Poster 168

MEASLES FUSION MACHINERY IS DYSREGULATED IN NEUROPATHOGENIC VARIANTS

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Abstract: Paramyxoviruses, including the human pathogen measles virus (MV), enter host cells by fusing their viral envelope with the target cell membrane. This fusion process is driven by the concerted action of the two viral envelope glycoproteins, the receptor binding protein (hemagglutinin (H)) and the fusion protein (F). H attaches to specific proteinaceous receptors on host cells; once receptor engages, H activates F to directly mediate lipid bilayer fusion during entry. In a recent MV outbreak in South Africa several HIV positive people died of MV central nervous system (CNS) infection. We analyzed the virus sequences from these patients and found that specific intra-host evolution of the F protein had occurred and resulted in viruses that are "CNS-adapted". A mutation in F of the "CNS-adapted" virus (leucine to tryptophan present at position 454) allows it to promote fusion with less dependence on engagement of H by the two known wild-type (wt) MV cellular receptors. This F is activated independently of H or receptor, and has reduced thermal stability and increased fusion activity compared to the corresponding wt F. These functional effects are the result of the single L454W mutation in F. We hypothesize that in the absence of effective cellular immunity, such as HIV infection, MV variants bearing altered fusion machinery that enabled efficient spread in the CNS underwent positive selection.
Disclosure of Interest: None declared

Poster 169

CHD1 CHROMATIN REMODELER IS A POSITIVE MODULATOR OF INFLUENZA VIRUS REPLICATION THAT PARALLELS RNAP II DEGRADATION IN THE INFECTED CELLS

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Abstract: Influenza A virus polymerase associates with chromatin components of the infected cell, such as the CHD6 chromatin remodeler. Here we show that CHD1, a member of the same family, also interacts with the viral polymerase complex and positively modulates viral replication. Silencing of CHD1 causes reduction on viral polymerase activity, viral RNA transcription and production of infectious particles. Similar results are obtained during infection with H1N1 and H3N2 influenza virus subtypes, but not with Vesicular stomatitis virus or Adenovirus 5, indicating that CHD1 is an important protein for influenza virus replication and that chromatin plays a significant role on influenza virus life cycle.

Influenza virus transcription requires a functional coupling with cellular transcription for the cap-snatching process. Despite that, the RNAP II is degraded during the infection in a process triggered by the viral polymerase, once viral transcription is finished and on-going cellular transcription is not required. Since CHD1 specifically modulates influenza virus RNA transcription, and associates with Mediator, a transcriptional coactivator complex of RNAP II–mediated transcription, its possible degradation during influenza virus infection, was evaluated. Reassortant viruses from strains that induce or not RNAP II degradation have allowed the identification of PA and PB2 subunits as responsible for the degradation process, the involvement of specific residues within these subunits and the correlation between absence of RNAP II degradation and attenuation of pathogenicity in mice. Here we show that CHD1 associates with RNAP II and strictly parallels its degradation pattern during influenza virus infection, suggesting that degradation of both host factors are involved in viral pathogenicity.
Disclosure of Interest: None declared

Poster 170

RESPIRATORY SYNCYTIAL VIRUS NS2 INDUCES MONO-UBIQUITINATION OF HOST PROTEINS

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Abstract: Respiratory syncytial virus (RSV) belongs to the Paramyxoviridae family and is a major cause of severe lower respiratory tract infection in young children worldwide. The nonstructural NS2 protein is one of the first and most abundantly expressed proteins during infection. NS2 is a multifunctional protein important for viral replication and disease pathogenesis, and is essential for RSV-induced proteasomal degradation of host STAT2. Here, we further explore the interaction of NS2 with the host ubiquitin machinery. A549 cells infected with wild-type recombinant RSV showed an increase in levels of ubiquitinated host proteins compared to mock-infected cells or cells infected with RSV lacking the NS2 protein. Using a transfection system, we have found that NS2 induces monoubiquitination of host proteins. In addition, we have defined domains and residues of NS2 required for host protein ubiquitination. Using mass spectrometry, we identified several host proteins that are ubiquitinated to a greater extent in the presence of NS2 in infected cells. NS2-induced ubiquitination likely targets host anti-viral processes and can be limited through mutation of important NS2 residues.
Disclosure of Interest: None declared

Poster 171

CELL-TYPE SPECIFIC REGULATION OF ADHERENS JUNCTION PATHWAYS IN RESPONSE TO FILOVIRUS INFECTION

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Abstract: Filoviruses, namely Ebolavirus and Marburgvirus, are known to cause hemorrhagic fever in humans. These viruses cause acute disease with multiple hemorrhaging occurring within a few days and mortality as high as 90%. There are currently no FDA approved vaccines and specific antiviral therapy remains largely supportive. The recent outbreak of EBOV in West Africa has highlighted the unpredictability of the geographic range of the virus. Antivirals may prove especially useful for such outbreaks since they are sporadic and it is difficult to predict populations at risk. Though vascular leakage and barrier disruption are well known symptoms of infection, the exact mechanisms of these manifestations have not been determined. Specifically, the effect of filovirus replication on specific cell adhesion signaling pathways remains uncharacterized. Determining this role will have important implications for understanding viral pathogenesis and can allow identification of new targets for antiviral therapy.
Adherens junctions mainly consist of E-cadherin, a transmembrane protein primarily responsible for the adhesion function. The cytoplasmic domain of E-cadherin associates with cytosolic proteins called catenins (α, β, and p120), which in turn provide anchorage to the actin cytoskeleton to form stable cell–cell contacts. AJs are crucial for the initiation and maintenance of intracellular adhesion. Using the Caco-2 and HUVEC cell model, our transcriptional analysis reveals a differential response to infection, on the basis of cell type. Preliminary data also shows the involvement of beta catenin during filovirus infection, with the protein accumulating 48hrs p.i. compared to mock infected controls. We further aim to characterize the mechanism of this regulation, and also the viral factor(s) involved in this process. Elucidating this mechanism can help identify targets manage the hemorrhagic manifestations of the disease.
Disclosure of Interest: None declared

Poster 172

MAPPING THE IMMUNOSTIMULATORY ACTIVITY OF A SENDAI VIRUS DEFECTIVE VIRAL GENOME

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Abstract: Introduction: Sendai virus defective copy-back genomes have strong immunostimulatory activity. These genomes induce potent expression of type-I interferons (IFN) and other cytokines upon binding to the intracellular viral sensor RIG-I. In this work, we set out to identify the molecular motif that makes a prototypic Sendai virus defective copy-back genome a superior immunostimulator. Methods: We generated a series of deletion mutants of the parental defective viral genome and tested their ability to induce type I IFNs upon transfection. The secondary RNA structure of each mutant was modeled in silico and used to identify candidate motifs essential to confer potent immunostimulatory potential. A predicted motif was further studied through deletions, point mutations and engineering into an inert RNA molecule. Results: Modeling of the mutants identified a stem loop domain that is essential for type I IFN induction. Consistent with this prediction, we demonstrated that mutants lacking this region lost their stimulatory activity, while mutants that kept intact this region preserved it. Both structural, as well as, sequence compositions were identified as essential to maintain the stimulatory activity of the motif. Remarkably, this motif kept its strong immunostimulatory potential when transferred to inert RNA molecules. Conclusion and discussion: We have identified a virus-derived potent immunostimulatory motif that is critical for the potent immunostimulatory activity of SeV defective copy-back genomes. Together with preliminary evidence that indicates that these types of oligonucleotides preserve their immunostimulatory activity when injected in vivo, we propose that oligonucleotides including such region may represent novel alternatives to be harnessed as adjuvants for vaccination. **Disclosure of Interest**: None declared

Poster 173

JUNIN VIRUS BLOCKS PKR-MEDIATED PHOSPHORYLATION OF EIF2ALPHA

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Abstract: Arenaviruses are important human pathogens for which FDA-approved vaccines and effective antiviral therapeutics do not exist. We comprehensively mapped the arenavirus nucleoprotein-human protein interactome as a means to uncover critical host-pathogen interactions that can be therapeutically targeted. Bioinformatic analysis of interacting host protein partners revealed that host translation appears to be a key biological process engaged by the arenaviruses during infection. In particular, dsRNA activated protein kinase (PKR), a well characterized inhibitor of translation initiation via phosphorylation of eIF2α in response to virus infection, was identified in the screen. Infection with the New World Junin virus Candid 1 (JUNV C#1) leads to increased expression of PKR as well as its redistribution to viral replication factories. Functional characterization of the role of PKR in Junin virus infection revealed that PKR becomes phosphorylated following infection, yet it is unable to phosphorylate eIF2α, even in response to high doses of the synthetic dsRNA poly(I:C). This blockade of PKR function is highly specific as the Old World lymphocytic choriomeningitis virus (LCMV) was unable to inhibit eIF2α phosphorylation. Interestingly, siRNA silencing of PKR, a protein with well documented antiviral activity, resulted in a modest inhibition in the production of infectious virus particles compared to cells expressing normal levels of PKR. Not only does this indicate that JUNV C#1 is able to potently inhibit the antiviral activity of PKR, it suggests that the hijacking of this kinase may be beneficial to the viral life cycle.
Disclosure of Interest: None declared

Poster 174

KNOCKDOWN OF THE CELLULAR PROTEASE TMPRSS2 INHIBITS INFLUENZA A VIRUS GROWTH IN HUMAN CELL CULTURES

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Abstract: Current human therapeutics against influenza virus infections target viral gene products and are of only moderate efficacy. Further, viruses resistant to approved drugs are now in circulation, compounding the need for the development of novel antiviral agents. Cleavage of the influenza virus hemagglutinin (HA) by host cell proteases is crucial for viral infectivity and represents a process of interest for potential drug targeting. The protease TMPRSS2 is present in human airway epithelial cells and cleaves HA having a monobasic cleavage site. Peptide-conjugated phosphorodiamidate morpholino oligomers (PPMO) are single-stranded DNA-like antisense agents that enter cells readily and can modulate gene expression by sterically blocking complementary RNA. In this study, we targeted TMPRSS2 with PPMO in two different cell types infected with various strains of influenza A virus. A PPMO (T-ex5) designed to interfere with pre-mRNA splicing of TMPRSS2 was effective at inhibiting the HA cleavage of several influenza A viruses known to infect humans. Treatment of Calu-3 airway epithelial cell line or differentiated cultures of primary human airway epithelial cells with T-ex5 markedly reduced the growth and spread of H1N1 and H3N2 viruses, at concentrations that were not cytotoxic to uninfected cells under similar conditions. Overall, T-ex5 was an effective inhibitor of viruses having HA with a monobasic cleavage site, but ineffective against those with a multi-basic cleavage site. The data further support the importance of TMPRSS2 to the HA cleavage of various influenza viruses in human airway cells. PPMO appear to represent a useful reagent for investigating HA-activating proteases and a promising strategy for the development of novel therapeutics to address influenza infections.
Disclosure of Interest: None declared

Poster 175

THE CELLULAR STRESS RESPONSE AS AN ANTIVIRAL MECHANISM DURING EBOLA VIRUS INFECTION

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Abstract: Replication of the highly pathogenic Ebola virus (EBOV) takes place in the cytoplasm of infected cells, making the virus prone to innate immune sensors and cellular antiviral responses. One such mechanism includes the formation of stress granules (SGs), which leads to the aggregation of stalled translation pre-initiation complexes and RNA binding proteins, and culminates in global translational arrest. Many RNA viruses interfere with SG formation to avoid the detrimental effects of stalled translation. Here we examined the dynamics of the cellular stress response during EBOV infection. SG formation was not observed at any time point p.i. in EBOV-infected cells. When infected cells were treated with sodium arsenite (Ars) to induce oxidative stress, SGs formed at early time points p.i, mirroring SG formation observed in Ars-treated, mock-infected cells. However, at later stages of infection SG formation was reduced. To investigate this late disruption of SGs, individual EBOV proteins were tested for their ability to block SG formation in Ars-treated cells. EBOV VP35, known to inhibit the IFN response, colocalized with SGs upon Ars treatment and at high concentrations was able to disrupt SG formation. When expressed at high levels VP35 also interfered with the formation of nucleoprotein derived viral inclusions and disrupted the aggregation of proteins associated with neurodegenerative diseases. Collectively, these data suggest that VP35 may interfere with the nucleation of self-aggregating proteins. Staining of newly synthesized proteins during EBOV infection demonstrated that these nascent proteins localize to viral inclusions in the absence of stress but are prevented from doing so under Ars treatment. This indicates that although VP35 is able to disrupt SG formation induced by Ars, viral replication is still sensitive to cellular stress. Currently, we are exploring the opportunity for therapeutic intervention aimed at inducing the stress response in infected cells.
Disclosure of Interest: None declared

Poster 176

UNDERSTANDING THE ROLES OF PHOSPHORYLATION OF RSV P IN VIRUS REPLICATION: IDENTIFICATION OF AKT PHOSPHORYLATION SITES

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Abstract: The importance of respiratory syncytial virus phosphoprotein (RSV-P) phosphorylation in RSV RNA synthesis continues to be elucidated. It has been shown that the host kinase AKT has a role in the RNA synthesis of RSV and inhibition of AKT reduces RSV protein expression. In addition, inhibition of AKT results in a significant reduction in RSV-P phosphorylation in RSV infected cells, suggesting that RSV-P is the target of AKT in the virus. To further investigate the role of AKT phosphorylation of RSV-P in viral RNA synthesis we conducted an *in vitro* kinase assay with unphosphorylated recombinant RSV-P and active AKT1. Mass spectrometry analysis of the AKT phosphorylated RSV-P identified the serine at position 86 (S86) to be phosphorylated. This serine is well conserved among phosphoproteins of the *Pneumoviridae*. A S86A mutation reduced the level of phosphorylation of recombinant P by AKT *in vitro*. Using mass spectrometry analysis we found S86 is also phosphorylated in virus infected cells. To study the effect of mutating this AKT phosphorylation site on virus growth we incorporated the S86A mutation into the RSV genome. Surprisingly, there was no effect on RSV growth kinetics following infection, suggesting that S86 may not be the only target of AKT. We have now identified residues within RSV-P that are critical for virus replication by mass spectrometry of *in vitro* phosphorylated recombinant S86A P protein.
Disclosure of Interest: None declared

Poster 177
THE INFLUENZA VIRUS PROTEIN PB1-F2 INTERACTS WITH CALCOCO2 (NDP52) TO MODULATE INNATE IMMUNE RESPONSE
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Abstract: PB1-F2 is a viral protein encoded by Influenza A viruses (IAV). PB1-F2 is implicated in virulence by triggering immune cells apoptosis and by enhancing inflammation. To get insight into the molecular mechanisms of the PB1-F2-mediated virulence, we used the yeast two-hybrid system to find new PB1-F2 cellular interactors. This allowed us to identify CALCOCO2 (NDP52) and PSMC3 as binding partners of PB1-F2. Binding of PB1-F2 to CALCOCO2 was confirmed by pull-down and confocal microscopy. Surface Plasmon Resonance binding experiments enabled us to estimate the dissociation constant (Kd) of the two partners to be around 20 nM. The integration of these interactions data into a transcriptomic and interactomic cell network perfectly fitted with the previously described exacerbation of the inflammatory pathway mediated by PB1-F2. NF-kB reporter assays in which CALCOCO2, MAVS and PB1-F2 were co-expressed showed a cooperation of the three proteins to increase the inflammatory response. By contrast, PB1-F2 inhibits the TBK1-dependent activation of an ISRE reporter plasmid. We also demonstrated that the signal transducer TRAF6 is implicated in the enhancement of the NF-kB activity mediated by the PB1-F2-CALCOCO2 binding. Altogether, this report provides an interaction map between PB1-F2 and human proteins and allows a better understanding of the involvement of PB1-F2 in the pathologic process mediated by IAV.
Disclosure of Interest: None declared

Poster 178
DISSECTING THE INTERACTION BETWEEN NIPAH VIRUS P GENE ENCODED PROTEINS AND NF-KB PATHWAY
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Abstract: The Nipah virus (NiV), from the Henipavirus genus, is a zoonotic Paramyxovirus that is continuously reemerging in South Asia since the end of the 90's. Henipaviruses can be distinguished

from all other Paramyxoviruses by their broad species tropism and the ability to cause severe diseases among a large range of mammalians. In Humans, NiV causes acute respiratory troubles and encephalitis, associated with a high case fatality rate and inter-human transmissions observed in last outbreaks. NiV produces three nonstructural proteins, V, W and C, from the phosphoprotein (P) gene, which, by analogy with other Paramyxoviruses, mostly act to control the innate immune response. NF-kB pathway is the main regulator of the initiation of innate and adaptive immune response and can be triggered by numerous pathogen recognition receptors. Since some recent data suggest that NiV may modulate the NF-kB pathway, we undertook the analysis of interactions between NiV non-structural proteins and effectors of the NF-kB pathway, using a protein complementation assay and by co-immunoprecipitation. We also investigated the ability of NiV proteins to affect the NF-kB mediated activation using a firefly luciferase reporter assay after either transient protein expression or viral infection. We particularly focused on the canonical activation of the NF-kB pathway and the interaction of NiV proteins with the IkB kinases (IKK) complex. Our data suggest that the IKK complex may be a potential partner for the NiV non-structural proteins, associated with a modulation of the NF-kB pathway. The mapping of the P protein region responsible for this interaction is currently in progress and will be reported. Obtained results and comparison with other Paramyxoviruses should reveal the mechanisms used by NiV proteins to modulate the NF-kB pathway and possibly give some clues to understand the molecular basis of high pathogenicity of NiV.
Disclosure of Interest: None declared

Poster 180

A MOLECULAR SENSOR TO CHARACTERIZE ARENAVIRUS ENVELOPE GLYCOPROTEIN CLEAVAGE BY SUBTILISIN KEXIN ISOZYME 1 (SKI-1)/SITE 1 PROTEASE (S1P)

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Abstract: Arenaviruses are rodent-borne emerging viruses that include several causative agents of severe hemorrhagic fevers with high mortality in humans. The advent of next-generation sequencing has greatly accelerated the discovery of genetic evidence for novel arenavirus species in rodents and other animals. However, many of these novel viruses have not been isolated and their zoonotic disease potential remains unknown. During the arenavirus life cycle, processing of the viral envelope glycoprotein precursor (GPC) by the cellular subtilisin kexin isozyme 1 (SKI-1)/site 1 protease (S1P) is crucial for productive infection. The ability of newly emerging arenaviruses to hijack human SKI-1/S1P appears therefore as a crucial determinant for zoonotic transmission and their human disease potential. Here, we implement a newly developed cell-based molecular sensor for human SKI-1/S1P to characterize the processing of known and putative arenavirus GPC-derived sequences by human SKI-1/S1P. Requiring sequence information for only 8 amino acids flanking the putative cleavage site, our sensor accurately recapitulates the efficiency and subcellular location of arenavirus GPC processing. The sensor further allowed to correctly predict the efficient processing of the GPC of the newly emerged pathogenic Lujo virus by human SKI-1/S1P and to define the exact cleavage site, providing proof-of-concept. Lastly, we used our sensor to examine the efficiency of GPC processing of a panel of human pathogenic and non-pathogenic New World arenaviruses. Our quantitative analysis revealed that all NW arenavirus GPCs underwent efficient processing by SKI-1/S1P, regardless of human disease potential, indicating that human SKI-1/S1P represents no barrier for zoonotic transmission of these pathogens.
Disclosure of Interest: None declared

Poster 181

NOVEL HOST FACTOR-DEPENDENT INFLUENZA A VIRUS VRNA SYNTHESIS FROM ITS CRNA

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Abstract: Replication of the influenza virus genome RNA (vRNA) is catalyzed by viral RNA-dependent RNA polymerases (RdRP). In the first step, RNA complementary to vRNA (cRNA) is copied from vRNA,

and in the second step the progeny vRNAs are greatly amplified from cRNA. The viral RdRP and vRNA are minimal requirements for replication, but efficient replication could not be reproduced in a *cell-free* system using only these viral factors. Previously we found that the replication process for cRNA synthesis is activated by a host factor, IREF-1/MCM. Here, we established a biochemical complementation assay system for vRNA replication from cRNA template. Using this we identified and purified from nuclear extracts prepared from uninfected HeLa cells, a novel activity that allows robust unprimed vRNA synthesis from cRNA template, and designated as influenza virus replication factor (IREF)-2. IREF-2-dependent RNA products were found to be of full length and contain a triphosphate moiety at the 5'-terminus. We found that a regulatory target of IREF-2 appears to be a free form of the viral RdRP trimeric complex. IREF-2 was shown to up-regulate vRNA synthesis from cRNA template preferentially rather than cRNA synthesis from vRNA template. siRNA-mediated knock-down experiments indicated that IREF-2 is involved in viral replication *in vivo*. Based on the results obtained here and in previous studies, a plausible function(s) of IREF-2 will be discussed in terms of an initiation process of vRNA replication.
Disclosure of Interest: None declared

