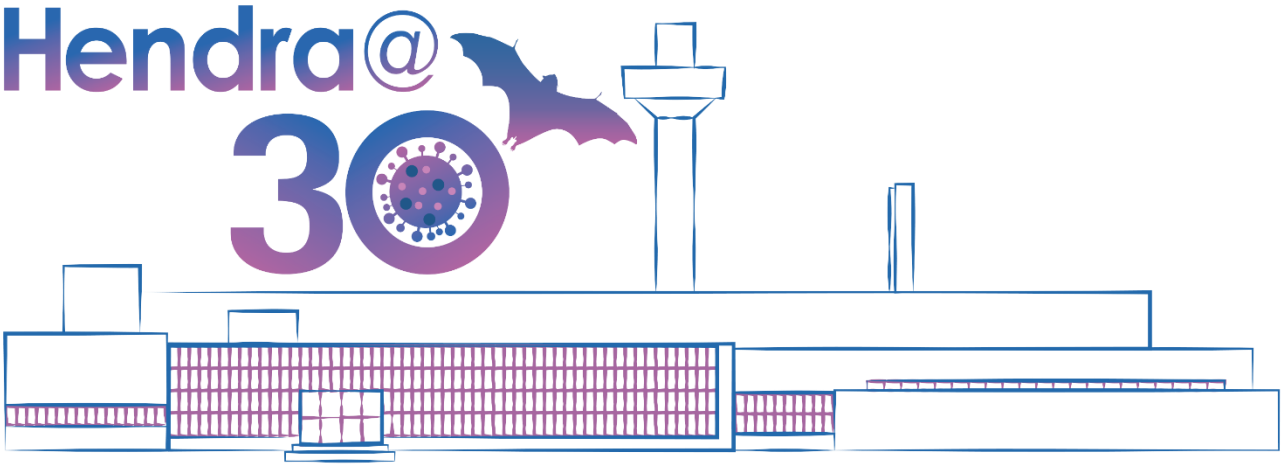

Proceedings from Hendra@30

Henipavirus International Conference

8th – 11th

December 2024

Geelong

Victoria, Australia

CEPI

CSIRO

Welcome to Hendra@30 – Monday 9th December

The Conference was inaugurated with the Traditional Owners, the Wadawurrung people of the Kulin Nation, welcoming delegates to Australia and to the Waterfront location with a traditional smoking ceremony. This was followed by a formal welcome by CSIRO and CEPI, the co-sponsors of Hendra@30. From CSIRO, Brett Sutton described the conference as a "Call to arms" in which it was important to bring a broader set of disciplines to the collective thinking. He highlighted that the first BSL4 vaccine has not yet been taken up to the full extent, which poses the question: How do we make sure whatever innovations come in the future are adopted? Also from CSIRO, Debbie Eagles described Hendra as the pathogen that made the first big changes to personal and work safety measures (BSL4) in the veterinary field. Finally, Amy Shurtleff, from CEPI, presented CEPI's mission and vision, its vaccine portfolio, and what is needed for the 100-day mission.

History of Hendra virus

Chair: Kim Halpin
Monday 9th December, 10 AM – 11 AM

Beth Cookson "Reflections from the CVO"

Beth Cookson shared her ***Reflections as the Chief Veterinary Officer in Australia***. She made reference to the deep expertise in the room, focussing on the reflections and lessons learnt from the first Hendra outbreak and the influence this had on subsequent policy and practices. An important shift in the landscape has been the One Health approach. This is significant, considering that 30 years ago we didn't know about the various bat-borne viruses. The One Health approach has brought together cross-disciplinary think tanks, such as the one formed by Peter Black, Ian Douglas and Hume Field in 2015. Beth also reflected on human and government attitudes towards bats, citing a special badge she received from the Singapore government to help teach and to promote the role of bats as pollinators. In spite of the strides in the One Health approach, the threat of misinformation continues. For example, due to the fears and perceived risk of Hendra virus, some countries are still requiring seronegative results from various animals prior to export. Lastly, Beth reflected on how field epidemiology has become more relevant across disciplines, shifting from reactive to proactive and to preventative.

