rodents and shrews, but three of the them, namely the Dobrava, Dobrava–Kurkino and Puumala orthohantaviruses, cause disease in humans, with significant differences in the severity of symptoms. The aim of our study was to gain detailed insight into HFRS dynamics by analysing sequential (mostly daily) values of selected clinical and laboratory parameters in patients.

Methods: Analysis of clinical and laboratory parameters obtained serially in 81 patients with HFRS, of whom 15 were infected with Dobrava virus and 66 with Puumala orthohantaviruses.

Results: The initial symptoms, appearing on median day 1 of illness, were fever, headache, and myalgia. These were present in 86%, 65%, and 40% of patients and had a median duration of 4, 4, and 5.5 days, respectively. They were followed by myopia (day 5), insomnia (day 6), oliguria/anuria (day 6), polyuria (day 9), and sinus bradycardia (day 9.5). These were present in 35%, 30%, 28%, 91%, and 35% of patients; their median duration was 2, 2, 2, 7, and 1 day, respectively. Laboratory abnormalities were ascertained on admission to hospital (day 5 or 6 of illness) and they included: thrombocytopenia (95%; median duration (MD) 4 days), elevated alanine aminotransferase (87%; MD 3 days), CRP (99%; MD 7 days), procalcitonin (91%; MD 3 days), creatinine (94%; MD 9 days), diminished glomerular filtration rate (87%; MD 8 days) and leucocytosis (55%; MD 2 days). Comparison of patients infected with Dobrava and Puumala orthohantaviruses found several differences in the frequency, magnitude, and duration of abnormalities, indicating that Dobrava orthohantavirus causes the more severe HFRS.

Conclusions: In the majority of patients, the classic clinical distinction into febrile, hypotonic, oliguric, polyuric, and convalescent phases of illness is unclear.

- **A comprehensive and comparative study of SISPA-, RNA-access, and multiplex PCR-based next-generation sequencing for *Hantaan orthohantavirus* in *Apodemus agrarius* lung tissues**

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Context: Orthohantaviruses pose a global public health threat. Orthohantavirus infections cause hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome in humans. *Hantaan orthohantavirus* (HTNV), harbored by the striped field mouse (*Apodemus agrarius*), was identified as an etiological agent of HFRS in East Asia. Defining of whole-genome sequencing helps in identification and characterization of the virus outbreaks. Next-generation sequencing (NGS) has been widely applied to various fields in virology, e.g. metagenomics of virome, tracking of virus spread, and development of therapeutics. Molecular enrichment methods enable to identify whole-genome sequences of the viral genome from environmental specimens containing low amounts of the infectious particles.

Objectives: To analyze and compare the coverage of viral genome sequences using different molecular enrichment-based NGS, the whole-genome sequencing of HTNV was performed from lung tissues of wild rodents independently of cultivating viruses.

Methods: From the lung tissues of 14 A. agrarius, targeted viral RNA was enriched by sequence-independent, single-primer amplification (SISPA), RNA access-, and multiplex PCR-based methods, respectively, prior to the NGS library preparation. NGS was performed on the Illumina MiSeq benchtop sequencer. Whole-genome sequences of HTNV were recovered by using the CLC Genomics Workbench 5. The coverage rate of HTNV genomes was examined and analyzed on the basis of lung tissues classified by viral RNA copies per μL.

Results: SISPA-based NGS showed the low coverage and sensitivity for whole-genome sequencing of HTNV from the rodent lung tissues containing low viral RNA loads. RNA access- and multiplex PCR-based NGS demonstrated high coverage rates of genomic sequences for HTNV up to 103-104 copies/μL of the viral RNA. Furthermore, the multiplex PCR-based NGS had a 10-fold (102 copies/μL) higher sensitivity than the RNA access-based method.

Conclusion: This study provides useful insights into target-enrichment NGS for the identification and characterization of orthohantavirus outbreaks without isolating the viruses.

• Positive urine glucose predicts the overall severity of acute Puumala hantavirus infection

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Nephropathia epidemica (NE) caused by Puumala virus (PUUV) is common in Finland. The clinical feature includes fever, thrombocytopenia, acute kidney injury (AKI) and increased capillary leakage. Proteinuria and hematuria are found in most patients and they predict the severity of upcoming AKI. Glucosuria is a rare finding according to two previous studies among adult and pediatric patients with NE.

We analyzed dipstick urine glucose (U-Gluc) from 195 patients with serologically confirmed NE on admission to Tampere University Hospital during 1994 to 2014. None of the patients had diabetes. Patients were divided into glucosuria group (U-Gluc 1+, 2+ or 3+, n=24) and no-glucosuria group (U-Gluc 0, n=171). The severity of AKI was measured by the maximum serum creatinine (crea-max), inflammation by the maximum leukocytosis, and thrombocytopenia by the minimum platelet count during hospital care. Maximum hematocrit, minimum plasma albumin, and the change in body weight reflected the capillary leakage, while the length of hospital stay the overall severity of the disease.

Urine glucose test predicted the severity of AKI: median crea-max was 459 µmol/L (range 78-1041) in the glucosuria group and 166 µmol/L (range 51-1499) in the no-glucosuria group ($p<0.001$). Glucosuria associated with leukocytosis (16.0x109/L vs. 10.2x109/L, $p<0.001$) and trombocytopenia (41x109/L vs. 62x109/L, $p=0.006$), as well as with all parameters reflecting capillary leakage and with the length of hospital stay (7.5 days vs. 6 days, $p=0.009$).

In the glucosuria group, only 1/24 patient had glucosuria also in the second urine test during hospital care. None of the patients in no-glucosuria group, had glucosuria in later control urine tests at hospital.

Glucosuria is a relatively rare and transient finding in NE. When present it associates with all markers of disease severity. Urine dipstick test is cheap and easily available at clinical work and can be used for risk stratification in the treatment of patients with acute NE.

- **Development and validation of a European orthohantavirus microneutralization test**

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Four different orthohantaviruses are associated with human disease in Europe: Dobrava/Belgrade virus (DOBV), Puumala virus (PUUV), Seoul virus (SEOV) and Tula virus (TULV). Of these, PUUV, SEOV and TULV are circulating in the Netherlands, with PUUV assumed to have the highest infectious burden in humans. Each year approximately 25-30 human orthohantavirus infections are reported in the Netherlands. The infecting species cannot be determined conclusively using routine serological assays due to existing cross-reactivity within and between the two main orthohantavirus serogroups, while sampling typically takes place too late to warrant molecular detection. Consequently, most infections in the Netherlands are attributed to PUUV without definitive confirmation by Gold Standard serology, i.e. virus neutralization. However, as zoonotic source and disease severity differ between orthohantavirus species, conclusive determination of infecting species is of importance for forecasting clinical outcomes and supportive treatment, for laboratory and syndromic surveillance, for source attribution (e.g. pet rats as source for SEOV) and monitoring of the effectiveness of implemented risk management.

Orthohantaviruses can only be typed by RT-PCR or comparative virus neutralization assays (VNT). The focus reduction neutralization test is generally viewed as the Gold Standard for orthohantavirus VNTs, however, the more high-throughput microneutralization test (MNT) was recently described a good alternative for serotyping HTNV and SEOV infections. Here, we describe the successful development and validation of a MNT for orthohantavirus species circulating in Europe. The assay was validated using a panel of rodent and human sera RT-PCR and/or routine serology positive for PUUV, SEOV, DOBV or TULV. Comparative MNTs could confirm PUUV (n=5), SEOV (n=5) or TULV (n=3) infection in all but one RT-PCR positive rodents based on a $\geq$ 4-fold titer difference. 100% of RT-PCR confirmed human PUUV cases (n=9) were confirmed by comparative MNT. 94% of clinically suspected cases (PUUV, SEOV, DOBV; n=54), previously indicated as probable orthohantavirus infections based on routine serology, were confirmed and typed by MNT. The added value of comparative MNT for orthohantavirus species determination was clearly illustrated by the confirmation of two previously not recognized SEOV cases.

In summary, we present the development and validation of a robust European orthohantavirus MNT.

- **Increased secretion of urinary kidney injury molecule-1 in patients with hemorrhagic fever with renal syndrome caused by Puumala virus**

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Kidney injury molecule-1 (Kim-1) is a transmembrane receptor from the T cell immunoglobulin and mucin domain family, whose members have critical roles in various immune responses like allergy, autoimmunity, viral infections as well as kidney injuries including diabetic and chronic kidney disease or acute kidney injury. In patients with hemorrhagic fever with renal syndrome (HFRS), kidneys are severely affected, sometimes with complete renal failure. KIM-1 has been identified as a modulator of the immune response in the kidney and protects the tissue from further trauma. In addition, it is secreted and elevated in urine of various diseases, affecting physiological kidney functions and acting as an early biomarker for kidney injury.

We aimed to detect potential secretion of urinary KIM-1 in HFRS patients infected with Puumala virus (PUUV) and asses KIM-1 as a non-invasive biomarker of kidney injury.

Paired urine samples of 61 male HFRS patients with confirmed PUUV infection, have been collected at hospitalization and before discharge. Patients were hospitalized at the University Hospital of Infectious Diseases in Zagreb and clinical and laboratory data were collected retrospectively from the patients history. Urine samples of HFRS patients and 20 age matched male healthy controls have been tested for the presence of KIM-1 according to the manufacturer's protocol (R&D Systems, Minneapolis, MN, USA). Nonparametric statistical analysis was used for analysis.

The levels of secreted KIM-1 were significantly (p value: < 0.001) increased in urine of HFRS patients, in both time points, compared to healthy controls. In the convalescent urine samples, the overall KIM-1 excretion was significantly (p value: < 0.001) lower then during the acute phase of disease. Due to a majority of mild cases (76 %), with no or only moderately increased serum creatinine (mean: 123 µmol/L, range72 – 893 µmol/L) and urea values (mean: 6,05 mmol/L, range: 2 – 47,5 mmol/L), no signifacant correlation between KIM-1 and these traditional markers of kidney function have been observed. Our study also indicate that KIM-1 could be more sensitive biomarker of kidney injury in HFRS than urea and creatinine.

- **Fatal case of Puumala virus infection and attempt to treatment with icatibant**

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A 79 year old male patient with a history of previous smoking, obstructive sleep apnea, hypertension and well-regulated type II diabetes was admitted in our department after 1 day of fever and rigors. A diagnosis of Puumala virus infection was established by serology and PCR. The patients condition deteriorated with development of cardiopulmonary failure requiring treatment with noradrenalin and invasive mechanical ventilation on day 3 post onset of fever. The following day we attempted treatment with bradykinin inhibitor icatibant as a one-time dose of 30 mg given subcutaneously but without any apparent effect and the patient died 12 hours later in refractory shock and multiorgan failure. Previous case descriptions has suggested beneficiary effects of icatibant in severe Puumala virus infection (Antonen et al. Scand J Infect Dis 2013, Vaheri et al. Antiviral Res 2014, Laine et al. Infect Dis 2015) but this could not be documented in the current case. The autopsy revealed extensive tissue edema in internal organs together with effusions in pleura and abdominal viscera, indicating vasculatory leakage in the pathophysiology.

We will perform and present additional analyses and discuss the role of icatibant as treatment in severe hantavirus infection.

- **Hantavirus or leptospirosis? A case report from the United Kingdom**

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Herein we present a case of hantavirus in the United Kingdom (UK) which was initially misdiagnosed as leptospirosis. This case illustrates the potential diagnostic difficulties associated with differentiating between these conditions, especially in a country where Hantavirus is traditionally rare and not well known by medical professionals.

A 33 year old Caucasian male presented to a District General Hospital in Wales, UK, with a four day history of myalgia, headache, fever, diarrhoea and vomiting. He lived in a caravan at his place of work – a local farm that produced rats for pet feed. Admission blood tests revealed acute kidney injury and deranged liver function. The presumptive diagnosis was leptospirosis and he was managed as such. However, his renal function further deteriorated and he required transfer to a tertiary renal centre for specialist input. Further investigations revealed negative leptospira serology and positive Hantavirus serology. Both hantavirus and leptospirosis cause flu-like syndrome with high fever, haemorrhage and acute renal failure.

This was the second case from this particular rat facility. As hantavirus is a rare cause of acute kidney injury in the UK, the diagnosis had not been considered. However, with increasing incidence of hantavirus in Europe, this case demonstrates the importance of a full occupational and social history and consideration of hantavirus as the diagnosis if risk factors are present.

Lightning Talks

ECOLOGY - LIGHTNING TALKS

- ***Jemez Springs orthohantavirus* diversification in the *Sorex monticola* complex reflects host response to climatic cycling**

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Context: Comparative phylogeography of multi-species host systems can provide valuable insights into the biotic and abiotic forces responsible for viral diversity, geographic structure, and distribution. Orthohantaviruses, like other members of *Hantaviridae*, are inextricably linked to the ecology and evolutionary history of their mammalian hosts.

Objectives: We hypothesized that in regions with dramatic climate shifts, orthohantavirus diversity and evolution are shaped by host responses (e.g., population contraction, expansion, and contact) to environmental change through processes such as divergence in isolation, host switching, and reassortment. Jemez Springs virus (JMSV), an orthohantavirus harbored by the dusky shrew (*Sorex monticola*) and five close relatives distributed widely in western North America, was used to test this hypothesis.

Methods: Total RNAs, extracted from liver or lung tissue from 69 *S. monticola*, 37 *S. vagrans*, 35 *S. trowbridgii*, 10 *S. palustris*, 1 *S. bairdi*, and 1 *S. haydeni* collected from western North America during 1983–2007 and archived at the Museum of Southwestern Biology and Portland State University Museum of Natural History, were analyzed for hantavirus RNA by RT-PCR. Phylogenies were inferred from the L-, M- and S-segment sequences of 30 JMSV strains using RAxML 8.2.12 and were compared with host mitochondrial cytochrome b in R using PhyTools, as well as TreeMap.

Results: Phylogenetic reconstruction and demographic analyses showed that JMSV has an evolutionary history similar to its host species. Viral clades largely corresponded to host clades, which were largely structured by geography. Divergent JMSV lineages in the Pacific Northwest were consistent with the Coastal Refugium Hypothesis, with expansion of independent northern and southern continental clades from the Pacific Northwest following the Last Glacial Maximum. Despite an overall congruence between host and viral phylogenies at deeper scales, phylogenetic signals were recovered that also suggested a complex pattern of host switching, and at least one reassortment event in the evolutionary history of JMSV.

Conclusion: A fundamental understanding of how orthohantaviruses respond to periods of host population expansion, contraction, and secondary host contact is key to establishing a framework for a more comprehensive understanding of orthohantavirus evolutionary dynamics and disease emergence and can provide broader insights into host-pathogen systems.

- ## Hantavirus ecology and evolution in Chile

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Zoonotic diseases have major impacts in the society, with viruses representing an important group as human pathogens. For understanding the phenomenon of the emergence of a specific pathogen, it is essential to understand the epidemiology, ecology and evolutionary processes responsible for the appearance and spread of that pathogen.

