Antiviral Research 176 (2020) 104733

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Meeting report: Eleventh International Conference on Hantaviruses

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A B S T R A C T

The 2019 11th International Conference on Hantaviruses (ICH 2019) was organized by the International Society for Hantaviruses (ISH), and held on September 1–4, 2019, at the Irish College, in Leuven, Belgium. These ICHs have been held every three years since 1989. ICH 2019 was attended by 158 participants from 33 countries. The current report summarizes research presented on all aspects of hantavirology: ecology; pathogenesis and immune responses; virus phylogeny, replication and morphogenesis; epidemiology; vaccines, therapeutics and prevention; and clinical aspects and diagnosis.

1. Introduction

The 11th International Conference on Hantaviruses (ICH) was held in Leuven, Belgium, on September 1–4, 2019. Previous conferences have been held every three years since 1989 in Seoul, Beijing, Helsinki, Atlanta, Annecy, Seoul, Buenos Aires, Athens, Beijing, and Fort Collins, respectively. Each ICH offers scientists from around the world an opportunity to meet, and hear the most up-to-date advances on a broad area of topics in hantavirology, hemorrhagic fever with renal syndrome (HFRS) and hantavirus cardiopulmonary syndrome (HCPS). The organizers tried to select an international group of dynamic speakers to present key talks on various topics divided over 6 classical ICH sessions: *(i)* Ecology, *(ii)* Pathogenesis and Immune Responses, *(iii)* Virus Phylogeny, Replication and Morphogenesis, *(iv)* Epidemiology, *(v)*

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https://doi.org/10.1016/j.antiviral.2020.104733
Received 29 January 2020; Accepted 1 February 2020
Available online 15 February 2020

Fig. 1. Picture of the attendants of ICH 2019, after the reception at the historical town hall in Leuven, Belgium. The president (Jan Clement MD) of the Internatinal Society for Hantaviruses (ISH) figures up in the middle of the stairs. Standing to the left are three pioneers from the early 1980s on in hantavirus research: in front Prof (ret.) Guido van der Groen (Belgium), behind him Dr. (ret.) Evgeniy A. Tkachenko (Russia) and Prof. Tatjana Avšič-Županc (Slovenia), who both during a sabbatical in Guido's lab in the Institute of Tropical Medicine, Antwerp, Belgium, isolated a novel PUUV and the first DOBV strain, respectively.

Vaccines, Therapeutics and Prevention, and *(vi)* Clinical Aspects and Diagnosis. As a result, the ICH 2019 organizers could offer the audience a total of 10 keynote lectures, 47 oral presentations, 27 so-called "lighting talks" (short presentations of selected posters) and 56 poster presentations (see Fig. 1).

At the start of each triennial ICH, it is the tradition to offer two lectureship awards: a Ho-Wang Lee Life-time Achievement Award, and a Joel M. Dalrymple Memorial Award, presented respectively to a senior hantavirologist, who has made exceptional contributions to the field of hantavirus research, and to an early-to mid-career hantavirologist, who has performed innovative, forward-thinking research, reflecting the legacy of Dr. Dalrymple of The United States Army Medical Research Institute of Infectious Diseases. In this 2019 conference, the lectureship awards were granted to Prof. Antti Vaheri (Finland) and Prof. Boris Klempa (Slovakia), respectively.

Antti Vaheri, 2019 recipient of the Ho Wang Lee award: *Effect of hantaviruses on endothelial cells.*

Hantaviruses can infect many types of established cell lines and primary human cells, all without cytopathic effect (Temonen et al., 1993). Endothelial cells and monocyte/macrophages are generally considered to be the principal target cells of hantaviruses. Early studies by Vaheri and coworkers showed that in monocyte/macrophages interferon can induce tissue plasminogen activator (tPA) (Hovi et al., 1981), and more recently in human endothelial cells, when Puumala orthohantavirus (PUUV)-infected interferon-mediated induction of tPA was seen (Strandin et al., 2016). However, plasminogen activator inhibitor type 1 (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, and generalized increased capillary hyperpermeability, 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 high-molecular-weight kininogen by the kallikrein-kinin proteolytic system. BK receptor 2 can be blocked by icatibant, and has been successfully used in some severe cases of PUUV infection with capillary leakage and shock (reviews: Mustonen et al., 2013; Mustonen et al., 2017; Vaheri et al., 2013; Vaheri et al., 2014). Moreover, Vaheri and coworkers have described fatal PUUV cases with capillary leakage, fibrinolysis and complement activation (Sironen et al., 2017). Thrombocytopenia and altered coagulation are key elements in the pathobiology of all hantavirus diseases, both HFRS and HCPS. The mechanism of thrombocytopenia remains elusive, but expectedly altered platelet-endothelial cell interaction is involved.

The Finnish group has also studied and detected a number of clinical and laboratory parameters such as creatinine, leukocytosis, C-reactive protein (CRP), von Willebrand factor antigen, fibrinogen, decreased fibronectin, regulatory T-cell response and indoleamine 2,3-dioxygenase activity, circulating cell-free DNA, histones and neutrophil elastase (presumably from neutrophil extracellular traps), 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 (if present) glucosuria predict the severity of acute kidney injury (AKI). The severity of thrombocytopenia was found to be associated with biomarkers reflecting the degree of inflammation, such as interleukin (IL)-6, tumor necrosis factor (TNF)-α, pentraxin-3, soluble urokinase-type plasminogen activator receptor (suPAR), and variables reflecting capillary leakage, but not with the severity of AKI in PUUV disease (reviews: Hepojoki et al., 2014; Mustonen et al., 2013; Mustonen et al., 2017). Interestingly, however, glucosuria predicts thrombocytopenia (Tietäväinen et al., 2019).

Boris Klempa, 2019 recipient of the Joel M. Dalrymple award: *Hantaviruses: Of mice, shrews, moles, bats and men.*

Boris Klempa is currently the head of the Department of Virus Ecology at the Biomedical Research Center of the Slovak Academy of Sciences in Bratislava, Slovakia. Until 2018, he was associated with the Institute of Virology of the Charité University Hospital in Berlin, Germany, where he performed most of his hantavirus-related research. In his lecture, he summarized his studies focused on the discovery, molecular evolution, and epidemiology of several hantaviruses. First, he focused on the diversity of Dobrava-Belgrade orthohantavirus (DOBV) as the most virulent hantavirus in Europe. He showed that the virus is hosted by several species of *Apodemus* mice, and forms host-specific lineages, which we nowadays recognize as DOBV genotypes inducing disease of various severity (Klempa et al., 2013). He also reported on the generation of several virus isolates including reassortants, characterization of their receptor usage and innate immunity modulation (Kirsanovs et al., 2010; Klempa et al., 2003, 2005, 2008; Popugaeva et al., 2012).

He then reported on the efforts to identify African hantaviruses, which led to discoveries of Sangassou virus as the first autochthonous African hantavirus (Klempa et al., 2006), the shrew-borne Tanganya virus (Klempa et al., 2007), as well as the bat-borne Magboi and Makokou viruses (Weiss et al., 2012; Witkowski et al., 2016). These findings played a substantial role in uncovering the parallel universe of the non-rodent-borne hantaviruses, which seem to be evolutionarily older than the rodent-borne hantaviruses. However, while the latter group still remains to be a serious health threat for humans, the former has hitherto not yet been shown to constitute a similar threat.