Peter Reid "The original Hendra virus outbreak in horses"

Peter Reid was the vet involved with the first outbreak in 1994. Here he recounted his experiences. In 1994, a mare presented with unusual symptoms, ultimately leading to the cancellation of racing and causing $6 million losses to the racing industry. The horses, loved by their owners, showed signs of deterioration. Clinical signs included not eating, being depressed and unstable, some swelling around the eyes and head. There was rapid deterioration, and death. The horses that recovered only had mild signs. Blood samples were taken to a private vet lab in Queensland, and the cause was explored. Possible causes included African horse sickness, although at the wrong time of year (no midges), or tetanus toxoid. Queensland police were involved due to

poisoning scares. A Morton Bay fig tree was observed in the paddock at Cannon Hill. Samples were sent to AAHL, and a new novel virus was discovered.

Paul Selleck "Hendra virus: the laboratory response"

Abstract O-01

In September 1994, the Australian Animal Health Laboratory (AAHL) in Geelong was alerted to a possible exotic disease outbreak in horses in Hendra, a Brisbane suburb. When samples arrived, rapid antigen detection tests for African horse sickness and equine influenza were performed and were negative. Tissues from the horses were inoculated on to cell cultures and CPE (cytopathic effect) was first seen on day 3 post-inoculation. At first, the CPE was thought to be caused by an equine herpes virus; however, electron microscopy showed the virus to be a novel paramyxovirus. Naive horses were infected with the new virus and Kock's postulates fulfilled, thus identifying the new virus as the causative agent of the Hendra outbreak. Diagnostic tests for the new virus were developed and used to prove freedom from infection with the virus in the horse and human populations. A second outbreak occurred in Mackay in August 1995, leading to further investigation and identification of flying foxes as the host species. Subsequent serological testing showed that the person infected in the Mackay outbreak was infected prior to the September 1994 outbreak at Hendra.

Linfa Wang "Reflecting on HeV research over the last 30 years"

Peter Reid was the first vet see Hendra. Paul Selleck was the first person to see Hendra virus in the laboratory. Linfa was the first person to sequence the genome. They were all key figures in the fight against the Hendra virus. They were the first to isolate the virus and sequence its genome. They also discovered the first known outbreaks of the Nipah virus in 1998/9 and were the first to develop a PC4-based vaccine in 2007. Success often comes from preparedness, and the virus continues to spread. The movie *"The Host"* is set to release in 2025, highlighting the ongoing race for vaccine development.

Personal stories

Chair: Linfa Wang
Monday 9th December, 11.30 AM – 12 PM

Natalie Beohm

Natalie was a 21-year-old working in her dream job as a veterinary nurse, when she became infected with Hendra virus while caring for horses. She was hospitalized for six weeks due to encephalitis, losing her ability to walk and talk. Doctors warned her she would be unlikely to survive. Natalie had never heard of Hendra virus. She continues to be monitored by an infectious diseases' physician with ongoing neurological issues. Natalie's colleague veterinarian Ben Cunneen died from the disease during the outbreak in 2008. Natalie's love for horses is evident. Natalie is left to deal with the emotional impact on her daughter. Natalie had to take antiviral

treatment for a long period of time and still has high levels of antibodies against HeV and so was worried about the impact that would have on her daughter.