Hantavirus cardiopulmonary syndrome (HCPS) is an emerging infectious disease, first reported in the Americas in 1993. *Andes Orthohantavirus* (ANDV; *Hantaviridae*) is a major ethiologic agent of HCPS in Chile and Argentina, that produces a variable number of human cases every year, with a case-fatality ratio between 30 and 50%. Strikingly, person-to-person transmission of hantavirus has only been demonstrated for ANDV infection. In Chile, the first case of HCPS date from 1995, and since then 1092 new HCPS have been confirmed until 2017 ranging from Valparaíso region to Aysén region. The Sigmodontinae rodent O. longicaudatus (the pigmy rice rat) is the main reservoir inhabiting in contrasting geographic features and landscapes including Mediterranean, deciduous Temperate Forests, and Patagonia. In Chile, O. longicaudatus has higher abundances in mesic areas in the Temperate forests and Patagonia, but spillover has been reported to other sympatric rodent species, although with no apparently epidemiological importance.

Using molecular tools, we describe the phylogeographic structure of ANDV and its associated rodent host for understanding ANDV viral ecology and evolution, integrated with spatio-temporal reconstruction of ANDV lineages. Also, an 18-years study allowed us to found significant differences in the relative prevalence of anti-ANDV antibodies in rodent samples across the ecoregions. Results will help us to gain new insights about the understanding of the ANDV disease dynamics, and identifying emerging epidemiological patterns for disease control and prevention.

PATHOGENISIS AND IMMUNE RESPONSES - LIGHTNING TALKS

• Pulmonary endothelial glycocalyx degradation in *orthohantavirus* infection

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Objective: The endothelium plays a central role in the pathogenesis of viral hemorrhagic fevers. It regulates inflammation, adhesion of immune cells and platelets, and vascular permeability. Puumala virus (PUUV) is a rodent-borne orthohantavirus which is transmitted to humans via inhalation. PUUV causes hemorrhagic fever with renal syndrome. Previously, we have shown that PUUV causes systemic glycocalyx degradation associated with disease outcome and platelet activation and consumption (Connolly-Andersen et al., Open Forum Infect Dis 2014 & J Infect Dis 2015). In addition, PUUV infection constitutes a significant risk factor for acute myocardial infarction, stroke and venous thromboembolism (Connolly-Andersen et al., 2014, Circulation; 2018, Clin Infect Dis.). In the present study, we aimed to investigate pulmonary endothelial glycocalyx degradation during PUUV infection.

Methods: Pulmonary biopsies were obtained through bronchoscopy in 16 hospitalized PUUV patients and in 15 age, sex and smoking-matched healthy volunteers and stained for thrombomodulin (TM) and EN-4. Endothelial glycocalyx degradation markers (soluble TM, heparan sulphate and syndecan-1) were determined in patient plasma obtained at different time points during disease using an ELISA.

Results: The ratio of TM-stained blood vessels was significantly lower in PUUV-patients compared to controls. Furthermore, the markers for endothelial glycocalyx degradation (syndecan-1, heparan sulphate and soluble TM) were all significantly higher during acute disease compared to follow-up.

Conclusions: Our results show pulmonary endothelial glycocalyx degradation during orthohantaviral disease, which normalizes during the follow up period. The endothelial dysfunction may result in plasma leakage and pulmonary complications during hantavirus infection: Consequently, the endothelium warrants further notice when investigating pathogenesis and designing future therapeutic interventions.

- **Andes virus nucleocapsid protein binding to RhoGDI activates RhoA and directs endothelial cell permeability**

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Andes virus (ANDV) causes acute pulmonary edema termed hantavirus pulmonary syndrome (HPS). In HPS patients virtually every pulmonary microvascular endothelial cell (PMEC) is infected and nonlytically results in vascular leakage and highly lethal pulmonary edema. The mechanism of hantavirus induced vascular permeability and edema remains to be resolved. We observed that ANDV activation of RhoA directs the dissociation of inter-EC adherens junctions resulting in PMEC permeability. Persistent expression of the ANDV nucleocapsid protein (N) in primary hPMECs similarly resulted in RhoA activation that was not observed in PMECs expressing the TULV N protein. We found that ANDV N protein failed to co-precipitate RhoA but instead bound to the RhoA inhibitor RhoGDI (Rho GDP-dissociation inhibitor). ANDV, but not TULV, N protein co-precipitated endogenous RhoGDI in PMECs and when co-expressed ANDV N dose dependently bound RhoGDI. Expression of RhoA failed to inhibit ANDV N binding to RhoGDI, while expressing RhoGDI blocked RhoA activation directed by the ANDV N protein. Thus ANDV N forms complexes with RhoGDI that block its ability to inhibit RhoA. Phosphorylation of RhoGDI on S34 and S96 inhibits RhoGDI binding to RhoA and prenylated RhoA interactions with membranes that activate RhoA. Recent findings that ANDV N protein is phosphorylated suggest that N protein interactions with RhoGDI may activate RhoA by directing RhoGDI phosphorylation. Roles N protein directed interactions with RhoGDI S34 and S96 mutants establish roles for phosphorylated RhoGDI in directing N protein adherence and RhoA release. These findings demonstrate that the ANDV N protein activates RhoA by binding the RhoA inhibitor RhoGDI through domains in RhoGDI distinct from RhoA-RhoGDI binding sites. We reveal potential mechanisms for ANDV N protein to direct PMEC permeability via interactions with RhoGDI and suggest potential therapeutic targets for resolving RhoA directed endothelial cell barrier dysfunction during hantavirus infection.

- **Role of soluble mediators in the immune response in patients with hemorrhagic fever with renal syndrome**

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Background: The pathogenesis of hemorrhagic fever with renal syndrome (HFRS) is poorly understood, but it has been suggested that host's immune mechanism is an important factor influencing the disease. The key element of the antiviral immune response is the release of cytokines, chemokines and other soluble mediators, that can in cases of uncontrolled release lead to more severe clinical manifestation. The aim of our study was to investigate cytokine and chemokine responses in HFRS patients and to expose possible biomarkers for clinical outcome.

Methods: Forty soluble mediators of the immune, coagulation, and endothelial system were measured in acute serum samples (first 7 days after onset of symptoms) in 100 HFRS patients and 30 healthy controls. Among HFRS patients 50 were infected with DOBV and 50 infected with PUUV; 52 patients had mild and 48 severe disease.

Results: As anticipated, in both HFRS groups patients had significantly elevated levels of cytokines/chemokines associated with innate and adaptive Th1 immune response (GM-CSF, INFα2, INFγ, IL-6, IL-8, IL-12p70, IP-10 and TNFα) in comparison to control group. In addition, HFRS patients had higher concentrations of serum MIP-1α and MIP-1b, which promote activation of macrophages and NK cells. Patients infected with PUUV had higher levels of GROα and IL-10 than those infected with DOBV. In the subgroup of DOBV infected patients with severe disease IL-6, IL-8, IL1Rα, TNFα and MCP-1 were significantly elevated while in PUUV infected patients with severe disease IP-10 was also higher than in those with mild disease. Factors indicating endothelial dysfunction (sVCAM-1, Angiopoietin-2, Fibrinogen, Thrombomodulin, Tissue Factor) were significantly elevated in HFRS patients while sCD40 ligand, which is considered to contribute to the promotion of prothrombotic responses and production of angiogenesis-associated factor, was significantly downregulated in HFRS patients in comparison to controls.

Conclusions: Our study supports the hypothesis that the major players in the immunopathogenesis of HFRS are pro-inflammatory mediators, which mediate vascular dysfunction, disseminated intravascular coagulation, organ failure, and shock.

- **Immunoregulatory responses as mediators of persistent hantavirus infections in reservoir hosts as determined with a novel reservoir host cell-isolated Puumala hantavirus**

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Hantaviruses are zoonotic pathogens that cause transient human infections often leading to severe immunologically mediated illness. This is in contrast to hantavirus reservoir hosts where the infections are persistent and without pathological changes. While the immunological response of humans to hantavirus infection is relatively well characterised, the mechanisms facilitating virus persistence in their reservoir hosts are less explored. Here we report on a study using experimental infections to investigate the ability of Puumala hantavirus to induce immunoregulatory responses in the bank vole (*Myodes glareolus*), its reservoir host, and examined adaptive cellular and humoral responses by quantifying changes in T-cell related gene expression in the spleen and IgG responses in the blood, respectively. Since existing Vero E6-cell adapted hantavirus isolates are believed to have lost their wild-type infection characteristics, infections were carried out with a novel Puumala hantavirus isolated on a bank vole cell line. We found that only minor genetic changes occurred during the isolation process, and that the new isolate did produce infections similar to natural vole infections. A delayed virus-specific humoral response occurred in experimentally infected animals, which could allow for more efficient virus replication and the establishment of persistent infection. Furthermore, infections on bank vole splenocytes in vitro demonstrated that Puumala hantavirus infection in its reservoir host promotes immunoregulatory responses by inducing IL-10, a cytokine strongly associated with chronic infections. Our results indicate that hantavirus isolation in its reservoir host cells retains natural virus phenotype and that host immunoregulation is likely to play a part in maintaining hantavirus infections in nature.

- ***Orthohantavirus* infection triggers differentiation and subsequent polarization of human blood monocytes into macrophages**

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Orthohantaviruses (HTV) are enveloped RNA viruses, belonging to genus *Orthohantavirus. Puumala orthohantavirus* (PUUV) is pathogenic causing mild to moderate forms of hemorrhagic fever with renal syndrome (HFRS) in Euroasia, an acute viral disease. *Tula orthohantavirus* (TULV) is considered apathogenic due to limited evidence of pathogenesis in humans. In Croatia, HFRS is endemic rodent-borne disease of high public health importance. Outbreaks affecting the continental part of the country occur every few years. Monocytes, macrophages and dendritic cells (DC), as innate immune system cells, are target cells for HTV potentially contributing to dissemination of the virus in the body and development of the disease. Cytokines are considered to play role in HFRS immunopathogenesis and possibly may drive different influence on clinical picture. The mechanisms which lead to HFRS development are still very poorly understood and little is known about the host immune response to the orthohantavirus infection.

The aim of this *in vitro* study was to analyze the expression dynamics of selected cell surface molecules on monocytes infected with PUUV or TULV, as well as the secretion patterns of selected cytokines and chemokines, in order to investigate whether HTV infection triggers differentiation of monocytes and subsequent cell polarization.

Primary human monocytes were infected in vitro with PUUV or TULV and cultured for seven days post infection. Immunophenotyping was done in three time points using in-house created polychromatic screening panel with antibodies specific for cell surface molecules on monocytes, macrophages and DC. Expression levels of 21 cytokines/chemokines in cell culture supernatants in six time points were determined by multiplex immunoassays with magnetic beads.

Differences have been identified between infected and uninfected cells in cell surface molecules expression levels and concentrations of some cytokines and chemokines in supernatants (TNF-a, IL-1RA, IL-6, CCL2, CCL22, CD206). Differences were also found between infections with pathogenic and apathogenic HTV (CXCL8, CXCL10, CD206). Our findings indicate that HTV induce activation and differentiation of primary human monocytes toward macrophages in the time course.

VIRUS PHYLOGENY, REPLICATION AND MORPHOGENESIS - LIGHTNING TALKS

• Delineating the interplay of host factors in New World hantavirus entry into endothelial cells

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Hantaviruses are globally emerging pathogens causing two distinct zoonotic diseases in humans: hemorrhagic fever with renal syndrome (HFRS) in the Old World and hantavirus pulmonary syndrome (HPS) in the New World. HPS is highly lethal (40%) and higher hantaviruses incidence is likely due to climate change. However, no FDA-approved vaccines or antivirals are available and their development is challenged by crucial gaps in our understanding of hantavirus entry. Our lab recently identified protocadherin-1 (PCDH1) as a critical determinant of attachment, entry, and infection by New World hantaviruses. In addition, integrins (β1, β2, and β3), decay accelerating factor (DAF/CD55) and gC1qR/p32 have been proposed as hantavirus receptors. We hypothesize that the described host factors act as receptors/co-receptors to orchestrate hantavirus entry. Because hantaviruses target capillary endothelial pulmonary cells in vivo, primary human umbilical or pulmonary endothelial cells are considered the gold standards for in vitro studies. However, these primary cells are not amenable to efficient genetic engineering. Here, we employed an immortalized human pulmonary microvascular endothelial cell (HPMEC) line, HPMEC-ST1.6R, to establish a tractable in vitro model to study hantavirus entry and infection. Using CRISPR/Cas9 genome engineering, we generated clonal cell lines deficient in PCDH1, αvβ3 integrin, and DAF to investigate their specific roles in hantavirus entry. Because hantaviruses may use more than one receptor, we also generated double-knockout cell clones. Together with receptor-specific antibodies, soluble receptors and recombinant vesicular stomatitis viruses encoding hantavirus Gn/Gc glycoproteins, these knockouts and their respective cDNA-complemented cell lines provide a unique opportunity to dissect the interplay of proposed receptors in hantavirus attachment, internalization and membrane fusion.

- ## Insights into Andes virus mRNA synthesis

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Andes virus (ANDV) has a tri-segmented single-stranded RNA (ssRNA) genome with negative polarity. Thus, the synthesis of viral mRNA can be considered a limiting step in the early stages of its life cycle. The presence of 5´-capped extensions derived from the host mRNA in the viral transcripts suggests the use of a cap-snatching mechanism to initiate transcription. However, the cytoplasmic confinement of all RNA species implies the use of a novel non-canonical system.

We have identified the 200 residues at the N-terminus of ANDV L protein as the endonuclease involved in the cap-snatching process. Due to difficulties in the production of the soluble wild-type protein, we worked with single amino acid mutants. These variants were assessed for their thermal stability and nuclease activity. Despite the absence of a 7-methylguanosine cap in the substrate, the unit exhibited an Mn2+-dependent endoribonuclease with specificity for ssRNA. To evaluate the molecular similarities between equivalent domains, we solved the structures of mutants K127A and K124A. The superimposition of both structures generated a pseudo wild-type (wt) model of the protein. The tridimensional arrangement of atoms revealed an active site that is conserved throughout evolution and contributed to elucidate the roles played by the mutated residues. Together, our functional and structural data add a piece of the puzzle to comprehend how the transcriptional machinery of orthohantaviruses works.

- **Identifying the patterns and drivers of Puumala hantavirus enzootic dynamics using reservoir sampling**

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Hantaviruses are zoonotic hemorrhagic fever viruses for which prevention of human spillover remains the first priority in disease management. Tailored intervention measures require an understanding of the drivers of enzootic dynamics, commonly inferred from distorted human incidence data. Coupling sequence data with epidemiological metadata can provide more insights into spatiotemporal dispersal during hantavirus enzootic expansion, but this critically depends on the availability of reliable substitution rate information. Here, we use longitudinal sampling of approximately three decades of *Puumala orthohantavirus* (PUUV) evolution in isolated reservoir populations to estimate PUUV evolutionary rates and apply these to study the impact of environmental factors on viral spread. We find that PUUV accumulates genetic changes at a rate of ~10-4 substitutions per site per year and that land cover type defines the dispersal dynamics of PUUV, with forests facilitating and croplands impeding virus spread. By providing reliable short-term PUUV evolutionary rate estimates, this work facilitates the evaluation of spatial risk heterogeneity starting from timed phylogeographic reconstructions based on virus sampling in its animal reservoir, thereby side-stepping the need for difficult-to-collect human disease incidence data. These results can assist in identifying intervention points and improve the efficiency of public health resource allocations.