Poster 182
IDENTIFICATION OF A NOVEL INTERACTING PROTEIN WITH NIPAH VIRUS V PROTEIN.
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Abstract: Nipah virus (NiV) is highly pathogenic to several animals including human beings, but the molecular mechanism of its high pathogenicity is poorly understood. We previously reported two accesory proteins V and C had key roles in high pathogenecity in vivo. The recombinant NiV, which was deleted the unique sequence for V protein by inserted artificial stop codon following the editing site was significantly attenuated in experimentally infected goldenhamster. In this study, we searched host proteins interacting with V protein. By immunoprecipitation of transiently expressed V protein and mass spectrometry analysis, UBX domain containing protein 1 (UBXN1) was identfied as a novel interacting protein. By interaction analysis using various deletion mutants of V protein and UBXN1, we revealed binding domains of each protein. Co-expression of V protein upregulated expression level of UBXN1, and surppressed degradation of UBXN1. UBXN1 has been reported to have multifunctions containing a negative regulator of IFN induction. Functional analysis of the interaction is now under investigation.
Disclosure of Interest: None declared

Poster 183
INVOLVEMENT OF DNA DAMAGE RESPONSE IN BORNA DISEASE VIRUS INFECTION
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Abstract: Many environmental and physiological factors, such as ionizing radiation, reactive oxygen species and various chemicals, can directly induce genome DNA damages and activate DNA damage response (DDR) pathways. Infection of mammalian viruses, especially DNA viruses, is also known to induce DNA damages and subsequent DDR. Some viruses are interacted with central players in the DDR and employ them to facilitate viral replications. For example, in herpes simplex virus 1 (HSV-1) infection, several members of DDR factors, such as γH2AX and phosphorylated ataxia-telangiectasia mutated (ATM), are known to accumulate in the viral DNA replication compartment and then play beneficial roles in HSV-1 replication.
Borna disease virus (BDV), a non-segmented, negative strand RNA virus, is capable of establishing persistent infection in the cell nucleus. BDV generates viral speckles of transcripts (vSPOTs) in the nucleus as the viral replication compartment. Unlike DNA viruses, persistent infection in the nucleus is uncommon for animal RNA viruses. Therefore, interaction between RNA virus and DDR pathways has been largely unknown.
In this study, we investigated whether BDV infection associates with DDR pathways. We found that γH2AX co-localizes with vSPOTs. On the other hand, neither acute nor persistent infection of BDV

could activate DDR in infected cells. These observations suggested that BDV may locate preferably at the DDR focus occurred under the physiological condition. We further determined that the induction of DDR by X-ray (10 Gy) activates BDV replication in the infected cells, suggesting that BDV may interact with DDR to enhance its replication. We are now investigating the molecular mechanism of how DDR pathways activate BDV replication.
Disclosure of Interest: None declared

Poster 184
TRANSCRIPTIONAL REGULATORY NETWORK FOR COMPREHENSIVE DOWNREGULATION OF HOUSEKEEPING GENES INDUCED BY MORBILLIVIRUS INFECTION
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Abstract: Morbillivirus infection causes several severe and common syndromes including transient strong immunosuppression. *In vitro* morbillivirus-induced immunosuppression and immune responses are cell-type dependent.
To clarify the infection-induced cell type specific responses and implication of the viral accessory protein, we previously performed microarray analysis with recombinant measles viruses and rinderpest viruses. We found that the infection caused comprehensive downregulation of housekeeping genes, and that the accessory C protein repressed the downregulation signal completely specific for lymphoid cells.
Transcriptional regulatory network for the comprehensive downregulation are thought to be driven by a defined set of master transcription factors (TFs) which represent the most upstream of the network. To focus on construction of the network, we employed the CAGE (cap analysis of gene expression) analysis with a next generation sequencer for quantification of promoter activities. Alteration of the TF activities over the infection time course was analyzed with the MARA (motif activity response analysis) for prediction of the network. Based on the obtained information, we reconstructed the transcriptional regulatory network after the infection. As a result, we revealed that the downregulation signal was triggered by two master TFs, transmitted to mediator TFs, and then propagated to nine terminal TF families which conduct the expression of peripheral housekeeping genes. We further validated the network with recombinant viruses, and confirmed that the C protein repressed the alteration of master TF motifs in lymphoid cells.
These findings uncover a novel host strategy of the defense system for suppressing viral propagation, and a unique activity of C protein as a cell-type specific virulence factor.
Disclosure of Interest: None declared

Poster 185
INTERACTION BETWEEN THE NIPAH VIRUS NONSTRUCTURAL C PROTEIN AND PROTEIN PHOSPHATASE 2A INHIBITOR SUPPRESSES INFLAMMATORY CYTOKINE INDUCTION
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Abstract: Nipah virus (NiV) is a member of the genus *Henipavirus*, which emerged in Malaysia in 1998. NiV causes severe encephalitis in human with highly mortality. We had previously reported that the NiV nonstructural C protein (NiV-C) played a key role in pathogenesis and the roles were independent of interferon antagonist activity. Recently, it is becoming clear that NiV-C regulates proinflammatory response. However its mechanism is still unknown. In present study, we used affinity purification to isolate human proteins that bind to the NiV-C. One of the NiV-C binding proteins is inhibitor of serine/threonine protein phosphatase 2A. We determined the region of NiV-C that was important for the interaction with it. Moreover, NiV-C significantly increased the total cellular PP2A activity. Stable expression of NiV-C suppressed the induction of proinflammatory cytokine by poly(I:C) treatment. These results suggest that interaction between NiV-C and I2PP2A results in increased PP2A activity and inhibited proinflammatory cytokine induction

Disclosure of Interest: None declared

Poster 186

UTILISATION OF HIGH-THROUGHPUT SEQUENCING TO INVESTIGATE A RABIES EPIZOOTIC FOLLOWING CROSS-SPECIES TRANSMISSION OF VIRUS FROM DOGS TO FOXES

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Abstract: Cases of lyssavirus cross species transmission (CST) are generally quite poorly characterized. A well-documented epizootic event in the late 1990's in Turkey, resulted in a long term enzootic spread of rabies virus (RABV) within the wild fox population. Intervention using oral vaccination failed to prevent the spread of RABV in foxes, which continues to the present day. Based on epidemiological, tempo-spatial and phylogenetic data, the fox epizootic is postulated to have resulted from a CST event from domestic dogs. The genetic data available (N-gene 400bp) from both dogs and foxes positive for rabies, was sufficient to conclude that the viruses involved were closely related but the direction of transmission and a potential mechanism for the establishment of infection in the naïve fox population was unclear. Full genome consensus sequences were obtained from both fox and dog samples and Bayesian analyses were used to determine the time to most recent common ancestor (TMRCA) in comparison with available data. The analyses concluded that the most likely source of the RABV fox epizootic was from domestic dogs. Deep sequence data was generated from a cohort of original dog and fox brain tissues sampled between 1999 and 2012. This data was analysed to investigate the initial CST event, track the virus and determine the relative viral heterogeneity within samples. The data has enabled a transmission pathway from domestic dogs to foxes to be characterized, allowing the genetic heterogeneity of the virus to be assessed through the spread of virus in a new host. A reduction in viral heterogeneity was seen through a phase of the enzootic, potentially as a result of a vaccination campaign instigated in 2008. Interestingly, a number of non-synonymous changes were observed between the dog and fox sequences that may suggest adaptation and the level of genetic variation and its potential impact are discussed.

Disclosure of Interest: None declared

Poster 187

INVESTIGATING THE MITOCHONDRIAL FUNCTIONS OF THE PB2 SUBUNIT OF THE INFLUENZA VIRUS POLYMERASE

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Abstract: Influenza viruses infect a wide range of hosts including humans, waterfowl, poultry and swine. The viral RNA polymerase consists of three subunits PB1, PB2 and PA, which act in concert in the nucleus to catalyse transcription and replication of the viral RNA genome. In line with this it has been shown that strains from non-human hosts, the 1918 and 2009 human pandemic strains and human isolates of H5N1 strains encode PB2s which exhibit a solely nuclear localisation. However, in a large proportion of human seasonal strains PB2 also localises to the mitochondria due to the presence of asparagine at position 9 of PB2 in place of the aspartic acid encoded in other strains. We have shown the presence of threonine at position 9, which is also frequently found in human seasonal strains, also leads to the mitochondrial localisation of PB2. Using APEX (Enchanced Ascorbate Peroxidase) technology we have demonstrated that mitochondrial-localising PB2 is imported into the mitochondrial matrix. Affinity purification of PB2 from isolated mitochondria has identified matrix localising factors involved in metabolism, interferon regulation and mitochondrial translation as potential interactors of mitochondrial PB2. Ongoing work focuses on the importance of these interactors in viral replication and the effects of mitochondrial PB2 on cellular metabolism.

Disclosure of Interest: None declared

Poster 188

A COMPARATIVE STUDY OF RESPIRATORY SYNCYTIAL VIRUS (RSV) INFECTION OF MURINE MACROPHAGE CELL LINES REVEALS REMARKABLE DIFFERENCES IN SUSCEPTIBILITY.

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Abstract: RSV is responsible for severe bronchiolitis in children and elderly and is also linked with chronic pulmonary problems, like asthma. For the link between the pathology and the immune response, and since macrophages are prominent cells of the lung immune system, the macrophages are studied during infection. Macrophages appear to be permissive for RSV infection, but the obtained results are often inconclusive and contradictory. A viable explanation is that diverse types and cell lines of macrophages were used. The aim of this study was to evaluate the susceptibility for RSV of different macrophage cell lines.
The macrophage cell lines MH-S, RAW 264.7 and J774 were infected with the RSV strain A2. To have a general idea of both RSV entry and infection, cells were fixed at 2 and 24h p.i. Cells were permeabilized and RSV antigens were visualized with a goat polyclonal anti-RSV serum followed by an AF488-labelled conjugate. As a negative control mock-infected cells were used. Both MH-S (2%) and RAW 264.7 (0,4%) were infected, showing staining of RSV-antigens in the cytoplasm of the cells 24h p.i. This staining was more intense compared to the staining at 2h p.i., indicating that new RSV antigens were synthesized. J774 cells showed no clear positive signal of RSV antigens at 24h p.i.
Further analysis revealed that there are no RSV-antigens expressed on the surface of RAW 264.7 cells in contrast to MH-S cells. This suggests that RSV knows an abortive infection in RAW 264.7 cells. This notion was explored by inoculating HEP-2 cells with supernatants of infected RAW 264.7 cells and MH-S cells, collected 24 and 72h p.i. The percentage of infected HEP-2 cells varied from 1,5 to 5% when inoculated with supernatants of MH-S from 24 or 72h p.i. This in contrast to HEP-2 cells inoculated with supernatants of RAW 264.7 cells, where the percentage of infected cells varied between 1,4 and 1,2%. In conclusion, the RSV infection with the A2 strain varies among macrophage cell lines.
Disclosure of Interest: None declared

Poster 189

IDENTIFICATION OF ANP32B/APRIL AS A NOVEL HOST PROTEIN INTERACTING WITH INFLUENZA VIRUS RIBONUCLEOPROTEINS

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Abstract: Influenza A viruses are negative stranded RNA viruses causing annually recurring respiratory disease in humans with high mortality and morbidity. During infection, influenza A viruses are highly dependent on the interaction with specific cellular proteins in order to manipulate the host intracellular environment to allow completion of the viral life cycle and evasion of the intracellular antiviral response system. Because of this dependency, host cell proteins have come into focus as promising targets for the development of new antiviral strategies. We are especially interested to discover virus-host interfaces during the early phase of infection. Using a recombinant influenza A virus carrying a strep-tag at the PB2 Protein and subsequent purification, we were able to identify novel host proteins that interact with influenza A virus vRNPs 4 hours p.i. Aside from known vRNP interaction partners, we identified the multifunctional protein ANP32B, also referred to as APRIL. APRIL is involved in diverse cellular processes like nuclear export of cellular mRNA via the HuR-dependent export pathway, apoptosis, and regulation of gene expression. Knock down of APRIL enhances viral replication of the influenza A virus WSN indicating a potential inhibitory property. Overexpression of APRIL results in a concentration dependent down-regulation of reporter protein expression in the polymerase reconstitution assay. Strikingly, primer extension analysis revealed that reduced reporter expression is not mediated by inhibition of the viral polymerase activity but rather occurs on the posttranscriptional

level by a yet unsolved mechanism. Mutation of the NES and NLS in APRIL, respectively, suggests that the nuclear localization but not export of APRIL is required for this phenomenon. We are currently investigating the functional mechanism of the APRIL-mediated protein expression using different molecular biology and biochemical approaches.
Disclosure of Interest: None declared

Poster 190
THE ADAPTATION OF AVIAN INFLUENZA VIRUSES TO THE RESPIRATORY EPITHELIUM OF PIGS
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Abstract: Pigs are an important host for influenza A viruses and may play a crucial role in the interspecies transmission. Primary target cells for influenza viruses are cells of the respiratory epithelium. Recently we have established precision-cut lung slices (PCLS) from the porcine lung as a culture system for differentiated respiratory epithelial cells. In PCLS, the differentiated epithelial cells are maintained in their original setting.
Here we used PCLS as a culture system to analyze the adaptation of avian influenza viruses to the respiratory epithelium of pigs.Avian influenza virus of the H9N2 subtype was subjected to several passages in PCLS. The changes in the viral properties that are associated with the adaptation process were characterized by analyzing: duration of the growth cycle; amount of infectious virus released into the supernatant; extent of the ciliostatic effect. Sequence analysis revealed which amino acid changes occurred during the different virus passages.
Adaptation of the avian viruses to growth in porcine cells was evident in a shortening of the growth cycle. Sequence analysis revealed that few amino acid changes occurred during the different virus passages. The importance of the individual mutations has been analyzed by generating recombinant viruses that contain the respective mutated proteins. The functional importance of individual mutations will be reported. Our study helps to understand the processes involved in the adaptation of H9N2 influenza viruses to new hosts.
Disclosure of Interest: None declared

Poster 191
L-SIGN AS AN ATTACHMENT FACTOR FOR PHLEBOVIRUSES
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Abstract: The *Bunyaviridae* constitute a large family of RNA viruses distributed worldwide. Aside from hantaviruses, bunyaviruses are transmitted to vertebrate hosts by arthropods and cause severe pathologies in humans and livestock. With the spread of vectors to new areas and an increasing number of outbreaks, arthropod-borne bunyaviruses are considered emerging agents of diseases and represent a global threat to public health. Our lab has previously identified the C-type lectin CD209 as an authentic entry receptor for many bunyaviruses into dendritic cells, which are arguably the first cells to encounter the viruses in the skin following arthropod bites. In further rounds of infection, it is apparent that bunyaviruses use other receptors to infect a wide spectrum of tissues, most of which do not express CD209, such as the liver. Our hypothesis is that CD209L, which is closely related to CD209 and abundantly expressed on liver sinusoidal endothelial cells, is important for the liver tropism of some bunyaviruses. Our results show that CD209L significantly enhances infection by Uukuniemi (UUKV) and Toscana viruses, only slightly that by Rift Valley fever virus, whilst Punta Toro virus does not seem to use the lectin. We observed that UUKV replication increases over time in CD209L+ cells and strongly

depends on CD209L expression. Moreover, treatment with inhibitors such as EDTA and blocking antibodies decreases the infectivity. To further define the CD209L-bunyavirus interactions, we have investigated all steps of UUKV entry into CD209L+ cells, including virus binding, intracellular trafficking, and penetration. In contrast to CD209, our results suggest that CD209L merely serves as an attachment factor rather than an endocytic receptor. Together our data sheds new light on possible strategies used by bunyaviruses to target the liver and lays the basis for the development of new anti-viral strategies.
Disclosure of Interest: None declared

Poster 192
LASSA VIRUS INFECTION IN HUMAN BRONCHIAL EPITHELIAL CELLS
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Abstract: Lassa virus (LASV), a member of the family Arenaviridae, is a highly pathogenic hemorrhagic fever virus that is endemic in West Africa and can cause severe systemic infections in humans. The primary transmission route of LASV from its natural reservoir, the rodent *Mastomys natalensis*, to humans is by direct exposure to virus-containing rodent excretions. Infection may occur via the respiratory tract through inhalation of infected particulates, or via ingestion of contaminated food. Although the respiratory tract clearly represents an important virus entry site, knowledge about the interaction between LASV and human respiratory epithelial cells is limited. In this study, we used human bronchial epithelial cells (16HBE14o-) as a model system to study LASV infection in the respiratory tract. Our data showed that 16HBE14o- cells were highly susceptible to LASV infection. 16HBE14o- cells cultured on microporous membrane filters differentiated into a polarized cell phenotype, characterized by the formation of a high transepithelial electrical resistance and an intact permeability barrier, as well as the development of distinct apical and basolateral membrane domains separated by *adherens junctions* and *tight junctions*. Further, 16HBE14o- cells formed a pseudostratified epithelium that is similar to the human bronchial epithelium. Polarized 16HBE14o- cells were then used to determine the site of virus entry and release. Our results indicate that LASV can infect human airway epithelial cells via the apical or basolateral membrane, and progeny virus particles are released bidirectionally, with higher virus titers observed in apical compartments. These data suggest that LASV infection of the respiratory epithelium may play an important role in systemic dissemination of LASV in humans as well as serving as a source of viruses involved in further spread from human to human via aerosol transmission.
Disclosure of Interest: None declared

Poster 193
DC-SIGN ENHANCES TICK CELL-DERIVED UUKUNIEMI VIRUS INFECTION
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Abstract: The *Bunyaviridae* constitute a large family of enveloped animal viruses distributed worldwide. Several cause serious diseases in vertebrates, including humans and livestock. During natural transmission, many bunyaviruses are introduced into the host skin through bites by infected ticks. However, the viral particles produced in ticks remain largely uncharacterized at a molecular level and the early interactions between tick-derived bunyaviruses and vertebrate host cells are virtually unknown. To reproduce host switch *in vitro*, we established a reverse genetics system in BHK-21 cells to rescue the tick-borne bunyavirus Uukuniemi (rUUKV) with a genome identical to that of the virus isolated from ticks. We found that the *Ixodes ricinus* tick cell lines IRE/CTVM19 and IRE/CTVM20 are both sensitive to the mammalian cell-derived rUUKV and enable amplification of the rescued virus. Using tick cell-derived rUUKV, we found that the surface glycoproteins of the virus progeny had no *N*-glycosylation site remaining resistant when subjected to treatment with PNGase F and Endo H. The virus produced in tick cells was in addition able to exploit the C type lectin DC-SIGN to infect mammalian

cells. Together, our results indicate that viral glycoproteins produced in tick cells present high-mannose carbohydrates, a major advance in the understanding of the transmission of tick-borne pathogens to vertebrate hosts.
Disclosure of Interest: None declared

Poster 194
USE OF INTERFERON-STIMULATED GENES CDNA OVEREXPRESSION TO SCREEN INFLUENZA VIRUS-HOST INTERACTIONS IN PRIMARY HUMAN RESPIRATORY EPITHELIAL CELLS
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Abstract: Influenza virus infections are a major cause of respiratory disease in humans. Influenza A viruses (IAV) are responsible for annual epidemics and occasional pandemics with potentially fatal outcome. The differences in the severity of the disease among individuals infected by IAV could be due to pre-existing health conditions, genetic factors, differences in the virulence of the circulating viruses or a combination of these elements. Thus, a comprehensive understanding of viral-host interactions, host innate immune responses and viral evasion strategies is pivotal to understand viral pathogenesis. Published genetic screens performed to identify host factors involved in IAV replication were designed to identify host proteins required for viral replication. In order to identify key early genes involved in the restriction of IAV infection, we are performing a functional genomic screen in primary human respiratory epithelial cells. We are using an interferon-stimulated genes (ISGs) overexpression approach, which will provide a comprehensive elucidation of innate host proteins that block early stages in IAV infection. In addition, we are developing a high-throughput screening platform to interrogate subcellular (trans)-localization events with the aim of evaluate the impact of IAV infection in the localization of different ISGs. We are using these two screen platforms to compare systematically three clinically relevant human IAV strains that differ in their virulence (H5N1, seasonal H3N2 and 2009 pandemic H1N1), which will enable us to identify important viral-host networks that are predictive of viral pathogenesis.
In summary, we are using the high-throughput cDNA overexpression technology in different ways in order to elucidate the relationship between the host innate immune system and influenza virus. The proteins identified in our screens could be critical to better understand the host-IAV interactions and provide new targets for therapeutic approaches.
Disclosure of Interest: None declared

Poster 195
ESTABLISHING A HUMAN EX VIVO LUNG TISSUE MODEL TO CHARACTERIZE HOST FACTORS ESSENTIAL FOR INFLUENZA A VIRUS INFECTION AND IDENTIFY NOVEL DRUG TARGETS
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Abstract: Influenza A virus (IAV) is an RNA virus encoding up to 13 viral proteins. Due to this limited coding capacity IAV needs to use the host cellular machinery to complete its lifecycle. Host proteins that play a role in IAV replication and interact with IAV proteins are subject of our investigation. To date, the roles of host proteins in IAV infection have predominantly been characterized using immortalized cell lines like A549 as model systems for IAV infection. However, the use of these cell lines has several drawbacks including differences in host regulatory networks compared to non-tumorigenic cells.