Wee Chee Yap

Wee Chee is currently a research assistant on Nipah virus (NiV). She recounted how she was personally affected by NiV during the1998-1999 Malaysian outbreak. She was born and raised in a pig farming village near Nipah. When the outbreak first hit, and when pigs and people became sick with respiratory disease and encephalitis, it was incorrectly assumed it was due to Japanese encephalitis virus (JEV). But epidemiologically, this was a novel virus that killed adults and not children; it was also a virus that was killing primarily the Chinese population involved in pig farming. During the outbreak, Wee Chee and her family had to leave their home. Her father continued to look after the sick pigs following advice of the health authorities that it was okay and subsequently became ill and died. After the outbreak, her family lost their economic sources and had to go to their grandparents' place. When she was in secondary school, she had an interest and looked into the cause of death of her father, and saw it was Nipah virus on the certificate. She felt inspired to prevent this from happening to others, and this was a drive for her scientific journey. This is also why she was presenting at the conference. She ended by reminding scientists that their work has the power to change lives: *"Every discovery, no matter how small, brings us closer to a world where diseases like Nipah encephalitis no longer take away the people we love."*

Disease Ecology Session 1

Chair: Raina Plowright and Belinda Linnegar
Monday 9th December, 12 PM – 1 PM

Raina Plowright "Ecological perspectives on the emergence of Hendra virus"

Co-chair Raina Plowright opened the session with an overview on ***Ecological perspectives on the emergence of Hendra virus*** (HeV) and the hope to build upon the collaboration of Bat One Health. She recounted how fieldwork, done in 2006, revealed that a cyclone had destroyed the habitat for many bats. Bats were feeding on what is described as "starvation avoidance foods." And there was a high amount of viral shedding. Beekeepers could neither find nectar for their bees, nor work out where to put their hives. During this nectar shortage, there were fewer bats; and those that survived were not as healthy and were also shedding virus. Raina further presented some data from her seminal work on pathogen spillover driven by rapid changes in bat ecology.[1] In brief, climate oscillation, food shortage and viral shedding are so closely connected that Bayesian network models can be developed to accurately predict clusters of spillovers in a 25-year period. She described bats, the HeV hosts, as nomadic nectarivores that constantly move to find food, thereby spreading nectar. Her observations indicate that bats currently stay in urban and agricultural areas and do feed on starvation avoidance foods (sub-optimal feeding conditions) because they have lost the habitat where they feed in winter and food is too uncertain otherwise. Bats are making the decision that it is too difficult to be nomadic. However, whenever

[1] https://www.nature.com/articles/s41586-022-05506-2

a remnant patch of winter rainforest flowers, bats leave the agricultural/urban areas and there wasn't any HeV spillover events. Therefore, in an effort to reduce spillover events, she advocates to replant the winter forest for bats to live. Her colleagues have also studied food shortages and spillovers, as well as the diversity of virus being shed.[2],[3] During El Niño and other dry periods resulting in food shortages, her colleagues have observed higher loads and "big pulses" of HeV shedding. The observations suggest that bats live on the "energetic edge" (some bats replace half of their fat stores each night) and mostly shed HeV in times of stress. In this model, bats experience an "allostatic overload," i.e. when energy (food) available in the environment is less than that required, diversion of energy shifts away from immunity, and this results in lower immunity and higher HeV shedding. When asked about if there is spillover of other species, Raina answered that there likely is but they only test for HeV.

Abstract O-02

Thirty years of Hendra Virus Spillover: A synthesis of ecological insights and future directions

Alison J. Peel1, Raina K. Plowright2, Peggy Eby3,4, Hamish McCallum3, Tamika J. Lunn5,6

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Since its emergence in horses and people 30 years ago, Hendra virus has become a model system for One Health investigations into bat viral spillover. Three decades of research have shaped our conceptual understanding of spillover as a multilayered One Health process; encapsulating reservoir host ecology (animal health) through to patterns in host exposure (human health), underpinned by environmental change (environmental health). At this milestone, and in the context of current efforts towards primary pandemic prevention, we critically review the cumulative evidence underpinning current understandings of Hendra virus ecology and spillover. Using the framework presented in Plowright et al. (2017), we examine the evidence supporting currently accepted views and perceptions at each level of the Hendra virus spillover process, from reservoir host ecology through to host exposure. Through extensive review of the published literature, we update the current knowledge on the pathways and multifaceted drivers of Hendra virus spillover, compare expectations and knowledge gaps for a recently identified novel genotype (HeV-g2) relative to the original genotype (HeV-g1), and highlight key future areas to understand the connections between human, animal, and ecosystem health.