- **Complete genome sequences from hantavirus pulmonary syndrome cases involved in person-to-person transmission, southwestern Argentina, 2014**

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Context: In 1995, 3 clustered cases of hantavirus pulmonary syndrome in the Andean region of Patagonia led to the characterization of Andes virus (ANDV) in Argentina, since then around 1200 cases have been reported in the country. Hantaviruses are enveloped single-stranded RNA viruses with tripartite negative sense genomes: S (small, 1.8-2.1kb), M (medium, 3.6-3.8kb), L (large, 6.5-6.7kb). They are maintained in nature by small mammals and humans usually become infected through inhalation of aerosolized excreta produced by infected rodents. Among all hantaviruses known worldwide, ANDV is unique due to its ability to be transmitted from person-to-person.

Objectives: The aim of this study was to analyze and compare complete viral genome sequences from a cluster of 3 cases reported in 2014 and suspected of person-to-person transmission from El Bolsón, Rio Negro.

Methods: Two patients living in the same house began symptoms with a difference of 15-days from each other (P1, P2), and a third case (P3) who only performed medical assistance to one of those patients during their prodromal phase. Not related cases were also included in the analysis. Next-generation sequencing techniques were used. RNA sequencing libraries were prepared and viral enrichment was performed by specific probes designed for ANDV. FASTQ files were analysed by in house pipelines for viral genomes. Nucleotide sequence analysis was performed with Mega 6 and BioEdit v7.0.5.3.

Results: Viral genetic analysis showed that P1 presented 100% nucleotide identity in the complete S and M segments with P2 and P3, but 2 nucleotide changes were detected in the L segment (99.96% of nucleotide identity in the L segment, from which 84% could be sequenced). However, P2 and P3 showed 100% nucleotide identity in the complete S, M and L segments.

Conclusions: We could confirm person-to-person transmission among P2 and P3 (100% nucleotide identity). To accurately define if P2 was infected by co-exposure or by person-to-person transmission more complete genomes of ANDV are needed to accurately establish the nucleotide variation rate in person to person transmission chains and also within the natural rodent reservoir population. These will help to differentiate between rodent exposure and person-to-person transmission mechanism.

- **Phylogeography of American rodent-borne hantaviruses**

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Hantaviruses (*Bunyavirales, Hantaviridae*) are important zoonotic pathogens. They are associated with numerous species of rodents, shrews, moles and bats. Because of the great diversity of their reservoir hosts, hantaviruses are excellent models to evaluate the dynamics of virus-host co-evolution. To understand the mechanisms behind the evolutionary history of hantaviruses through virus-reservoir interactions, it is important to know how the radiation and diversity of hantaviruses occurred. In this work, we used phylogenetic analyses involving complete S segment sequences of hantaviruses from different regions, focusing on the American continent, to infer the genetic and geographic relations between rodent-borne hantaviruses and their reservoirs. The phylogenetic relationships of the complete S segments were estimated using (a) ML phylogenetic inference and (b) a Bayesian Markov Chain Monte Carlo (MCMC) method. Phylogenetic analyses revealed a high degree of phylogeographic structure and a surprising pattern of geographical distribution of New World hantaviruses. In order to analyze possible recombination events, the sequence alignment was analyzed with Bootscan, implemented in Simplot and RDP4. The sequences for Bootscan analysis were grouped according to clustering of the nominal taxa seen in the phylogenetic tree for each sequence. The available data suggest that hantaviruses related to the Arvicolinae rodent subfamily in North America probably emerged and initially adapted from a shared common ancestor of the Tula virus. The first clade of hantaviruses associated with Neotominae occupied a stem lineage, especially those that emerged in Central America or Mexico. Hantaviruses from Central America and Mexico found in Neotominae rodents spread northward and probably gave rise to the first phylogroup of hantaviruses associated with Sigmodontinae in North America. The sequences for bootscan analysis were evaluated one by one and grouped according to clustering seen in the phylogenetic tree based on the complete S segment sequences, but no sign of recombination was found. Two preferential host-switching transmissions in hantaviruses apparently gave rise to two different paraphyletic group in Neotominae and Sigmodontinae. Our study supports a probable epicenter of diversification in Central America and/or Mexico for hantaviruses related to both the Neotominae and Sigmodontinae subfamilies.

- **Imprint of negative selection evidenced on hantaviral S genetic segment**

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Context: Hantaviruses, members of the order *Bunyavirales*, family *Hantaviridae*, are enveloped viruses with a negative sense three-segmented RNA genome. Genetic drift, homologous recombination and reassortment have been main proposed mechanisms for genetic diversity of hantaviruses. However, natural selection is also known to be important mechanism for hantavirus evolution. Diversity of hantaviruses has been shaped by negative selection, resulting in proportionally less substitutions affecting phenotype than expected.

Objectives: The aim of the study was to estimate the overall selection pressure in both, DOBV and TULV S segment sequences.

Methods: The study included two datasets; first consisting of 151 DOBV S segment sequences and the second consisting of 137 TULV S segment sequences. Both datasets were aligned using MEGA 6 software package. To examine the nature of codon selection, the single likelihood ancestor counting (SLAC) method based on NJ tree was employed.

Results: The number of examined aa sites in DOBV alignment, using per-site SLAC analysis, was 164. At the protein level, there were 22 amino acid variable sites. The overall value of the dN/dS ratio based on SLAC analysis was dN/dS = 0.0225, log L= - 4653.26 for $p < 0.1$. Number of calculated codon sites under negative selection was 118. The analyzed number of sites in the TULV alignment was 181. At the protein level, there were 51 amino acid variable sites. The overall value of the dN/dS ratio based on SLAC analysis was dN/dS = 0.0197, log L= -10470.52 for $p < 0.1$. Number of calculated codon sites under negative selection was 170.

Conclusion: The type of detected aa changes and evidence of negative selection ($p < 0.1$) in action on this region both imply a high tendency for protein structure conservation, most probably linked to its function related to RNP assembly. The analyzed N protein region in both datasets comprised 15 highly conserved lysines/arginines residues.

EPIDEMIOLOGY - LIGHTNING TALKS

• A human Dobrava-Belgrade virus infection in France

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Background: Three zoonotic hantavirus species have been reported in France: Puumala, Seoul and Tula viruses. An average of 100 human cases is detected per year, mostly associated with Puumala virus, and generally in the North-East part of France. Here we report the case of a patient presenting an acute kidney injury caused by Dobrava-Belgrade virus (DOBV).

The case: A 48-year-old male patient with a history of hypertension presented in hospital 3 days post day of onset (D4) with a fever at 39°C associated with an abdominal clinical picture. The examination found a naked fever without point of infectious call. The biological assessment showed a hepatic cytolysis without cholestasis. No inflammatory syndrome was noticed while a persistent thrombocytopenia was observed. The evolution was marked by an acute kidney injury, serum creatinine rising from 50 to 680 µmol/L. There was a major proteinuria, significant hematuria and leukocyturia. The patient developed significant headache episodes and skin rash. Interestingly, a percutaneous renal biopsy exhibited a macroscopically hemorrhagic medulla suggesting a hantavirus infection. Histology showed acute hemorrhagic interstitial nephritis. An initial oliguric phase was followed by a polyuric episode five days after. The evolution was rapidly favorable without need of dialysis.

The hantavirus infection was confirmed by the detection in a serum sample collected at D12 of IgM and IgG against Seoul virus antigens. However, using a Nested RT-PCR assay, a DOBV strain was detected retrospectively in a blood sample collected at D9.

The patient was a plumber living near Lyon. He spent one month in Bulgaria and returned in France 3 weeks before D1, suggesting an importation of the virus.

Conclusion: This is the first detection of DOBV in France. Epidemiological and virological investigations are currently carried out to look for exposure and confirm the importation of the virus or not (the rodent Apodemus flavicollis, one of the virus reservoir, is reported in this part of France). The macroscopic haemorrhagic appearance of the renal biopsy played an important role in suggesting the diagnosis. Acute kidney damage caused by hantavirus infections remains a diagnostic challenge.

- **Andes hantavirus mother to child transmission and role of breastfeeding: epidemiological and virological data**

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Andes orthohantavirus (ANDV) is the etiological agent of hanta cardiopulmonary syndrome (HCPS) in Chile. ANDV is acquired by inhalation of *Oligoryzomys longicaudatus* ´s infected feces and is the only hantavirus transmitted person-to-person.

Aim: Present two cases of ANDV person-to-person transmission from mother to breastfed children.

Methods: Two pairs “mother and child” infected with ANDV were included. Clinical and epidemiological data were registered. ANDV RT-PCR was performed in blood, and for one woman, in breast milk.

Results: Pair-A: A 30 years-old woman was hospitalized after four days of fever, night sweats and weakness. She died 48h later with hantavirus cardiopulmonary syndrome. After 20 days of her first symptoms, her 9-month-old baby developed fever, and presented two tonic-clonic seizures. He was hospitalized with HCPS and discharged after 13 days. ANDV was positive by RT-PCR in blood, respiratory secretion, urine during the acute-phase. Concerning epidemiological data, his mother closely cared and breastfed him when she was symptomatic before hospital admission. Both also visited for one night a rural area 29 and 49 days before onset of symptoms respectively. Pair-B: A 21 years old woman was hospitalized after eight days of fever, headache and myalgia. She had a mild presentation of hantavirus infection and was discharged after one week. ANDV-genome was detected in blood and breastmilk samples 2 weeks after her admission. Since the mother´s diagnosis, her 22 days-old newborn was hospitalized in isolation and followed with weekly ANDV RT-PCR in blood. Viremia was detected at day 18 of incubation period (IP), when she was still asymptomatic. After 2 days she developed HCPS and 4 days later she died. Virologic test in acute-phase showed ANDV-genome in blood, respiratory secretions and urine samples.

Conclusion: Here we highlight the ability of ANDV to be transmitted by person-to-person route. Pair A was exposed to environment risk, but incubation time was beyond the known IP. However, for baby´s couple B the only risk was close contact with her mother and breastfeeding. Saliva or respiratory secretion can be the vehicle of transmission, however breastmilk and the gastrointestinal port-of-entry cannot be rule out as responsible of infection. (Fondecyt 1161197, PIA ACT1408).

- **A small-scale epidemiologic survey and phylogenetic analysis of rodent- and shrew-borne orthohantaviruses in Western Poland**

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Context: Rodents and shrews of multiple species harbor orthohantaviruses (family *Hantaviridae*). In Eurasia, *Dobrava-Belgrade orthohantavirus* (DOBV) is hosted by the yellow-necked mouse (*Apodemus flavicollis*), striped field mouse (*A. agrarius*), and Black Sea field mouse (*A. ponticus*); *Puumala orthohantavirus* (PUUV) by the bank vole (*Myodes glareolus*); and *Seewis orthohantavirus* (SWSV) by the common shrew (*Sorex araneus*). DOBV, PUUV and SWSV are widely distributed across the geographic ranges of their reservoir hosts. Limited data are available about DOBV, PUUV and SWSV in western and northern Poland.

Objectives: This small-scale epidemiologic survey investigated the geographic distribution and molecular phylogeny of rodent- and shrew-borne orthohantaviruses in western and northern Poland.

Methods: A total of 42 *A. agrarius*, 25 *A. flavicollis*, 27 *M. glareolus* and 10 *S. araneus* were captured between 2009 and 2014 in northern Poland (Gdańsk in Pomeranian province) and western Poland (Osobowice and Milicz in Lower Silesia province). RNAlater®-preserved lung or spleen tissues were analyzed for orthohantavirus RNA by reverse transcription-polymerase chain reaction, using specific primers for DOBV, PUUV and SWSV. Phylogenetic analyses were performed by the maximum likelihood method.

Results: DOBV was detected in 4.8% (2/42) *A. agrarius* and SWSV in 30% (3/10) *S. araneus*, captured in recreation areas in western Poland. *A. flavicollis* and *M. glareolus* tested negative for orthohantaviruses. Phylogenetic analyses of partial L and S segments of DOBV showed a shared common ancestry with the Kurkino genotype from Slovakia, Russia and Hungary, while phylogenetic relationships based on the SWSV L and S segments demonstrated a genetic lineage with SWSV strains from central Poland (Kurowice and Huta Dłutowska), Czech Republic, and Germany.

Conclusions: DOBV has been reported in *A. flavicollis* from southeastern Poland, PUUV from northeastern Poland, and SWSV from central Poland. This study is the first to report DOBV in *A. agrarius* in Poland and SWSV in *S. araneus* in western Poland. Continued surveillance and large-scale investigations will aid in understanding the epidemiology, phylogeny, and disease risk of rodent- and shrew-borne orthohantaviruses in Poland.

- **Castelo dos Sonhos hantavirus: an unnoted etiological agent of HPS in Brazilian Amazon**

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Hantaviruses are etiological agents of hantavirus pulmonary syndrome (HPS), an emerging zoonotic disease. In Brazil, HPS has been reported with increasing frequency in some regions, but information about viral genetic identification is still limited, as diagnostic rely in serological findings. To date a total of nine hantavirus genotypes were reported in Brazil, six related to HPS cases. Castelo dos Sonhos virus (CASV) was first detected from a fatal HPS from Pará state, Amazonian region, in 1995 and further studies this virus found in a species of pygmy rice rat (*Oligoryzomys utiaritensis*). Since 1995, only eight additional cases of CASV have been reported, all from Brazilian Amazon region. In this study, molecular analysis was performed in order to characterize HPS causing hantaviruses from Mato Grosso state, Brazil during 2015 and 2018. A hundred and eighteen serum samples of HPS suspected cases were included in the study. Partial hantaviral S segment were PCR amplified producing 460nt amplicons. Hantavirus specific PCR products were DNA sequenced in the obtained sequences phylogenetically confirmed and analyzed. CASV was identified in HPS cases. Confirmed cases ranged from 9 to 49 years, four were male, reporting to be enrolled in agriculture, mining or outdoor activities (fishing and hunting). All patients needed to be hospitalized, presenting fever, dyspnea and acute respiratory failure. Non-specific laboratory tests showed increased serum creatinine and urea levels, hemoconcentration and thrombocytopenia. Gastrointestinal tract manifestations were found in >50% of the cases, potentially confounding diagnosis and leading to inappropriate therapy, five cases were treat with antibiotics. The case-fatality rate was 50%. The results of this work highlight the emergence of new hantavirus genotype, indicating CASV as an important HPS causing virus in Brazilian Amazon. We also emphasize the need for molecular confirmation of the hantaviruses genotypes of human cases for a better understanding of the mortality-related factors associated with HPS cases in Brazil.

- **Molecular detection of *Puumala orthohantavirus*: struggling with high nucleotide sequence variability**

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Puumala orthohantavirus (PUUV), member of the family *Hantaviridae*, is the most common causative agent of hemorrhagic fever with renal syndrome (HFRS) in Europe. To date, eight genetic lineages of PUUV have been described, all associated with the bank vole (*Myodes glareolus*) as reservoir. Our investigations in Germany showed that PUUV of the Central European lineage is associated with the presence of the Western bank vole phylogroup. Human and reservoir host PUUV infections are absent in northern and northeastern Germany, but occur in western and southern areas of the country. In contrast, bank voles in Poland belong to the Carpathian and Eastern evolutionary lineages carrying the Latvian and Russian PUUV lineages. The nucleotide sequence divergence within the S-segment of different PUUV strains reaches up to approx. 20% causing problems to select primer and probe sequences for the detection of all PUUV lineages with one PUUV real-time RT-PCR. Currently, we are evaluating a real-time RT-PCR assay for the detection of PUUV strains of different origin. Perspectively, we intend to design a diagnostic platform allowing comprehensive and timesaving molecular bed-side and pen-side diagnostics of PUUV and pathogens causing similar symptoms.