Therefore, better models are urgently needed to perform comprehensive studies on human host factors that influence IAV infection.
Human *ex vivo* lung tissue is a particularly useful model system that applies authentic human tissue in which different cell types are still connected in 3D structure and able to interact with each other. We successfully established an *ex vivo* human lung tissue model and confirmed that it supports replication of several H1 and H3 IAV strains, exhibiting up to 3-log titer increase upon 48 hours of infection. Furthermore, we demonstrated that IAV titers were decreased to non-detectable levels by the addition of antiviral drugs such as oseltamivir, which underlines that *ex vivo* human lung tissue is a valuable tool to analyze the effects of drugs on viral replication. Future experiments will focus on silencing selected host genes in human *ex vivo* lung tissue using peptide-conjugated phosphodiamidate morpholino oligomers (PPMOs) and identifying their roles in IAV infection.
In summary, our project concentrates on characterizing proteins involved in replication and pathogenicity of different IAVs in physiologically relevant human lung tissue. This approach will provide critical insight into the roles of selected proteins in the IAV lifecycle and will serve as a model for the development of novel antiviral drugs targeting human host proteins.
Disclosure of Interest: None declared

Poster 196
A GENOME-WIDE RNAI SCREEN IDENTIFIES A ROLE FOR WNT/BETA-CATENIN SIGNALING DURING BUNYAVIRAL REPLICATION
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Abstract: Rift Valley Fever virus (RVFV) is an arthropod-borne zoonotic pathogen within the *Bunyaviridae* family capable of causing serious morbidity and mortality in both humans and livestock. To identify new host factors involved in bunyavirus replication, we employed genome-wide RNA interference (RNAi) screening as a functional genomic approach for comprehensive analysis of virus-host interactions. RNAi screening identified 381 cellular genes whose knockdown reduced viral infection. The Wnt signaling pathway was the most represented pathway when gene hits were functionally clustered. RNAi induced silencing of beta-catenin expression reduced RVFV replication and indicated a role for canonical Wnt/beta-catenin signaling during RVFV infection. Further investigation revealed RVFV infection: 1) activated Wnt signaling in a beta-catenin reporter assay; 2) stabilized active beta-catenin protein; 3) increased expression of Wnt/beta-catenin responsive genes; 4) was enhanced when Wnt signaling was pre-activated; and 5) was blocked using chemical inhibitors of Wnt signaling. Similar results were found using distantly related buynaviruses, which indicates a conserved mechanism involving Wnt signaling and bunyaviral replication. Finally, we propose a model where bunyaviruses activate Wnt responsive genes to regulate optimal cell cycle conditions needed to provide abundant cellular mRNAs for priming viral transcription. We anticipate this new understanding of the fundamental mechanisms of RVFV infection combined with the development of Wnt inhibitors for cancer treatments will aid in the design of efficacious host-directed anti-RVFV therapeutics.
Disclosure of Interest: None declared

Poster 197
PPMO-KNOCKDOWN OF CELLULAR GENE EXPRESSION AFFECTS INFLUENZA VIRUS GROWTH IN THE LUNGS OF MICE
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Abstract: Diminishing the expression of specific host genes during a viral infection can help elucidate their roles in virus growth and pathogenesis. We used antisense peptide-conjugated morpholino oligomers (PPMO) to reduce the expression of cellular genes TRIM6 and UBR4 in the lungs of mice, then evaluated the effect on influenza virus growth. Initially, to validate specific activity by the PPMO, uninfected cell cultures were treated with PPMO designed to

interfere with the pre-mRNA splicing of TRIM6 or UBR4. Immunoblots showed specifically reduced levels of protein of the targeted genes. In mice infected with influenza virus (PR8/H1N1), intranasal administration of PPMO likewise produced substantial reduction in the level of TRIM6 or URB4 protein in lung tissue. The knockdown of TRIM6 resulted in an increase in virus growth in the lungs, whereas the knockdown of UBR4 resulted in reduced virus growth. These studies provide insights into the biological roles of TRIM6 and UBR4, specifically in the context of *in vivo* influenza virus infection, and further establish PPMO as effective reagents for knockdown of gene expression *in vivo*. **Disclosure of Interest**: None declared

Poster 203

THE NIPAH VIRUS P/V/W/C COMPLEX CONTRIBUTES IN MULTIPLE WAYS TO THE PATHOGENESIS AND DISEASE COURSE IN THE FERRET MODEL.

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Abstract: Nipah Virus (NiV) is a paramyxovirus that recently emerged as a causative agent of febrile encephalitis and severe respiratory disease in humans with outbreak fatality rates as high as 92%. The NiV P gene encodes four different proteins including the P, V, W, and C proteins. Plasmid-based studies have identified multiple potential targets for these proteins involved in blocking various aspects of innate immune signaling including RIG-I, MDA-5, STAT1, STAT2, TLR3 and TLR7/9 through IKKα, IKKε, and other unidentified mechanisms. Little is known about which of these viral proteins and cellular targeted pathways are important in the context of *in vivo* infection with NiV. We have created various recombinant (r)NiV strains and performed a series of *in vivo* studies in ferrets and complementary studies in primary human lung and brain microvascular endothelial cells to identify which viral proteins contribute to the respiratory or to the neurological component of NiV-mediated disease in the ferret model. Disturbance in the expression patterns/levels of these viral innate immunomodulatory proteins resulted in observable changes in disease course.

Disclosure of Interest: None declared

Poster 204

SYSTEMIC POLYARTERITIS NODOSA AS THE CAUSE OF SUDDEN-ONSET BILATERAL SENSORINEURAL DEAFNESS IN A PRIMATE AFTER EXPERIMENTAL INFECTION WITH LASSA VIRUS

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Abstract: Lassa virus is the etiologic agent of Lassa fever, a severe, rodent-borne hemorrhagic disease endemic to West and Central Africa. Unilateral or bilateral sudden-onset sensorineural hearing loss occurs in approximately 30% of recovering Lassa fever survivors. Though it is clear that a cause-and-effect relationship exists between Lassa fever infection and hearing loss, the mechanism has not been identified. Here, we describe a macaque experimentally infected with Lassa virus that survived infection and developed bilateral sensorineural hearing loss, confirmed by brainstem auditory evoked response (BAER). Another experimentally infected macaque that survived infection experienced unilateral hearing impairment based on tuning fork and other tests, but did not screen deaf with BAER analysis at 80dB. Upon analysis of tissues collected from these animals, systemic polyarterisis nodosa (PAN), an autoimmune vasculitis disorder that has been associated with sudden hearing loss, was the pathologic diagnosis. There is precedent for PAN and other vasculitis syndromes resulting from viral infections, including HepB, HepC, HIV, CMV, EBV and Parvovirus. Severe PAN lesions were identified in medium and small vessels surrounding the vestibulocochlear nerve in the deaf and to a lesser degree, the hearing-impaired animal. Furthermore, PAN lesions were in most organ systems, including

the heart, pancreas, liver and spleen, but not the lungs. The distribution and severity of lesions in these animals are consistent with a diagnosis of PAN. A review of past studies conducted at USAMRIID revealed that similar lesions were present in these animals as well, indicating that PAN-like vasculitis is a consistent finding in surviving animals. We demonstrate, for the first time, that development of systemic PAN is the mechanism of sudden sensorineural hearing loss with Lassa virus infection in an experimental model. This study was funded by the Military Infectious Disease Research Program (MIDRP).
Disclosure of Interest: None declared

Poster 205
COMPARATIVE PATHOGENESIS OF MARBURG VIRUS STRAINS IN SYRIAN GOLDEN HAMSTERS
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Abstract: ABSTRACT
Background: Phylogenetic comparisons of known Marburg virus (MARV) strains reveal nucleotide variances over 20% among across known MARV genomes suggesting differing virulence between lineages may accompany this genetic divergence. To date, there exists limited systematic experimental evidence of pathogenic differences between Marburg strains.
Methods: Uniformly lethal outbred hamster models of MARV-Angola (MARV-Ang) and MARV-Ci67 were developed by serial adaptation. Changes in genomic sequence, weight, temperature, histopathology, immunohistochemistry, hematologic profiles, circulating biochemical enzymes, coagulation parameters, viremia, cytokine, eicanasoid, and nitric oxide production were compared between strains.
Results: MARV-Ci67 demonstrated delayed differences in circulating inflammatory and prothrombotic elements, notably lower viremia, less severe histologic alteration and a delay in mean time of death compared to MARV-Ang. Both strains produced more marked coagulation abnormalities than previously seen in MARV-infected small animal models including mice and guinea pigs, including hallmark feature of striking rashes at the skin surface.
Conclusions: Although both strains exhibit great similarity to pathogenic markers of human and nonhuman primate MARV infection, these data highlight several key differences in pathogenicity that may serve to guide choice of strain and model used for development of vaccines or therapeutics for Marburg hemorrhagic fever.
Disclosure of Interest: None declared

Poster 206
CORTICOSTEROIDS DO NOT PREVENT PATHOGENESIS IN THE SYRIAN HAMSTER MODEL OF HANTAVIRUS PULMONARY SYNDROME
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Abstract: Andes virus (ANDV) is associated with a lethal vascular leak syndrome in humans termed hantavirus pulmonary syndrome (HPS). The mechanism for the massive vascular leakage associated with HPS is poorly understood, however immune dysregulation is often suggested as a possible cause. A small number of studies have suggested that treatment of human HPS with corticosteroid may provide some protection from lethal disease arguing that limiting inflammation and immune responses may prevent pathogenesis. This has never been formally tested in an adult lethal disease animal model of HPS. In hamsters, ANDV causes a respiratory distress syndrome closely resembling human HPS. To determine whether corticosteroid treatment would prevent lethal disease, hamsters were challenged with ANDV and then were treated with corticosteroids at various times after challenge. Neither dexamethasone nor methylprednisolone prevented disease in hamsters following a high dose (2000 pfu) intramuscular ANDV challenge when steroids were administered in a dose-escalating manner, or when steroids were front-loaded and then administered in a dose-deescalating manner. Similar results were also obtained when steroid dosage and timing of administration were

altered following a low dose (80 pfu) challenge. In all cases, corticosteroid treatments did not prevent pathogenesis or prolong disease despite evidence of reduced adaptive immune responses in animals receiving corticosteroids as measured by flow cytometry. These data indicate that the immune responses suppressed by corticosteroids are not necessary for HPS pathogenesis in the hamster model of HPS and suggest that corticosteroid treatment alone is insufficient to prevent HPS.
Disclosure of Interest: None declared

Poster 207
INNATE IMMUNE RESPONSES ELICITED BY SIN NOMBRE VIRUS PROTECT HAMSTERS FROM LETHAL ANDES VIRUS-INDUCED HANTAVIRUS PULMONARY SYNDROME
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Abstract: Sin Nombre virus (SNV) and Andes virus (ANDV) cause hantavirus pulmonary syndrome in humans. Both SNV and ANDV infect Syrian hamsters, but only ANDV causes disease. We tested if dual-infection with SNV and ANDV would result in lethal disease, or asymptomatic infection. When SNV was injected 1 day before ANDV injection, hamsters did not develop disease. However, when both viruses were injected at the same site concurrently the hamsters succumbed. The same experiment was performed using two SNV/ANDV reassortant viruses, including a novel monoreassortant. Both reassortants were SNV-like in their capacity to asymptomatically infect hamsters; however, these reassortants were incapable of preventing lethal ANDV infection when administered 1 day prior. ASA reassortant virus was able to protect hamsters from lethal ANDV infection when administered 3 days prior, while the other reassortant virus, SAS, was not. Polyl:C administered to hamsters 1 day before ANDV challenge induced expression of type I interferon, and was protective. Combined, these results suggest that the dramatic difference in the pathogenicity of SNV and ANDV in hamsters involves differences in early host-pathogen interactions and resultant anti-viral immune responses.
Disclosure of Interest: None declared

Poster 208
AVIAN RECEPTOR BINDING INFLUENZA VIRUS ADAPTS RAPIDLY IN FERRETS TO TRANSMIT BY AEROSOLS
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Abstract: Influenza A viruses pose a major public health threat worldwide. Their epidemiological success relies on the ability to transmit from person-to-person by respiratory droplets (RD); however, the viral properties governing aerosol transmission of influenza viruses are complex. Infection by influenza viruses is mediated via binding of the viral surface glycoprotein HA to terminally attached α2,3 or α2,6 sialic acids (SA) on cell surface glycoproteins. Human influenza viruses preferentially bind α2,6 linked SA while avian influenza viruses bind α2,3 SA-containing complex glycans on airway epithelial cells. Historically, influenza viruses with preferential α2,3-linked SA association have not transmitted efficiently by RD in the ferret model. We engineered a 2009 pandemic H1N1 virus (H1N1pdm) to preferentially bind α2,3 SA and were surprised to find that it transmitted by RD as efficiently as the wild-type H1N1pdm virus. We show that transmission was associated with rapid evolution of the hemagglutinin (HA) gene at a single site which conferred binding to long-chain α2,6 SA, without loss of α2,3 SA binding. The transmissible virus emerged in the experimentally infected ferret within 24 hours post-infection and was remarkably enriched in some tissues of the respiratory tract, where long-chain α2,6 SA predominate. We further demonstrate that expression of long-chain α2,6 SA is conserved in the corresponding tissues of pigs and humans. Thus, using a “loss-of-function” approach, we have identified an anatomical site of rapid adaptation and emergence of a virus with dual α2,3 and α2,6 SA binding that is capable of efficient RD transmission. These results enhance our understanding of the properties necessary for aerosol transmission of influenza viruses.

Disclosure of Interest: None declared

Poster 209

INCREASED PATHOGENESIS OF INFLUENZA A H1N1 VIRUS LED BY A PA RESIDUE DETECTED IN A FATAL CASE

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Abstract: Pandemic 2009 H1N1 (pH1N1) influenza viruses caused mild symptoms in most infected patients. However, a greater rate of severe disease was observed in healthy young adults and children without comorbid conditions, suggesting that viruses with different pathogenicity could cocirculate. Our previous data indicated that a strain of pH1N1 virus isolated from a fatal case presented enhanced pathogenicity compared to a virus isolated from a mild case, which circulated during the 2009 pandemic. PB2 A221T, PA D529N, HA S127L changes appeared as particularly interesting and suggested that one or combination of these changes could play an important role in increased pathogenicity. Biological properties of recombinant viruses (pH1N1 California/04/) carrying each of these residues or combination of them have been analyzed both *in vitro* (human lung alveolar epithelial cells) and *in vivo* (murine model). Wild-type recombinant virus and viruses carrying PA529N, PB221T or both changes replicated at similar rate, but HA recombinant virus had a slightly higher replication rate at 9 and 12 hpi in cell culture. In vivo analysis showed a significantly decreased LD50 of 50 and 10 fold for PA and PA/PB2 recombinant viruses, respectively, compared to that of the control virus. Viral titer in lungs of PA recombinant virus infected mice was higher up to 7 dpi., moreover a high proportion of mice presenting infectious virus in the heart, was found in these infected animals whose replication was detected by the presence of NEP (Nuclear Export Protein) mRNA. Analysis of CD45+ cells in lungs of infected mice showed higher percentage of neutrophils and dendritic cells by 1 and 2 dpi, as well as rapid loss of alveolar macrophages by the 2 dpi in PA and PA/PB2 recombinant viruses infected mice compared with the control virus infected mice. These results indicate that PA529N residue leads to increased pathogenicity of influenza A H1N1 virus mediated by several biological processes.
Disclosure of Interest: None declared

Poster 210

A SINGLE AMINO ACID CHANGE IN THE MARBURG VIRUS MATRIX PROTEIN VP40 IMPROVES VIRAL FITNESS IN A SPECIES-SPECIFIC MANNER

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Abstract: Marburg virus (MARV, family *Filoviridae*) induces a severe haemorrhagic fever in humans and non-human primates but only transient non-lethal disease in rodents. However, sequential passages of MARV in rodents boost infection to a lethal disease. Selection of adapted highly pathogenic MARV variants in guinea pigs needed only eight passages. Adapted MARV contained besides three mutations in the viral polymerase L, one in the matrix protein VP40 at residue 184 (D184N).
MARV VP40 plays an important role in viral assembly, budding and suppression of the interferon signalling. We wanted to analyse whether the mutation D184N in the VP40 influences its function in a species-specific manner. We cloned and rescued recombinant MARVs containing either wtVP40 (recMARVWT) or guinea pig-adapted VP40 (recMARVD184N) and analysed growth kinetics. RecMARVD184N grew to substantially higher titers compared to recMARVWT in guinea pig cell lines than in human cells. Furthermore, recMARVD184N showed higher infectivity and displayed efficient assembly in guinea pig cells at a ultrastructural level.
To study in more detail what function of VP40 was affected by the D184N mutation, known functions of ectopically expressed VP40WT or VP40D184N were analysed by different approaches. The capabilities of VP40WT and VP40D184N to bind membranes, to form virus-like particles (VLPs), to attract nucleocapsid proteins into VLPs, and to inhibit interferon signalling were comparable in human and guinea pig cells. Only the suppressive effect of MARV VP40 on the transcription and replication in the

minigenome or infectious VLPs assay was attenuated by the D184N mutation in guinea pig compared to human cells.
Our data suggests that the improved viral fitness of recMARVD184N in guinea pig cells is not due changes in the assembly capabilities or the IFN suppression functions of VP40D184N, rather than a reduced inhibition on viral replication and transcription in guinea pig cells.
Disclosure of Interest: None declared

Poster 211
THE ROLE OF NATURAL KILLER CELLS IN HANTAVIRUS-INFECTION
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Abstract: Hantaviruses cause hemorrhagic fever with renal syndrome (HFRS) and hantavirus cardio-pulmonary syndrome (HCPS), two human diseases with high case fatality rates. Endothelial cells are the main targets for hantaviruses and vascular permeability is a hallmark of HFRS/HCPS. An intriguing observation in patients with HFRS/HCPS is that the virus infection leads to strong activation of cytotoxic lymphocytes, CD8 T cells and Natural Killer (NK) cells, but no obvious destruction of infected endothelial cells.
Here, we provide a possible explanation for this dichotomy by showing that hantavirus-infected endothelial cells are protected from NK cell-mediated killing. This protection was attributed to inhibition of granzyme B and caspase 3 by the viral nucleocapsid protein. Next, we sought to explain the strong NK cell activation. Hantavirus-infected cells were shown to strongly activate NK cells in a cell-cell contact-dependent way, this was due to virus-induced IL-15 and IL-15Rα on infected cells and the response was blocked with anti-IL-15 antibodies. A consequence of this IL-15-dependent NK cell response was that it led to killing of uninfected cells despite expression of normal levels of HLA class I. Our findings provide a tentative explanation for the hantavirus-mediated block of cytotoxic granule-mediated killing, and hence the protection of infected cells from cytotoxic lymphocytes. Taken together with the activation of NK cells and killing of uninfected cells, our data add further insights into mechanisms behind the immunopathogenesis of hantavirus infections in humans and identify new possible targets for intervention.
Disclosure of Interest: None declared

Poster 212
INCREASED RISK FOR CANCER FOLLOWING HEMORRHAGIC FEVER WITH RENAL SYNDROME
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Abstract: Increased risk for cancer following hemorrhagic fever with renal syndrome
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Hantaviruses cause two severe acute diseases; hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). In Sweden, Puumala virus (PUUV) causes a relatively mild variant of HFRS. Using register studies, we recently analyzed long-term consequences of PUUV-infection in Sweden, showing that there is a 73% increased risk for lymphoma following HFRS-diagnosis. This prompted us to study the cause of death pattern in previously HFRS-diagnosed

individuals in Sweden. During 1997 – 2013, 6,757 individuals were diagnosed for HFRS, whereof totally 474 individuals had died. Surprisingly, while the observed overall standardized mortality rate (SMR) was significantly lower than expected, we observed significantly increased SMR for one specific form of cancer. The findings of increased risks for lymphoma, and of dying from another form of cancer, after being diagnosed with HFRS suggests a possible role for hantaviruses in cancer, or alternatively that (yet undiagnosed) cancer might affect the risk of developing HFRS after PUUV-infection. Hantavirus-mediated regulation of apoptosis and inflammation might play important roles: we have recently observed that hantaviruses strongly affect these important cellular and immunological responses in previously unknown manners indicating a potential role for hantaviruses in cancer. These findings will be presented and discussed.
Disclosure of Interest: None declared