Keynote lecture 1, Connie S. Schmaljohn (USA), ISH Past President's lecture: *Hantavirus vaccines: Promise and pragmatism.*

DNA vaccines for HFRS, expressing the Gn and Gc genes of Hantaan orthohantavirus (HTNV) and PUUV, were developed and clinically tested. After several trials with intramuscular electroporation delivery, a Phase 1 study of a more pragmatic delivery method is being conducted, using the needle-free, PharmaJet Stratis® disposable-syringe, jet-injection device to deliver DNA vaccines intramuscularly. This device, which is cleared by the US Food and Drug Administration and by several other countries for commercial use, has been used to deliver conventional vaccines to more than 100,000 people worldwide. In the Stratis® Phase 1 trial, the seroconversion rates were ≥78%, resulting in plans for a Phase 2 trial. Results to date demonstrate both the promise and pragmatism of DNA vaccines for HFRS.

Keynote lecture 2, Jason Botten (USA): *Host factors that regulate virus infectivity.*

Jason Botten (USA) recounted his group's discovery that the human protein ERGIC-53 is incorporated into viral particles and is required for the infectivity of several families of enveloped RNA viruses (Klaus et al., 2013). In particular, he explained how virion-packaged ERGIC-53 is required for virus particles to efficiently attach to permissive host cells. Dr. Botten then described more recent findings, including the identification of a minimal domain of ERGIC-53 that is required for virion infectivity, as well as a second human protein that binds to this critical region of ERGIC-53 and inhibits ERGIC-53's ability to confer infectivity to viral particles. He concluded by showing that extracellular, virion-associated-ERGIC-53 could be targeted to neutralize virion infectivity. Because ERGIC-53 is not essential for human health, it could serve as a promising broad-spectrum antiviral target.

1.1. Session 1: Ecology. Oral presentations

This session was chaired by Herwig Leirs and Rainer G. Ulrich. Prediction models for human hantavirus outbreak years are in general based on the assumption that the frequency of infections in humans is associated with reservoir abundance (Reil et al., 2017). Herwig Leirs (Belgium) reported findings from an extensive field study in Central Finland where, surprisingly, the higher bank vole densities did not necessarily result in a higher prevalence of PUUV in the reservoir, nor in an increased incidence of associated nephropathia epidemica (NE) cases (Voutilainen et al., 2016). His Belgian-Finnish-Dutch team developed a novel individual-based spatially-explicit model of the infection in the vole population, which showed that the reduction of the PUUV prevalence in bank voles during peak vole years can be explained by the transient maternal antibody-mediated immunity in newborn voles (Kallio et al., 2010). In contrast to the team's expectations, the density-dependent spatial vole behavior plays only a minor role. This model might be applicable not only for PUUV, but also for other rodent-borne pathogens.

The knowledge of hantavirus transmission pathways is important as well for the development of predictive models. Bryce M. Warner (Canada) investigated the Sin Nombre orthohantavirus (SNV) transmission mechanisms in the deer mouse (*Peromyscus maniculatus*). SNV is the main cause of HCPS in North America, with a case fatality rate of up to 50% (Krüger et al., 2015). Deer mice were housed together with naïve uninfected animals, or naïve uninfected animals were placed into “contaminated” cages, where infected deer mice were housed before. Direct transmission of SNV was found to be the main driver of SNV transmission; exposure to “contaminated” cages did not result in infection of deer mice.

A field study on the marsh rice rat (*Oryzomys palustris*) and the Bayou orthohantavirus (BAYV), a hantavirus causing few HCPS cases in the USA (Knust and Rollin, 2013), was performed after the 2010 Deepwater Horizon oil spill (Wallace et al., 2019). Anna Perez-Umphrey (USA) reported on the potential influences of the ecological disturbances on the population dynamics of the marsh rice rat and its BAYV prevalence. For this purpose, a capture-mark-recapture study was performed during 2013–2017 in coastal saltmarshes of Louisiana. First

results of the investigation revealed a lower survival rate for rice rats at "oiled" sites. BAYV-reactive antibodies were detected in rice rats at two of four "oiled" sites, but also at one of three "unoiled" sites. In addition, the seroprevalence did not differ between both types of sites. Interestingly, male rats were found to have a higher infection rate at both site types, and have a lower survival at "oiled" sites. The authors plan a rice rat immunome study to determine population genetic structure, and immune gene diversity.

Seoul orthohantavirus (SEOV) is the only hantavirus with a global distribution, but paradoxically also the most underestimated and often missed pathogenic hantavirus (Clement et al., 2019a), being described in, and isolated from, laboratory and commensal wild rats since nearly four decades (Clement et al., 2019b). Indeed, in addition to wild rats of different *Rattus* species, this hantavirus has now been (re-) detected in laboratory, pet and breeder rats in different countries. Barry Rockx (The Netherlands) described a study on feeder rats from a breeding farm. SEOV-positive rats were investigated by real-time RT-PCR. In all RT-PCR-positive rats, the lung sample was found to be SEOV RNA positive. In the lungs and conductive airways, including tracheas, no inflammation, nor SEOV antigen was detected in epithelial cells. Interestingly, in the liver of persistently infected rats a mild inflammation was observed. This finding raises again questions on the consequences of orthohantavirus infections on the fitness of the rodent reservoir.

Tula orthohantavirus (TULV) is a broad geographically distributed hantavirus species with several deep phylogenetic clades (Saxenhofer et al., 2017). Gerald Heckel (Switzerland) reported a parallel study of the common vole (*Microtus arvalis*) and TULV in a hybrid geographical zone of two evolutionary lineages of the common vole. In contrast to hybridization and local gene flow between the common vole lineages, there was no indication of genetic interaction between the TULV lineages, i.e. reassortment or recombination within this hybrid zone. TULV genomes of the different clades indicated a nucleotide sequence divergence of up to 22%; interestingly, the M-segment region, encoding the N-terminal region of the envelope glycoprotein, showed evidence of positive selection. Of further interest, TULV has only recently been molecularly confirmed as a (hitherto contested) human pathogen, resulting however in a very mild HFRS form, i.e. without noticeable renal function impediment (Reynes et al., 2015), explaining perhaps its current underestimation as a human pathogen (Clement and Van Ranst, 2016).

There is an increasing list of newly described hantaviruses in bats (Arai and Yanagihara, 2019; Witkowski et al., 2016). Satoru Arai (Japan) reported a multi-national study on bat-associated mobatviruses in Myanmar and Vietnam. Lung tissues of about 500 specimens of six bat families were investigated for hantavirus RNA by RT-PCR. Hantavirus RNA was detected in black-bearded tomb bats (*Taphozous melanopogon*), family Emballonuridae, and Pomona roundleaf bats (*Hipposideros pomona*), Stoliczka's Asian trident bats (*Ascelliscus stoliczkanus*) and ashy leaf-nosed bats (*Hipposideros cineraceus*), all family Hipposideridae. Determination of the complete S-, M- and L-genome segments resulted in the identification of three distinct mobatviruses: Đakrông virus in *A. stoliczkanus*, Láibĩn virus in *T. melanopogon*, and Xuân Sơn virus in *H. pomona* and *H. cineraceus*. The phylogenetic analyses indicated a shared common ancestry of mobatviruses and a basal position in the phylogenetic tree, suggesting that ancestral bats might have represented early mammalian hosts of ancient hantaviruses.