Hendra virus, a zoonotic pathogen, has become a model system for One Health investigations into viral spillover from bats. This understanding has evolved over three decades, encompassing reservoir host ecology, human health, and environmental health. The current understanding of

[2] https://www.biorxiv.org/content/10.1101/2023.09.06.556454v1.full

[3] https://www.tandfonline.com/doi/full/10.1080/22221751.2019.1661217

Hendra virus ecology and spillover is critical in the context of primary pandemic prevention efforts.
Using the framework presented in Plowright et al. (2017), the evidence supporting currently accepted views and perceptions at each level of the Hendra virus spillover process, from reservoir host ecology through to host exposure was examined. Pathways to zoonotic spillover must clear all barriers, including reservoir and pathogen release, and pathogen survival which Hendra virus does. However, there is a lack of understanding of how the virus impacts bats. Horizontal transmission is the primary form of transmission between flying foxes. All four flying fox species are reservoir hosts in Australia, with Black and Spectacled flying foxes being the primary reservoir hosts. Grey-headed and little red flying foxes are non-maintenance reservoir hosts.

The review highlights the importance of understanding the various layers of Hendra virus spillover, including barriers, changes across time, and the importance of addressing the lack of evidence on the impact of bat abundance and density. E.g. it is unclear if higher density of bats leads to higher spillover risk. Future research should focus on systematic screening of bats and understanding the interactions between human, animal, and ecosystem health.

Abstract O-03

Hendra virus in Australian bats show a diffuse spatio-temporal structure

Claude Kwe Yinda1, John-Sebastian Eden2, Alison Peel3, Raina Plowright4, Bat One Health Team, Vincent Munster1

1Rocky Mountain Laboratories, Division of Intramural Research, National Institutes of Health Hamilton, Montana, USA, 2Westmead Institute for Medical Research - Centre for Virus Research, Sydney Institute for Infectious Diseases, The University of Sydney, 3Griffith University, Nathan, Queensland, Australia, 4College of Veterinary Medicine, Cornell University, NY, USA.

The Henipavirus genus includes viruses that are both zoonotic and highly pathogenic, including the Hendra virus (HeV) and Nipah virus (NiV). These viruses can cause respiratory distress and fatal encephalitis, with case fatality rates ranging from 40-100%. Despite the availability of an effective vaccine since 2012, equine spillover of HeV has continued in non-vaccinated horses since its discovery in 1994. Spillover of HeV occurs through close association between the natural bat reservoir and horses, and then onward transmission to humans in close contact with the horse. To evaluate the temporal and spatial risks of HeV spillover in eastern subtropical Australia, we sampled five bat roosts monthly for approximately three years and screened 9853 samples for Hendra virus. We then sequenced full genomes of positive samples with higher viral loads using next-generation sequencing techniques. Our data showed that viral RNA could be detected year-round, but high viral loads predominantly occurred in winter (June-September). We recovered forty-eight bat HeV genomes (currently only 15 full genomes are available in GenBank). We classified these, and nine additional horse HeV genomes from other past outbreaks, into four lineages with additional cryptic lineages. We also found that some bat strains are closely related to those causing fatal outbreaks in humans and horses, while others constitute novel lineages. Moreover, we showed that individual roosts have multiple circulating lineages, but with each genetic lineage covering a much larger spatial footprint, probably reminiscent of bat mobility patterns. Our results suggest that bats, which fly long distances and often aggregate in mixed-

species roosts, may maintain a more diverse population of HeV variants than was previously known.