Poster 213
GAME BIRDS AS A POTENTIAL RESERVOIR FOR THE SPREAD OF NEWCASTLE DISEASE VIRUS
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Abstract: Newcastle disease, caused by Newcastle disease virus (NDV), an avian paramyxovirus type 1 (APMV-1), is one of the most devastating diseases of poultry. Game birds, such as pheasants and partridges, are susceptible to infection with NDV. Two of the last incursions of NDV into the UK have been in the game bird industry. The susceptibility to NDV infection and the ability of NDV to transmit between pheasants and partridges was assessed using two ND viruses – APMV-1/pheasant/GB/739/05 (739/05) and PPMV-1/partridge/UK(Scotland)/7575/06 (7575/06), a pigeon paramyxovirus type 1 (PPMV-1), an antigenetic variant of APMV-1. Pheasants and partridges were infected with one of three doses of 739/05 or 7575/06. Oropharyngeal and cloacal swabs were taken daily and birds were selected randomly for examination *post mortem.* Virus was detected in swabs and tissues by real-time RT-PCR and in tissues by immunohistochemistry. NDV in game birds was highly infectious, causing systemic spread of virus, and high levels of virus shedding for three weeks. Infection of both pheasants and partridges with NDV resulted in minimal/complete absence of clinical signs. Transmission studies performed between birds infected directly with these two viruses and naïve, contact birds showed that the PPMV-1, classified as ND in domestic poultry including game birds and endemic in European wild *columbiformes,* was able to transmit between pheasants. Although 739/05 NDV infected both pheasants and partridges successfully, no transmission of this particular isolate to contact birds occurred. However, field observations from the two separate UK outbreaks in this sector along with subclinical infections of NDV in game birds observed in the present study, indicates that these birds pose a significant risk pathway for introduction of ND to the *galliforme* production species, game bird poultry industries and mixed sector environments through subclinical propagation and spread of this notifiable disease.
Disclosure of Interest: None declared

Poster 214
MUTATIONS IN THE M SEGMENT OF AN ORTHOBUNYAVIRUS COMPENSATE DEFECTIVE S-SEGMENT ATTENUATED MUTANTS IN VIVO.
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Abstract: Schmallenberg virus (SBV) is an *Orthobunyavirus* of ruminants that emerged in Europe in 2011. SBV infection is associated with malformations in stillborn or newborn lambs and calves.
We have previously shown that the NSs protein of SBV is a determinant of virulence due to its ability to counteract the innate immune system of the host. Here we used a forward genetics approach to further identify SBV determinants of virulence. Unexpectedly, we found that a serially tissue culture passaged

SBV strain (called SBVp32) harboring mutations in all three genome segments displays increased pathogenicity in 10-day old NIH-Swiss mice compared to wild type SBV (SBVwt). By *in vivo* assays, we found that the M segment contains the determinants of increased virulence of SBVp32. A reassortant containing the M segment from SBVp32 and the remaining genome segments from SBVwt (SBV-M32) was more infectious than SBVwt *in vitro* and reached higher titers in the brains of 10-day old mice compared to SBVwt. On the other hand, we found that a reassortant containing the S segment of SBVp32 and the remaining genome segments from SBVwt (SBV-S32) is attenuated *in vivo*, due to its inability to block the innate immune system of the host. Interestingly, SBV-M32 mutations can compensate the attenuated phenotype of SBV-S32. The results obtained in this study suggest that the design of live attenuated vaccines based on deletions of the SBV non-structural proteins might require careful consideration.

Disclosure of Interest: None declared

Poster 215

SEQUENCE PLASTICITY OF PB2-627 POSITION OF INFLUENZA A VIRUS DEMONSTRATES DISTINCT REPLICATION PROPERTIES IN VITRO AND IN VIVO

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Abstract: The 627 position of polymerase basic protein 2 (PB2) of influenza A virus is well known as a genetic marker responsible for pathogenesis and host adaptation. Sequence analyses of the PB2 genes indicate that the 627 position almost exclusively contains either lysine (K) or glutamic acid (E), suggesting a high sequence constraint at this genetic marker. Here, we applied a site-directed random mutagenesis method to demonstrate that the PB2-627 position has a high sequence plasticity. Recombinant viruses carrying various amino acid residues at this position are viable in cell cultures. These PB2-627 mutants showed various polymerase activities and replication kinetics in mammalian and avian cells as well as pathogenicity in mice. Serially passaging these mutants in MDCK cells generated some compensatory mutations at PB2 that can restore polymerase activities of the PB2-627 mutants. Of these compensatory mutations, PB2-D309N was identified as a novel mutation responsible for polymerase activity increase. Besides showing that influenza virus can tolerate a wide range of amino acid residues at the PB2-627 position, this study also demonstrates a potential strategy to identify novel mutations that can enhance viral polymerase.

Disclosure of Interest: None declared

Poster 216

INTERCHANGING GENE ORDER OF INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS (IHNV) TO GENERATE LIVE ATTENUATED VIRUSES

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Abstract: To date, there is no commonly used live-attenuated vaccine against fish viruses due to ecosafety reasons. The Infectious Hematopoietic Necrosis Virus (IHNV) is a novirhabdovirus responsible for massive economic losses in salmonid aquaculture. As all negative-sense single-stranded RNA viruses, IHNV replication is dependent on a decreasing transcription gradient of the viral genes along its genome 3'-N-P-M-G-NV-L-5'. Thus, as described for Vesicular Stomatis Virus, interchanging the gene order within its genome alters the level of expression of each viral protein consequently affecting viral replication (Flanagan et al., 2001). Furthermore, as no homologous recombination has been clearly documented, changes in gene order are irreversible in contrast to single point mutations which might easily revert. Therefore, an artificially-designed attenuated IHNV with reordered genes would be stable, safe and would provide an effective candidate vaccine against IHNV infection. In the current work, using the reverse genetics system for IHNV previously established in our laboratory (Biacchesi et al., 2000), we have interchanged the gene order of N, P, M and G genes to engineer 6 recombinant IHNV: N1G2, N1G3, N2G3, N2G4, N3G4 and N4G1. The expression profile of each gene for these recombinant viruses has been evaluated by RTqPCR. Characterization of

cytopathic effect, kinetics of replication and viral titers have also been measured and in vivo attenuation of each recombinant virus has been evaluated in rainbow trout (Oncorhynchus mykiss) by bath immersion. Among these viruses, N2G3 displayed an interesting balance between attenuation and immunogenicity. This virus did not induce any mortality and conferred an efficient protection against a lethal challenge with the wild-type virus. This study is a first step towards the generation of a safe live-attenuated vaccine for fish farmers and constitutes an important tool for better understanding gene regulation in IHNV pathogenicity.
Disclosure of Interest: None declared

Poster 217
HEPARIN-MEDIATED INHIBITION OF HENIPAVIRUS INFECTION
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Abstract: The *Henipavirus* genus includes two closely related, highly pathogenic paramyxoviruses, Nipah and Hendra virus, which cause prominent morbidity and mortality in animals and humans. Pathogenesis of these viral infections is poorly understood and efficient antiviral treatment is still missing. Both viruses use ephrin-B2 and B3 as cell entry receptors and following the initial entry into an organism, they are capable of rapid spread throughout the host. We have previously reported that Nipah virus can use another attachment receptor, different from its entry receptors, to bind to non-permissive circulating leukocytes thereby promoting viral dissemination within the host. We report here the identification of this intriguing attachment molecule as heparan sulfate for both Nipah and Hendra virus. Cells devoid of heparan sulfate were not able to mediate Henipavirus trans-infection and showed reduced permissivity to infection. Virus pseudotyped with Nipah virus glycoproteins bound heparan sulfate and heparin but no other glycosaminoglycans in a surface plasmon resonance assay. Furthermore, heparin was able to inhibit the interaction of the viruses with the heparan sulfate and to block cell-mediated trans-infection of Henipaviruses. Moreover, heparin was shown to bind to ephrin-B3 and to restrain infection of permissive cells *in vitro*. Consequently, treatment with heparin devoid of anticoagulant activity, significantly improved the survival of Nipah-infected hamsters. Altogether, this study demonstrates previously unrecognized heparan sulfate-henipavirus interaction involved in both viral infection and dissemination within the host, playing thus an important role in viral pathogenesis. Finally, these results reveal heparan sulfate as a potential therapeutic target for the development of novel treatment and possibly metaphylaxis approaches against these highly lethal viral infections.
Disclosure of Interest: None declared

Poster 218
SWAPPING OF NP BETWEEN LASSA AND MOPEIA VIRUSES SHED LIGHT ON THE CONSERVATION OF EXONUCLEASIC FUNCTION BETWEEN THOSE TWO VIRUSES.
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Abstract: Lassa virus (LV) is responsible for severe hemorrhagic syndrome which causes up to 6000 deaths yearly in West Africa. To date, no vaccine is available for human use, and the sole treatment is based on Ribavirine administration, but its efficiency is limited due to difficult access to care in LV endemic areas. LV belongs to the Arenaviridae family, as well as Mopeia virus (MV), a virus which is phylogenetically close to LV, but non pathogenic for non human primates (NHP), and no human infection has been reported so far. Furthermore, it was previously reported that infection with MV induces protection against LV in NHP. Genetic proximity between MV and LV allows generation of chimeric viruses, containing components of LV and MV. Up to now, pathogenic features of LV were mainly associated with exonucleasic function of NP, which allows digestion of double stranded RNA (dsRNA), thus inhibiting induction of interferon response. We set up a reverse genetic system for MV, and rescued 2 chimeric viruses, one bearing the LV-*np* gene in MV backbone, and the other one containing half genome of LV (Small Segment, s) and half genome of MV (Large Segment, l). Viruses

were characterized, and their immune phenotypes were assayed. Interestingly, introduction of the main LV virulence factor in MV background does not restore a LV-like phenotype, indicating that exonucleasic function of LV-NP is not sufficient to explain LV/MV differences regarding pathogenicity.
Furthermore, biochemical assays revealed that MV-NP is also able to digest dsRNA, and to circumvent IFN response. Site-directed mutagenesis was performed to knock-down this function in MV. Resulting virus showed a strongly attenuated phenotype compared to LV but also to MV. All together, these results indicates that (i) NP and its capacity to subvert IFN response is not sufficient to explain LV-induced pathogenicity, and (ii) the exonucleasic function of NP of Arenavirus probably has another function in virus replicative cycle.
Disclosure of Interest: None declared

Poster 219
CHARACTERIZATION OF THE CELLULAR IMMUNE RESPONSE TO NIPAH VIRUS INFECTION
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Abstract: Nipah virus (NiV), a BSL-4 classified zoonotic paramyxovirus, causes a severe respiratory disease in pigs and fatal encephalitis in humans. Several studies have shown that NiV is able to evade the innate immune response by an interferon antagonistic activity. However, how the adaptive cellular immune system of different hosts responds to NiV infection remains poorly understood. With regards to the porcine host, NiV-infected pigs undergo seroconversion and produce NiV-specific neutralizing antibodies. Thus, the humoral CD4+ helper T-cell dependent immunity is a critical factor in surviving NiV infection and future protection. Interestingly, only CD8+ T lymphocytes are permissive to NiV infection. However, the role of the CD8+ cytotoxic T cells in response to NiV infection in the porcine host is not known.
Aim of this study is to investigate if NiV is able to trigger a MHC class I-restricted CD8+ cytotoxic T-cell response in the model system mouse and in the porcine host. For the mouse studies, mice will be inoculated with NiV VLPs consisting of the two surface glycoproteins G and F followed by several boosts. At 21 dpi and 42 dpi, splenocytes will be isolated, restimulated ex vivo with the NiV antigens and the CD8+ response will be measured. Data obtained from mice experiments will provide a basis for the follow-up studies in pigs. Again, priming of naïve CD8+ T cells by VLP incubation or NiV infection will be in the amplifying host based on the natural immune response cascade followed by *in vitro* restimulation and measurement of the CD8+ T-cell response.
Altogether, our findings will improve our knowledge on how the immune system of mice and swine respond to NiV infections and moreover, whether this response has beneficial or harmful effects for the host.
Disclosure of Interest: None declared

Poster 220
PRELIMINARY RESULTS FROM MASTOMYS NATALENSIS EXPERIMENTALLY INFECTED WITH OLD WORLD ARENAVIRUSES
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Abstract: *Mastomys natalensis*, the small Natal multimammate mouse, is widely distributed in sub-Saharan Africa. In West Africa, it is the natural host of Lassa virus, which causes haemorrhagic Lassa fever in humans. In East Africa, it carries other Old World arenaviruses, such as Mopeia, Morogoro, and Gairo virus, which are not associated with human disease. However, little is known about the course of infection of these arenaviruses in their natural host *M. natalensis*.
We present preliminary results from *M. natalensis* experimentally infected with a range of Old World arenaviruses, some of which are hosted by the rodent. *In vivo* experiments were complemented by *in vitro* infection studies in primary hematopoietic and stromal cells from *M. natalensis*. The data indicate that *M. natalensis* is a potent reservoir host for a variety of arenaviruses. The course of infection depends on the virus species and the age of the animals at the time of infection.

Disclosure of Interest: None declared

Poster 221

VIRULENCE OF RECOMBINANT PIGEON PARAMYXOVIRUS ALTERED AFTER A CHICKEN BRAIN PASSAGE

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Abstract: Avian paramyxovirus type 1 in pigeon (PPMV-1) causes a serious disease with high rates of pigeon illness and death and it can affect other avian species including poultry. Most PPMV-1 are characterized by a polybasic amino acid sequence motif at the fusion protein (F) proteolytic cleavage site. The German PPMV-1 isolate R75/98 possesses five basic amino acids (^{112}RRKKR^{116}) at the F cleavage site and has an intracerebral pathogenicity index (ICPI) of 1.1 that is characteristic for a mesogenic (intermediate virulence) strain. In contrast, the resulting recombinant virus that possesses the same sequence motif at the F cleavage site has an ICPI of only 0.28, indicating a lentogenic (low virulence) pathotype. While ten virus passages of recombinant R78/98 on embryonated chicken eggs did not result in any alteration of virus characteristics, re-isolation of recombinant virus from the brain of an intracerebrally inoculated chicken resulted in a mesogenic virus with an ICPI of 0.93. Sequence determination by Next Generation Sequencing of original recombinant and re-isolated recombinant R75/98 and their comparison identified only two amino acid differences, one in the F protein (N472K) and the second in the large RNA dependent RNA polymerase (K2168R), indicating that only very few amino acid alterations are sufficient to modulate virus virulence in the presence of a polybasic amino acid sequence at the proteolytic F protein cleavage site.
Disclosure of Interest: None declared

Poster 222

ENGINEERING IMMUNOCOMPETENT MOUSE MODELS FOR EBOLA VIRUS DISEASE THROUGH BONE MARROW TRANSPLANTATION

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Abstract: A top priority in the filovirus community is the design of small animal models susceptible to filovirus infection. Moreover, in order to study immunity and pathogenesis, it is required that the models are able to mount a competent immune response. Inbred laboratory mice such as C57BL/6 and BALB/c are resistant to filovirus infection and only knockout mice with different degrees of immunosuppression are susceptible to non-adapted filoviruses. Yet, filovirus infection in these models does not fully recapitulate some of the key features of the human disease, including for example the coagulation abnormalities observed in human EVD.
Using transplantation of hematopoietic stem cells from bone marrow or human cord blood, we have generated chimeric mice harboring fully competent hematopoietic immunity from mouse or human origin respectively. Our strategy was to use recipient mice with defects on stromal immunity, e. g. IFNAR$^{-/-}$ and NOD-scid. Transplantation of immunocompetent hematopoietic stem cells in these mice, rendered them susceptible to Ebola virus infection, and yet, allowed them to mount a fully functional adaptive immune response. An additional advantage of our models was the generation of double transgenic recipient mice expressing human HLA-A2 and HLA-B7, which allowed investigations on the kinetics of HLA-restricted human T cell responses to EVD *in vivo*.
Our findings in these novel models indicate that EVD dissemination from the site of inoculation requires infection of migratory dendritic cells, and that both functional CD4 and CD8 T cells are essentially required to prevent virus dissemination. In addition, reconstitution of human, but not mouse, immunity in NOD-scid mice resulted in high mortality, virus replication in peripheral organs, and liver dysfunction. Thus, we have generated several models allowing dissection of the physiology of EVD immunity as well as testing of vaccines and antivirals in a fully competent immune system.
Disclosure of Interest: None declared

Poster 223

KEY SET UP OF BRAIN ORGANOTYPIC CULTURES FOR EFFICIENT STUDIES OF NEUROTROPIC VIRUSES TARGETING THE CENTRAL NERVOUS SYSTEM

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Abstract: During the last decades, organotypic brain cultures (OBC) have been developed and commonly used for their relevance in the fields of neurodevelopment, neurodegenerative diseases and neuropharmacology. Indeed, these OBC maintain the three-dimensional tissue architecture of the brain with survival of many if not each cell type including neurons, oligodendrocytes, microglial cells and astrocytes. Furthermore, multiple groups have demonstrated the broad spectrum of applications of the OBC to investigate different aspects of CNS infections. However, during our use of hippocampal slices of human CD150 transgenic mice to investigate the efficacy of fusion inhibitory HRC derived peptides against measles virus, we noticed progressive tissue structure and cellular composition rearrangement with tissue culture duration. In order to work with OBC in a standardized and repeatable way, we undertook a detailed analysis of the evolution with time of tissue culture of essential parameters such as mortality induced by the slicing, remodeling of the cell populations and their activation that have never been investigated so far. We also recorded the induction the type 1 interferon response that could be potentially induced by the tissue damage during brain slicing and it effect on the viral growth/infection to determine the best moment to infect OBC. We thus propose for the first time a method highlighting the minimal requirements, including statistical analysis of the different slicing parameters depending on the substructure used, to set up OBC for relevant studies of neurotropic viruses such as measles virus within two different substructures of the CNS.
Disclosure of Interest: None declared

Poster 224

CHIMERIC MICE WITH COMPETENT HEMATOPOIETIC IMMUNITY REPRODUCE KEY FEATURES OF SEVERE LASSA FEVER

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Abstract: Lassa fever (LASF) is a highly pathogenic viral syndrome endemic to west African countries. Despite the high morbidity and mortality caused by Lassa fever yearly, very little is known about the pathophysiology of the disease. One of the reasons that has precluded basic research on LASV has been the lack of relevant small animal models that reproduce human disease.

Immunocompetent laboratory mice are resistant to infection with Lassa virus (LASV) and, to date, only immunodeficient mice have shown some degree of susceptibility to experimental infection. Since immunopathology is thought to be a key component of LASV pathogenesis, we sought to develop a susceptible mouse model with intact adaptive, hematopoietic-driven immune response. Using transplantation of wild-type bone marrow cells into lethally irradiated type I interferon receptor knockout mice ($IFNAR^{-/-}$), we generated chimeric mice that reproduced the main features of severe Lassa fever in humans, including high lethality, T cell-mediated immunopathology, and inflammation-mediated organ damage.

Our strategy allows easy generation of a suitable small animal model to test new vaccines and antivirals and to dissect the basic components of the immune response to LASV.
Disclosure of Interest: None declared

Poster 239

RSV REPORTER VIRUSES FOR IN VITRO AND IN VIVO NON-INVASIVE IMAGING

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Abstract: Respiratory Syncytial Virus (RSV) is the most important cause of lower respiratory tract infections in young children worldwide. Yet neither vaccine nor efficient curative treatments are available. Mouse model remains the most popular to evaluate antiviral molecules or vaccine strategies. However monitoring the curse of infection requires animal sacrifice followed by RSV quantification using labor intensive, time consuming and expensive technologies such as plaque assays, quantitative RT-PCR or ELISA tests.

To make easier screening assays for antiviral compounds or neutralizing antibodies, we set up a new recombinant human HRSV reverse genetics system derived from HRSV Long strain and inserted the red fluorescent protein (mCherry) or the firefly luciferase (Luc) genes into the HRSV genome between P and M genes. Expression of mCherry and Luc are correlated to infection rate, allowing the monitoring of HRSV multiplication in cell culture using automated plate reader. Cherry expression was also detected by microscopy or cytometry analyses in mouse precision-cut lung slices and in human neonatal immune cells infected *ex vivo* showing that HRSV-mCherry may also be used to investigate the cellular targets of RSV. As a proof of concept, both mCherry and Luc expressing HRSV were used to assess antiviral activities of two RSV inhibitors. Replication of the HRSV-Luc in adult or neonatal living mice can be visualized by bioluminescent imaging, bioluminescence being detected in the snoot and lungs of infected mice after nasal inoculation. The intensity of bioluminescence correlates with the viral load, whereas little or no clinical signs are observed. We showed that HRSV-Luc can be used to monitor antiviral drug efficiency against RSV in mouse model. We propose that these recombinant viruses are valuable tools for screening of compounds active against RSV, and can be used as an extremely sensitive readout for studying effects of antiviral therapeutics in living mice or tissues.