Sabrina Weiss (Germany) reported on the collection of bats of two families, Vespertilionidae (banana pipistrelle, *Neoromicia nanus* and white-winged serotine, *Neoromicia tenuipinnis*) and Molossidae (Angolan free-tailed bat, *Mops condylurus*) in two different parts of Africa. In the two *Neoromicia* species two different hantaviruses, i.e., Mouyassué virus and Ponan virus, were detected with a stable virus prevalence of 20% over five years. In *M. condylurus*, a novel, so far unknown hantavirus was discovered, tentatively named Kiwira virus. The collection of infected animals within or in vicinity of human settlements suggests potential human exposure.

Brno loanvirus is the only currently known bat-associated hantavirus in Europe (Straková et al., 2017). Since Petra Straková (Czech Republic) unexpectedly could not attend the conference, her contribution was presented by Boris Klempa (Slovakia). The initial detection of Brno hantavirus in the common noctule (*Nyctalus noctula*), family Vespertilionidae, was confirmed by an additional RNA-positive noctule, collected again in Brno city. Surprisingly, Brno virus RNA was also detected in a lesser horseshoe bat (*Rhinolophus hipposideros*), family Rhinolophidae, collected in a nature reserve north of Brno. This finding might be interpreted as a spillover infection, but further investigations are needed to understand the geographic distribution of this hantavirus in Europe and its host specificity.

Keynote lecture 3, Nicole D. Tischler (Chile): *The hantavirus surface: Molecular insights into glycoprotein dynamics and cell entry.*

Nicole Tischler reviewed the role of the Gn and Gc glycoproteins in viral cell entry and summarized the molecular and functional features of the hantavirus Gc fusion protein (Acuña et al., 2015; Barriga et al., 2016; Guardado-Calvo et al., 2016; Kleinfelter et al., 2015; Willensky et al., 2016) in comparison to other class II fusion proteins. Based on the 15.6 Å cryo-electron tomography map (Li et al., 2016), and 2-fold contacts observed in Gc crystals at neutral pH (Guardado-Calvo et al., 2016), she showed that it is possible to cross-link spikes by engineering of disulfide bonds (Bignon et al., 2019).

Additional mutations at this inter-spike interface showed that the ionic contacts orchestrate particle assembly, the overall spike stability, and also membrane fusion to establish inter-chain salt bridges in the stable Gc post-fusion trimer. Temperature-dependent experiments using native gels and coflotation assays revealed that the viral spikes display a temperature-dependent dynamic behavior at neutral pH, alternating between "open" and "closed" forms. In the closed form, the fusion loops are concealed, while in the open conformation, the fusion loops are reversible (Bignon et al., 2019). Viral infectivity and virus particle-liposome fusion experiments showed that this later conformation cannot drive membrane fusion and viral cell infection. Conformational dynamics have been observed for a number of unrelated viruses and challenged vaccine design to elicit neutralizing antibody responses (Graham, 2017; Kotecha et al., 2015; McLellan et al., 2013; Rey and Lok, 2018). In this context, the presented data suggest that the design of a subunit vaccine that exposes an inert "closed spike" conformation of hantaviruses would elicit the strongest antibody response.

1.2. Session 2: pathogenesis and immune responses. Oral presentations

This session was chaired by Detlev Krüger and Alemka Markotić. **To understand infection-related pathogenesis, virus interactions with monocytes play a central role. Grazielle Esteves Ribeiro (Chile) and collaborators presented some initial data on** transcriptome characterization of the immune response in monocytes of Andes orthohantavirus (ANDV)-infected patients between day 1 and 6 after onset of cardiopulmonary symptoms. The identified genes were related to activation of immune response and control of apoptosis. So-called blood transcriptional modules, associated with immune response, were found to be over-represented on different days in severely ill patients. In a parallel approach, Lidija Cvetko-Krajinović (Croatia)

and colleagues analyzed transcriptomic expression profiles in primary human blood monocytes of HFRS patients infected with PUUV. The most significant over-represented pathways were MHC class I mediated antigen processing and presentation, as well as interferon type I signaling. The study revealed that many functional alterations are happening in monocytes during their interaction with PUUV during the early phase of HFRS. Sindhu Vangeti (Sweden) and co-workers showed that monocyte and dendritic cell (DC) subsets were significantly reduced in the circulation of HFRS patients (especially $CD16^+$ non-classical monocytes that patrol vasculature, aiding or controlling inflammation), but they are normalizing over time to similar frequencies as observed in healthy individuals. 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 toll-like receptor stimulation. Moreover, Petra Svoboda (Croatia) and colleagues showed some differences between chemokines and cell surface molecules on monocytes, infected with PUUV or TULV.

Other presentations forwarded new findings on the virus interaction with B cells. Marina Garcia (Argentina) described both direct and indirect effects of hantaviruses on B cells. It was found that a human B cell line (BJAB) and primary B cells (pBC) are susceptible to both HCPS- 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.

Jussi Matias Hepojoki and coworkers (Finland) showed that acute orthohantavirus infection induces increase in serum free light chains (FLC). Their findings suggest that orthohantavirus infection induces activation of B cells, possibly due to active replication, which leads to overproduction of FLC. Since overproduction of FLC can be connected to various forms of kidney disease, they suggest the AKI in HFRS could be in part attributed to high titers of FLCs (Hepojoki et al., 2019). Andy Dernstedt (Sweden), looking at the small fraction of CD27-IgD-atypical B cells (ABC), demonstrated an association between reduced kidney function and accumulation of ABC in circulation. Moreover, their data emphasize that an increase of circulating ABC may result in a productive antiviral response in HFRS patients.

Daniel Bourquain (Germany) presented some findings related to the innate immune response. They showed that RIG-I-like receptors (RLR), including RIG-I and melanoma differentiation antigen 5 (MDA5), are essential pattern recognition receptors, which sense distinct dsRNA molecules that accumulate during PUUV infection. Although PUUV is able to delay the early interferon (IFN) response to allow successful replication, late IFN-induction via the RLR pathway still hampers viral replication. Continuing their recent apoptosis research, Charles Solà Riera and Jonas Klingström (Sweden) found out 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 (Solà-Riera et al., 2019a, 2019b). Alemka Markotić (Croatia) and colleagues were focused on changes of the IgG glycosylation profile in peripheral circulation of HFRS patients infected with PUUV, which may have an influence on dynamic of the inflammatory process during the course of disease, and could be used as useful biomarkers of HFRS severity.

In addition, Clas Ahlm (Sweden) and coworkers showed pulmonary endothelial glycocalyx degradation during HFRS, which normalizes during the follow-up period. Erich Mackow (USA) revealed potential mechanisms for ANDV N protein to direct pulmonary microvascular endothelial cell permeability via interactions with Rho GDP-dissociation inhibitor, suggesting potential therapeutic targets for resolving RhoA-directed endothelial cell barrier dysfunction during hantavirus infection. In support to previous immunopathogenesis studies, Miša Korva (Slovenia) showed that the major players in HFRS immunopathogenesis are pro-inflammatory mediators, which may mediate vascular dysfunction, disseminated intravascular coagulation, organ failure, and shock (Korva et al., 2019). Tomas Strandin (Finland) demonstrated that PUUV infection in its reservoir host (bank vole) promotes immunoregulatory responses by inducing IL-10, a cytokine strongly associated with chronic infection.