VACCINES, THERAPEUTICS AND PREVENTION - LIGHTNING TALKS

• TIM-1 contributes to entry of HTNV in human airway epithelial cells

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Context: Hantaviruses are emerging human pathogens leading to serious diseases and represent a challenge for public health. Currently there are no effective antivirals, vaccines, or immuno-therapeutics to treat hantavirus diseases. The development of novel strategies for anti-viral therapeutic intervention is therefore an urgent need. The identification of cellular factors required by hantaviruses to enter host cells is a promising approach for the development of novel strategies to combat pathogenic hantaviruses.

Objectives: Viral attachment and entry represent the first steps in virus transmission and are promising targets for anti-viral therapeutic intervention. We investigated the largely unknown receptor used in human airway epithelium of the Old World prototypic hantavirus Hantaan virus (HTNV) and the South American Andes virus (ANDV). We hypothesized that hantaviruses may use apoptotic mimicry to invade human respiratory epithelial cells and examined the role of the cellular phosphatidylserine (PS) receptors of the T-cell immunoglobulin and mucin (TIM) and Tyro3/Axl/Mer (TAM) family.

Methods: Since hantavirus cell attachment and entry are mediated exclusively by the viral envelope, we employed a validated pseudotype system based on replication-competent and propagation-deficient recombinant vesicular stomatitis virus (VSV) bearing the glycoprotein of HTNV and ANDV.

Results: Combining antibody perturbation with specific inhibitors, and other techniques, we provide evidences for differential receptor use by HTNV and ANDV in human respiratory epithelial cells. TIM-1 strongly contributes to entry of HTNV and to a lesser extend in ANDV. In line with previous studies, HTNV, but not ANDV was able to use the glycosaminoglycan heparan sulfate and αvβ3 integrin as co-receptors.

Conclusion: In sum, our studies demonstrate for the first time that hantaviruses use PS receptors and hence apoptotic mimicry to invade human airway epithelium, which may explain why these viruses can easily break the species barrier.

- **Alpaca polyclonal IgG antibodies demonstrate protection against lethal Andes virus hamster infections**

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Context: Hantaviruses remain a global health issue as the number of infections continues to rise from year to year. These viruses were originally thought to be transmitted only through inhalation of infected rodent excreta. However, cases for the South American Andes virus (ANDV) confirm person-to-person transmission. ANDV demonstrates a fatality rate of 30-40% and there is currently no approved treatment or vaccine available. Recent animal studies have documented the potential of using antibodies as an effective treatment.

Objective: The purpose of this study is to evaluate the protective role of alpaca polyclonal IgG antibodies against lethal ANDV infection. Alpaca IgG antibodies include heavy-chain only IgG's, which possess unique properties that demonstrate greater virus recognition and binding than conventional antibodies.

Methods: Three alpacas were vaccinated using a DNA-based vaccine that expresses the ANDV glycoprotein. Over a period of 4 months, plasma was collected from each animal and the neutralizing antibody titre was determined using PRNT assays (Plaque Reduction Neutralization Test). Plasma with a PRNT 80 titre of 1:2560 was purified and evaluated as a treatment source in the lethal Syrian hamster model. Two groups of hamsters were challenged with 150 FFU each of ANDV, and hamsters were given either naïve polyclonal IgG or neutralizing polyclonal IgG at days +1 and +3 post challenge.

Results: All animals treated with neutralizing polyclonal IgG survived the challenge. Tissues and blood collected from 3 hamsters per group were evaluated for viral load using RT-PCR. Results show positive Ct values for the naïve polyclonal IgG-treated animals, while animals treated with neutralizing polyclonal IgG demonstrated negative Ct values.

Conclusion: Neutralizing polyclonal alpaca IgG demonstrates to be an effective treatment for lethal ANDV infections within the Syrian hamster model.

- **Evaluation of adjuvants efficiency in the HFRS vaccine**

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Low immunogenicity and/or short-term duration of the protective effect are known to be key disadvantages of inactivated vaccines. Increasing their immunogenicity is of current interest. Selection of appropriate adjuvants can enhance these vaccines.

The aim of this study was to identify the ability of various adjuvants to improve immunogenicity of the PUUV based vaccine.

We have tested: 1 - vaccine (AG) without adjuvants; 2 - AG+Al (aluminum hydroxide, 1,0 mg/ml); 3 - AG+LPS (low endotoxic lipopolysaccharide derived from the Shigella sonnei, 50 μg/ml); 4 - AG+Al+LPS; 5 - AG+SPs (spherical particles of temperature remodeled Tobacco mosaic virus, 300 μg/ml); 6 - AG+SPs (150 μg/ml); 7 - AG+HLB (heat-labile recombinant protein, 0,2 μg/ml); 8 – 0,85% NaCl (control group). These vaccine variants were investigated undiluted and in 1/8 dilution. To study immunogenicity 7-8 weeks female Balb/c mice were immunized intramuscularly 3 times with a 2-week intervals. FRNT50 and IFA methods were perform for specific antibodies determination. ELISA kits (Bender MedSystems, Cusabio) were used to determine the cytokines IL-1β, IL-12, INF-ɣ.

No side effects were observed when tested vaccine preparations. PUU-TKD strain based vaccine (AG) with a specific activity of 3.8×105 copies of RNA/ml, 8 times diluted, provided neutralizing antibodies production in 100% of immunized mice with an average titer of 8.67±0.2 log2 and also a strong Th-1 immune response according to IL-1β, IL-12, INF-ɣ rising. AG+Al caused the same immune response like AG. SPs are not acceptable as an adjuvant, since the immune stimulating effect disappears when their concentration decreased below 300 μg/ml.

HLB and LPS may be considered as promising adjuvants: their presence in the vaccine contributed to an increase of the neutralizing antibodies geometric mean titer in 2.2 times as compared to AG. An additional advantage of LPS is that it has been tested in the clinical trials that confirmed its safety.

• Pre-clinical studies of inactivated combined HFRS vaccine

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Hemorrhagic fever with renal syndrome (HFRS) is the most common natural focal disease in the Russian Federation. About 98% of HFRS cases associated with the Puumala virus (PUUV), other HFRS cases are caused by Hantaan (HTNV), Amur, Seoul, Kurkino and Sochi viruses.

We have developed candidate inactivated aluminum hydroxide adsorbed combined vaccine on the basis of the PUU-TDK, HTN-P88 and DOB-Sochi virus strains, propagated in Vero cells. After Vero cells infection (MOI 0.02 – 0.05) culture fluids of each virus separately were collected from day 5 to 9. Culture fluids pool of each virus was clarified by filtration (filter PPG060B01BA, Cuno), followed by virus concentrated by tangential flow filtration (Pellicon 2 mini cassette, Millipore), purified by gel filtration (Sepharose 6FF). Purified stocks of viruses were diluted to 4.5±0.5 lg FFU/ml and inactivated with beta-propiolactone (1:6000) followed by pooled in equal volumes. Before sorption on aluminum the vaccine specific activity has been determined by the RT-PCR with strain specific primers. The number of RNA copies in the vaccine was: PUU-TDK – 2.8±0.3×105, HTN-P88 – 3.6±0.4×104 and DOB-Sochi–1.8±0,5x104. Vaccine contains: total protein – 78 μg/ml (by Lowry's method); cellular DNA – <10 ng/ml (by RT-PCR), aluminum hydroxide – 1.0 mg/ml.

For immunogenicity investigation of vaccine 7-8 weeks female Balb/c mice were immunized intramuscularly 3 times with a 2-week intervals. Blood was taken from eyes of the mice before and 2 weeks after immunization. FRNT50 and IFA methods were performed for specific antibodies determination. ELISA kits (Bender MedSystems, Cusabio) were used to determine the cytokines IL-1β, IL-12, INF-ɣ.

PUUV, HTNV and Sochi virus neutralizing antibodies were identified in 10/10 mice immunized with a 1/4 dilution of the vaccine. Neutralizing antibodies geometric mean titers were: PUUV – 8.2±0.08, HTNV – 7.17±0.09, Sochi virus – 6.09±0.24. IL-1β, IL-12 and INF-ɣ in the blood sera of mice increased on the average 2.5, 4.8 and 40 times respectively, which reflects a pronounced stimulation of Th-1 immunity. The vaccine was well tolerated and safe in experiments on laboratory animals (mice, rats, guinea pigs, rabbits).

- **Chloroquine, an anti-malaria drug as effective prevention for hantavirus infections**

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We investigated whether chloroquine can be used as prevention against hantavirus infections in vitro and in vivo, using the Hantaan virus newborn C57BL/6 mice model and the Syrian hamster model for Andes virus. In vitro antiviral experiments were performed with Vero E6 cells and several hantavirus species. Hantavirus genomes were detected using quantitative RT-PCR. For all hantavirus species tested, results indicate that the inhibitory concentration (IC50) of chloroquine (mean 10.2 ± 1.43 µM) was significantly lower than the CC50 (mean 260 ± 2.52 µM), yielding an overall selectivity index of 25.5. We also investigated whether chloroquine could prevent death in newborn mice after Hantaan virus infection and for its antiviral effect in the hantavirus Syrian hamster model. Therefore, C57Bl/6 mother mice were treated subcutaneously with daily doses of chloroquine. Consequently, 1-day-old suckling mice were inoculated intracerebrally with 5 x 102 Hantaan virus genomes. In litters of untreated mothers, none of the pups survived challenge. The highest survival rate (72.7% of pups) was found when mother mice were treated with a concentration of 10 mg/kg chloroquine. Survival rates declined in a dose dependent manner, with 47.6% survival when treated with 5 mg/kg chloroquine, and 4.2% when treated with 1 mg/kg chloroquine. Assessing the antiviral therapeutic and prophylactic effect of chloroquine in the Syrian hamster model was done using 2 different administration routes (intraperitoneal and subcutaneous using a small osmotic release vessel). A delay in onset of disease was noted and for the small release vessel, 60 % survival was observed. Our results show that chloroquine can be highly effective against Hantaan virus infection in newborn mice and Andes virus in Syrian hamsters.

CLINICAL ASPECTS AND DIAGNOSIS - LIGHTNING TALKS

• Hantavirus infections and Crimean-Congo hemorrhagic fever in Bulgaria

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Context: Hantavirus infections and Crimean-Congo hemorrhagic fever (CCHF) are two viral hemorrhagic fevers spread in Bulgaria.

Objectives: To reveal current hantavirus and CCHF virus (CCHFV) circulation in the country. To present clinical manifestations and diagnosis of ше two viral hemorrhagic fevers in the country.

Methods: Commercially available ELISA kits, hantavirus immunoblot test, TaqMan RT-PCR

Results: Hantavirus seroprevalence in the country, based on results of the immunoblot, was estimated as 3.1% and was much higher for Puumala virus (PUUV) than for Dobrava-Belgrade virus (DOBV). Contact with livestock was found as a risk factor. So far in Bulgaria, only DOBV infections have been confirmed by PCR and sequencing. However, using immunoblot test, out of 23 patients with hemorrhagic fever with renal syndrome (HFRS), diagnosed within two years in Bulgaria, DOBV was detected as etiological agent in 16 (70%) patients and PUUV in 30% patients. Comparative analysis of clinical manifestations of DOBV and PUUV infections revealed that patients with DOBV infection were much more likely to present arthromyalgia, severe headache, severe asthenoadynamia, abdominal pain, vomiting, hypotension and nervous system disorders and required more often and for a longer time hemodialysis than patients with PUUV infection. CCHFV seroprevalence rate of 3.7% was detected. Anamnesis for tick bites, contact with livestock, age over 40 years and residency in Southeastern Bulgaria were found as risk factors. Molecular analysis of CCHFV strains isolated from patients in the country showed that they belong to lineage Europe 1, while CCHFV isolated from Rhipicephalus sanguineus s.l. ticks clustered in Europe 2 lineage.

Conclusion: Results of the seroprevalence studies showed that although rare, CCHF and hantavirus infections appeared to be much more wide-spread in the country than previously thought but may often present with mild manifestation or even go asymptomatic. Increased awareness of various forms of HFRS and CCHF among physicians is needed.

- ## Development of a pan-hantavirus enrichment protocol for next generation sequencing

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Context: The family *Hantaviridae* exhibits worldwide distribution and is carried by numerous rodents, moles, shrews and insecticivous bats. Given this broad temporal and geographic distribution, the family *Hantaviridae* is estimated to be 20 to 30 million years old and likely co-evolves with their hosts. Hantaviruses are generally categorized into New World and Old World strains based on the geographic distribution of the rodent reservoir, however, given the ancient nature of hantaviruses, the possibility exists that additional species exist, which have not been detected or fully characterized.

Objectives: Hantaviruses are difficult to isolate by cell culture (although a limited number of hantavirus isolates exist), and since we cannot easily culture them, our knowledge of these viruses are based solely on the quality of viral sequence that one can generate. As a viral diagnostic lab, we frequently receive human specimens for diagnostic testing and given the diversity of hantaviruses we wanted to explore whether our diagnostic assay may miss unidentified hantavirus cases.

Methods: To better explore hantavirus diversity, we developed a pan-hantavirus enrichment protocol for next generation sequencing. Using synthetic samples, we assessed the sensitivity of our enrichment assay. We also assessed the specificity of our assay using diverse hantavirus strains sequenced directly from human clinical specimens.

Results and Conclusion: Without any prior knowledge of the hantavirus strain, we can perform full genome sequencing directly from limited clinical material collected from human diagnostic specimens. Herein, we will review the NGS protocol and the phylogeny of hantavirus strains we have sequenced directly from human clinical material.

• Molecular diagnosis of hemorrhagic fever with renal syndrome

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Background: Haemorrhagic fever with renal syndrome (HFRS) is endemic in Slovenia with yearly recorded sporadic cases and seasonal epidemic outbreaks. The diagnosis of HFRS is based on clinical symptoms, epidemiological information and laboratory confirmation. Routine laboratory diagnosis of acute HFRS is mainly based on serology, since in most cases antibodies are already present at the onset of symptoms. There are two major shortcomings in using the serological approach: high cross-reactions among different hantaviruses and misidentification of early cases. Therefore, the detection viral RNA can be a valuable addition to the existing serological approach.

Methods: A prospective study was performed in 2 epidemic years (2012 and 2017), when we tested 1187 HFRS suspected cases. RNA isolation was performed in different clinical samples (serum, plasma, EDTA blood or urine) using automated methods. One-step multiplex qRT-PCR assay for simultaneous detection of both DOBV and PUUV was developed based on the whole S segment of Slovenian strains. Molecular results were correlated with serology results obtained with IgM/IgG EIA or with rapid ReaScan Point-of-Care test.

Results: Using standard serological methodology, 263 patients were found HFRS positive (244 PUUV and 19 DOBV) and 249 (94.7%) were also positive with multiplex RT-PCR. Additionally, we detected PUUV RNA in 4 patients which were initially serologically negative and seroconverted 3 days later. Moreover, the correlation between PCR and ReaScan POC was 98%. Viral load in PUUV-infected patients was ranging from 0.79 - 6.74 log10 RNA/ml and in DOBV-infected patients from 1.54 - 8.63 log10 copies RNA/ml. In acute HFRS stage, viral RNA was detected in all sample types, but urine was positive only in 12% of tested samples.

Conclusions: A molecular diagnosis of HFRS is a very useful tool for routine diagnostics in epidemics, when early appearance of cases is expected and, especially in areas, where different hantaviruses are circulating.