Disclosure of Interest: None declared

Poster 240

LIVE RECOMBINANT MEASLES-M2 VACCINE INDUCES BROAD-SPECTRUM PROTECTIVE IMMUNITY AGAINST INFLUENZA VIRUSES

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Will be disclosed after the congress.

Poster 241

TARGETING THE RSV N0/P COMPLEX WITH CONSTRAINED ALPHA-HELICAL PEPTIDES

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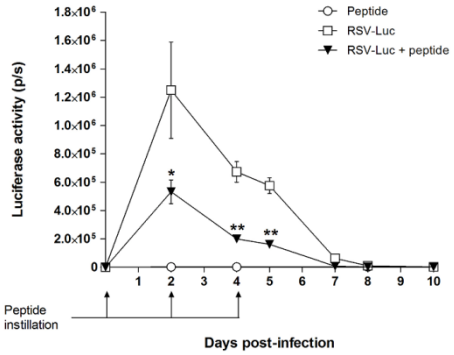
Abstract: Each year, the infection of infants with RSV is the cause of a large number of hospitalizations and deaths worldwide. The current standard of care, Synagis, is used as a preventive medicine for premature children only, and there is no drug available to treat the patients who are suffering from this infection. Targeting the polymerase replication complex is a strategy that has been used successfully to develop drugs against other viruses such as HIV, HCV and HBV. In this project, we wish to inhibit the replication complex by disrupting the N^0/P interaction. N^0 is the nucleoprotein species that is bound as a complex to the phosphoprotein prior to nucleic acid encapsidation. Recently, the N-terminal region (1-29) of the RSV P protein has been identified as sufficient to bind to N^0, and the importance of each

P residues has been assessed through alanine scanning mutagenesis experiments. Additionally, NMR studies have provided evidence that P (13-25) has a propensity to fold into an alpha helix in solution. We used these data to design stabilized alpha-helical peptides, with the aim to screen for dominant negative inhibitors of the RSV N^0/P interaction. The peptides were stabilized as macrocyclic peptides using the stapled peptide technology, because this technique can improve their potency, proteolytic stability and cellular permeability. The putative P alpha-helix was plotted onto an alpha-helical wheel, and the amino-acids located on the non-interacting site of the helix were selected for modification with the non-natural amino-acids required for stapling. We will present the results of this stapled peptide scan by reporting i) the conformational analysis by circular dichroism, ii) the biological activity in a novel N^0-P fluorescence polarization assay, a viral replication assay and a cytotoxicity assay and iii) the *in vivo* proof of concept by intranasal administration of a hit peptide, HEVS 124, to BALB/c mice inoculated with a Luc-encoding RSV.

Picture:



Disclosure of Interest: None declared

Poster 242

ONCOLYTIC ACTIVITY OF A RECOMBINANT SLAM-BLIND MEASLES VIRUS FOR COLORECTAL CANCER CELLS

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Abstract: Oncolytic virotherapy is a distinct antitumor therapeutic approach that is based on the cancer-specific infectivity and replication capacity of the virus within tumor cells, exerting consecutive antitumor effect with a few shots. We previously generated a recombinant measles virus (MV) that uses poliovirus receptor related-4 (PVRL4)/Nectin-4 as its receptor but cannot bind its original receptor, signaling lymphocyte activation molecule (SLAM). In this study, we evaluated the oncolytic activity of the recombinant MV (rMV-SLAMblind) on colorectal cancer cells. Of 10 cell lines analyzed, 6 lines, including anti-cancer drug resistant cell lines, expressed PVRL4/Nectin-4. rMV-SLAMblind infected and lysed the cell lines in a PVRL4/Nectin-4-dependent manner. The tumor progression in xenograft models was also abrogated by the virus. These results suggest that rMV-SLAMblind can be a novel therapeutic agent in colorectal cancer.

Disclosure of Interest: None declared

Poster 243

GENERATION OF LIVE ATTENUATED INFLUENZA VACCINE BY CODON USAGE BIAS

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Abstract: Seasonal influenza epidemics in many areas of the northern hemisphere in 2014/15 winter, partly due to the low effectiveness of the vaccine against the dominant antigenic drifted H3N2 virus, has once again raised the need for a better, more cross-protective influenza vaccine. Much hope has been built on live attenuated influenza vaccine which can more effectively stimulate humoral and cellular immune response. Previous studies have found that the codon usage patterns were different between influenza viruses of different subtypes, hosts, and time of isolation. This observation might be related to host adaptation and therefore, we hypothesized that changing the codon usage of a seasonal human influenza virus from human to avian could attenuate the virus and it could serve as a vaccine candidate that is compatible with egg-based production system.

We introduced synonymous mutations into the genome of seasonal H1N1 influenza virus to give a mutant virus with avian codon usage bias. The mutant virus was significantly attenuated in human, canine cells and mice but had comparable growth to wild-type virus in embryonated eggs. A single dose of intranasal vaccination of the mutant virus induced high level of neutralizing serum antibodies and conferred protection against challenge of a mouse-adapted homologous virus, a lethal heterologous virus, and a heterosubtypic mouse-adapted H3N2 virus. The mutant virus could also work as a vaccine master strain by substituting hemagglutinin and neuraminidase of other influenza viruses. These results suggested that the virus attenuation strategy of altering codon usage bias was successful and could be applied to rapid generation of seasonal and pandemic vaccines.
Disclosure of Interest: None declared

Poster 244

A SLAM-BLIND RECOMBINANT MEASLES VIRUS IS A NOVEL EFFICACIOUS TOOL FOR CANCER TREATMENT

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Abstract: Oncolytic viruses are promising tools for cancer treatment. We previously reported that a wild-type measles virus (MV) efficiently infects and lyses breast cancer cells. Since MV is known to enter immune cells using signaling lymphocyte activation molecule (SLAM), which is a key event to exert its pathogenicity in host animals, we generated a recombinant MV selectively unable to use SLAM (rMV-SLAMblind). The rMV-SLAMblind lost infectivity to SLAM-expressing lymphoid cells, but maintained infectivity to breast cancer cells, because it uses another receptor of MV, PVRL4 (poliovirus receptor related-4)/Nectin-4. Recent studies suggested that Nectin-4/PVRL4 is up-regulated in various types of tumor cells, including breast and lung cancer, which proposed that Nectin-4/PVRL4 is a good marker of these types of tumors. By screening of permissive cell lines for rMV-SLAMblind, we revealed that the virus shows oncolytic activity against various cell lines derived from refractory cancers including lung cancer, colorectal cancer, and pancreatic cancer, in which Nectin-4/PVRL4 expression is observed on surface of the cells. The virus effectively suppressed tumor mass growth in xenograft models. Thus, rMV-SLAMblind is a promising candidate of a novel therapeutic agent for caner treatment.
Disclosure of Interest: None declared

Poster 245

INVESTIGATING LYSSAVIRUS GLYCOPROTEIN ANTIGENICITY AND NEUTRALISATION.

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Abstract: Rabies is the only viral disease that causes 100% fatality following the onset of clinical disease and globally causes over 65,000 deaths per year. Alongside rabies virus, numerous related lyssaviruses exist that cause fatal encephalitis clinically indistinguishable from rabies. Whilst the human burden of these non-rabies lyssaviruses remains unclear, fatalities have been reported.

The lyssavirus glycoprotein is the sole target for virus neutralising antibodies and several antigenic sites have been strongly associated with virus neutralisation. Lyssaviruses are genetically and antigenically categorised into phylogroups that indicate the level of protection afforded by current vaccines. Rabies vaccination affords protection against all viruses within phylogroup I but is unable to confer protection against more divergent viruses. In this study both lentivirus based pseudotypes and reverse genetics techniques have been utilised to investigate intra- and inter-phylogroup neutralisation. To this end a panel of wildtype lyssavirus pseudotype particles were generated and used to assess neutralisation by standardised human and canine sera. Using these lyssavirus pseudotypes we have confirmed a strong level of intra-phylogroup neutralisation in addition to very limited inter-phylogroup neutralisation. Alongside this we have generated mutated lyssavirus pseudotypes that have had antigenic sites exchanged between phylogroup I and phylogroup II viruses. These mutants have indicated residues key to glycoprotein functionality and have identified regions capable of eliciting cross phylogroup neutralisation. Where pseudotype data has indicated mutations of interest, lyssaviruses expressing divergent wildtype and recombinant glycoproteins have been rescued. The growth kinetics of rescued recombinant viruses has been studied both *in vitro* alongside an investigation into the pathogenicity and degree of vaccine induced protection against these viruses *in vivo*.

Disclosure of Interest: None declared

Poster 246

VACCINE CANDIDATE FOR CONGO-CRIMEAN HAEMORRHAGIC FEVER

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Abstract: Congo-Crimean haemorrhagic fever (CCHF) is a severe tick-borne human disease, caused by a virus classified within the *Nairovirus* genus of the family *Bunyaviridae*. It is the most wide spread agent of severe haemorrhagic fever known; being endemic over much of Asia, extending to focal endemic areas across Africa and increasing regions of Europe. Humans usually acquire the virus through an infected tick bite or from contact with infected blood, or other tissues from patients or livestock. Severe disease, manifest as haemorrhagic fever with frequently high mortality rate, only occurs in humans. Between 15-70% of reported cases are fatal. Neither an approved vaccine nor an effective therapeutic are available. Consequently the virus is categorized as a hazard group 4 agent mandating maximum biological containment for laboratory work with infectious materials. In light of its continued spread into Europe and frequent incursions into non-endemic areas (through modern air transport of holidaymakers and professionals visiting and working in CCHF endemic zones), an effective vaccine approach which can alos meet international regulatory standards is urgently needed. We have used an attenuated poxvirus vector, (Modified Vaccinia virus Ankara), to develop a recombinant candidate vaccine expressing the CCHF virus glycoproteins. Using an established small animal model of CCHF, our research confirmed the development of cellular and humoral immunogenicity. Additionally this vaccine was able to protect all recipient animals from an otherwise lethal challenge dose of CCHF virus, adapted to represent infection via a tick bite. Histopathology and viral load analysis of protected animals confirmed that they had been exposed to challenge virus, even though they did not exhibit clinical disease. This is the first demonstration of efficacy of a CCHF vaccine.
Disclosure of Interest: None declared

Poster 247

MULTIVALENT, HETEROLOGOUS PRIME-BOOST VACCINE REGIMENS PROTECT AGAINST SEVERAL FILOVIRUS SPECIES

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Abstract: A broadly applicable filovirus vaccine should protect against all currently known strains, including the current outbreak Zaire ebolavirus variants. We investigated heterologous prime-boost vaccine regimens based on vectors of low-seroprevalence replication-incompetent human adenoviruses Ad26 and Ad35, and modified vaccinia Ankara (MVA) expressing glycoproteins of Marburg virus, Sudan-Gulu and Zaire ebolavirus for protection against filovirus infection in non-human primates. Immunization gave strong filovirus glycoprotein-specific humoral and cellular immune responses covering multiple strains that were associated with full protection against Marburg and Ebola Zaire challenge and partial protection against Ebola Sudan-Gulu challenge. Our results demonstrate that it is feasible to generate a multivalent filovirus vaccine with extended cross-species protection. A similar vaccine approach to target Ebola Zaire alone is currently in clinical phase 1 trials as countermeasure for the current outbreak in Africa.
Disclosure of Interest: None declared

Poster 248

EBOLAVIRUS SPECIFIC ANTIBODY THERAPIES AND THE ROLE OF NEUTRALIZING ANTIBODY

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Abstract: Antibody therapies for filovirus infection have garnered much attention in recent years after our group and several others reported successful treatment of filovirus infected nonhuman primates (NHPs) using virus-specific monoclonal or polyclonal antibodies. We previously reported that fractionated Ebola virus (EBOV)-specific IgG purified from serum of convalescent NHPs protected naïve NHPs against EBOV infection, even when treatment was initiated as late as 48 hours post-exposure. Here, we expand on our previous efforts to evaluate the protective efficacy of fractionated EBOV-specific IgG purified from the serum of NHPs vaccinated with a protective EBOV vaccine. Characterization of vaccinee IgG (VIgG) and convalescent IgG (CIgG) products by ELISA revealed approximately 10 fold lower EBOV GP-specific antibodies in VIgG product versus CIgG product. Unlike CIgG, which contained antibodies specific for multiple EBOV proteins, VIgG was specific for only the GP of EBOV. Most interestingly, CIgG was 30 times more potent at neutralizing virus compared to VIgG. Treatment of EBOV infected NHPs with VIgG did not provide protection despite using a treatment regimen that successfully protected CIgG treated NHPs. Relative to CIgG treatment, VIgG treatment resulted in similar EBOV glycoprotein (GP)-specific serum antibody titers in two of the three NHPs, however, neutralizing antibody titers in serum of VIgG treated NHPs remained undetectable throughout the duration of the study. These results suggest that antibody quality may be more important than antibody quantity for controlling viral replication and providing protective efficacy against filovirus infection.
Disclosure of Interest: None declared

Poster 249

H5 AND H7 PANDEMIC LIVE ATTENUATED INFLUENZA VACCINES PRIME FOR A ROBUST ANTIBODY RESPONSE AND INDUCE LONG-TERM IMMUNITY

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Abstract: Human cases of H7N9 and highly pathogenic avian influenza A (H5N1) virus infection underscore the need for control strategies to prevent an influenza pandemic. We generated pandemic (p) live attenuated influenza vaccines (LAIV) with the hemagglutinin (HA) and neuraminidase genes from H5N1, H7N7 and H7N9 viruses and the internal protein genes of the licensed seasonal LAIV. The pLAIVs were promising in preclinical studies but were severely restricted in replication and variably immunogenic in humans. However, administration of a dose of inactivated subunit vaccine (ISV) 2-5 years after pLAIV unmasked immunity in H5N1 and H7N7 pLAIV recipients with a rapid, high titer, high quality antibody response that was broadly cross-reactive across several clades or lineages, even in

the absence of detectable antibody responses to primary vaccination. We conducted a series of clinical trials with H7N9 pLAIV and ISV to determine the optimal number of priming doses of pLAIV and optimal interval between pLAIV and subsequent ISV. Either 1 or 2 doses of pLAIV priming induce robust antibody responses but the frequency of seroconversion was greater following 2 doses of pLAIV. An interval of 4 weeks between the 2nd dose of pLAIV and ISV appears to be as effective as 8 and 12-week intervals. Studies of the immune mechanisms underlying this phenomenon are under way. In summary, with 3 different pLAIV-ISV combinations we have demonstrated evidence that pLAIVs effectively prime the immune system to respond to a subsequent dose of unadjuvanted ISV with robust, high quality, cross-reactive antibody responses, highlighting the potential for vaccination schedules that combine different influenza vaccine platforms.
Disclosure of Interest: None declared

Poster 250

ANTIBODY-MEDIATED PROTECTION AGAINST LETHAL JUNIN VIRUS CHALLENGE REQUIRES FUNCTIONAL FC-DOMAINS BUT IS NOT DEPENDENT ON COMPLEMENT IN GUINEA PIGS

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Abstract: We recently found that DNA vaccination of geese produces neutralizing antibodies against Andes virus (ANDV) glycoproteins that protect hamsters from disease. Goose IgY antibody is not compatible with mammalian Fc-effector functions indicating that antibody-mediated protection against ANDV is Fc-independent. Here we tested whether antibodies produced in geese could protect against Junin virus (JUNV), where Fc-effector functions are reported to play a role in antibody-mediated protection. Six geese were vaccinated with a JUNV GPc-based DNA vaccine using a Pharmajet intramuscular delivery device. IgY antibodies produced in the geese neutralized JUNV strain candid#1 and Romero with GMT PRNT50s of 51,606 and 6,451, respectively. The protective efficacy of anti-JUNV goose antibodies was tested in guinea pigs challenged with JUNV strain Romero. 15000 therapeutic units (TU)/kg of antibody were given subcutaneously starting on either day -1, 2 or 4 and again three days later. These antibodies failed to protect animals from lethality; however, there was a significant delay in time to death compared to control animals. In contrast, 15000 TU/kg of anti-JUNV antibodies produced by DNA vaccination of rabbits protected guinea pigs against lethal disease when given two days post-challenge. We next explored the role of complement in antibody protection by transient disruption using cobra venom factor (CVF). Guinea pigs were infected with JUNV and treated with CVF (300 mg) or PBS on day 1 then administered a dose (15000 TU/kg) of anti-JUNV rabbit antisera targeting glycoprotein on day 2. CVF and antibody were readministered on days 6 and 7, respectively. There was no negative impact on protection observed in animals receiving CVF and anti-JUNV antibodies, and all animals survived. These findings indicate that antibody Fc-domains are critical for antibody-mediated protection against JUNV in guinea pigs, however complement does not appear to play a significant role.
Disclosure of Interest: None declared

Poster 251

ESTABLISHMENT OF A NOVEL RSV POLYMERASE ASSAY TO DISCOVER AND CHARACTERIZE RSV POLYMERASE INHIBITORS

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Abstract: Respiratory Syncytial Virus (RSV) is the leading cause of lower respiratory tract infections in young children and other high-risk populations. Finding an effective RSV treatment is a public health priority. The RSV L protein functions as an RNA-dependent RNA polymerase, capping enzyme, and methylase to synthesize viral RNA and capped mRNAs, making it an attractive target for antiviral therapy. The lack of robust RSV polymerase assay has hindered the discovery and characterization of RSV polymerase inhibitors.

Here we report the establishment of a novel non-radioactive RSV polymerase assay, using recombinant RSV L and P protein complex and SYBR-Green II fluorescent dye detecting the *in vitro* synthesized RNA. This is the first assay reported to date allowing direct quantification of compound effect on RSV polymerase activity in a convenient plate-based format. This assay format could be readily adapted to other RNA viruses to establish similar viral polymerase assays. Two different classes of RSV L protein inhibitors, AZ-27 and compound D (Boehringer-Ingelheim), were characterized in both a gel-based RSV polymerase assay detecting ^{32}P-ATP substrate incorporation and the new plate-based fluorescence assay. The dose-dependent effects of the compounds on RSV polymerase activity were in good agreement in both assays. Interestingly, AZ-27 directly inhibited the RSV polymerase activity whereas compound D, a reported RSV capping enzyme inhibitor, enhanced the synthesis of short RNA transcripts. These data validated the novel RSV polymerase assay, demonstrated its potential for antiviral discovery, and also brought new insight into the mechanism of action for the RSV L inhibitors tested.
Disclosure of Interest: None declared

Poster 252
ENGINEERING OF RESPIRATORY SYNCYTIAL VIRUS F GLYCOPROTEIN EXPRESSED BY A PARAINFLUENZA VIRUS VECTOR FOR ENHANCED IMMUNOGENICITY
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Abstract: A live-attenuated chimeric bovine/human parainfluenza virus type 3 (B/HPIV3) expressing wild-type (wt) respiratory syncytial virus (RSV) strain A2 fusion (F) glycoprotein has been under development as a bivalent intranasal vaccine against RSV and HPIV3, two major pediatric respiratory viruses. In a clinical study in seronegative children performed by others, this vaccine candidate was immunogenic for HPIV3, but induction of RSV neutralizing serum antibodies (NAb) was less than expected. In the present study, we explored whether the RSV NAb response could be improved by (i) increasing RSV F expression (up to 10-fold) by codon-optimization and modifying the amino acid sequence to be identical to that of an early passage of strain A2, (ii) enhancing the packaging of RSV F into the vector virion by substituting the cytoplasmic tail alone (CT) or together with the transmembrane domain (TMCT) with their counterparts from the vector F protein, and (iii) including previously described mutations in the RSV F protein (DS, or DS/Cav1) that stabilize it in the pre-fusion conformation. In hamsters, TMCT, or DS, or DS/Cav-1, but not CT, independently resulted in an increased level of high-quality RSV neutralizing antibodies. Surprisingly, packaging of the wt or pre-fusion form of RSV F did not significantly impair vector replication in vitro. However, in rhesus macaques, vector expressing TMCT/DS was 100-1000 times more attenuated than vectors expressing the wt or DS RSV F; nevertheless, it induced 100- and 30-fold higher titers of serum RSV NAb than vectors expressing the wt and DS form of RSV F, respectively. Thus, TMCT-mediated packaging and the pre-fusion conformation contributed independently to the enhanced NAb response in primates, with TMCT having the greater effect. Generally, the RSV F insert was genetically stable during vaccine virus replication in vitro and in vivo. B/HPIV3 expressing the TMCT/pre-fusion form of RSV F is under development for clinical evaluation.
Disclosure of Interest: None declared