Keynote lecture 4, Jan Clement (Belgium), ISH President's lecture: *Proteinuria is an early, but rapidly evanescent diagnostic sign in probably all hantavirus infections*

.Jan Clement (Belgium) pointed out in his key talk that Old World hantavirus infections have as principal diagnostic signs different degrees of AKI, a renal lesion consisting in fact of three ± concomitant laboratory anomalies: 1) ↓glomerular filtration rate (GFR), 2) proteinuria, and 3) microhematuria (Clement, 2015). Since these three initial renal anomalies are completely asymptomatic, they can easily be missed, particularly in mild cases. Moreover, AKI is too often considered as synonymous to ↓GFR, but in these same mild cases, the "kidney injury" can consist of only very transient proteinuria and/or microhematuria, i.e. AKI presenting with normal serum creatinine levels (Swanink et al., 2018). Endothelial hyperpermeability is often described as the main mechanism for transient capillary leak (Maes et al., 2004; Vaheri et al., 2013), causing acute symptoms in both HFRS and HCPS. However, in all affected organs, including the kidney, this hyperpermeability involves in fact at least three successive layers, all functioning normally as leaking barriers: endothelium (plus its glycocalyx), basement membrane, and epithelium. Concomitant breaching of all these three barriers necessitates an intense paracrine crosstalk, of which hitherto little is known, but DCs, monocytes and macrophages, activated by hantaviral infection, all play a critical modulatory role. For instance, intense macrophage activation can result in a variant of another immunopathological affection, called hemophagocytic lymphohistiocytosis, marked likewise by transient AKI and massive proteinuria (Clement et al., 2016b, 2016c).

Of note, the renal capillary tuft is covered with a unique epithelial layer, consisting of podocytes, which are equally covered by an "external" glycocalyx. Podocytes are highly specialized cells, which have the "slit diaphragm" as inter-podocyte bridging structure, responsible for most, if not all, renal ultrafiltration, and which is temporarily disturbed in its function ('acute podocyte injury' or API) by the transient pro-inflammatory cytokine storm, typical for human hantaviral infection (Maes et al., 2004). This API results in often-massive glomerular proteinuria and microhematuria (Chand et al., 2020; Clement, 2015; Clement et al., 2014; Mantula et al., 2017). However, these asymptomatic urinary abnormalities have a life span of mostly only 2 weeks (Clement et al., 2014; Mantula et al., 2020), as described already in 1964 in 32 HFRS cases, probably induced by SEOV, and spread by wild commensal rats in Osaka, Japan (Tamura, 1964). Moreover, this novel concept of API could also explain the hitherto ill-known mechanisms of AKI with proteinuria in other emerging zoonotic infections (Clement et al., 2016b).

Consequently, presumptive diagnosis of a hantaviral infection can be quickly confirmed on admission by demonstrating both thrombocytopenia, plus proteinuria and microhematuria, the latter by simple, but preferentially daily urine dipstick examination. This easily obtained

bedside information is valid for both Old and New World hantaviral human infections. In the latter, whenever early proteinuria and microhematuria were eventually examined, they were confirmed indeed, not only in ANDV infections (Lázaro et al., 2000; Peters and Khan, 2002), but also in SNV (Passaro et al., 2001) and in New York virus infections (Fernando et al., 2019). Very recently, the eight[h] SEOV-HFRS case in the USA was reported in Colorado, being initially considered as non-fatal HCPS with mild interstitial infiltrates on RX chest, with a high SNV-specific Ig M titer, but without molecular confirmation by viral sequencing. Nevertheless, the case was reported as being SEOV-induced, since presenting in a farmer with a history of recent rat exposure, and showing a self-remitting 2-weeks course with mild AKI, but with nephrotic-range proteinuria on sequential urine samples (Chand et al., 2020).

Keynote lecture 5, Jonas Klingström (Sweden): *Immune response during human hantavirus infection: immunopathogenesis and keys to treatments.*

Hantaviruses infect endothelial cells, but also other types of cells, where they manipulate important cellular functions including apoptosis, stress granule formation and innate immune responses (Christ et al., 2020; Gupta et al., 2013; Solà-Riera et al., 2019a, 2019b; Stoltz et al., 2007). In turn, infected cells can activate cytotoxic lymphocytes (Braun et al., 2014). This strong capacity to manipulate infected as well as bystander cells is likely involved in shaping the pro-inflammatory responses observed in HFRS and HCPS (Klingström et al., 2019). A hallmark of HFRS and HCPS is strong immune responses, including high levels of pro-inflammatory cytokines and vigorous natural killer cell, CD8 T cell, and B cell responses (Garcia et al., 2017; Klingström et al., 2019; Maleki et al., 2019). Recent observations, indicating an important role for hantavirus-mediated immune responses in driving immunopathology, can hopefully provide new insights into HFRS and HCPS disease pathogenesis and possible treatment strategies, including aggressive anti-inflammatory treatment (Klingström et al., 2019).

1.3. Session 3: Virus phylogeny, replication and morphogenesis. Oral presentations

This session was chaired by Colleen B. Jonsson and Richard Yanagihara. In the first presentation, Amar Dhananjai Parvate (USA) reported on the optimization of a glutaraldehyde-fixation protocol to inactivate biosafety level 3/4 (BSL-3/4) classified New World orthohantaviruses for high-resolution cryo-electron microscopy and tomography (Parvate et al., 2019). New World orthohantaviruses exhibited a variety of morphologies, with Black Creek Canal orthohantavirus (BCCV) showing predominantly tubular structures. Cryo-tomography studies with a target resolution of 20 Å showed that the spikes of BCCV and ANDV resembled that of Old World orthohantaviruses. The fixation protocol, which has been replicated successfully in multiple laboratories, makes many hitherto difficult-to-study BSL-3/4 viruses amenable to analysis by cryo-electron microscopy.

Won-Keun Kim (Korea) and colleagues used multiplex PCR-based next-generation sequencing (NGS) (Kim et al., 2016) to investigate the epidemiologic and phylogenetic characteristics of SEOV infection in six cases of HFRS and in *Rattus norvegicus* collected in HFRS-endemic areas in Korea. Multiplex PCR-based NGS revealed nearly whole-genome sequences of SEOV from HFRS patients and reservoir hosts. Comparative analyses of the SEOV M and S segments demonstrated distinct genotypes and a possible genetic reassortment. Phylogeographic analysis will aid in tracking SEOV and in mitigating disease risks in outbreaks of SEOV infection.

Florian Binder (Germany) investigated the role of PUUV S-segment non-structural (NSs) protein by conducting large-scale sequencing of PUUV in infected bank voles (*Myodes glareolus*) trapped during 2010–2014 in Baden-Wuerttemberg and North Rhine-Westphalia. The NSs open reading frame (ORF) showed a higher number of non-synonymous mutations than the overlapping and non-overlapping nucleocapsid (N) ORF. Further analysis revealed positive selection for the NSs ORF and a negative (purifying) selection for the non-overlapping N ORF. As assessed by mutational studies, NSs showed inhibiting activity of the interferon-ß promoter as previously found by others (Jääskeläinen et al., 2007). The results suggested an influence of bank vole population dynamics on the molecular evolution of PUUV.