- **Case report: severe Seoul hantavirus infection during peripartum period**

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We present the case of severe Seoul hantavirus infection which onset concurrented with peripartum period. The 36-year-old pregnant woman, with complicated obstetric and gynecological anamnesis was admitted to the hospital with complaints for lower abdominal pain, backache, anuria, yellowness of the skin and fever 38.6°C during last three days. Diagnosis at admission was: Pregnancy 37-38 weeks, first stage of labor. Severe preeclampsia, HELLP syndrome, multi-organ failure. The patient has residency in a small rural city located in the endemic area of hantavirus infection of Russian Far East. Low platelets level (40x109/l), high serum levels of aminotransferases, creatinine and urea, pathological findings of coagulation tests, slight neutrophilic leukocytosis and anemia partially supported preliminary diagnosis. Ultrasonic examination revealed diffuse changes in the liver and kidneys parenchyma, splenomegaly, effusion into the abdominal and pleural cavities and fluid in the lower pelvis. After spontaneous vaginal delivery the patient's condition worsened, anemia and thrombocytopenia persisted and due to atonic postpartum hemorrhage the hysterectomy was performed. Based on clinical symptoms and epidemiological data the hantavirus infection was supposed and confirmed by detection of specific IgM antibodies (titer 1:400) via ELISA. Hantavirus RNA was isolated from patient blood, L-segment fragment (2969-3314 bp) was subsequently sequenced, and genotype Vladivostok of Seoul virus was identified as ethiological agent. After 2 months of the patient’s intensive care (including but not limited repetitive plasma exchanges and hemodialysis) her condition gradually improved. By the time of release, the coagulation system parameters were near normal with moderate anaemia (red blood cells 2.96x1012 g/l, hemoglobin 90 g/l, hematocrit 26.7%) and lymphocytosis. However, signs of renal failure (serum creatinine level 366 mmol / l, serum urea level 15.34 mmol / l, proteinuria 0.3 g / l) persisted. The final diagnosis: Severe hantavirus infection, renal-liver-failure. Acute renal damage stage 3 according to AKIN, class I by RIFLE. Symptomatic arterial hypertension. Secondary anemia. This case is a fascinating example of how difficult to differentiate hantavirus infection during peripartum with other pathological conditions in patients with high-risk pregnancy and delivery. The case also reinforces the fact that genotype Vladivostok of Seoul virus can cause severe, life threatening disease with multisystem failure.

- **Next-generation approaches for full-length sequencing *Prospect Hill orthohantavirus***

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Next-generation sequencing (NGS) provides researchers the ability to rapidly and accurately identify virus strains circulating in clinical or environmental samples, or for basic research. In this study, *Prospect Hill orthohantavirus* (PHV), a BSL-2 pathogen, was used to develop NGS approaches with various applications. We will present methods developed for the Oxford Nanopore Technologies MinION and the Illumina MiSeq sequencers. For each of these platforms, we used purified PHV genomic RNA (vRNA) as starting material. We examined three amplification approaches, cDNA, 7-cycle PCR and 30-cycle PCR, and two pooling approaches using an amplicon tiling scheme. Libraries were assessed for quality reads and depth. MiSeq results showed that directly sequencing cDNA gave the lowest vRNA coverage (51-78%), while the 7-cycles produced much greater coverage (93-95%). The best coverage (97-100%) was demonstrated with the 30-cycles of amplification, however, this approach had greater PCR and primer bias which also resulted in unequal genome coverage. For the MinION, multiplexing of primers showed an equal genome coverage for the cDNA and 7-cycle, which varied by segment (44-99%). The 30-cycle PCR led to much higher coverage across all segments (92-99%). Both platforms suggested that the best amplification method for detection was the 30-cycle PCR and a multiplex-primer approach. Lastly, we tested the ability of the MinION platform to directly sequence PHV vRNA. Using <1% of the total reads available, we were able to cover 76% of the PHV genome (56% L segment, 100% of M and S segments). In summary, the methods developed should be applicable for detection of hantaviruses in clinical and environmental samples. We are currently evaluating our approaches for studies of intrahost and interhost genetic variation.

Poster Presentations

Please note that all lighting talks are also presented as a poster. For the detailed abstracts, please consult the lightning talk abstracts.

- **Ecoepidemiology of hantavirus diseases in Panamá, 1999-2019**

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Context: Hantavirus pulmonary syndrome (HPS) in Panama is caused by the Choclo virus, which is transmitted by the rodent *Oligoryzomys fulvescens* of the subfamily Sigmodontinae. The first outbreak of hantavirus occurred between the end of 1999 and the beginning of 2000.

Objective: To strengthen epidemiological surveillance of hantavirus in Panama for control and prevention of disease.

Methods: From 1999 to 2019, cases of hantavirus disease were included in this study following protocols established by the Ministry of Health of Panama. Human sampling was conducted in rural communities during and after outbreaks in agroecosystems in which hantavirus is endemic. Concurrently, the ecology, dynamic population and hantavirus prevalence of the rodent community were characterized. The human and rodent samples were analyzed by enzyme-linked immunosorbent assay (EIA), strip immunoblot assay (SIA) and reverse transcription polymerase chain reaction (RT-PCR).

Results: During a period of 20 years, 335 cases of HPS have been reported in west-central Panama with an accumulated case-fatality rate of 14.6%(49/335). The prevalence of IgG antibodies is high (32.9%), varying from 16.5% and 60.4% in rural communities of Los Santos with an annual increase of 5%(P=0.0014). The ratio of total HV infections to moderately severe HPS of 9:1 was similar to the ratio of annual seroconversions to hospitalized HPS of 14:1 calculated in these communities. The O. fulvescens has a monophyletic lineage and has been captured consistently in hantavirus-endemic agroecosystems in west-central Panama.

Conclusion: Fundamental evidence has been generated in terms of public health that has allowed the comprehensive management of hantavirus disease through: i) training of health personnel in primary care (2000), ii) the decentralization of the IgM-ELISA test, which has allowed physicians to confirm the diagnosis for proper management (2008), iii) the preparation of guidelines and norms to strengthen surveillance (2016), and iv) the establishment of a warning system based on the dynamics population of rodents at the microscale level (2006). The study of this zoonosis established an important paradigm and the next phase is to develop cost-effective interventions at the community level with a One Health approach to the control and prevention of hantavirus disease and other zoonoses.

• Identification of novel parechovirus and paramyxoviruses in bank voles in HFRS-endemic areas in Russia

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Hemorrhagic fever with renal syndrome (HFRS) is one of the most important zoonotic disease. HFRS caused by hantaviruses and is transmitted by respiratory or alimentary routes with rodent secrets. The main natural reservoir of HFRS is voles (*Myodes glareolus, Microtus arvalis*) and mice (*Apodemus* spp.). The viruses, which have the similar to hantaviruses ecology, are of particular interest, since they are constantly exposed to humans.

Here we report results of genetic and phylogenetic studies of novel virus, named Oltush virus (OLTV), isolated from kidney tissues of bank vole (*M. glareolus*) in HFRS-endemic areas in Russia. Genome of OLTV was sequenced by NGS and subsequent analysis revealed that it is a new member of the genus *Parechovirus* (*Picornaviridae*). Comparative analysis has shown greatest similarity (82% n. o. comparing full genomes) of OLTV with Ljungan virus, isolated from bank voles in Sweden.

Second part of the work was devoted to the study of prevalence of previously described Bank vole virus (BaVV, ungroup *Paramyxoviridae*) in HFRS-endemic regions. We have developed genus-specific PCR primers for detection of BaVV and related paramyxoviruses. The primer set was designed to amplify a conservative region (557 n. o.) of N gene. We tested samples from 169 bank voles from Volga region and found 4 positive samples. These samples were sequenced and genetic analysis revealed that they belong to two new paramyxoviruses different from, but closely related to BaVV. These novel paramyxoviruses have 89% n.o. identities with each other and with BaVV in the analyzed region of the N gene. Phylogenetically the viruses fall in the BaVV lineage alongside with Mossman and Narriva viruses, two other only known members of this group of the rodent paramyxoviruses.

In summary, we have shown that the bank voles distributed in HFRS-endemic regions in Russia harbor diverse population of paramyxoviruses as well as parechoviruses. Since these viruses were isolated from kidneys and lungs of the voles, their ecology and rout of transmission probably are similar to hantaviruses. Their role in human pathology in the region remains to be studied.

- **Detection and sequencing of new french Puumala virus isolates**

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The PALADIN project aims to better understand the evolution and current diversity of a zoonotic virus (Puumala virus, PUUV) circulating in France in bank vole populations and responsible in humans for nephropathy epidemic (NE). A recent study has shown the presence of distinct French PUUV "sublineages" in endemic and non-endemic regions. However, the presence of PUUV has never been investigated in many regions, including in areas between regions where the virus is known to circulate. This prevents to reconstitute precisely the evolutionary history of the virus in France. In this study, we combine rodent sampling, serological analyses and virus sequencing to investigate the epidemiological situation of these areas, analyze PUUV strains diversity and understand the links between PUUV epidemiology and evolutionary history. We already have collected samples from various localities in Franche-Comté, Jura, Loiret, Aube, Alsace and Ardennes. During the project, we will sample bank voles in 3 in-between forests (Morvan, Fontainebleau and Tronçais) where no presence of PUUV has been previously reported. The datas collected during these sampling will be used for phylogeographic analyzes that will provide a better understanding of PUUV spread in recent years. Altogether, these results may highlight some of the genetic features accompanying / favoring PUUV pathogenicity in humans, and their potential geographic variations. We present here the first results of this project concerning the forest of Morvan.

• Functional consequence of atypical B cells in patients with acute Hantavirus infection

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Circulating B cells in healthy individuals comprise a small fraction of CD27-IgD- atypical B cells (ABCs). These cells accumulate in patients with Systemic Lupus Erythematosus (SLE) with nephritis. In vitro studies imply that ABCs are dysfunctional or exhausted but their in vivo biological function remains poorly understood. Hantavirus infections that cause hemorrhagic fever with renal syndrome (HFRS) lead to transient kidney dysfunction in patients, as shown by increased serum creatinine levels. We hypothesized that (i) development of ABCs is associated with reduced kidney function, and (ii) that studies of HFRS could be used to assess if accumulation of ABCs is detrimental to the development of antiviral humoral immunity. Moreover, we assessed a potential mechanism for shedding of CD27.

Using longitudinal HFRS-patient blood samples stratified based on the median creatinine level, we show by flow cytometry that ABCs preferentially accumulate in circulation of patients with high serum creatinine levels. Phenotypical analysis showed that HFRS-induced ABCs had lower expression of activation markers and showed reduced capacity for antigen presentation to T cells. Moreover, we found the same expression pattern of ABC associated surface markers identified for SLE and other chronic infections, also in ABCs in acute HFRS. In addition, these cells had decreased expression of the complement regulatory protein CD55.

Since extracellular ATP can cleave membrane bound CD27 in mice, we hypothesized that also human CD27 could be cleaved. We here demonstrated that addition of extracellular ATP is effectively cleaving CD27 from the cell surface, and that the shedding is blocked when co-incubating with a specific MMP-8 inhibitor. Further, levels of soluble CD27 correlates both with kidney dysfunction and levels of ATP breakdown products in patient plasma. Finally, we found that increased kidney dysfunction in patients was correlated to longitudinal development of Gn/Gc-targetting neutralizing antibodies.

Collectively, this study demonstrates an association between reduced kidney function and accumulation of ABCs in circulation. Moreover, our data shed light on the potential impact that accumulation of circulating ABCs may result in a productive antiviral response in HFRS-patients.

- **Hemorrhagic fever with renal syndrome: outbreak caused by Sochi virus**

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Recently we have discovered Sochi virus, a new genotype of the Dobrava-Belgrade virus, which is carried by the Black Sea Field Mouse (*Apodemus ponticus*) as its natural reservoir. In humans, the virus causes severe HFRS with a case fatality index as high as about 15 percent. This makes Sochi virus the most lethal genotype of *Dobrava-Belgrade orthohantavirus* (DOBV) and the most lethal hantavirus in Europe at all.

Here we describe an HFRS outbreak among seasonal workers of a private construction company in the Blue Bay community (Gelendzhik city, Krasnodar region) in Southern European Russia which has occurred in the autumn of 2013. Detailed clinical and diagnostic data of 3 patients will be presented. After acute febrile onset, they developed gastrointestinal and hemorrhagic symptoms, together with drop in blood pressure. Acute renal failure (3 patients) and acute respiratory failure (2 patients) occurred. In all cases, severe HFRS was found; one patient with a particularly fulminant clinical course died at day 2 after onset. The other patients were discharged from the clinic at day 50 and 12, resp.

ELISA and IFA serology of acute sera showed infection by DOBV. Fine typing of convalescent sera of the two surviving patients demonstrated neutralizing antibodies against Sochi virus with 2-4 fold titer difference when compared to other human pathogenic DOBV genotypes (Kurkino virus, Dobrava virus). In the deceased patient, hantavirus RNA was amplified from heart, lung, liver, kidney, spleen, pancreas, brain, and lymph node. Nucleotide sequence determination of S and L segment regions and their molecular phylogenetic analysis clearly demonstrated infection by Sochi virus and clustering of the sequences with those obtained from A. ponticus trapped in the same geographical region.

Sochi virus infections are a serious human health threat in regions where *A. ponticus* is occurring.

- **Interaction of different viral proteins from pathogenic and non-pathogenic orthohantaviruses with the interferon pathway**

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Orthohantaviruses are emerging viruses of the order *Bunyavirales*. They do not produce any obvious symptoms in their natural rodent hosts but can provoke diseases when occasionally transmitted to humans by aerosol. Clinical traits vary from no symptoms to hemorrhagic fever with renal syndrome (HFRS) in Europe and Asia or hantavirus cardiopulmonary syndrome (HCPS) in the Americas. In some cases, the disease can evolve to death at various rates depending on *Orthohantavirus* species. In the course of infection, viruses have first to antagonize the antiviral interferon (IFN) response (mainly IFN-a/b) to replicate and spread in the infected organism. We checked whether proteins encoded by the pathogenic Puumala virus (PUUV), responsible for many cases of a mild to moderate form of HFRS in Europe, and low or non-pathogenic Tula virus (TULV) or Prospect Hill virus (PHV) could differentially interfere with the IFN signaling pathway. In contrast to many other bunyaviruses, some orthohantaviruses could express a non-structural protein NSs. Indeed, those specifically infecting rodents of the subfamily Arvicolinae (i.e. PUUV, TULV and PHV) possess an open reading frame encoding a short putative NSs which overlaps the nucleocapsid (N) encoding sequence. Moreover, the cytosolic tail of the Gn envelope glycoprotein of orthohantaviruses has been proposed to interact with elements of the IFN pathway. Therefore the N, the putative NSs and the cytosolic tails of Gn of PUUV, TULV and PHV, were cloned in mammalian expression vectors and transfected into HEK293T cells, together with a reporter gene plasmid where the luciferase is expressed under the control of constitutively activated IFN-b promoter. Our results show different levels of inhibition of the IFN pathway by the different viral NSs and N proteins depending on their orthohantavirus origin. We investigated which step(s) of the pathway the viral proteins could affect. A differential antagonistic effect of the RIG-I induced IFN activation was observed at the level of the TBK1 kinase. This could account for differences in the virulence of orthohantaviruses that could rely on the viral protein interacting with human innate immunity.

- ## Colonic mucosa proliferation processes in hemorrhagic fever with renal syndrome

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The present paper presents the findings of morphological, histochemical, morphometric and electron-microscopic study of colonic mucosa (CM) biopsies of rectosigmoid colon from 20 HFRS patients obtained at an early stage of the disease.