Poster 253
ATTENUATED HUMAN PARAINFLUENZA VIRUS TYPE 1 EXPRESSING THE FUSION F GLYCOPROTEIN OF HUMAN RESPIRATORY SYNCYTIAL VIRUS AS A BIVALENT HPIV1/RSV VACCINE
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Abstract: Respiratory syncytial virus (RSV) is the leading viral cause of acute bronchiolitis and pneumonia in infants and children. It is a major cause of childhood morbidity and mortality with an estimated 199,000 annual deaths worldwide. No licensed vaccine or effective antiviral drug is available. Live attenuated human parainfluenza virus type 1 (rHPIV1) was investigated as a vector to express the respiratory syncytial virus (RSV) fusion (F) glycoprotein, to provide a candidate bivalent vaccine against RSV and HPIV1. The RSV F gene along with the HPIV1 transcription signals was inserted individually at three genome locations in each of the two attenuated rHPIV1 vectors. Each vector backbone contained a single previously-described attenuating mutation that was stabilized against de-attenuation: specifically, a non-temperature-sensitivity deletion mutation involving six nucleotides in the overlapping P/C ORFs ($C^{\Delta 170}$), or a temperature-sensitivity missense mutation in the L ORF (L^{Y942A}). The insertion sites were pre-N (F1), N-P (F2), or P-M (F3). High levels of RSV F expression were observed with rHPIV1-$C^{\Delta 170}$-F1, -F2, and -F3, and rHPIV1-L^{Y942A}-F1. *In vitro*, the insertion of F gene reduced the rate of virus replication, but the final titers were the same as wt HPIV1. In hamsters, the rHPIV1-$C^{\Delta 170}$-F1, -F2, and F3 vectors were moderately restricted in the nasal turbinates and highly restricted in the lungs, and were genetically stable after *in vivo* replication. Among the $C^{\Delta 170}$ vectors, the F1 virus was the most immunogenic and protective against wt RSV challenge. The rHPIV1-L^{Y942A} vectors were highly restricted *in vivo* and were not detectably immunogenic or protective, indicative of over-attenuation. The $C^{\Delta 170}$-F1 construct appears to be suitably attenuated and immunogenic for further development as a bivalent intranasal pediatric vaccine.
Disclosure of Interest: None declared

Poster 254

CLINICAL ASSESSMENT OF A BIVALENT DNA VACCINE FOR HEMORRHAGIC FEVER WITH RENAL SYNDROME DELIVERED BY INTRAMUSCULAR ELECTROPORATION

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Abstract: Hemorrhagic fever with renal syndrome (HFRS) is endemic in Asia, Europe and Scandinavia, and is caused by infection with the hantaviruses Hantaan (HTNV), Seoul (SEOV), Puumala (PUUV), or Dobrava (DOBV) viruses. We developed candidate DNA vaccines for HFRS expressing Gn and Gc genes of HTNV or PUUV and evaluated them in an open-label, single-center Phase 1 study. Three groups of nine subjects each were vaccinated on days 0, 28 and 56 with the DNA vaccines for HTNV, PUUV, or mixture of both vaccines using Ichor Medical Systems intramuscular electroporation (IM-EP) delivery device. Each dose consisted of 2.0 mg DNA in an injected volume of 1 mL saline. For the combined vaccine, the mixture contained equal amounts (1 .0 mg) of each DNA vaccine. There were no study-related serious adverse events (SAEs). Neutralizing antibody responses were detected in 5/9 and 7/9 of individuals who completed all three vaccinations with the HTNV or PUUV DNA vaccines, respectively. In the combined vaccine group, 7/9 of the volunteers receiving all three vaccinations developed neutralizing antibodies to PUUV. The three strongest responders to the PUUV vaccine also had strong neutralizing antibody responses to HTNV. These results demonstrate that the HTNV and PUUV DNA vaccines delivered by TDS-IM separately or as a mixture are safe. In addition, both vaccines were immunogenic, although when mixed together, more subjects responded to the PUUV than to the HTNV DNA vaccine, suggesting immunological interference. Consequently, we have developed an optimized HTNV DNA vaccine that shows no interference in hamsters when mixed with the PUUV vaccine. A Phase 2a study is in progress to evaluate the optimized mixed vaccine delivered by IM-EP. The 120 study subjects are receiving 1 mg or 2 mg of DNA two times eight weeks apart or three times at four week intervals. An additional Phase 1 study is being planned to compare IM-EP delivery to intradermal-EP delivery with these DNA vaccines.
Disclosure of Interest: None declared

Poster 255

CONSTRUCTION, IN VITRO CHARACTERIZATION AND IMMUNOGENICITY OF A RECOMBINANT ISFAHAN VIRUS VACCINE VECTOR

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Abstract: Recombinant vesicular stomatitis virus (rVSV) vectors expressing foreign antigens have demonstrated efficacy and utility as live attenuated vaccines in many animal disease models. Advantages of the rVSV vector platform include a genome that can be readily manipulated to robustly express target antigens, inherent high level antigenic properties, low seroprevalence in the human population and the ability to vigorously propagate in many continuous cell lines. Recently, an optimized rVSV vector expressing the HIV-1 gag protein has successfully completed Phase I clinical evaluation (HVTN 090). However, commensurate with these advances, remains the question of potential immunity generated against vector specific proteins that may interfere with subsequent immunizations using the same rVSV vector. Serotype switching of rVSV vectors, achieved by swapping the surface G protein with that of a different vesiculovirus serotype can enhance immunogenicity in prime-boost regimens in mice. However, cross-reactivity of cellular immune responses directed towards rVSV core proteins may limit this approach. In view of these observations and potential limitations, we have established a reverse genetics system for the generation of recombinant Isfahan virus (rISFV), another vesiculovirus, which is serologically distinct and phylogenetically divergent from VSV. As for the rVSV vector system, we were able to attenuate rISFV vectors using N gene translocation and G protein cytoplasmic tail truncation. In addition, we have demonstrated reduced immunological cross reactivity between the N proteins of both viruses and enhanced immunogenicity of a model antigen following rVSV/rISFV vaccination compared to rVSV/rVSV vaccination regimens.
Disclosure of Interest: None declared

Poster 256
IDENTIFICATION OF NOVEL EBOLAVIRUS INHIBITORS THAT BLOCK MACROPINOCYTOSIS.
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Abstract: We have screened a 350,000 molecule library using a *Marburgvirus* pseudotype by quantitative high throughput screening (qHTS) at National Center for Advancing Translational Sciences (NCATS). Hits identified through this screen were counter-screened using a second pseudotype and then wild type Marburg and Ebolaviruses at BSL4. 22 broad-spectrum *Filovirus* inhibitors were identified. One of the problems faced after identification of inhibitors was how to understand the mechanism of action for each of the hits using wild type virus in the BSL-4 setting. Our solution has been to develop a high resolution 3D imaging and modeling approach to rapidly characterize compounds as binding, macropinocytosis, trafficking and endosomal pH inhibitors. Mechanism of action for 10 of the hits was determined using this approach. Here we report findings for two of these compounds, 555232 and 762907. Quantitative modeling using Ebola and Marburg virus or virus like particles (VLPs) revealed that cells treated with 555232 and 762907 have: (i) increased number of viral particles bound to cell surface (ii) reduced co-localization with early endosomal marker (iii) reduced number of viral particles in cytoplasm and (iv) a reduction in vesicular uptake of fluorescent dextran - A hallmark of macropinocytosis; compared to untreated cells. Using a separate microscopy based assay we determined that 555232 and 762907 do not inhibit entry by blocking endosomal acidification. Taken together our findings indicate that 555232 and 762907 act by inhibiting macropinocytic uptake of *Filoviruses* without affecting receptor binding. Each represents a new class of macropinocytosis and virus entry inhibitor, from which we can gain deeper insights into cell and *Filovirus* biology. We are currently evaluating derivatives and medicinal chemistry for improved activity.
Disclosure of Interest: None declared

Poster 257
PASSIVE IMMUNOTHERAPY FOR THE TREATMENT OF WEST AFRICAN EBOLA VIRUS INFECTION IN NONHUMAN PRIMATES
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Abstract: The filoviruses, Marburg virus and Ebola virus, cause severe and often fatal hemorrhagic fever in humans and nonhuman primates (NHP). The current *Zaire ebolavirus* (ZEBOV) outbreak in West Africa has highlighted the need for effective, approved therapeutics and vaccines to respond and/or prevent future outbreaks. Amazing progress has been made toward anti-ZEBOV monoclonal antibody cocktails as therapeutics, culminating in the most recent report detailing the successful treatment of nonhuman primates (NHPs) with ZMapp. While this result is groundbreaking, a full response to an outbreak the level of 2014 ZEBOV-Makona using this method of treatment would require production levels currently not available. The World Health Organization has released guidance on the use of ZEBOV convalescent whole blood or plasma in West Africa (clinical trials ongoing/starting in Liberia, Sierra Leone, and Guinea). This is not a new consideration for the treatment of ZEBOV infection as there was limited use of convalescent serum in 1976 and convalescent whole blood in 1995 in patients infected with ZEBOV. Whether these treatments were successful or not is still unknown as there were many confounding factors such as additional care and timing of treatment given to patients. This method of treatment has previously been tested in NHPs using ZEBOV convalescent whole blood which afforded no protective efficacy against ZEBOV challenge. However, this study did not use blood from the same convalescent phase; years after versus proximal to convalescence as used in the 1995 outbreak (36-67 days post onset). We therefore sought to investigate whether 28 day post-challenge convalescent sera from siRNA therapeutic ZEBOV-Makona or SEBOV-Gulu studies could protect NHPs when treated at days 3, 6, and 9 post-challenge. Convalescent sera were assessed for anti-ZEBOV Immunoglobulin G (IgG) titers and quality as well as for therapeutic efficacy against ZEBOV-Makona challenge in NHPs.
Disclosure of Interest: None declared

Poster 258

THE GENETIC STABILITY OF PIV5-VECTORED RSV VACCINE CANDIDATES AFTER IN VITRO AND IN VIVO PASSAGE

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Abstract: Human respiratory syncytial virus (RSV) is the leading cause of lower respiratory tract infections in children, yet no licensed RSV vaccine exists. Our laboratory developed two parainfluenza virus 5 (PIV5)-based RSV vaccine candidates that confer robust protection against RSV infection in mice. The PIV5 genome was engineered to express either the RSV fusion protein (F) or the RSV major attachment glycoprotein (G) between the HN and L genes. We examined the stability of the two vaccine candidates (rPIV5-RSV-F and rPIV5-RSV-G) *in vitro* by passaging them in Vero cells for eleven generations and comparing the genome sequences of the resulting viruses with those of the parent viruses. Although mutations were found within clonal populations of the vaccine candidates and in the PIV5 backbone of rPIV5-RSV-G, there were no mutations in the consensus sequences of the gene insertions for both candidates. We also investigated the *in vivo* stability of the vaccine candidates after a single passage in African green monkeys. No mutations were observed in the consensus sequences of viruses collected from the BAL fluid. These results indicate that foreign gene insertions are stable in the PIV5 genome.
Disclosure of Interest: None declared

Poster 259

GENERATION OF A G-DEFECTIVE NOVIRHABDOVIRUS AS A POTENTIAL SAFE VACCINE FOR TROUT.

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Abstract: Trout aquaculture has to face to *Novirhabdovirus* infections, like the Viral Hemorrhagic Septicemia Virus (VHSV), which destroy tons of fish every year. To fight against VHSV, availability of vaccines is crucial for fish farmers. Trout fingerlings are extremely sensitive to VHSV and so vaccines have to be administrated at that life stage, implying the use of live attenuated vaccines deliverable by bath immersion. We have investigated the possibility of generating by reverse genetics a recombinant VHSV lacking the external glycoprotein G leading to a virus unable to spread. A helper EPC cell line constitutively expressing the VHSV-derived G was established and used to recover recombinant rVHSV-ΔG expressing the green fluorescent protein (GFP) or the Renilla luciferase (RLUC) genes. These rVHSV-ΔG were readily recovered, amplified, purified and characterized through SDS-polyacrylamide gel electrophoresis, indirect immunofluorescence and Western-blot assays and electron microscopy observations. The rVHSV-ΔG-GFP was mass produced and used to inject juvenile trout twice at 2-week interval before challenging immunized trout with wild-type (wt) VHSV infection. The rVHSV-ΔG-GFP was shown to efficiently protect fish against wtVHSV since 97% of the trout survived to the infection. As the best way for vaccine administration to juvenile trout is bath immersion, rVHSV-ΔG-LUC at $5x10^4$ PFU/mL was given in a tank containing 50 fish. A month later, vaccinated trout were challenged with wtVHSV. None of the trout were protected from VHSV challenge. To investigate if the lack of protection was due to the absence of rVHSV-ΔG-LUC fish infection or if fish have been infected with rVHSV-ΔG-LUC but without inducing any immune response, luciferase activity was measured in fish shortly post rVHSV-ΔG-LUC bath infection. No luciferase activity was detected in fish, indicating that no rVHSV-ΔG-LUC infection occurred, although this virus was shown to be able to enter and to replicate in fish cell.
Disclosure of Interest: None declared

Poster 260

DEVELOPMENT OF CLINICAL DIAGNOSIS KIT FOR EBOLA VIRUS DISEASE

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Abstract: Ebola Virus Disease (EVD) is one of the most sever viral hemorrhagic fevers (VHF) with high case fatality rate and EVD outbreak in West Africa including Sierra Leone, Liberia and Guinea since March, 2014 has caused more than 23,000 cases and nearly 9000 deaths, constituting an 'extraordinary event' worldwide. Currently, Ebola vaccine and specific drug treatment are undergoing clinical trials, early laboratory diagnosis is critical for Ebola control.
National Institute for Viral Disease Control and Prevention (IVDC), China CDC has successfully developed Ebola virus nucleic acid, antigen and antibody detection reagents, which were preliminary tested in Pasture Institute, laboratory of Unit of Biology Emerging Viral Infection (UBIEV), National Reference Center for Viral Hemorrhagic Fever, WHO Reference Laboratory for Viral Hemorrhagic Fever and Arborvirus in Lyon, France. The Ebola virus RNA test line of the Real-time RT PCR kit for Zaire Ebola virus NP/GP gene detection are equivalent to the method used in the Reference laboratory in Lyon. Recently, 'Detection Kit for Zaire Ebola Virus RNA
(PCR-Fluorescence Probing)', which was developed by IVDC, China CDC and prepared by DAAN Gene Co., Ltd. Of Sun Yat-sen University has completed clinical trials in Sierra Leone, and has gotten Certificate for Ebola Virus Real-time PCR Diagnostic Kit issued by CFDA (Reg. No. 20143402058, 25/11/14). It is the first issued clinical use diagnostic kit for EVD in China and has been provided to China CDC Lab team, used for clinical diagnosis of EVD in Sierra Leone and to 31 province CDC in China, used for detection of Ebola virus in serum samples from EVD observing cases in clinic.
Ebola Virus Real-time PCR Diagnostic Kit developed by IVDC, China CDC plays critical role in our aids to fight against epidemic of EVD in West Africa and in China's response to EVD.
Disclosure of Interest: None declared

Poster 261

DEVELOPMENT OF A HANTAAN VIRUS MINIREPLICON SYSTEM FOR DRUG-SCREENING

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Abstract: Newly emerging pathogenic viruses represent a serious challenge for Global Health. Hantaviruses are an important group of viruses that can cause serious diseases in humans and poses an increasing threat. Human pathogenic hantaviruses are associated with two severe diseases: Hemorrhagic Fever with Renal Syndrome (HFRS) in Asia and Europe and Hantavirus Cardiopulmonary Syndrome (HCPS) in the Americas, with case mortality rates up to of 15% and 40%, respectively. The geographical distribution of hantaviruses is determined by their natural primary host, rodents, shrews, moles and bats, that carry the virus as asymptomatic persistent infection. Human infection occurs accidentally, mainly by inhalation of aerosolized rodent excreta. Currently no effective preventive vaccine, immunotherapeutics, or antiviral drugs are licensed for specific treatment of hantavirus diseases.
Replication and transcription of the viral RNA genome represent crucial steps of viral multiplication, which are highly conserved among hantaviruses and therefore represent promising drug targets for the development of effective broad-spectrum antivirals. Over the past decade, minireplicon systems have emerged as powerful experimental surrogates to study replication and transcription of many highly pathogenic RNA viruses. The major goal of our study is to develop a Nanoluciferase reporter-based minireplicon system for hantaviruses that can be used in high-throughput screening (HTS) of synthetic small molecules libraries. After their initial discovery, novel candidate anti-viral drugs will be validated with live pathogenic hantaviruses in the new high containment facilities at Spiez Laboratory.
Disclosure of Interest: None declared

Poster 262
DEVELOPMENT OF A NOVEL VACCINE AGAINST LASSA VIRUS
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Abstract: Lassa virus (LASV) causes a severe hemorrhagic fever with high mortality in humans and represents a serious public health problem in Africa. Currently, there are no FDA approved vaccines available and treatment options are limited, making the development of a safe and efficacious LASV vaccine an urgent need. Neutralizing antibodies (nAb) represent a major correlate of protection in many successful vaccines. However, the nAb response in human LASV infection is generally delayed and of low magnitude, posing a challenge for vaccine development. Here we evaluate a novel vaccine platform based on polymersomes (PS) coupled with biosynthetic antigens for its potential to elicit a protective antibody response against LASV. Our PS platform is based on the assembly of an amphiphilic block copolymer composed of polyethylene glycol (PEG) and polypropylene sulfide (PPS) and offers several advantages as a vaccine platform, such as delivery of immunogens to dendritic cells. Their aqueous interior permits delivery of immunogens without chemical modification, preserving their native conformation. First, we engineered a LASV immunogen derived from the receptor-binding domain of the viral envelope glycoprotein (GP)1. To achieve sufficient expression levels and to ensure proper folding, LASVGP1 was expressed as a C-terminal fusion with human IgG1 containing an enterokinase processing site, allowing cleavage of the protein. When expressed in human cells, the LASVGP1 immunogen showed an N-glycosylation pattern similar to authentic LASVGP1 present on the virus and bound the LASV receptor dystroglycan with high affinity. LASVGP1 immunogen was efficiently encapsulated into PS. Immunization of mice with LASVGP1 loaded PS combined with adjuvants induced a robust antibody response to LASVGP1, which was significantly higher than immunization with the recombinant protein alone. Profiling of IgG subtypes revealed predominantly IgG2a and IgG3. Neutralizing properties are currently characterized.
Disclosure of Interest: None declared

Poster 263

ONCOLYTIC EFFICACY OF THE MEASLES VIRUS SELECTIVELY BLIND TO SLAM (RMV-SLAMBLIND) AGAINST PANCREATIC CANCER CELLS EXPRESSING PVRL4 (NECTIN-4)

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Abstract: PVRL4 is a tumor cell marker which is known to be highly expressed on the surface of cancer cells such as breast cancer, lung cancer and colorectal cancer. Based on the fact that the measles virus uses PVRL4 as the epithelial receptor, there is a potential to use measles viruses (MVs) as oncolytic agents for cancer treatment. Wild-type MVs are also known to use SLAM as the main receptor, which is expressed in cells of the immune system. For the specific infection to PVRL4 expressing cells, we have generated recombinant measles virus, rMV-SLAMblind, which lost the binding capacity to SLAM. In this study, we evaluated the anti-cancer efficacy of rMV-SLAMblind against the pancreatic cancer in vitro and in vivo. The rMV-SLAMblind efficiently infected and killed the pancreatic cancer cell lines which express PVRL4 in vitro. The intratumoral inoculation of the rMV-SLAMblind suppressed the growth of pancreatic cancer xenografts in mice. The sequence analysis of isolated MVs from the tumor revealed that the mutation site of H protein of the rMV-SLAMblind has been maintained for 84 days after the first inoculation. From these results, it is considered that the rMV-SLAMblind is an useful oncolytic agent to treat pancreatic cancer.

Disclosure of Interest: None declared

Poster 264

MEASLES VIRUS HAS THE ABILITY TO INFECT CHICKEN EMBRYONIC FIBROBLASTS THROUGH CHICKEN NECTIN-4

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Abstract: Laboratory adapted, and vaccine strains of measles virus (MV) can infect human cells through human CD46, SLAM and nectin-4 molecules. In addition to human cells, vaccine strains of MV can infect primary chicken embryonic fibroblasts (CEFs) through an unidentified receptor. It is known that nectin-4 is highly expressed on human and mouse embryonic cells. We demonstrated that CEFs express a molecule homologous to the human nectin-4 (chicken nectin-4). A flow cytometry analysis showed that CEFs and CEF-derived cell line, UMNSAH/DF1, have sub-populations expressing chicken nectin-4. Although the amino acid sequence of chicken nectin-4 showed low similarity with human nectin-4, amino acids critical to bind hemagglutinin (H) protein of MV found in human nectin-4 are conserved in chicken nectin-4. To compare the ability of MV in using different receptors, we used CHO cell lines expressing human SLAM, human nectin-4, and chicken nectin-4. GFP-expressing recombinant MVs that possess an H protein of a vaccine strain, AIK-C (IC/AIKC-H-EGFP), and a wild-type strain, IC (IC-EGFP) used chicken nectin-4 efficiently. A nectin-4-blind mutation in the H protein of MV reduced infectivity in CHO expressing chicken nectin-4, UMNSAH/DF1, and CEF cells. Furthermore antibodies and shRNA against chicken nectin-4 reduced infectivity of MV in UMNSAH/DF1 and CEF cells. These data revealed that MV has the ability to infect CEFs through chicken nectin-4 and improved our understanding of MV vaccines, which have been produced using CEFs.