Jörg Hofmann (Germany) reported on the potential of using an L-segment screening PCR (Klempa et al., 2006) to ascertain the spatial distribution of PUUV. Of 93 PUUV-infected patients with positive L-segment screening PCR, only 60 (65%) were also PCR positive for the S segment. Segment-specific phylogenetic trees resulted in well-supported clusters corresponding to the geographical origin of PUUV. Thus, analysis of sequences directly obtained from a diagnostic L-segment screening PCR allowed for PUUV typing and could be used to assess the molecular epidemiology of PUUV-associated HFRS (Weiss et al., 2019).

Giulia Gallo (France) described the *in vitro* interactions of pathogenic PUUV and low- or non-pathogenic TULV and Prospect Hill orthohantavirus (PHV) with cellular factors of the human and rodent reservoir hosts. In assessing the differential susceptibility of cell lines, only simian Vero-E6 kidney cells and human HuH7 hepatocyte and THP1 monocyte cells were permissive to the three orthohantaviruses. By contrast, cell lines derived from bank vole and common vole (Binder et al., 2019) showed species specificity to PUUV and TULV, respectively. As evidenced by virus infectivity titer and viral RNA load, differential virus-host interactions aligned with the virus species and cell type. These results complement proteomics and interactomics approaches that demonstrate differential interactions between viral proteins and host cellular factors.

Boris Klempa (Slovakia) used an in-solution hybridization-capture approach followed by NGS to obtain all three genomic segments of Tigray virus (TIGV) from *Stenocephalemys albipes*, *S. albocaudata* and *S. griseicauda* in Ethiopia, thus confirming that *Stenocephalemys* mice are the reservoir hosts (Meheretu et al., 2012). Phylogenetic analyses, using maximum-likelihood and Bayesian methods, confirmed the conflicting placement of TIGV in segment-specific trees, suggesting that TIGV might have emerged from ancient reassortment events, involving ancestral rodent- and shrew-borne hantaviruses.

Lorraine M. McElhinney (UK) reported on the whole genome of Tatenale virus (TATV), an orthohantavirus identified recently in field voles (*Microtus agrestis*) from the United Kingdom (Pounder et al., 2013). A hybrid TATV sequence, constructed by combining TATV-specific reads obtained from tissues of two field voles, was used to realign reads and assemble the TATV genome. The S, M and L segments of the TATV genome were 1,994, 3,501 and 6,446 nucleotides in length. Nucleic acid sequence similarity with other group 3a hantavirus genomes was 60.4–64.8% for S, 65.1–72.9% for M and 74.0–76.0% for L, reinforcing that TATV represents a new orthohantavirus species.

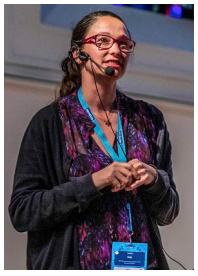
Felix A. Rey (France) reported on the X-ray structures of the Gn/Gc ectodomain of ANDV and Maporal orthohantavirus (MAPV), at 2.6 and 3.2 Å resolution, respectively.

Protocadherin-1 (PCDH1) has been identified recently as a critical determinant of attachment, entry, and infection by New World orthohantaviruses (Jangra et al., 2018). In addition, integrins (β1, β2, and β3), decay accelerating factor (DAF/CD55) and gC1qR/p32 have been

proposed as hantavirus receptors (Mittler et al., 2019). Maria Eugenia Dieterle (USA) reported on the use of HPMEC-ST1.6R, an immortalized human pulmonary microvascular endothelial cell line (HPMEC), to establish a tractable *in vitro* model to study hantavirus entry and infection. Using CRISPR/Cas9 genome engineering, single- and double-knockout clonal cell lines deficient in PCDH1, αvβ3 integrin and DAF were generated to investigate their specific roles in hantavirus entry. Together with receptor-specific antibodies, soluble receptors and recombinant vesicular stomatitis viruses (VSV), 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.

Yaiza Fernández-Garcia (Germany) reported on insights into ANDV mRNA synthesis. Residues 1–200 at the N-terminus of the ANDV L protein were identified as the cap-snatching endonuclease. Due to difficulties in producing the soluble wild-type L protein, single amino acid mutants were assessed for their thermal stability and nuclease activity (Fernández-García et al., 2016). The structures of mutants K127A and K124A generated a pseudo wild-type model of the L protein, which revealed an active site that is conserved throughout evolution of orthohantaviruses.

Lies Laenen (Belgium) reported on the patterns and drivers of PUUV enzootic dynamics (Laenen et al., 2019). Longitudinal sampling of isolated reservoir populations over approximately three decades was used to estimate PUUV evolutionary rates, which were applied to study the impact of environmental factors on viral spread. PUUV was found to accumulate genetic changes at a rate of $\sim 10^{-4}$ substitutions per site per year and land cover type defined 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.



Keynote lecture 6, Cecilia Vial (Chile): *Understanding hantavirus cardiopulmonary syndrome (HCPS).*

Cecilia Vial talked about how the patient's response to ANDV infection is participating in the 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 and cause leakage (Mertz et al., 2006). After analyzing patient's immune response, she found that this was a typical viral response with great involvement of CD8 T-cells.

When comparing patient's genetic variation to understand why some patients have a mild or severe response to ANDV infection, two copy number variants, that involve two deletions in genes participating in innate immune response, where associated to clinical course. The first one is a deletion that includes complement factor H-related protein (CFHR1 and 3) genes. These proteins regulate the complement-signaling pathway (Heinen et al., 2009; Jòzsi et al., 2019), and its deletion is a risk factor to have a severe clinical course. The second one is a deletion that includes the signal-regulatory protein beta-1 (SIRPB1) gene, which has been shown to regulate neutrophil transepithelial migration (Liu et al., 2005). The deletion of this gene is a protective factor, and was found more frequently in mild HCPS patients (Ribeiro et al., 2019).

Finally, when analyzing laboratory tests at admittance and sociodemographic characteristics of patients, three different characteristics were found in severe patients: platelets < 115 μL (Lopez et al., 2019), being of European ancestry, and living in an urban area (Vial et al., 2019). The initial degree of proteinuria and microhematuria was however not evaluated.

1.4. Session 4: Epidemiology. Oral presentations

This session was chaired by Anna Papa and Hiroaki Kariwa. The first speaker of the session was Detlev Krüger (Germany), who described the epidemiology of hantaviruses in Germany where the disease is notifiable since 2001. Since then, more than 12,000 human cases have been reported in the country, with major outbreaks in 2010, 2012 and 2017 (2000–3000 cases each). It is of interest that the majority of cases is caused by PUUV, and occur mainly in early summer in the western and southern part of the country, while few sporadic cases are caused by DOBV-Kurkino genotype, and occur in the northeastern part of the country, with a peak of cases in early winter. A clear spatial geographic clustering is observed in both PUUV and DOBV-Kurkino sequences, while sequences from clinical cases cluster together with those of the respective rodent hosts, trapped in the regions where the human cases occurred (Faber et al., 2019).