CM biopsy specimens of rectosigmoid colon were fixed in 10% neutral formalin and embedded in paraffin. Serial sections were stained by Ehrlich's hematoxylin and eosin; neutral mucopolysaccharides were identified by PAS-reaction, acidic mucopolysaccharides – by Alcian blue staining with additional hematoxylin staining of nuclei. For IHC study, paraffin-embedded sections processed using streptavidin-biotin methods and monoclonal commercial antibodies to Ki-67 antigen were used. Proliferative index was determined after counting of 1000 cells in the average. Research results were assessed using a semi-quantitative method and Mann-Whitney test with Statistics 10.0 software. CM biopsy specimens (10 observations) of normal healthy control male subjects aged 18 to 21 were used for comparison.

A morphological study of distal colonic mucosa in HFRS patients within 10 days from the onset of the disease revealed signs of catarrhal inflammation with single erosions and focal hemorrhage. Histologically, colonic mucosa presented with dystrophy and necroses of enterocytes, vacuolization and desquamation of superficial epithelium, increase in the number of goblet cells with signs of hypersecretion, migration of lymphocytes into foveolar epithelium. Deep mucosa of the colon presented with edema, stases, minute vessel plethora, focal hemorrhage, perivascular lymphoid histiocytic infiltration admixed with plasma cells and neutrophilic leukocytes.

An analysis of proliferation processes in CM showed a marked expression of Ki-67 antigen in the intestinal glandular epithelium. The parameter was statistically higher as compared to the group of control subjects (U Z = -4.09: p=0.0002).

Thus, it is at an early stage of the disease that HFRS agent – Hantanvirus – causes epithelial damage to colonic mucosa and lymphoid-macrophagal infiltration of the stroma. In addition, activation of proliferation processes is observed, which is evidenced by an elevated proliferative index of Ki-67.

• Dynamics of hantavirus infection in complex food-webs

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Human encroachment on wildlife habitats has contributed to the emergence of several zoonoses, such as hantaviruses. The present study evaluates the role of complex food webs in the enzootic cycle of hantavirus. Field studies were carried out in two different biomes of Brazil. The first site comprises extensions of Goiás and Minas Gerais states. Most of these areas are occupied by cattle farms covered with exotic pasture vegetation, although there are small patches of original Cerrado (savanna-like) vegetation. The second site is a research station in the south region of the Pantanal biome, in Mato Grosso do Sul state. Hantavirus antibodies were detected, using an EIA, in animals from both regions with a higher seroprevalence in domestic dogs than in wild carnivores (41.45% vs. 14.29%). In the domestic group, 51 (69.87%) of the 73 dogs from Cerrado biome and 29 (24.37%) of 119 dogs from Pantanal biome were reactive to Andes hantavirus recombinant nucleoprotein. Sera from 80 wild carnivores included in this study were tested, and 11 (13.75%) showed reactivity. Eight (21.05%) crab-eating fox (*Cerdocyon thous*) of 38 examined animals had hantavirus IgG antibodies, seven from Cerrado and one from Pantanal biomes. In Cerrado biome, were also captured 11 specimens of Hoary fox (*Lycalopex vetulus*), 4 Maned Wolf (*Crysocyon brachyurus*), 3 Striped Hog-nosed skunk (*Conepatus semistriatus*), 2 Puma/Montain lion (*Puma concolor*) and 1 Crab-eating raccoon (*Procyon cancrivorus*). One (9.10%) Hoary fox specimen, 1 (25%) Maned Wolf and 1 (100%) Crab-eating raccoon were reactive to hantavirus. Twenty specimens of South American coati (*Nasua nasua*) and one specimen of Puma were captured Pantanal biome, none of them were seroreactive. We found a high prevalence of antibodies in domestic dogs, suggesting that these animals may have an important role in reducing human disease risk (acting as a possible barrier) in agricultural and peridomestic areas. Differently, wild carnivores seems to be under influence of a positive association between biodiversity and infection prevalence for hantavirus. These mammals are part of “healthy” ecosystems with higher diversity leading to lower contact rate between predators and reservoirs, thus exhibiting low antibody titers.

- **Hemorrhagic fever with renal syndrome (HFRS) in Poland**

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Context: Hemorrhagic fever with renal syndrome (HFRS) constitutes the only case of viral hemorrhagic fever on the territory of Poland. Until now, the south-eastern part of Poland (Podkarpacie) has been recognized as an endemic region of the disease. The infections occurring in this region have been caused by the Puumala and Dobrava serotypes.

Objectives: The aim of the study was to confirm the hantavirus infection in patients with acute symptoms characteristic for HFRS admitted to the Nephrology Wards in Lublin and Warsaw (the region of Eastern Poland neighbouring the Podkarpacie region and Central Poland). The results presented below refer to 10 acute cases and they constitute a preliminary report from the study planned until 2021.

Methods: The serological method (screening ELISA) was applied to detect the presence of IgM and IgG antibodies against hantavirus. The positive results in the IgM and IgG classes were confirmed in the indirect immunofluorescence test (IIFT).

Results: The conducted studies show that in 3 patients high titres in the IgM class were detected and their values were: 3.57, 7.25 and 6.6. In 2 out of these 3 cases the level of IgG was above 200 RU / ml, while in the third - 157 RU / ml. This may indicate an acute infection with the Hantavirus. In one case, the titre of antibodies determined in the IgM class was questionable, while the IgG antibody level was above 200 RU / ml. In yet another case, only a high level of IgG 190.53 RU / ml was detected, which may indicate a long-term exposure to the virus and previous convalescence. The IIFT confirmation test conducted in this 5 cases showed that one of the sera reacted most strongly with the Puumala virus antigen and the other 4 with the Dobrava-Belgrade virus antigen. Five patients did not have any antibodies to the Hantavirus.

Conclusion: The preliminary data shows that the area of HFRS in Poland is wider than current report would suggest and requires further research.

- **Protocadherin-1-mediated cell entry by New World hantaviruses**

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Accidental transmission of rodent-borne hantaviruses to humans in the New World causes a highly fatal respiratory disease, hantavirus pulmonary syndrome (HPS). No vaccines or therapies exist for these viruses and host factors required for their entry and in vivo pathogenesis are poorly characterized. Using a genetic screen in human haploid cells, we recently identified the human asthma-associated gene protocadherin-1 (PCDH1) as an essential determinant of entry and infection in pulmonary endothelial cells by multiple species of New World hantaviruses, including the HPS-causing Andes virus (ANDV) and Sin Nombre virus (SNV). Genetic ablation of PCDH1 by CRISPR/Cas9-mediated genome engineering rendered Syrian golden hamsters highly resistant to a usually lethal ANDV challenge highlighting its in vivo requirement. PCDH1, a member of the cadherin superfamily, is expressed on cell surface and directly recognizes New World hantavirus surface glycoproteins via its outermost extracellular domain (EC1). To understand the mechanism of PCDH1-mediated hantavirus entry, we assessed the role of its cytoplasmic tail in viral infection and uptake by using PCDH1 variants. We also analyzed the endosomal colocalization of PCDH1 and ANDV during viral entry using confocal microscopy. Taken together, our findings elucidate the mechanism(s) by which PCDH1 orchestrates New World hantavirus entry.

[RKJ, ASH, RL & LTJ are co-first and TRB, ZW, JMD & KC are co-senior authors]

- **Co-infections of *Tula orthohantavirus* and *Leptospira spp.* in common voles**

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Rodents harbor several pathogens relevant for public health including species-specific pathogens in terms of rodent host species such as hantaviruses. The common vole (*Microtus arvalis*) harbors a number of rodent-borne pathogens incl. *Tula orthohantavirus* (TULV) - a common vole-specific pathogen and *Leptospira spp*., a pathogen found not only in common voles, but also other vole and rodent species. This study aimed to determine the prevalence of TULV RNA and *Leptospira spp*. DNA in common voles and search for co-infections with both pathogens.

Common voles were trapped in the “Thüringer Becken”, a central German region known for intensive large-scale agriculture where outbreaks of the common vole occur about every 2-5 years. Previous studies showed that both pathogens are generally present in common voles in this area.

We sampled 867 voles and analysed lung samples for TULV using a standard S-segment-specific RT-PCR with subsequent sequence determination and kidney samples for *Leptospira spp*. using a lipl32 screening PCR followed by a secY-typing PCR.

First results show strong differences between pathogen prevalence in the common vole depending on the habitat. Prevalences ranged between 0% and 58% for TULV and Leptospira prevalences ranged between 0% and 64% in common vole.

We were able to detect both pathogens in common voles in this area and strong differences between pathogen prevalence in the common vole depending on the habitat was observed. First analysis indicated not only single infections, but also co-infections in common voles.

- **Inhibition of hantavirus replication by passive transfer of immune sera in a Syrian hamster model**

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Hantaan orthohantavirus (HTNV) belonging to the family *Hantaviridae* causes hemorrhagic fever with renal syndrome in humans. To develop medical countermeasures against HTNV, it is imperative to assess their efficacy in animal models. Here we show the effects of vaccine-immune human sera on HTNV replication in an immunocompetent Syrian hamster model. After injected with vaccine-immune human sera via the intraperitoneal route, Syrian hamsters were infected with HTNV (2 x 105 plaque forming unit). We then investigated HTNV replication in lung, kidney, and spleen of Syrian hamsters using plaque assay and qRT-PCR. In the plaque assay, HTNV replication was inhibited in all the tested organs of the immune sera-injected Syrian hamsters in a dose-dependent manner, compared with PBS-injected controls. Consistently, much lower viral RNA copies were detected in the immune sera-injected Syrian hamsters than in the PBS-injected controls. These results demonstrate the inhibitory effects of the passive transfer of vaccine-immune sera on HTNV replication and suggest the potential of our animal model as a tool for investigating end-point protective serum titers in prophylactic and therapeutic measures against HTNV infection in humans.

- **Active surveillance to identify hantavirus outbreaks**

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Context: Endemic emergence of hantaviruses becomes a critical public health threat worldwide. *Hantaan orthohantavirus* (HTNV) is an etiologic agent for hemorrhagic fever with renal syndrome (HFRS) in humans. Comparative genomic analyses of HTNV from humans and rodents enable to localize the putative infection sites for HFRS patients. To identify the infectious origin of HTNV outbreak, partial genomic sequences might not reflect precise phylogenetic positions over the whole-genome sequence; finer granularity of rodent sampling reflects more precisely the circulation of strains.

Objectives: This study aimed to investigate infection locations of HFRS patients with HTNV, using epidemiologic surveys, targeted rodent trapping, and multiplex PCR-based next-generation sequencing (NGS).

Methods: Epidemiologic interviews were performed with five HFRS patients during the hospitalization. Laboratory diagnosis confirmed HTNV infection by indirect immunofluorescence antibody test and reverse-transcription polymerase chain reaction. Active surveillance was conducted by trapping small rodents at suspected HFRS outbreak areas. Multiplex PCR-based NGS was applied to obtain the genomic sequence of HTNV from patients and rodents. Phylogenetic trees of human- and rodent-derived HTNV strains were analyzed by the maximum likelihood method.

Results: Targeted rodent trappings were conducted at Paju-si and Yeoncheon-gun where HFRS outbreaks occurred. A total of 11 (15.9%) of 69 small mammals sera were positive for anti-HTNV IgG. Seven (63.6%) of 11 lung tissues of the seropositive rodents harbored HTNV RNA. Nearly whole-genome sequences of HTNV were recovered from the sera of HFRS patients and lung tissues of A. agrarius. The phylogeographic analyses demonstrated genetic clusters of HTNV strains from clinical specimens with the HTNV circulating in rodents at suspected sites of patient infections.

Conclusion: This study describes a major shift in molecular epidemiological surveillance of HTNV. Active surveillance at sites of suspected infections unveiled the location of emergence of hantaviruses. The integrated implementation of epidemiology, active surveillance, and multiplex PCR-based NGS provides an enhanced resolution of putative infection locations for HFRS patients. This novel modus operandi will help in the identification, disease risk assessment, and control strategies of hantavirus outbreaks.

- **Analysis of the integrin β3 receptor for pathogenic orthohantaviruses in rodent host species**

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Hantaviruses are associated with specific host animals via an unknown mechanism. The entry via receptors is an important step in the viral replication cycle and may determine host specificity and organ tropism. Integrin β3 was identified as receptor for pathogenic hantaviruses in primate cell culture models. Furthermore, the aspartic acid residue at position 39 (D39) within an N-terminal domain of human integrin β3 was described to be crucial for primate infection and is absent in *Mus musculus* as a non-susceptible species. We analyzed the integrin β3 sequence from tissue samples of striped field mice (*Apodemus agrarius*) and bank voles (*Myodes glareolus*), the host species of Hantaan (HTNV) and Puumala (PUUV) virus, respectively. The sequencing of integrin β3 of both host species revealed that an asparagine is present instead the aspartic acid at position 39 corresponding to the sequence of the non-susceptible species *Mus musculus*. No differences were observed between the sequences derived from lung and kidney tissue samples or PUUV-infected and uninfected bank voles.

Analyzing the transcription and expression levels in BKV168 cells, a cell line established from bank vole kidney, revealed that transcription level of integrin β3 was 100-fold lower in BVK168 cells than in Vero E6 cells and integrin β3 protein expression was not detectable in BVK168 cells. Despite the lack of amino acid residue D39 and the absence of detectable integrin β3 expression, BVK168 cells are susceptible to infection with both PUUV and HTNV. These findings demonstrate that integrin β3 does not determine hantaviral host species specificity and that the mechanism of hantaviral entry in rodent species differs from the requirements that were described for the entry in primate cells. Further studies are necessary to analyze the receptor usage and entry mechanism of hantaviruses in host species and the possible role in host specificity.

- **The characteristics of current natural foci of hemorrhagic fever with renal syndrome in Shandong Province, China, 2012-2015**

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Background: Hemorrhagic fever with renal syndrome (HFRS), an infectious disease caused by hantaviruses, is endemic in China and remains a serious public health problem. Historically, the reported HFRS cases in Shandong Province accounted for approximate one third of total cases in the whole country and had the largest HFRS burden in China. However, we do not have a comprehensive and clear understanding of the current epidemic foci of HFRS in Shandong Province. The study conducted a systematic analysis of surveillance information on HFRS in Shandong Province from 2012 to 2015 to assess the severity of the situation and determine the new epidemic foci in Shandong Province.

Methodology/Principal Findings: The incidence and mortality rates were calculated, and a phylogenetic analysis was performed after laboratory testing of the virus in rodents. Spatial epidemiology analysis was applied to investigate the epidemic foci, including their sources. A total of 6,206 HFRS cases and 59 related deaths were reported in Shandong Province in 2012-2015. The virus carriage rates of the rodents *Rattus norvegicus, Apodemus agrarius* and *Mus musculus* were 10.24%, 6.31% and 0.27%, respectively. The phylogenetic analysis indicated that two novel viruses isolated from *R. norvegicus* in Anqiu City and Qingzhou City were dissimilar to the other isolated strains, but closely related to strains previously isolated in northeastern China. Three epidemic foci were defined based on patients, rodents and molecular epidemiology characteristics. two of which were derived from the Jining and Linyi epidemic foci, respectively, while the other was the residue of the Jining epidemic focus.