Disclosure of Interest: None declared

Poster 265

A MOUSE MODEL FOR SEVERE FEVER WITH THROMBOCYTOPENIA SYNDROME

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Abstract: Severe fever with thrombocytopenia syndrome (SFTS) caused by SFTS virus, a novel Phlebovirus in the family bunyaviridae, was first reported in 2011, and has been reported to be epidemic in Asia. SFTS virus causes systemic infection accompanied by thrombocytopenia and leukocytopenia in humans, with a case fatality rate of 10-30%. The pathogenesis of SFTS virus in humans is poorly understood, and it is desired to establish the animal model and effective antiviral therapies for SFTS. In this study, we inoculated SFTS virus to various strains of mice, and evaluated the susceptibility of each mouse strain to SFTS virus infection. Among mouse strains we tested, C3H mice showed remarkable thrombocytopenia and leukocytopenia, especially when infected with SFTS virus intradermally. SFTS virus grew well in spleens of C3H mice, and virus antigen was detected in macrophages of splenic white pulp, showing a “starry sky” appearance. Furthermore, we examined the efficacies of favipiravir (T-705) and ribavirin against SFTS virus infection in this C3H mouse model. T-705 effectively inhibited the virus growth in mouse spleens, whereas it did not improve the thrombocytopenia and leukocytopenia. On the other hand, ribavirin showed little antiviral effect against SFTS virus infection *in vivo*.Above all, we conclude that C3H mice provide a useful animal model to elucidate the pathogenesis of SFTS and the efficacy of antiviral drugs.
Disclosure of Interest: None declared

Poster 266

IDENTIFICATION OF RIFT VALLEY FEVER VIRUS INHIBITORY COMPOUNDS USING CELL-BASED HIGH-THROUGHPUT SCREENING, BASED ON VIRAL REPORTER GENE EXPRESSION

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Abstract: Abstract:
Rift Valley Fever (RVF) is an emerging infectious disease, considered as one of the most important viral zoonoses in Africa. It is an acute, febrile mosquito-borne disease caused by RVF virus (RVFV). Humans acquire RVF through bites from infected mosquitoes or by exposure to blood, body fluids or tissues from infected animals. RVF causes abortions and perinatal mortality (>95%) in livestock herds used for meat and dairy production. In humans, RVF is an acute febrile illness with occasional complications of hepatitis, encephalitis, retinitis and haemorrhagic syndromes. The overall mortality in humans is around 1.0%, however, if the illness progress to a more severe disease a case fatality up to 30% is observed. Presently there are no antivirals to treat RVFV infection.
In our study, we have used a whole cell based high-throughput drug-screening assay to screen ~27,000 small chemical compounds from two different libraries, by using a replication competent red fluorescent protein (Katuska) expressing NSs-deleted RVFV virus (rRVFV-ΔNSs:Kat). This unique assay allowed measuring the fluorescence changes as a direct result of rRVFV-ΔNSs:Kat genome expression. After primary screening, 641 compounds were identified that inhibited RVFV expression in vitro by ≥80%, at 50µM concentration. Later 63 compounds were confirmed as dose dependent inhibitors of RVFV. Finally we identified several lead compounds that are promising to inhibit RVFV in µM concentration and can be further developed as antivirals against RVFV.
Disclosure of Interest: None declared

Poster 267

DEVELOPMENT OF MONOCLONAL ANTIBODIES SPECIFIC FOR H7 HA AND THEIR APPLICATION TO RAPID DETECTION OF INFLUENZA A/H7N9 VIRUS

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Abstract: *Background*

The avian influenza A/H7N9 virus caused first human infection in early 2013 in China. The H7N9 virus infection is sporadic persistent in humans and has the potential to cause serious future pandemic. There are a number of commercially available rapid influenza diagnostic tests for influenza A and B virus infections. However, these are indistinguishable from seasonal influenza and H7N9 influenza. To detect H7N9 virus sensitively and specifically by immunoassay, we produced monoclonal antibodies (mAbs) recognizing linear epitopes specific for H7 hemagglutinin (HA).

Materials and methods

To obtain specific mAbs for HA of H7N9 virus, mice were immunized severally with two synthesized peptides conjugated to keyhole limpet hemocyanin (KLH). These amino acid sequences of two peptides were highly conserved among HAs of H7N9 virus. The screening of mAb was conducted by ELISA using denatured HA antigens of A/Anhui/1/2013 (NIBRG-268) virus, which was lysed with 0.025% SDS treatment. The lysates were diluted with ELISA-coating buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, pH 9.6) and coated ELISA plates. Following ELISA method was according to the conventional protocol. To assess the specificity of mAbs for H7 HA antigen, these mAbs were tested by ELISA using H1, H3 and H5 virus antigens.

Results

When two peptides having conserved to linear epitopes of H7 HA immunized to mice, some mAb clones were obtained by the screening ELISA. These clones were a highest reactivity in ELISA was shown in a condition of buffer with 0.025% SDS, and reacted with H7 virus antigen specifically, not with H1, H3 and H5 virus antigens.

Conclusions

The mAb clones specific for H7N9 virus were obtained from mice immunized with peptides having conserved linear epitopes of H7 HAs. Development of a highly sensitive H7N9 virus detection system is in progress using these mAbs and immunoassay.

Disclosure of Interest: None declared

Poster 268

DEVELOPMENT OF NUCLEOCAPSID PROTEIN MONOCLONAL ANTIBODY BASED ANTIGEN-CAPTURE ELISA FOR DETECTION OF SEVERE FEVER WITH THROMBOCYTOPENIA SYNDROME VIRUS

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Abstract: Severe fever with thrombocytopenia syndrome (SFTS) is a tick-borne infectious disease with a high case-fatality and is caused by a novel bunyavirus, SFTS virus (SFTSV). SFTS has been reported in China, South Korea, and Japan so far. Safe diagnostic immunoreagents with high specificities are required to control the disease efficiently in the endemic area. In this study, we developed a SFTSV antigen (Ag)-capture enzyme-linked immunosorbent assay (ELISA) system using a novel monoclonal antibody (MAb) to the nucleocapsid (N) protein. The specificities of the MAbs were confirmed by indirect immunofluorescence and immunohistochemistry assays. By the sandwich Ag-capture ELISA using the MAb, various SFTSV strains from the culture supernatants was detected at least 350-1,220 $TCID_{50}$/100 µl. The method was evaluated for clinical diagnosis using serum specimens collected form SFTS suspected patients in Japan. In a total of 63 serum specimens, all of 24 specimens containing high copy numbers of viral RNA (more than 10^5 copies/ml) were positive by the Ag-capture ELISA. In contrast, 12 out of 15 specimens containing low copy numbers of viral RNA (less than 10^5 copies/ml) were negative by the Ag-capture ELISA. Among 12 specimens negative for the Ag-capture ELISA, 9 were positive for IgG antibodies against SFTSV (9/12, 75%). These results suggest that the Ag capture ELISA developed in this study may be useful for the diagnosis of SFTS during the acute phase with high viremia.

Disclosure of Interest: None declared

Poster 269

THERAPEUTIC EFFECT OF FAVIPIRAVIR AGAINST LASSA FEVER IN IMMUNOCOMPETENT CHIMERIC MICE

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Abstract: The pyrazinecarboxamide derivative T-705 (favipiravir) was published in 2002 by Toyama Chemicals (Japan) as an inhibitor of influenza virus replication and is currently in clinical development for the treatment of highly pathogenic flu as well as Ebola virus disease. In this study, we tested the effect of favipiravir against Lassa fever in a recently generated immunocompetent mouse model of disease. Favipiravir presumably acts as a nucleotide analog that selectively inhibits the viral RNA-dependent RNA polymerase or causes lethal mutagenesis upon incorporation into the virus RNA. Thus, besides influenza virus, favipiravir has shown potent antiviral activity against other segmented negative-strand RNA viruses such as arena- and bunyaviruses both *in vitro* and *in vivo*.

Following this rationale, we tested the therapeutic effect of favipiravir against Lassa fever (LASF), a severe febrile disease endemic to west African countries whose ethiologic agent is Lassa virus, a segmented, single stranded RNA virus. To model the disease in laboratory mice, we utilized recently generated immunocompetent models susceptible to the disease, in which stromal cells were $IFNAR^{-/-}$ and the hematopoietic compartment was fully immunocompetent.

Oral administration of favipiravir at 300 mg/kg after symptom onset (day 4 post-infection) rescued 100% of infected mice, and resolved viremia in 48 h. Furthermore, half dose of favipiravir was sufficient to successfully rescue mice from LASF when administered in combination with Ribavirin, the drug of choice for current treatment of LASV. Our results point out to favipiravir as a highly promising candidate for treatment of LASF, and indicate a synergistic effect between favipiravir and ribavirin.

Disclosure of Interest: None declared

Poster 270

IN SEARCH OF NOVEL METAL-CHELATING INFLUENZA VIRUS PA INHIBITORS: DIVERSE SCAFFOLDS AND EVEN MORE DIVERSE MECHANISTIC PROFILES

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Abstract:

The influenza virus PA endonuclease, which cleaves capped cellular pre-mRNAs to prime viral mRNA synthesis, is a promising target for novel anti-influenza virus therapeutics. The catalytic centre resides in the N-terminal part of PA (PA-Nter) and contains two (or possibly one or three) Mg^{2+} or Mn^{2+} ions which are critical for its catalytic function. The flexible PA active site can be targeted by compounds with metal-chelating scaffolds.

We designed new molecules from diverse chemical classes (i.e. β-diketo acid derivatives, 2-hydroxybenzamides, thiosemicarbazones, N-acylhydrazones and dihydroxyindole-2-carboxamides) and found several to cause strong inhibition of influenza PA-Nter in enzymatic assays. Our SAR analysis provided a relevant starting point for development of more potent and selective PA inhibitors (PAIs).

In addition, we demonstrated that the setup of the enzymatic assay (i.e. substrate, metal cofactor and type of readout) should be carefully chosen during PAI evaluation. Whereas most enzymatic studies with isolated PA-Nter have indicated that the enzyme is considerably more active in the presence of Mn^{2+} compared to Mg^{2+}, our novel molecular beacon-based assay works equally well with Mg^{2+} as with Mn^{2+}. Since the intracellular concentration of free Mg^{2+} is at least 1000-fold higher than that of Mn^{2+}, magnesium may be more biologically relevant, and evaluation of potential PAIs against both metals (as possible with our molecular beacon assay) seems recommended.

Some of the metal-chelating compounds displayed anti-influenza virus activity combined with favourable selectivity in cellular assays. However, although conceived as PAIs, for most of the inhibitors, the antiviral target in cell culture seems unrelated to PA, but rather associated with an early (virus entry) or late (maturation or release) event in the influenza virus life cycle. Clarification of the precise mode of action of these early lead compounds is currently ongoing.
Disclosure of Interest: None declared

Poster 271
PROTECTION OF POULTRY WITH MATERNALLY DERIVED ANTI-NDV ANTIBODIES AGAINST HIGHLY PATHOGENIC AVIAN INFLUENZA AND NEWCASTLE DISEASE
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Abstract: In the presence of antibodies against Newcastle Disease virus (NDV) due to vaccination or natural infection, the efficacy of NDV as a vaccine vector may be impaired in commercial poultry. A chimeric NDV with avian paramyxovirus 8 (APMV-8) surface proteins, expressing the hemagglutinin H5 (HA) of highly pathogenic avian influenza virus (HPAIV) (chNDVFHN$_{PMV8}$H5), overcomes this problem and protects chickens against HPAI also in the face of maternally derived α-NDV antibodies (αNDV-MDA+). However, the efficacy of vaccination with this virus in other important poultry species like turkeys is unknown. Therefore, commercial turkeys were vaccinated with chNDVFHN$_{PMV8}$H5 on day one after hatch, which was well tolerated by the animals. Although antibodies against HPAIV were not detected by HI assay three weeks after immunization, 70 % of vaccinated turkeys were protected while non-vaccinated controls showed high morbidity and mortality. These results indicate the potential of chNDVFHN$_{PMV8}$H5 as a vaccine virus also in other poultry species.
However, due to the absence of the major NDV immunogens F and HN, chNDVFHN$_{PMV8}$H5 fails to protect against ND, one of the most important infectious diseases of poultry. To achieve protection against HPAI and ND, we investigated an immunization pattern for αNDV-MDA+ chickens by vaccination with chNDVFHN$_{PMV8}$H5 and subsequent immunization with live attenuated NDV. Both vaccinations were well tolerated and induced immune responses. Three weeks after immunization, challenge infections with velogenic NDV and HPAIV H5N1 were carried out, resulting in death of non-vaccinated animals while vaccinated chickens survived. To summarize, a vaccination with chNDVFHN$_{PMV8}$H5 can be combined with NDV immunization to achieve protection of αNDV-MDA+ chickens from HPAI as well as ND, indicating that the antibody response against chNDVFHN$_{PMV8}$H5 does not interfere with subsequent live ND vaccination.
Disclosure of Interest: None declared

Poster 272
GENERATION OF A RECOMBINANT TRI-SEGMENTED MOPEIA VIRUS FOR ANTIVIRAL SCREENING
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Abstract: Arenaviridae is a family of viruses that can infect rodents as well as humans. Several arenaviruses can cause severe viral hemorrhagic fever (VHF) in humans and thus are of relevance for public health. Lassa virus (LASV) is endemic in West Africa and causes VHF. The LASV-related but nonpathogenic Mopeia virus (MPOV) may be used as a model to study the arenavirus life cycle and to test antivirals. The pathogenesis of the disease caused by arenaviruses is not well understood and treatment options are limited. Currently, only the broad-spectrum antiviral drug ribavirin has shown some therapeutic effect if given early after onset of illness. To facilitate medium-throughput screening for antivirals against arenaviruses, we established a recombinant MOPV system with tri-segmented antigenomes (r3MOPV): 1 L segment and 2 S segments expressing two reporter genes. The r3MOPV-based approach allows testing of antivirals in a biosafety level 2 setting. As a proof of principle, reporter gene expression was quantified under treatment with the antivirals ribavirin and favipiravir (T-705). As

expected, both compounds reduced reporter gene expression demonstrating inhibition of virus growth. The r3MOPV is an excellent tool to screen for inhibitors of the complete arenavirus life cycle *in vitro*.
Disclosure of Interest: None declared

Poster 273
EVALUATION OF IMMUNOGENICITY IN NON-HUMAN PRIMATES ELICITED BY MEASLES VECTORED HPV L1 VACCINE VERSUS ALUM ADJUVANTED RECOMBINANT PROTEIN VACCINE
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Abstract: Human papilloma viruses (HPVs) are the primary etiologic agent of cervical carcinoma; every year approximately half a million new cervical cancer cases are registered worldwide, particularly in developing countries where the access to screening programs is prohibitive due to high costs. The development of prophylactic HPV vaccines represent an important opportunity to prevent cervical cancer whilst a therapeutic immunization would be valuable in treating pre-malignant and malignant disease. At present, first-generation of commercial HPV vaccines composed of L1 virus-like particles (VLPs) are expensive to produce and deliver.
Live attenuated Measles virus (MV) vaccines have a well-established safety and efficacy record and are inexpensively delivered to the majority of developing countries. Furthermore, recombinant MV viruses produced by *reverse genetics* represent an attractive platform to generate candidates HPV vaccines to meet the needs of the developing world, due to low cost production and highly efficiency, safety and stability.
Here we present immunogenicity data of a combined vaccine based on recombinant MV expressing HPV antigens. Specifically, live attenuated Edmoston Zagreb strain of MV is used as viral vector to carry heterologous gene encoding the major capsid protein L1 of HPV type 16 and HPV type 18. Rescued recombinant attenuated MVEZ-HPV viruses, produced at high titer in MRC5 cell line, were used first to immunize transgenic CD46IFNAR mice susceptible to MV infection, and then immunogenicity was assessed in Rhesus monkey. Specific neutralizing antibodies against HPV were assayed in comparison with alum adjuvanted recombinant protein vaccine produced in *Pichia pastoris* KM71 strain.
Disclosure of Interest: None declared

Poster 274
STABILITY CHARACTERIZATION OF A FUSION GLYCOPROTEIN VACCINE FOR RESPIRATORY SYNCYTIAL VIRUS
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Abstract: Respiratory Syncytial Virus (RSV) infection is the leading cause of lower respiratory tract infection in both young children and older adults (>65 years). Currently, there is no vaccine available, and therapeutic options are limited. The infectious RSV particle is decorated with a type I viral fusion (F) glycoprotein that structurally rearranges from a metastable prefusion form to a highly stable postfusion form. In people naturally infected with RSV, the neutralizing antibodies primarily recognize the prefusion conformation. Therefore, we are evaluating a subunit RSV vaccine comprised of an engineered RSV F protein stabilized in its prefusion conformation (DS-Cav1, McLellan *et al.* 2013).
Long term stability at 4°C or higher is a desirable attribute for a RSV F subunit vaccine antigen. Accordingly, we evaluated the long-term stability of DS-Cav1 with a variety of biophysical and immunological assays. Our data suggested that DS-Cav1 undergoes conformational changes and forms intermediate structures upon long-term storage at 4°C. In addition, 4°C stored DS-Cav1 appears to lose binding to a prefusion-specific monoclonal antibody, D25, and gain the ability to bind an in-house generated antibody, 4D7, that recognizes the postfusion but not the prefusion form of F protein. Epitope

mapping was performed to determine the binding site of this stability-indicating antibody 4D7 and it appears to bind antigenic site I.
Structure-based design was performed to improve the stability of the RSV F subunit vaccine. We have identified additional mutations that stabilize RSV F protein in its prefusion conformation. We will combine the best stabilizing mutations with DS-Cav1 and evaluate if they confer improved long-term stability.
Disclosure of Interest: None declared

Poster 275
IMPROVING VIROLOGIC SURVEILLANCE FOR MEASLES
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Abstract: The World Health Organization (WHO) African Region has set a measles elimination goal by 2020. To achieve this goal, case-based surveillance, including laboratory confirmation of suspected cases and genotyping of circulating measles strains, needs to be widely implemented. In some settings, the main obstacle to virologic surveillance is the need for specimen storage and transportation via reverse cold chain. This study compared two methods for viral specimen storage and transport, i.e., the application of throat swab collections on Whatman FTA® cards versus a standard method of placing throat swabs in viral transport media. Specimens collected using the standard protocols were transported at 4-8°C, while samples spotted onto FTA cards were transported at ambient temperature. All specimens were collected in the Democratic Republic of Congo (DRC) in 2014. From each patient, 2 throat swabs, 2 oral fluid swabs, and a serum were collected. One throat swab and one oral fluid swab specimen was transferred onto an FTA card at the collection site. The FTA card was air dried and shipped to the national laboratory at ambient temperature. The other throat and oral fluid swabs were placed in viral transport media and shipped to the national laboratory on wet ice. Serum samples were tested for measles IgM at the DRC national laboratory. All throat and oral fluid specimens were transported to the CDC, where they were tested by quantitative real time RT-PCR to detect measles RNA. Of 212 patient samples, 146 (69%) were IgM positive for measles. For the RT-PCR, 165 (78%) TS and 170 (80%) OF samples were positive, while only 140 (66%) FTA TS and 150 (71%) FTA OF samples were positive. Samples collected by both methods were successfully genotyped. Though the detection rate was higher for the standard method, the results indicate that FTA cards can be used to transport samples for virologic detection when access to the reverse cold chain is a challenge.
Disclosure of Interest: None declared