Ellen Murthy (UK) presented the hantavirus surveillance in rodents (brown rats, field and bank voles), trapped in peri-domestic sites (pig farms, commercial premises and rural dwellings) in Great Britain (GB). SEOV was detected in 13 of 68 (19%) rats, mainly in pig farms; 12 sequences were highly similar to each other and to the previously reported Hunter strain of SEOV in the UK, while one sequence was more distant. In 7/23 (30.4%) field voles, TATV was detected, an orthohantavirus initially detected in 2013 in a field vole in GB (Pounder et al., 2013). Although the potential pathogenic risk of TATV is still unknown, SEOV is a known human pathogen, and it seems that it poses a potential risk to public health in the country (Murphy et al., 2019). Of note, the commensal wild rat is the only hantavirus rodent reservoir with a worldwide distribution, and SEOV-infected rats have been demonstrated since nearly 4 decades in all continents, including Africa (Clement et al., 2019b). Underestimation of SEOV-HFRS may partly be due to the often mild or atypical presentation of many patients, including cases with a throughout normal renal filtration function (Clement et al., 2014; Swanink et al., 2018), and/or to misleading serological cross-reactions in many commercial diagnostic kits (Chand et al., 2020; Clement et al., 2019b).

ANDV was first identified in 1995 from an HCPS patient in southwestern Argentina, and soon it was realized that the virus has a person-to-person transmission potential (Padula et al., 1998). Valeria Paula Martinez (Argentina) presented a major outbreak of 33 HCPS cases in Epuyén, a rural town in southwestern Argentina, which occurred in 2018–2019, and was caused by ANDV. All cases were epidemiologically related to one HCPS case and person-to-person transmission was confirmed, since the full-genome sequences from the patients were almost identical. Dr. Martinez presented also the strict control measures adopted, which led to the limitation of further viral transmission. The outbreak lasted 97 days, and 12 patients died.

In 2016, the first patient infected with SEOV was reported in the Netherlands and three other SEOV-infection cases were diagnosed in 2017. All patients had been in contact with either feeder rats or pet rats. Of note, this first Dutch index SEOV case showed once more no renal function impediment, only a proteinuria of 0.25 g/L. However, “transaminitis” and thrombocytopenia, both spontaneously remitting within 3 weeks, were recorded (Swanink et al., 2018). Miriam Maas (The Netherlands) presented results on SEOV infection in captive rat

populations in the country. Rats collected from commercial feeder rat breeding farms, ratteries, and individual rat owners were tested by serology and RT-qPCR. Two out of nine commercial farms had SEOV positive rats (5/10 and 6/10) and one out of eight ratteries had positive rats (2/8). In 29 rats of private owners, one rat was positive for SEOV.

A variety of hantaviruses have been identified in shrews, moles and bats (Arai and Yanagihara, 2019; Vaheri et al., 2013). Liudmila Yashina (Russia) presented insectivore-borne hantaviruses in Asian Russia. Investigation of insectivore-borne hantaviruses was conducted in 30 locations of Western, Eastern Siberia and Far East Russia. In 455 insectivore lungs, 73 samples were positive for hantavirus RNA. The phylogenetic analyses indicated that seven distinct insectivore-borne hantaviruses are endemic in shrews and moles of these areas. Those were Seewis orthohantavirus (SWSV) in *Sorex* species, Artybash virus in *S. caecutiens*, Kenkeme orthohantavirus in *S. roboratus*, Yakeshi orthohantavirus in *S. isodon* and *S. unguiculatus*, Altai virus in *S. araneus*, Lena virus in *S. caecutiens*, and a novel virus, named Academ virus in *Talpa altaica*.

Chronic kidney disease of uncertain etiology (CKDu) is a major public health threat in farming areas of Sri Lanka, and the exact etiology of CKDu has not yet been elucidated (Gamage and Sarathkumara, 2016). N. P. Sunil-Chandra (Sri Lanka) presented positive rates of antibodies to hantavirus and *Leptospira* in humans in CKDu high prevalent and low prevalent areas of the North Central province of Sri Lanka. 50 out of 179 (28%) CKDu patients, but also 16 out of 49 (32.6%) healthy blood relatives in a highly prevalent area, Anuradhapura district, had IgG antibodies to PUUV or HTNV, or to both viruses. However, 7 out of 48 (14.6%) healthy individuals, living in a low CKDu prevalent area, had also antibodies to hantaviruses. Positive rates of IgG antibody to *Leptospira* showed no difference between high and low prevalence CKDu areas. Moreover, co-infections with both *Leptospira* and hantaviruses were previously demonstrated in Sri Lankan patients, hospitalized with acute fever and AKI (Sunil-Chandra et al., 2015). Finally, it is now clear that the renal function impediment in proven hantavirus infections is of maximum 2–3 weeks duration (Clement et al., 2014; Mustonen et al., 2017; Outinen et al., 2015). Consequently, if all other concurrent causes of renal damage have been excluded, it seems now highly improbable that a hantavirus infection by itself could lead to any form of CKD (Clement et al., 2017).

Russia is known to be a highly endemic country for HFRS with a variety of epidemiology (Kariwa et al., 2009). Evgeniy Alexander Tkachenko (Russia) presented current status of HFRS in the country. A total of 137,590 cases of HFRS were reported between 2000 and 2017 in Russia. Most of the patients (98.4%) were reported in European Russia, and only 1.6% of the patients were from Asian Russia. The average fatality rate was 0.4%. Six hantaviruses were recognized as etiological agents of HFRS in Russia; HTNV, Amur virus, SEOV, PUUV, and two genotypes (Kurkino and Sochi) of DOBV having the principal hosts with *Apodemus agrarius, A. mantchuricus*, *A. peninsulae, Rattus norvegicus, Myodes glareolus, A. agrarius,* and *A. ponticus*, respectively. The incidence rate and pathology of HFRS vary, depending on the distribution of host animals and virus types in the different regions (Tkachenko et al., 2019).

In Europe, NE, a clinical form of mild HFRS, is caused by PUUV, which is carried by bank voles (Clement and Maes, 2015; Settergren, 2000). Sarah Madrières (France) presented PUUV infection in bank voles, experimentally infected with PUUV strains from endemic and peri-endemic areas in France. The results indicate that Hargnies-PUUV from the endemic area (Ardennes) has a higher capacity of replication in the infected animals than Vouzon-PUUV from the peri-endemic area (Loiret).

Five lightning talks were included in the epidemiology session. Abbas Deeb (France) presented a HFRS case with severe AKI, major proteinuria and significant hematuria in a plumber who returned to France after spending one month in Bulgaria. Serology initially suggested an acute SEOV infection, but RT-PCR showed in fact a DOBV viremia. This first demonstration of severe DOBV-HFRS in France might consequently be an imported case. Marcela Ferres (Chile) presented two pairs of HCPS cases (mothers and their breastfed children) in Chile caused by ANDV, and the role of breastfeeding was discussed as the virus was detected in blood and breastmilk of one mother. In the meantime, the youngest hitherto case of ANDV infection was documented in an Argentinean newborn, whose mother succumbed to a fatal HCPS. The possible routes of transmission considered were: intrauterine, perinatal during caesarean section, breastfeeding, or aerosol transmission from its ANDV-infected mother (Bellomo et al., 2019).