Conclusions/Significance: The southeastern and central Shandong Province are current key HFRS epidemic foci dominated by *A. agrarius* and *R. norvegicus*, respectively. The situation of HFRS epidemic foci in Shandong Province was clear. Our study could help local departments to strengthen prevention and control measures in key areas to reduce the hazards of HFRS.

- **Different expression of DNAM-1 and TIGIT on CD56dimCd16+ and CD56brightCD16- NK cell population in patients with hemorrhagic fever with renal syndrome**

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Hemorrhagic fever with renal syndrome (HFRS) is a multisystemic disease caused by hantaviruses (HTV). Immune response is involved in HFRS pathogenesis.

Natural killer (NK) cells are found to be activated during HFRS infection. Data regarding their immunological interaction with infected cells are still scarce. NK cells posses a variety of activating and inhibitory receptors sensing potential threats in their environment and engaging their effector function.

DNAM-1, an activating receptor, is important for formation of immunological synapses, cytokines secretion and differentiation of NK cells into memory cells. TIGIT, on the other hand, exhibits immunomodulatory function by competing with DNAM-1 for binding to their shared ligands on target cells: CD155 and CD112. The fine tuned balance between this receptors is crucial to achieve the appropriate immune response.

Our aim was to explore the dynamics of DNAM-1 and TIGIT expression on NK cells in PUUV infected patients. Blood samples were collected from 14 male patients at two stages of disease, early (upon hospitalization) and late at discharge. Expression of DNAM-1 and TIGIT were analyzed using flow cytometry on CD56dimCD16+ (predominate, more educated population) and CD56brigtCD16- NK cells. Expression in HFRS patients were compared with healthy, age and sex matched controls (n=9).

Percentage of CD56dimCD16+ was significantly decreased in early stage of disease, reaching the level of healthy controls at discharge. On the contrary, CD56brigthCD16- cells were significantly increased in early stage of disease.

Median fluorescence intensity of DNAM-1 receptor on CD56dimCD16+ population was significantly increased in both stages of disease, compared to healthy controls, as well as on CD56brightCD16- cells. Interestingly, in the late HFRS phase DNAM-1 was significantly higher on CD56brightCD16- cells compared to early stage. Expression of TIGIT was significantly increased in both NK cell population during the course of disease.

Different expression of activating receptor DNAM-1 and inhibitory TIGIT on NK cell population could play an important role in managing and directing early immune response to HTV infection in order to avoid excessive activation upon recognition of target cells.

- **Unique interferon pathway regulation by the ANDV nucleocapsid protein is conferred by phosphorylation of serine 386**

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Andes virus (ANDV) causes hantavirus pulmonary syndrome (HPS) and is the only hantavirus shown to spread person-to-person and cause highly lethal HPS-like disease in Syrian hamsters. The unique ability of ANDV N protein to inhibit IFNb induction may contribute to its virulence and spread. Here we analyzed IFNb regulation by ANDV N protein substituted with divergent residues from the nearly identical Maporal virus (MAPV) N protein. We found that MAPV N fails to inhibit IFNb signaling and that replacing ANDV residues 252-296 with a hypervariable domain (HVD) from MAPV N prevented IFNb regulation. In addition, changing ANDV residue S386 to histidine present in MAPV N, or alanine in other hantaviruses, prevented ANDV N from regulating IFNb induction. In contrast, replacing serine with phospho-serine mimetic aspartic acid (S386D) in ANDV N, robustly inhibited IRF3 phosphorylation and IFNb induction. Additionally, the MAPV N protein gained the ability to inhibit IRF3 phosphorylation and IFNb induction when ANDV HVD and H386D replaced MAPV residues. Mass spectroscopy analysis of N protein from ANDV infected cells revealed that S386 is phosphorylated, newly classifying ANDV N as a phospho-protein and phospho-S386 as a unique determinant of IFN regulation. In this context, finding that the ANDV HVD is required for IFN regulation by S386, but dispensable for IFN regulation by D386, suggests a role for the HVD in kinase recruitment and S386 phosphorylation. These findings delineate elements within the ANDV N protein that can be targeted to attenuate ANDV, and suggest targeting cellular kinases as potential ANDV therapeutics.

- **Dynamics of albuminuria in acute kidney injury caused by Puumala hantavirus infection**

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Puumala virus (PUUV) is spread by rodents and the infection in humans is characterized by acute kidney injury (AKI). Proteinuria is typical and sometimes massive in the acute phase of the disease. The amount of proteinuria is associated with the severity of AKI determined by the rise in plasma creatinine level. The outcome of AKI is favorable and no persistent proteinuria was found in studies of patients followed several years after the disease. We have previously examined the dynamics of proteinuria during the hospitalization until two weeks after the beginning of fever. After the peak, around the fifth day of the disease, proteinuria seemed to diminish. Now we evaluated the rate of disappearance of proteinuria by determining the amount of overnight albuminuria (cU-Alb) at different time points between acute phase and until six months after the hospitalization.

Study consists of 141 consecutive patients hospitalized because of acute PUUV infection, during years 2000-2014 in Tampere University Hospital, Finland. During hospitalization, 116/133 patients (87%) had albuminuria (cU-Alb >20 µg/min) and 76/133 patients (57%) had macroalbuminuria (cU-Alb >200µg/min). During the acute and convalescent phase, the median cU-Alb determined at 7 days or earlier after the beginning of fever was 311 µg/min (range 2.2-6460, n= 42), during 8-13 days 234.9 µg/min (range 6.8-5479, n=29), 14-20 days 2.8 µg/min (range 0.5-18.2, n=41), 21-30 days 2.5 µg/min (range 0.39-18.4, n=32), 31-40 days 2.1 µg/min (range 0.3-10.1, n=27), and 41-60 days 2.9 µg/min (0.2-35.4, n=47). At 6 months, the median cU-alb was 2.0 µg/min (range 0.6-14.5, n=36).

Albuminuria, regardless of the amount in the acute phase of PUUV infection, disappears rapidly. From 14 days after the beginning of fever on, none of the patients had significant albuminuria during the follow-up of six months.

- **Long lasting viremia in hantavirus pulmonary syndrome cases caused by Andes virus**

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Introduction: Hantavirus pulmonary syndrome (HPS) is a devastating disease with fatality rates up to 50%; in Argentina, more than 1200 cases were reported up today. The main causative agent of HPS in Argentina is Andes virus (ANDV) that has the particular property of person-to-person transmission.

There are not specific treatments or vaccines for HPS. Ribavirin, was tested for efficacy in patients or in animal models, without obtaining positive results for HPS cases, possibly because of therapeutic interventions that target viral replication may not be effective unless given early.

Recently, in Argentina, the largest person-to-person transmission outbreak of HPS was reported, involving 34 cases, 11 of which were fatal (32%). Four-link chains of viral transmission were identified, and the follow-up of the contacts by RT-qPCR allowed us early identification of new cases. Four cases from 34 (11%), who were confirmed around day 0 of onset of disease, were Ribavirin treated.

Objective: HPS cases showed high levels of viremia at the onset of pulmonary edema but there were no studies showing how long it is maintained. The objective was to establish a kinetic viral load in HPS cases linked to an outbreak of person-to-person transmission.

Methods: At least one blood sample was obtained daily form 33 of 34 patient involved in the outbreak and 150 samples were analyzed. IgM and IgG titles were quantified by ELISA test. Taq-Man RT-qPCR was performed to quantify a viral S-Segment fragment.

Results and Discussion: IgM titers were detectable since day 0 to 4 after onset of fever, while viral genome since onset of disease day. Viral genome was detected until day 34 after onset without clearance and with less than 22% of decrease in viral RNA values, even for patients discharged from the hospital. There were not significant differences in viral load between samples from Ribavirin treated (up to day 19 after onset of symptoms) and untreated patients.

Future studies of viral genome in samples such as urine, semen, etc., could be suggest the presence of immunological privilege sites and it would infer the implication of the prolonged viremia for the pathology, viral transmission and evolution.

- **T cell activation in patients with severe hantavirus pulmonary syndrome from Argentina**

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Hantaviruses are emerging human pathogens responsible of hantavirus pulmonary syndrome (HPS) in the Americas. Hantaviruses predominantly infect microvascular endothelial cells causing capillary leakage. The hallmark of the disease is the vascular permeability, leading to pulmonary edema in HPS patients.

In order to evaluate the role of the immune response on pathogenesis we performed T-cell phenotypic characterization in acute HPS patients (AP), and when possible, longitudinal analysis during convalescence (CONV). We also studied viral load, IgM/IgG titers and kinetics of neutralizing antibodies in blood samples. We obtained control samples from healthy adult volunteers (HV). Almost all patients presented a severe form of disease.

Analysis of PBMCs showed increased TCD8 and decreased TCD4 cells in HPS patients after 4 days of illness onset, resulting in alteration of the CD4/CD8 ratio. The phenotypic analysis of T-cell subpopulations showed an average of 39.1% CD8+/CD38+/HLA-DR+ cells (activated phenotype) in AP (average 9.6 days of illness); 12.3% in CONV (average 77.7 days) and 2.3% in HV. TCD4 cells showed 9.4% CD38+/HLA-DR+ in AP; 4.1% in CONV and 1.1% in HV. Statistic analysis show significant differences between AP and HV (ANOVA "Kruskal-Wallis test"). The average for CD8+/CD38+/CD28- was 13.41% in AP; 3.10% in CONV and 0.80% in HV (differences were not statistically significant). On the other hand, analysis of inhibitory markers PD1 and CTLA4 on T-cells did not show over-expression in AP.

Activated CD8T-cells phenotype did not show any correlation with viral load or severity grade. It is noteworthy that, in general, viral load was stable in blood samples during the acute phase, at discharged from hospital, and during early convalescence (up to 126 days after fever onset). Furthermore, the CD8T-cells activated phenotype persisted in elevated values at least up to 1 month after illness onset.

All patients had high IgM/IgG titers in AP. Longitudinal analysis showed decreasing IgM and increasing IgG titers but delay in the development of neutralizing antibodies.

Actually intracytoplasmic molecules are being analyzed in order to evaluate T-cell functionality.

Our analysis revealed an activated state of the immune system. Increased T-cell activation markers in acute patients show tendency to normalize during the convalescence.

- **Lactococcus lactis cell wall-derived particles containing Andes N hantavirus protein as a potential immunogen for vaccine design**

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Orthohantaviruses are responsible for zoonotic diseases, like hantavirus pulmonary syndrome (HPS) and hemorrhagic fever with renal syndrome (HFRS). In the Americas Sin Nombre virus (SNV) and Andes virus (ANDV) are the most common species causing HPS.

ANDV is the main HPS agent in Argentina, where the case-fatality rate can reach 36-40% in some endemic regions. The South variant of ANDV has frequently been associated with inter-human transmission, and this fact was evidenced in the 2 larger person-to-person transmission outbreaks; the first occurred in 1996 (16 cases) and recently in Epuyén, (34 cases) with a case fatality rate of 36-50%. As there is no specific treatment for this virus, vaccine development for hantavirus is highly desirable, being next-generation vaccine strategies the best option from the economic and safety points of view.

The nucleocapsid protein (N) encapsidates the genomic RNA and is highly immunogenic for both animals and humans. Moreover, it has been reported that immunization with recombinant N protein can protect animals from hantavirus infection with certain cross-protection. On the other hand, Lactococcus lactis is generally recognized as safe bacteria that has gained, in the last decade, significant relevance as a vehicle for antigen delivery to the mucosa.

Thus, the main objective of this work was to express in L. lactis the ANDV N protein and evaluate its immunogenicity in a mouse model. For that, the ANDV N coding sequence was RT-PCR amplified and cloned into the NICE expression system in such a way that the produced protein is exposed outside L. lactis anchored to its cell wall. Protein expression was optimized varying nisin concentration and incubation temperature, and analyzed by SDS-PAGE. Also, its identity was confirmed by Western blot analysis using anti-N antibodies. After expression was optimized, cell wall derived particles (CWDP) containing the recombinant protein were obtained, and N immunogenicity was assayed in a mouse model. Results confirmed its immunogenicity. Further studies will be conducted to evaluate the potential of these CWDP to induce antibodies that recognize ANDV in vitro and to induce protection in the ANDV/hamster model using different immunization routes.

- **Oral immune factors description in cases and close-household contact exposes to Andes Hantavirus (ANDV)**

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Background: *Andes orthohantavirus* (ANDV) is the sole etiological agent of HCPS in Chile, and its main reservoir is the long-tailed pygmy rice rat (*Oligoryzomys longicaudatus*). ANDV, until now is the only hantavirus transmitted through person-to-person, were case's sexual partners have 10 times more risks to become infected, specifically through saliva. In vitro, saliva component has been probed to inhibit ANDV infection. It´s still unknow the mechanism of why some people exposure to the same risk of infection became infected and other are resistant. Aim: We aim to show that differences in saliva composition of cases (exposed and infected) and close household contact (exposed but not infected), through detection and quantification of saliva components.

Methods: 79-cases and 113 close household contact were included. Secretory IgA (sIgA) was quantify by ELISA assay (Salimetrix), cytokines and interleukins (IL-1b; IL-12p70; TNFa; INF-y; IL-10; IL-6; VEGF; IP-10; IL-8; TGF) were measure through multiplex assay (Millipore, Merck). MUC5B and MUC7 were analyzed by western blot.

Results: Sixty-eight (68/79) and 38,9(44/113) % cases and close household contact were men, respectively ($p \leq 0,05$). sIgA mean concentration for cases was 0,0051± 0.61 (mg/ml) and 0.322 ± 0.46 (mg/ml) for close household contact. VEGF was higher in cases compared to close household contact (mean 554.24 ± 1081 y 72.41 ± 131.5 pg/mL respectively ($p = 0,06$). For mucins analyses we used dichotomized variables, for presence or absence and presence of 1 or more than 2 isoforms. Surprisingly, we found a higher frequency of MUC7 in close household contact that in cases, 62.6 and 40.5%, respectively ($p<0.05$). Likewise, the presence of MUC5B was higher in close household contact than in cases, 62.16 and 44.4%, respectively ($p<0.05$).

Conclusion: Three saliva components show differences between cases and close household contact (sIgA, VEGF and MUC7 isoforms). For MUC7, the isoforms show be more relevant that the quantity. This characteristic in saliva can be responsible to block more efficiently the ANDV infections or prevent infection itself. This work represents the first description of saliva component in ANDV infection, and can help, among other factors, to explained susceptibility to infection.

Funding: Fondecyt 1161197, ACT1408

- **Pathogenic Old World orthohantavirus Puumala infects cells of the human respiratory tract**

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Hantaviruses display a broad tropism to various tissues and viral antigen is detected in several organs of infected patients. Transmission of all known pathogenic hantavirus species usually occurs via inhalation of aerosols contaminated with viral particles derived from infected rodents. However, the role of pulmonary cells as primary target site for hantavirus infection is not well understood.

Therefore, we analysed the susceptibility and permissiveness of primary human pulmonary cells of different sites of the respiratory tract for Old World hantavirus Puumala (PUUV) in vitro. Productive infection with PUUV was observed for microvascular endothelial cells and epithelial cells derived from bronchi, bronchioles and alveoli. Quantification revealed an increase of infected cells over time. Interestingly, infection was also observed in bronchial and small airway epithelial cells, although expression of the hantaviral receptor integrin αvβ3 was not detectable in these cells. The receptor expression profiles of epithelial cells were confirmed using primary cells derived from a second donor. Despite of corresponding receptor expression, infection kinetics showed donor-specific variances in susceptibility to PUUV. However, release of infectious particles was observed for all cell types tested.