Poster 276
MMR VACCINATION IN HUMANS ELICITS A CROSS-NEUTRALIZING RESPONSE AGAINST A NOVEL MUMPS-LIKE VIRUS SEQUENCED FROM AFRICAN BATS
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Abstract: A mumps-like virus in African bats (afMuV) that is conspecific with human mumps virus (huMuV) (≥90% aa similarity across all genes) was recently identified (Drexler et al., 2012). The discovery of a possible mumps animal reservoir has implications for vaccination strategies and virus eradication, as elimination of circulating virus and subsequent cessation of vaccination might leave humans susceptible to disease from such a reservoir-borne virus. The genetic proximity of afMuV and huMuV raises the spectre of zoonotic spillovers. Thus, it is critical for global health efforts to know whether antibodies elicited by standard MuV vaccination have cross-neutralizing activity against afMuV. Remarkably, there are no international criteria for determining the level of protective neutralizing antibody responses against MuV. FDA- and EMA-approved tests for monitoring MMR vaccination only

assay for MuV-*reactive* titers, and often only for antibodies reactive against the N protein instead of the envelope F/HN glycoproteins that are more relevant to virus neutralization activity.
In order to evaluate whether the MMR vaccination in humans elicits cross-neutralizing antibody responses against afMuV, we cloned and rescued a recombinant GFP-reporter MuV vaccine strain (JL5) bearing the genes encoding F and HN of the afMuV in the homologous open reading frames. This chimeric JL5-afMuV-F/HN virus exhibited a hyperfusogenic phenotype compared with the parental JL5 virus, but grew to similar titers in Vero and DF-1 cells. Seroneutralization assays using a curated panel of human sera from donors in the U.S. showed that the majority of sera that were able to neutralize huMuV infection also had cross-neutralizing activity against the chimeric JL5-afMuV-F/HN (Mean IC_{50}, 1:250-1:500). Our data suggests that humans carrying neutralizing antibodies against huMuV might be protected from infection by afMuV. However, the extent and duration of MuV-specific MMR responses remain undefined.
Disclosure of Interest: None declared

Poster 287
COMMUNITY-WIDE SEQUENCE ANNOTATION STANDARDS TO IMPROVE USABILITY OF GENOMIC SEQUENCES OBTAINED DURING THE 2013-2015 EBOLA VIRUS DISEASE OUTBREAK
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Abstract: Genome sequence-based molecular epidemiology is being applied for the first time in filovirology during the current Ebola virus disease outbreak. In 2014, Gire *et al*. deposited 99 coding-complete Ebola virus genomes—effectively doubling the number of genome sequences available for all filovirus disease outbreaks since 1967 combined. It is expected that hundreds of additional genome sequences will be determined in the very near future. This wealth of data poses a formidable challenge for bioinformaticists, public-health officials, and the scientific community in general. Sequence data can provide information on the potential efficacy of antiviral drugs, such as antisense compounds or antibodies, but community-wide standards are critical to the usability of sequence data for efficacy prediction.
Here we report the progress of a working group tasked with developing such standards, beginning with the establishment of consistent filovirus nomenclatures employing experts of the ICTV and progressing to ongoing filovirus genome annotation standards with the support of NCBI, UniProt, Broad Institute, J. Craig Venter Institute, and USAMRIID. The formation of this working group not only facilitates solutions to long standing standardization issues within the field but also provides a platform readymade infrastructure to address emerging issues. For instance, in response to the initial submission of Ebola virus Makona sequences in 2014, NCBI rapidly constructed a value-added ebolavirus resource (www.ncbi.nlm.nih.gov/genome/viruses/ variation/ebola) that includes a specialized database. For this resource, reference sequences previously selected by the working group are used to guide annotation of all ebolavirus sequences, and metadata describing sequences are mapped to standardized terms. The result is a user-friendly interface that supports retrieval of ebolavirus sequences based on a variety of biological criteria.
Disclosure of Interest: None declared

Poster 288
DOES HOST EVOLUTION LIMIT THE DISTRIBUTION OF CENTRAL EUROPEAN PUUMALA VIRUS?
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Abstract: *Puumala virus* (PUUV) is a hantavirus that may cause a mild to moderate form of hemorrhagic fever with renal syndrome in humans. Its reservoir host, the bank vole (*Myodes glareolus*), is a widely distributed rodent species in Europe. In Germany simultaneous PUUV outbreaks have been described, mainly affecting regions in the western and southern part of the country. In contrast, only a very low number of human cases has been recorded in the northeastern part of Germany.
The objective of our ongoing studies is to find out potential reasons for the inhomogeneous distribution of PUUV infections in Central Europe. Our hypothesis is that the presence of different evolutionary lineages of the rodent host and their susceptibility for PUUV infection represents a major reason.
For this purpose, bank voles from relevant regions of Germany and the neighboring parts of Poland were tested for PUUV infection. Serological and molecular analyses demonstrated a usually medium to high PUUV prevalence in endemic areas. In contrast, bank voles from various sites in the northeastern part of Germany were seronegative. Similarly, the investigations showed an absence or low prevalence of PUUV in the Polish bank vole populations. Initial *cytochrome b* analyses of bank voles suggested the presence of the Eastern and Carpathian evolutionary lineages at the sites in Poland and the Northeast of Germany, but the Western evolutionary lineage in the endemic regions.
Future investigations will have to prove if the different genetic lineages of the bank vole differ in susceptibility to PUUV and might be a cause of the inhomogeneous distribution of this hantavirus in Central Europe.
Disclosure of Interest: None declared

Poster 289

ANTIGENIC AND GENETIC VARIATION OF H5 HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUSES IN DOMESTIC DUCKS IN VIETNAM

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Abstract: Since the emergence of H5N1 highly pathogenic avian influenza (HPAI) viruses in Asian countries, numerous efforts have been made worldwide to control outbreaks and eradicate the virus. The number of outbreaks has decreased, however, incidences of H5 virus infections among poultry have been reported sporadically in Vietnam. It has been suggested that HPAI viruses show moderate pathogenicity to domestic ducks while high pathogenicity to chickens. Therefore, domestic ducks are suggested as a potential source for HPAI viruses, playing a role in dissemination of viruses to environment. In the present study, virological surveillance has been carried out to elucidate constellation of avian influenza virus among domestic ducks in wet markets and duck farms in Vietnam.

A total of 1,000 and 1,260 swab samples (throat and cloacal) has been obtained from apparently healthy domestic ducks on farms in Nam Dinh province and on wet markets in Hanoi, Quang Ninh, Nha Trang and Long An provinces, and applied for virus isolation since September 2011. As of March 2015, total 329 influenza A viruses had been isolated (29 H3N2, 10 H3N6, 54 H3N8, 15 H4N6, 1 H4N9, 59 H5N1, 1 H5N2, 13 H5N6, 1 H5N8, 8 H6N2, 86 H6N6, 6 H6N8, 34 H9N2, 4 H9N6, 3 H10N2, and 5 H11N9). Phylogenetic analyses revealed that HA genes of almost H5 virus isolates were classified into clade 2.3.2.1. On the other hand, some H5 viruses isolated in 2013 in Quang Ninh province, located in border area with China, had HA genes belonged to clade 2.3.4.4, showing high homology with latest HPAI viruses in East Asian and North American countries. Subsequently, such virus has been also observed in Hanoi and Nha Trang provinces since 2014. Neutralizing test elucidated that antigenicity of clade 2.3.4.4 virus isolates was different from clade 2.3.2.1 viruses. These results suggested that novel H5 HPAI viruses are becoming major in domestic duck populations in Vietnam, and antigenic variation progressively occurs.
Disclosure of Interest: None declared

Poster 290

UTILITY OF ILLUMINA MISEQ WHOLE GENOME SEQUENCING FOR THE MOLECULAR EPIDEMIOLOGY OF MEASLES VIRUS

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Abstract: All six regions of the World Health Organization have adopted the goal of eliminating endemic measles. The genetic characterization of measles viruses is an important tool for measles surveillance as it helps to document chains of transmission, discriminate between imported or indigenous viruses, and monitor progress toward elimination. Lack of an endemic genotype is one of three essential criteria needed to verify measles elimination. Measles virus is divided into 24 genotypes based on sequence variations in the 450 nucleotides coding for the carboxyl terminal 150 amino acids of the nucleoprotein (N450). However, the resolution provided by N450 is not always sufficient to distinguish between continued, endemic circulation of the same viral lineage within a genotype and repeated importations of the same lineage. Sequencing the whole genome of measles virus provides maximum resolution, but is technically challenging. Sanger sequencing of DNA amplicons covering the highly variable, almost 16 kb RNA genome is time and labor-intensive, requires many sequence-specific primers, and is generally only feasible for virus isolates. Multiplexed Illumina MiSeq sequencing of measles genomes from virus isolates reduces the time to generate sequence information without reliance on sequence-specific primers. The applicability of Illumina sequencing for patient samples was examined. Samples of genotype B3 from recent outbreaks in the USA and from an outbreak in Ecuador in 2011-2012 showed near identity (0-1 nt difference) in N450 while the full genomes differed significantly (7-62 nt differences), demonstrating the potential utility of high-throughput sequencing for measles molecular epidemiology.

Disclosure of Interest: None declared

Poster 291

MOLECULAR EPIDEMIOLOGY OF MEASLES AND MUMPS VIRUSES DETECTED IN THE US DURING 2014

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Abstract: Genetic characterization of wild-type measles viruses can help to map the transmission pathways of the virus and is currently the only method available to confirm measles vaccine reactions. In the US, measles and mumps genotyping is performed at CDC, or at one of four Vaccine Preventable Disease Reference Laboratories located California, Wisconsin, New York, and Minnesota. In addition to genotype, sequences may be assigned to a specific named lineage which represent at least 50 identical sequences reported within the last 12 months. In 2014, the US reported 640* cases of measles from 23 outbreaks in 18 states. Genotypes were determined for 164 cases, which included representative viruses from all of the outbreaks. The majority of the cases were the result of importation of measles virus from the Philippines. Most of the viruses imported from the Philippines were genotype B3 of the Harare lineage (MVi/Harare.ZWE/38.09/). However, a second genotype, D9, was associated with a >300 case outbreak which began when unvaccinated individuals returned to Ohio after visiting the Philippines. At least 4 lineages of genotype D8 were detected in the US in 2014 including representatives of 2 named lineages, MVs/FrankfurtMain.DEU/17.11/ and MVs/Taunton.GBR/27.12/, which were frequently associated with European measles cases. Overall, genotype B3 was detected in 107 cases, genotype D8 in 18 cases, genotype D9 in 34 cases, and genotype H1 in 5 cases.
In contrast to measles, the genetic characterization of mumps viruses has not been established on a global scale, but genetic analysis has been used to trace transmission pathways and to identify vaccine strains. Genotypes of mumps virus are determined by sequencing of the gene coding for the small hydrophobic protein (SH). In 2014 there were 1151* cases of mumps reported in the US. The majority of the genotypes reported (165/170, 97%) were genotype G, but genotypes, C, D, H and K were also detected.
*provisional data

Disclosure of Interest: None declared

Poster 292

RAPID AND SENSITIVE DETECTION OF BAT INFLUENZA VIRUSES BY REAL TIME RT-PCR ASSAYS

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Abstract: Influenza (flu) viruses are important human and livestock pathogens and new reassortants of zoonotic origin have potential to cause pandemics. Aquatic birds harbor diverse flu A viruses and are recognized as major flu virus reservoirs in nature. However the recent discovery of flu viruses of a new H17N10 subtype in Guatemalan fruit bats and a new H18N11 subtype in Peruvian fruit bats, along with preliminary seroprevalence studies suggest that New World bats may carry divergent flu viruses and could be an unrecognized reservoir in nature. To understand how flu viruses are maintained in bat populations, systematic and global prevalence studies using more sensitive and efficient screening methods are needed. In this study, we developed two real time RT-PCRs targeting conserved regions within the NS and M gene segments of known bat flu viruses to enable sensitive and efficient detection from bat clinical samples. These assays are simple, rapid and at least 4X more sensitive for detection of bat flu viruses compared to the generic pan-flu PCR used previously. These assays were used to screen collections of bat swabs previously screened. A total of 803 rectal and 95 oral swabs from Guatemala (2009-2011) and Peru (2010) were tested by generic pan-flu PCR and rescreened with the bat flu M and NS real time RT-PCRs. In addition to the previously tested flu-positive bat samples, one additional rectal swab sample (Carollia perspicillata) from Guatemala (2010) was positive for bat flu virus by real time PCRs, but missed by generic flu PCR. Full genome sequencing was performed by Sanger and NGS methods. Phylogenetic analysis showed that this latest bat flu virus is more closely related to H18N11 (82.5%>96.3% nt identity to 8 orf segments of A/bat/Peru/10) rather than to H17N10 (53.5%>81.0% nt identity to 8 orf segments of A/bat/Guat/09). These new assays provide a rapid and sensitive tool to screen bat populations to better understand the ecology and evolution of bat flu viruses.
Disclosure of Interest: None declared

Poster 293

WITHIN HOST EVOLUTION AND CROSS-SPECIES TRANSMISSION OF EQUINE INFLUENZA VIRUS NON-STRUCTURAL PROTEIN NS1

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Abstract: Equine H3N8 influenza virus (EIV) emerged in 1963, causing a pandemic in horses. The virus is thought to be of avian origin, continues to circulate in horses and has undergone further cross-species transmission into dogs. Sequence analysis of the NS1 gene from a panel of virus isolates revealed variation between the avian-like prototype of H3N8 EIV, isolates from the 1980s-90s, the current Florida sublineage and canine H3N8 viruses, suggesting host-related adaptation. In particular, variation was observed at the C-terminus of NS1 with the terminal four amino acids changing from 1963 through to the 1990s, whilst NS1 of the Florida sublineage has been truncated by 11 amino acids. Interestingly, the NS1 of canine H3N8 was also truncated in early isolates, but more recent strains have full length NS1 with a novel C-terminus. The C-terminus of influenza NS1 has previously been shown to differ between avian and human isolates affecting virus replication and localisation of NS1. Here we demonstrate the effect of altering the C-terminal sequences of EIV NS1 on localisation properties of the NS1s in cells of avian, equine, canine and primate origin.
Disclosure of Interest: None declared

Poster 294

MOLECULAR EPIDEMIOLOGY AND GENETIC VARIABILITY OF HUMAN RESPIRATORY SYNCYTIAL VIRUS (HRSV) IN CROATIA, 2011-2014

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Abstract: Human respiratory syncytial virus (HRSV) is a paramyxovirus that causes common respiratory tract infections in infants, young children and among the elderly. The disease manifestation ranges from mild nonspecific respiratory symptoms to severe illness, such as bronchiolitis or pneumonia. By the age of 2 years, virtually all children have been infected at least once with HRSV. Reinfections are very common, even within the same epidemic season, due to very limited immune protection from earlier exposures.
HRSV strains are classified into two antigenic groups, A and B, representing separate genetic lineages. The molecular epidemiology of HRSV is rather complex as numerous genotypes exist, new genotypes emerge and some previously circulating genotypes appear to became extinct. To date, 15 HRSV A and 23 HRSV B genotypes have been established. Genotyping is based on sequences of the second hypervariable region of glycoprotein gene, located at the protein's C-terminus. Although this region accounts for just approx. 2% of the whole HRSV genome, it has been identified as the most reliable to describe the evolutionary changes of HRSV.
Molecular characterization of HRSV detected in clinical samples of severely ill patients hospitalized in Croatia in 2011-2014, revealed strong predominance of HRSV A strains (genotypes NA1 and ON1). Among group B strains, we detected strains belonging to genotypes BA9 and BA10. Especially broad genetic diversity, was detected among concurrently circulating NA1 strains, indicating high genetic plasticity of this globally dominant genotype.
Disclosure of Interest: None declared

Poster 295
EVOLUTIONAL ANALYSIS OF A RECOMBINANT SENDAI VIRUS EXPRESSING THE HUMAN MAVS PROTEIN
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Abstract: There is a constant battle between viruses and their hosts during their coevolution. For RNA viruses, the high mutation rate of the viral polymerase generates a cloud of potentially beneficial mutations which allows the viral quasispecies to evolve and adapt to new environments.
Using reverse genetic, a recombinant Sendai virus expressing the human MAVS protein, was constructed to follow its evolution under different conditions. The mitochondrial antiviral-signalling protein (MAVS) plays an essential role in the innate immune response, downstream of RIG-I and MDA5 and therefore represents an actor negatively impacting on virus multiplication.
This recombinant Sendai virus was passaged several times either in chicken embryonated eggs (interferon incompetent) or on interferon competent cell lines. The viral stocks obtained were tested for their ability to induce interferon. The results show that there is a decrease in interferon induction following passages, especially when done in interferon competent cells. INF induction was directly related to MAVS expression. Difference in INF induction was neither due to differences in viral replication, nor to quantitative differences in MAVS expression. However, a shorter form of MAVS appeared predominately in cells infected with late passaged viruses originating from interferon competent cells.
These results suggest an accumulation of mutations leading to the inactivation of MAVS. The next step will be the identification and the characterization of these mutations.

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Poster 296
MOLECULAR EPIDEMIOLOGY OF INFLUENZA VIRUSES IN SHOREBIRD IN CHILE.
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Abstract: Avian influenza viruses (AIV) are associated zoonotic events that can produce animal and human disease, and potentially of generate epidemics and occasionally pandemics in humans. The main reservoir for AIVs are wild birds. Chile has unique geographical barriers, and its governmental policies have allowed the country to be free from different animal pathogens. However, migratory birds can travel great distances, trespassing these barriers and might introduce pathogens such as AIV. In 2002 Chile had an outbreak with an H7N3 AIV that became highly pathogenic, and recent reports have confirmed the circulation of AIV in wild birds in South America, suggesting that genetic movement of these viruses might occur locally. To address this, through ongoing surveillance studies, we collected 540 fecal samples from shorebirds in two different locations in the Central Coast region of Chile, during spring and summer (2014-2015). Of these 25% of the samples were positive for AIV by Matrix qRT-PCR. Samples yielding low CT values were amplified directly and sequenced with the Illumina platform. We obtained complete genome sequences and isolated viruses from three Franklyn gulls, corresponding to the H13N2 subtype. These viruses clustered closely with a gull H13N2 AIV found in Chile in 2007, suggesting these AIVs might be endogenous from Chile. This provides information of potential ecological niches that contribute to the transmission and persistence of influenza viruses in wild birds in the region, and emphasizes the need of conducting and improving systematic AIV surveillance studies in Chile.
Disclosure of Interest: None declared

Poster 297
MOLECULAR CHARACTERIZATION OF EBOLA ZAÏRE ADAPTATION TO THE SYRIAN GOLDEN HAMSTER
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Abstract: Ebola virus (EBOV) causes severe hemorrhagic fever in human and non-human primates and belongs to the family Filoviridae, a group of enveloped, non-segmented, negative-strand-RNA viruses. Both a better understanding of viral pathogenesis and testing of therapeutics against EBOV infection require the use of an appropriate animal model. In this study in order to establish a small animal model for EBOV, we performed serial passaging of EBOV in Syrian golden hamsters (mesocricetus auratus). After 8 consecutive passages, the virus revealed a decrease in the mean time of death, an increase in lethality and also manifested by the appearance of skin haemorrhagy symptoms in approximately 25% of cases, in addition to gastrointestinal haemorrhages. The petechial haemorrhage correlated well with the areas of accumulation of red blood cells in the dermis. Histological analysis of tissues from the animals showed massive virus replication in the liver, pronounced lymphoid necrosis in the spleen and signs of apoptosis. Deep sequence characterization of the viral population from the last virus passage revealed the appearance of mutations in several viral genes including VP24, L and GP. While the same mutations in VP24 and GP genes were previously observed in mouse-adapted EBOV, mutations in the NP gene were not found in our hamster-adapted virus. The role and significance of individual mutations will be discussed in connection with the virus adaptation to a new animal host.
Disclosure of Interest: None declared

Poster 298
DIVERSITY OF SINGLE NUCLEOTIDE VARIANTS AND RNA EDITING OF PLUS AND MINUS RNA STRANDS FROM MEASLES VIRUS.
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Abstract: We optimized a Next Generation Sequencing (NGS) procedure to enable the analysis of the genome-scale genetic diversity, including single nucleotide variant (SNV), insertion and deletion (INDEL) events. Threshold levels and validity for SNV and INDEL were defined from sequencing data of a homogenous DNA plasmid source. To separately sequence plus and minus strands, strand specific reverse transcriptions were set up and validated. Rare INDELs corresponding to polyadenylation signals, P-V RNA edition and/or elongation of short homogenous nucleotide stretches, could be detected in both minus and plus strand, although occurring at a higher frequency in the later. This suggests that in addition to mRNA polyadenylation and edition of viral P transcripts to give rise mostly to the V mRNA, the polymerase may also sporadically stutter during the replication. Such a low level of stuttering may explain the onset of rare MeV genomes elongated by 6 nt with a repeat of a homogenous nucleotide stretch. Analysis of SNV distribution did not reveal a strand bias and different measles virus strains usually display a few tens of variable SNV pattern, mostly silent or in non-coding regions with a frequency rising up to 20-50% in very few positions. This SNV pattern remains usually stable after up to ten passages in the same cell host. Altogether our data further illustrate the remarkable stability of the genome of measles virus. **Disclosure of Interest**: None declared