A small-scale epidemiological survey and phylogenetic analysis of hantaviruses in western Poland was presented by Seung-Ho Lee (Korea). DOBV-Kurkino genotype and SWSV were detected in 2/42 (4.8%) *A. agrarius* and 3/10 (30%) *Sorex araneus*, respectively, while *A. flavicollis* (n = 25) and *M. glareolus* (n = 27) tested negative for hantaviruses. Jorlan Fernandes (Brazil) reported on Castelo dos Sonhos hantavirus (CASV). Although initially detected in 1995 from a fatal HCPS case in Brazilian Amazon, CASV seems to be unnoted in the region; it was shown that the virus causes several severe cases with 50% fatality. Finally, Rainer G. Ulrich (Germany) discussed the potential influence of coinfections on the outcome of hantavirus infections in reservoirs. He reported the complexity of PUUV phylogeny, which currently includes 8 genetic lineages, differing by approximately 20% at nucleotide level in the S segment. A real-time RT-PCR assay, capable to detect all PUUV strains, will be a very helpful diagnostic tool.

Keynote lecture 7, Richard Yanagihara (USA): *Genetic diversity, geographic distribution and evolutionary history of bat-borne loanviruses and mobatviruses.*

In his keynote talk, Richard Yanagihara (USA) provided an overview about the emerging insights into the evolutionary origins and phylogeography of hantaviruses harbored by bats, belonging to the suborders Yangochiroptera and Yinpterochiroptera, in Asia, Africa and Europe. Phylogenetic analyses, based on partial and full-length S-, M- and L-genomic sequences, using maximum-likelihood and Bayesian methods, showed that all 13 bat-borne loanviruses and mobatviruses discovered to date share a common ancestry, which is consistent with their host phylogeny. Moreover, the basal position of mobatviruses suggests that bats, rather than rodents, probably served as the primordial mammalian hosts of ancestral hantaviruses (Arai and Yanagihara, 2019). Finally, the fact that many more hantaviruses have been detected in rodents, shrews, moles and bats in Asia suggests an Asian origin of hantaviruses. Future investigations must focus on the myriad unanswered questions about the genetic diversity and geographic distribution, as well as the pathogenic potential, of bat-borne loanviruses and mobatviruses.

Keynote lecture 8, Kartik Chandran (USA): *Unravelling and inhibiting the cell entry mechanisms of hantaviruses.*

In his keynote, Kartik Chandran stressed that the mechanisms by which hantaviruses enter cells are still poorly understood, and that few

entry-related determinants of hantavirus virulence, host range, and tissue tropism have been identified so far (Jangra et al., 2018). Most significantly, a cadherin superfamily member, PCDH1, was recently identified as a critical entry factor for New World hantaviruses and a key determinant of ANDV virulence in the lethal Syrian hamster model (Jangra et al., 2018; Mittler et al., 2019). K. Chandran characterized the molecular mechanism by which hantavirus Gn/Gc engages PCDH1, the cell-biological roles of this interaction, and its specific functions vis-à-vis other known and putative hantavirus entry factors. He also presented findings suggesting that Gn/Gc:PCDH1 interactions can influence viral host range. Finally, he discussed the development of passive anti-hantavirus immuno-therapeutics that target the Gn/Gc entry machinery, and their interactions with PCDH1.

1.5. Session 5: Vaccines, therapeutics and prevention. Oral presentations

This session was chaired by Connie S. Schmaljohn and Mifang Liang. In the first talk of the session, Giulia Torriani (Switzerland) described studies using VSV pseudotypes with a GFP reporter to identify cellular factors involved in hantavirus entry into A549 cells. By screening a library of kinase inhibitors of major cell signalling pathways, she determined that both HTNV and ANDV likely enter cells through macropinocytosis, but that they use unique receptors and coreceptors. These results may help to explain the differing dependence on cellular factors that have been previously reported, and provide a new direction for investigating antiviral treatments for hantaviruses.

In the next talk, Jay Hooper (USA) described evaluation of a polyclonal antibody preparation derived from the serum of transchromosomic bovines, expressing fully human IgG genes. The bovines were vaccinated with a DNA vaccine for HTNV, and sera from the animals were tested for neutralization of HTNV, and for protection of Syrian hamsters from infection with HTNV. He found that passive transfer of the sera one day prior to HTNV challenge greatly limited HTNV replication. Further, in a newly developed marmoset infection model, he was able to show that the antibody partially clears the virus. These results demonstrated the potential of therapeutic anti-HTNV antibodies for treating or preventing HFRS.

In the third talk of the session, Maria Ines Barria (Chile) described ANDV neutralizing monoclonal antibodies derived from HCPS patients. She selected two highly neutralizing antibody clones from 400 IgG positive cells screened, and showed that they bind to different regions of the ANDV glycoproteins. A combination of these monoclonal antibodies, passively transferred to Syrian hamsters, prevented lethal disease, even when the antibodies were delivered up to five days after infection with ANDV. These results show great promise for treatment of HCPS patients, using an antibody-based approach.

Derek Stein (Canada) presented the fourth talk, which focused on recombinant VSV vaccines for SNV and ANDV. He found that both vaccines elicited cross-reactive IgG in hamsters, and that both vaccines provided cross protective immunity to Syrian hamsters from challenge with SNV or ANDV. This study indicates that a single vaccine might be sufficient to protect against multiple HCPS-causing hantaviruses.

The next talk of the session by Danny Noack (USA) described mouse monoclonal antibodies to ANDV. Mice were vaccinated with a DNA vaccine, and antibodies were screened using a VSV-ANDV pseudotype. Numerous monoclonal antibodies to Gn and Gc were obtained and used to generate antibody escape mutants, which are being studied by deep sequencing to identify important neutralizing epitopes on ANDV.

Erich Mackow (USA) presented the sixth talk of the session, in which he described screening of antiviral inhibitors of viral polymerase. In his studies, he identified a lead compound, Baloxovir (BXA) with potent inhibition of ANDV replication. Further studies are in progress to test 800 BXA analogues and provide a new strategy for therapeutic intervention on HCPS.

In the final talk of the session, Eva Mittler (USA) presented results of efforts to derive human monoclonal antibodies from convalescent PUUV patients. They isolated and screened approximately 180 antibody clones, and identified 50 of them with strong antigen binding affinity and pseudo-virion neutralizing activity. She further described efforts to identify the mechanism of neutralization. Among the findings were that potent neutralizing antibodies do not prevent binding, but that both Gn- and Gc-specific antibodies do block fusion. Continued down selection of the antibodies for cross-neutralization and eventual passive protection experiments in hamsters are planned.

1.6. Session 6: Clinical Aspects and diagnosis. Oral presentations

This session was chaired by Jan Clement and Clas Ahlm. In many countries, there are several human pathogenic orthohantaviruses circulating (Clement et al., 1996; Clement and Maes, 2015; Faber et al., 2019; Vapalahti et al., 2003). Cross-reactions are common in ordinary serological tests and molecular detection of virus is only possible in the early viremic stage of the infection. Therefore, additional diagnostic tools are needed for identification of which strain causes the infection, to predict disease severity and outcome, identify the potential source of infection/reservoir, and for purpose of surveillance. To be able to discriminate between the different orthohantavirus strains in Europe, Tabitha Hoorweg (The Netherlands) and collaborators developed a high-throughput microneutralization test (MNT). They successfully validated the test towards panels of human and rodent sera positive for PUUV, DOBV, SEOV, and TULV. In addition, the MNT could confirm 94% of the 54 clinically suspected cases that were included in the study.