The respiratory epithelium may represent the initial site of hantaviral infection and contribute to virus replication, dissemination and pathogenesis. The absence of detectable levels of integrin αVβ3 surface expression on bronchial and small airway cells indicates an alternate mode of hantaviral entry in these cells that is independent from integrin β3.

- ## Hantavirus infection in Brazilian patients suspicious of leptospirosis

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Brazil is endemic for leptospirosis, which shares clinical features with hantavirus infections. The investigation of hemorrhagic fever with renal syndrome (HFRS) in Brazil is incipient, where surveillance has been focused only on hantavirus pulmonary syndrome (HPS) agents. There are a few studies on Seoul virus (SEOV) investigated in rodents and there are no reports of SEOV infections in humans, suggesting lower medical awareness. Recognition of the HFRS, based on early signs and symptoms, can be confused with other prevalent diseases endemic in Brazil, such as, leptospirosis, dengue and rickettsiosis. This study provide a serological evidence of hantavirus infections in humans from urban areas in Brazil, where the clinical picture was first interpreted as leptospirosis. A total of 100 serum samples tested negative for leptospirosis from five Brazilian states of North and Southeastern region, were screened for IgM against recombinant nucleocapsid protein of the SEOV using immunochromatographic assay. Antibodies for rN-SEOV were detected in three samples from individuals living in urban areas of Rio de Janeiro state, with a seroprevalence rate of 3%. All positive samples also showed IgG seroreactivity. Patients were 34, 42 and 64 years and two individuals were males. All patients were hospitalized and presented with jaundice and gastrointestinal tract manifestations, with clinical characteristics of an acute febrile illness compatible with leptospirosis. The two fatal cases also showed myalgia, weakness, headache, respiratory and hemorrhagic signs and symptoms. Molecular analysis are under development to confirm the presence of SEOV. The serological evidence of hantavirus infections in patients from urban environment emphasizes the need of ecoepidemiologic surveillance of Rattus norvegicus rats and, highlights the importance of considering HFRS in the differential diagnosis of clinical pictures suggestive of leptospirosis.

- **Comparison of lymphocyte populations in DOBV and PUUV patients and their involvement in thepathogenesis of the disease**

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Background: In Slovenia HFRS is caused by Dobrava (DOBV) and Puumala (PUUV) orthohantavirus. DOBV is mainly responsible for more severe disease, whereas PUUV usually causes a milder form. However, the clinical severity of HFRS varies greatly and in Slovenia both severe and mild clinical courses of the disease have been observed, with an overall case fatality rate of 4.5%. The aim of our study was to investigate differences in PBMCs profile between Slovenian patients infected with DOBV and PUUV and to determine whether the observed difference in PBMCs profile is associated with HFRS severity.

Methods: PBMCs were isolated from EDTA blood samples of 36 HFRS patients during the acute phase of infection. We have analysed 15 patients infected with DOBV and 21 with PUUV. Ten patients infected with DOBV had the severe form of the disease and 5 the mild form, 7 patients infected with PUUV were categorized as having the severe disease and 14 as the mild form.

Results: Our results showed that NK cells are associated with HFRS severity. Higher percentage of NK cells were detected in DOBV infected patients in comparison to PUUV infected patients. Furthermore, PUUV infected patients with severe disease had higher concentration of NK cells, especially CD56dim NK cells, in comparison to patients with mild form. On the contrary, the highest percentage of T cells had PUUV infected patients with mild disease. PUUV infected patients had significantly higher concentration of activated T cell subsets, expressing markers CD25, CD69 and HLA-DR in comparison to DOBV infected patients.

Conclusions: Our study shows that higher activation of T cell subsets could be a contributor to milder HFRS, since higher immune response of activated T cells in patients infected with PUUV could contribute to more efficient virus clearance from the body and thus lower viral load.

- **GP-ANDV induced changes in the frequency and phenotype of CD4 T regulatory cells**

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Introduction: Andes hantavirus (ANDV) is one of the most pathogenic strains that generate hantavirus cardiopulmonary syndrome (HCPS) with a case fatality rate of around 35%. Despite its importance, little is known about the immune mechanisms involved on CD4 T regulatory (Tregs) in limiting the HCPS. Here we study the Treg response against ANDV-glycoprotein (GP) antigen.

Methods: Peripheral blood mononuclear cells (PBMCs) isolated from eighteen HCPS survivors and ten healthy donors (HD) were stimulated with ANDV-GP virus-like particles (VLP) for seven days. Memory CD4+ T cells were identified as CD4+CD45RO+ and memory Tregs (mTreg) as CD4+CD45RO+CD25hiCD127low/-FOXP3+. Moreover, Th-like Tregs were identified from mTregs using CXCR3, CCR4 and CCR6. In addition, suppression markers PD-1 and CTLA-4 were analyzed in memory Tregs before and after ANDV-GP VLP stimulation.

Results: Low frequency of memory CD4+ T cells was found in HCPS compared to HD group in baseline conditions (p=0,03), however no difference was observed regarding the percentages of mTreg between groups. Within mTregs, PD1 expression was increased in HCPS compared to HD (p=0,02), however CTLA-4 showed no difference between groups.

Upon VLP stimulus, PBMCs from HD resulted in a significant reduction of FOXP3 expression in mTreg compared to the mock (p=0,03). This depletion was not observed in the HCPS group. Moreover, no differences in frequencies of Treg PD1+ and Treg CTLA-4+ cells after VLP stimuli between groups were observed.

Interestingly, we found a predominance of Th17 and Th2-like Treg in HCPS survival subjects in baseline conditions. However, after VLP stimulation, the percentage of Th1-like Tregs was significantly reduced, whereas the percentage Tregs with a Th2-like phenotype was increased in comparison with mock.

Conclusion: Our data showed that ANDV-GP VLP reduced the expression of FOXP3 in mTregs from HD donor samples after antigen first encounter. In addition, ANDV-GP VLP stimulation in HCPS survivors induced a Th2-like phenotype within mTregs, suggesting that ANDV-GP promotes changes in the phenotype of mTregs in HCPS survivors. Furthermore, HCPS survivors expressed higher PD1 levels in mTregs ex vivo in comparison with HD.

- **Development of a multiplexed immunoassay to detect IgG and IgM antibody responses against pathogenic bunyaviruses within the country of Georgia**

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Background: Hemorrhagic fever with renal syndrome (HFRS) and Crimean-Congo hemorrhagic fever (CCHF) are serious and often deadly human diseases caused by infections with viruses in the *Hantavirus* or *Nairovirus* genera of the family *Bunyaviridae*, respectively. Hantaviruses and CCHFV are present in the country of Georgia and have caused human infections; however, there is limited information about the prevalence of disease or of the prevalence of hantaviruses in rodents and CCHFV in ticks.

Objective: We have initiated a project to develop and implement a Magpix-based multiplex system for ecology and epidemiology studies in Georgia. Traditionally, ELISA's have been used to assess seroprevalence in both clinical and field studies. Although ELISA's are robust immunological assays, they can be time-consuming, costly, and may deplete valuable samples when measuring multiple markers. To mitigate these shortcomings, we have developed a pan-hantavirus assay to measure IgG and IgM levels to a combination of nucleocapsid antigens of Puumala (PUUV), Dobrava (DOBV), Hantaan (HTNV), and Seoul (SEOV). To confirm assay performance, CCHFV-labeled beads were included.

Method: A mixture of purified recombinant viral nucleocapsid antigens were covalently linked directly to magnetic beads. The beads were incubated with human convalescent sera, an appropriate secondary antibody, and assayed for fluorescence output using the Magpix system.

Results: The mean fluorescence intensity (MFI) of the pan-hantavirus assay compared favorably with the MFI of individually labeled bead-sets. Beads coated with full-length CCHFV were included in the assay as a proof of concept for the multiplex format.

Conclusion: Our results demonstrate the successful development of a convenient and reliable multiplex assay for use in seroepidemiology studies in the country of Georgia.

- **Mapping the interface between New World hantaviruses and their receptor, PCDH1**

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We have recently shown that New World hantaviruses, including several agents that cause lethal hantavirus cardiopulmonary syndrome (HCPS), utilize protocadherin-1 (PCDH1) for endothelial cell entry and infection by directly engaging its first extracellular cadherin repeat (EC1) domain. Knockout of PCDH1 greatly reduced pulmonary infection and was highly protective in a Syrian hamster model of a lethal challenge with Andes virus (ANDV). To further understand PCDH1's role in hantavirus entry, we sought to map the binding interface between hantavirus Gn/Gc and PCDH1-EC1. Accordingly, we screened a panel of EC1 proteins, bearing point mutations in solvent-exposed residues, for their capacity to recognize Gn/Gc and block viral entry. EC1 mutations defective at Gn/Gc binding were engineered singly, and in combinations, into full-length PCDH1, expressed in PCDH1-knockout cells, and evaluated for their capacity to complement viral infection. We identified a surface in the PCDH1-EC1 domain, comprising contiguous residues, that was required for virus-PCDH1 recognition and PCDH1-dependent viral entry. This site forms a part of the epitope of an EC1-specific monoclonal antibody with antiviral activity. However, this region does not overlap with the EC1–EC4 heterodimer interface recently described by Modak and Sotomayor. Thus, it may provide an Achilles' heel for the development of host-directed antiviral drugs which do not interfere with PCDH1's endogenous function.

- **MMP9 associates with endothelial glycocalyx degradation during haemorrhagic fever with renal syndrome**

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Haemorrhagic fever with renal syndrome (HFRS) is characterized by fever, hypotension, vascular leakage, thrombocytopenia and renal failure. HFRS in Sweden is caused by the Puumala hantavirus and is spread by viral-infested droppings from bank voles. The health care system has little to offer these patients since there is no antiviral treatment and as of yet there is no vaccine prophylaxis available. We previously showed that a marker of endothelial glycocalyx degradation (Syndecan-1) was associated with disease severity and disseminated intravascular coagulation during HFRS (Connolly-Andersen et al., 2014, Open Forum Infect Dis.).

We analysed the levels of other endothelial glycocalyx degradation markers (heparan sulfate, soluble thrombomodulin, albumin), a potential “sheddase”: Matrix Metalloproinase 9 (MMP9) and neutrophil activation/tissue damage (neutrophil gelatinase-associated lipocalin, NGAL) in patient plasma from 44 HFRS patients collected consecutively following disease onset. We used the generalized estimating equation to analyse the association between endothelial glycocalyx degradation, MMP9 levels, neutrophil activation/tissue damage and HFRS disease outcome (need for oxygen, transfusion with blood components, need for intensive care unit (ICU) treatment and renal damage).

44 HFRS patients were included in this study (29 females (66%)); need for oxygen: 11 (25%); transfusion with blood components: 3 (7%) and stay at ICU: 2 (5%)). The levels of MMP9 were significantly associated with all markers of endothelial glycocalyx degradation. Neutrophil activation/tissue damage (NGAL) was also significantly associated with MMP9 and endothelial glycocalyx degradation markers (apart from albumin ($p = 0.053$). In addition degradation of endothelial glycocalyx associated with HFRS disease outcome.

Degradation of the endothelial glycocalyx could be a potential mechanism of HFRS pathogenesis, and potentially MMP9 could contribute to degradation of the endothelial glycocalyx.

- **An atypical outbreak of hemorrhagic fever with renal syndrome in Bosnia and Herzegovina in summer 2017**

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Context: Hemorrhagic fever with renal syndrome (HFRS) is endemic in Bosnia and Herzegovina (BIH), with sporadic cases that have been recorded yearly and rare epidemic outbreaks occurring every 5-10 years. In BIH majority of cases were caused by Dobrava (DOBV) and Puumala (PUUV) viruses. A spectrum of the clinical picture ranges from mild illness typical of PUUV infections to severe forms with fulminant hemorrhagic fever in DOBV infected patients.

Objectives: To present the basic epidemiologic data and clinical features of the last HFRS outbreak that took place outside and inside of endemic regions in BIH in summer 2017; to highlight influence of climatic factors on the outbreak of HFRS in BIH.

Methods: The epidemic, demographic, clinic and laboratory data of the patients hospitalized with HFRS in BIH from 01.01.2017. till 31.09.2017. as well as meteorological data have been collected and analyzed. The serological investigation was done with EUROIMMUN ELISA Hanta IgM and IgG. Confirmation test was done with EUROIMMUN Hantavirus profile 1 (PUUV, DOBV, HTNV).

Results: The epidemic year was extraordinary wet with the large amount of precipitation. During the period from 01.01.2017. till 31.09.2017 a total of 123 patients with HFRS were registered in BIH. The centre of the HFRS outbreak was located in the northwest part of BIH, where 95 infected persons were documented. Majority of infected persons were men (85,26%), and only 14,74% women, with a median age of 45 ±16,54 years. In the majority of patients (88,89%) PUUV infection was confirmed, whereas others were Dobrava infections, and 2 cases were non-typeable. Therapeutic hemodialysis was required in 8 (8,4%) of 95 patients with severe clinical symptoms.

Conclusion: HFRS outbreak that occurred in summer 2017, is not typical for Bosnia and Herzegovina, where a majority of HFRS cases arise in a period of late winter and early spring. Most of the infections occurred in the region of north and northwest BIH, in places close to border with Croatia. From the HFRS reports in Croatia it is evident that in 2017. PUUV infections were dominant in this country.

- **Kurkino and Dobrava genotypes of DOBV in rodents and humans in the Czech Republic, clinical and laboratory characteristics of human cases**

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Context: The data on hantaviruses in the Czech Republic are scarce, although increasing number of human cases is reported.

Objectives: The aim of this study was to elucide the distribution of hantaviruses in rodents in the Czech Republic and to determine the risk of their transmission to humans. Second objective was to compare the clinical severity and laboratory parameters in patients infected with Dobrava and Kurkino DOBV genotypes.

Methods: Extensive survey of hantaviruses in trapped rodents together with the examination of human clinical samples in the Czech Republic was realized between the years 2010-2018. The diagnosis of hantavirus infection in patients was based on clinical symptoms, serology and RT-PCR. Clinical and laboratory data were obtained from questionnaries sent to the physicians taking care of the patients.

Results: Out of 1,551 trapped wild rodents, 43 animals (2.77%) were tested positive for the presence of hantaviral RNA and revealed the presence of Dobrava-Belgrade (DOBV), Tula (TULV) and Seewis (SWSV) hantaviruses. Both DOBV genotypes were detected in local Apodemus mice. The diagnosis of hantavirus infection was confirmed in 61 patients. Out of them, 32 patients were RT-PCR positive for DOBV and sequence analysis revealed the presence of both genotypes of DOBV (DOBV-Kurkino and DOBV-Dobrava). In 2 patients RT-PCR and sequencing confirmed Puumala infection. 44 of 61 questionnaries (72%) were returned and out of those, 25 patients were RT-PCR positive. Sequencing revealed equal distribution of Kurkino and Dobrava DOBV genotypes (11 and 12 respectively) and 2 Puumala cases. Clinical and laboratory data confirmed more severe disease course and longer hospitalization in DOBV-Dobrava patients compared to DOBV-Kurkino and Puumala patients.

Conclusion: This is the first study reporting a local transmission of DOBV from infected mice to humans in the Czech Republic and also to demonstrate a presence of both DOBV-Kurkino and DOBV-Dobrava in human patients in this country. Differences in the clinical course of both DOBV genotypes in patients from the same area was observed.

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