In Slovenia, both DOBV and PUUV orthohantaviruses are present. Emil Pal (Slovenia) presented time-sequenced data from 81 patients of which 16 patients were infected by DOBV (Pal et al., 2018). One third of the patients had rapidly transient myopia, 28% had oliguria/anuria, 91% polyuria, and 35% sinusal bradycardia. Thrombocytopenia ($< 130 \times 10^9$/L) was present in 94% of the patients, while CRP and procalcitonin was elevated in 99% and 91%, respectively. The rapid appearance and disappearance of proteinuria and microhematuria was however not evaluated. When comparing the clinical and laboratory parameters, patients with DOBV infection had a more severe disease than PUUV-infected patients, i.e. more pronounced and longer duration of laboratory abnormalities. The authors also conclude that the classical clinical picture with five distinct phases, as formerly described in Asian HFRS (mostly HTNV-induced) cases, were not present in the majority of these European patients.

Severe PUUV infection may have similar clinical features as HCPS, i.e. hypotension and non-cardiogenic acute pulmonary edema (Clement et al., 1994; Clement et al., 2014; Gizzi et al., 2013; Rasmuson et al., 2011), sometimes requiring life-saving extracorporeal membrane oxygenation (ECMO) treatment (Vollmar et al., 2016). The suggested pathogenic mechanism, vascular leakage, is likewise the same in all hantavirus infections, and one potential mediator is bradykinin. Accordingly, treatment with icatibant, a bradykinin receptor antagonist, has been used successfully in a few PUUV-HFRS cases (Antonen et al., 2013; Laine et al., 2015; Vaheri et al., 2014). Johan Rasmuson (Sweden) presented preliminary data from another severe case of PUUV infection that died, despite of intensive care and one dose of subcutaneous treatment with icatibant.

AKI is common in HFRS. In Finland, PUUV virus causes a milder form of HFRS, also called NE. In a Finnish study presented by Jukka Mustonen, 195 patients with serologically verified PUUV infection were analyzed for urine glucosuria using dipstick, which was found in 24 (12%) of cases (Tietäväinen et al., 2019). Transient initial glucosuria predicted the overall clinical severity, and was significantly associated with the levels of serum creatinine, leukocytosis, and the degree of thrombocytopenia, and of all parameters reflecting capillary leakage. Glucosuria, if present, may therefore be used for a rapid risk stratification on admission.

Jin Sun No (Korea) showed the low coverage and sensitivity for whole-genome sequencing of HTNV from *Apodemus agrarius* lung

tissues containing low viral RNA loads, in contrast to the much higher yield by single-primer amplification-based NGS, RNA access- and multiplex PCR-based NGS. Furthermore, he demonstrated that the multiplex PCR-based NGS had a 10-fold (102 copies/μL) higher sensitivity than the RNA access-based method, concluding that target-enrichment NGS for the identification and characterization of orthohantavirus outbreaks might be possible, without isolating the viruses (No et al., 2019).

Ivan-Christian Kurolt (Croatia) demonstrated that levels of kidney injury molecule-1 (KIM-1), a transmembrane receptor from the T cell immunoglobulin and mucin domain family, were significantly increased in urine of 61 PUUV-HFRS patients, both at hospitalization and before discharge, compared to healthy controls. In the convalescent urine samples, the overall KIM-1 excretion was significantly lower than during the acute phase of disease. Due however to a majority of mild cases (76%), with no or only moderately increased serum creatinine (mean: 123 μmol/L; range: 72–893 μmol/L), no significant correlation between KIM-1 and these traditional markers of kidney function has been observed. Nevertheless, the study indicates that KIM-1 could be a more sensitive biomarker of kidney injury in HFRS than serum creatinine levels.

Finally, Martin Edward Winstanley (Wales, UK) detailed a HFRS case, which was initially misdiagnosed as leptospirosis. The patient was probably SEOV-infected, since he lived in a caravan at his place of work – a local farm that produced rats for pet feed, and since this was the second case from this particular Welsh rat facility. He presented worsening AKI and deranged liver function, prompting ultimately a transfer to a tertiary renal centre for specialist input. Further investigations revealed negative serology for *Leptospira*, but positive for a hantavirus infection. Both hantavirus and *Leptospira* cause a flu-like syndrome with high fever, pronounced liver participation, and AKI, which has led formerly in leptospirosis-suspected sera collections to the first serologically documented hantavirus infections, respectively in the New World (Recife, Brazil) (Hinrichsen et al., 1993), and in India (Clement et al., 2006). Moreover, patients can suffer from both concomitant zoonotic illnesses, as has been demonstrated in cases hospitalized for fever with AKI in Sri Lanka (Sunil-Chandra et al., 2015).

Four clinical “lightning talks” followed: 1) Iva Christova (Bulgaria) showed in a prevalence study, including ELISA, immunoblots and RT-PCR, that hantavirus infections (PUUV and DOBV) and Crimean-Congo hemorrhagic fever (CCHF) were two viral hemorrhagic fevers with a much higher spread (3.1% and 3.7% respectively), than anticipated in Bulgaria, probably due to many mild or even subclinical presentations. 2) Shannon Whitmer (USA) presented a pan-hantavirus enrichment protocol for NGS on limited clinical material, assessing sensitivity with synthetic samples, and specificity with diverse hantavirus strains CDC sequenced directly from human clinical specimens. 3) Misa Korva (Slovenia) presented one-step multiplex qRT-PCR assay for simultaneous detection of both DOBV and PUUV on the whole S segment of Slovenian strains. Molecular results correlated in 94.7% of 263 HFRS cases with results of IgM/IgG EIA, or with rapid ReaScan Point-of-Care test, but urine RNA was found in only 12% of samples. However, PUUV RNA was positive in 4 patients who seroconverted only 3 days later. 4) Mariah Taylor (USA) examined various applications of NGS approaches on PHV, a BSL-2 non-pathogenic orthohantavirus, to allow rapid and accurate identification of pathological hantavirus strains. The best amplification method for detection appeared to be the 30-cycle PCR and a multiplex-primer approach.

Of special clinical interest in the posters, Paula Mantula (Finland) et al. noted the amount of overnight albuminuria at different time points between the acute phase and until six months after the hospitalization in 133 PUUV-HFRS cases. Confirming an unexpected feature in nephrology (Clement et al., 2014; Clement, 2015), a “flash-like albuminuria” disappeared very rapidly, after a peak around day 5 (after onset of fever), up to day 14, regardless of the amount in the acute phase (Mantula et al., 2020). If confirmed, this would mean the clinician is mandated to follow, from the day of admission on, by daily urine dipstick the real magnitude of albuminuria in all HFRS (and HCPS?) cases: a simple, rapid, non-invasive, and cheap bed-side monitoring, which however is almost never performed.

Acknowledgments

We are grateful to Prof. Piet Maes (Rega Institute, Leuven, Belgium) for the group's picture in Fig. 1, as for all his other ICH 2019 pictures. We are also indebted to Ann Moerenhout, and her team from the KU Leuven Conferences and Event Office, for her skillful and professional assistance in the organization of this 11th International Conference on Hantaviruses. We are also grateful for the sponsoring by Life Science Research Partners (LSRP), the Research Foundation of Flanders (FWO), and the commercial firms Reagena, ApDia, and r-biopharm.

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