Summary: To evaluate the temporal and spatial risks of HeV spillover in eastern subtropical Australia, researchers sampled five bat roosts (in NSW and Queensland) monthly for approximately three years and screened 9853 samples for Hendra virus. 629 samples were positive for HeV. They then sequenced full genomes of positive samples with higher viral loads using next-generation sequencing techniques. The data showed that viral RNA could be detected year-round, but high viral loads predominantly occurred in winter (June-September). The researchers recovered forty-eight bat HeV genomes and nine additional horse HeV genomes from other past outbreaks, classifying them into four lineages with additional cryptic lineages. Some bat strains are closely related to those causing fatal outbreaks in humans and horses, while others constitute novel lineages. The results suggest that bats, which fly long distances and often aggregate in mixed-species roosts, may maintain a more diverse population of HeV variants than was previously known.

The study found that most of the genomes originated from the (flying fox) hosts, which did not display any signs of disease. The most recent common ancestor estimate is 1975 between the lineages. Virus replication kinetics in bat cells show some differences between clade B and D, suggesting some phenotypic differences between the different clades of HeV.

Abstract O-04

Periodic shifts in viral load increase risk of spillover from bats

Tamika J. Lunn1,2, Benny Borremans3,4, Devin N. Jones5, Maureen K. Kessler6, Adrienne S. Dale7, Claude K. Yinda8, Manuel Ruiz-Aravena9, Caylee A Falvo10, Daniel E. Crowley10, James O. Lloyd-Smith11, Vincent J. Munster8, Peggy Eby12,13, Hamish McCallum12, Peter Hudson14, Olivier Restif15, Liam P. McGuire16, Ina L. Smith17, Bat One Health Team, Raina K. Plowright10, Alison J. Peel12,18

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Prediction and management of zoonotic spillover requires an understanding of infection dynamics within reservoir host populations. Transmission risk is often assessed using prevalence of infected hosts, with infection status reflecting the presence of genomic material. However, detection of viral genomic material alone does not necessarily indicate the presence of infectious virus, which could decouple prevalence from transmission risk. We undertook a comprehensive and multi-faceted investigation of Hendra virus shedding in Pteropus bats (colloquially flying-foxes, the main reservoir hosts), including Hendra virus qRT-PCR of 6,151 urine samples collected from five sites over three years. We assessed longitudinal associations between viral prevalence, viral load proxies (Ct value and genome copies), and spillover using generalized additive models and a permutation analysis. In addition to seasonal and interannual fluctuation in prevalence, we found evidence for periodic shifts in the distribution of viral loads. The proportion of bats shedding high viral loads was higher during peak prevalence periods with spillover events, and lower during peak and non-peak periods when there were no spillovers. We suggest that prolonged periods of low viral load and low prevalence reflect prolonged shedding of non-infectious RNA, or viral loads that are insufficient or unlikely to overcome dose barriers to spillover. These findings show that incorporating viral load (or proxies) into longitudinal studies of virus excretion from bats will better inform predictions of spillover risk than prevalence alone. Our study provides key insights into the processes that facilitate spillover and a basis for further experimental studies to explore interacting mechanisms that drive high viral shedding in bats.

Tamika presented data on Hendra virus shedding in bat urine samples. Her work examined how detection of virus may be coupled or uncoupled with the risk of transmission or spillover. For instance, whilst low level shedding of virus is seen year round, this does not translate to year-round spillover. Therefore, prevalence alone is not predictive of transmission, and it is possible that higher viral loads may more accurately predict spillovers. Using real time PCR, Ct values from urine samples may be used as proxy values for high viral loads, i.e. samples with a lot of virus do not need as many cycles for the virus to be amplified, so they have a lower Ct. Although Ct values do not directly give information on whether the virus is infectious, they were used in a permutation analysis showing that Ct values were not normally distributed. Though her work has not yet been published, Tamika made reference to data that is now available online as pre-print on bioRxiv.[4]

Disease Ecology Session 2

Chairs: Alison Peel and Sarah van Tol
Monday 9th December, 2 PM – 3 PM

Abstract O-05

Bridging the knowledge gap in the Hendra virus: Exploring the role of horses in transmission dynamics.

Belinda Linnegar[1], Hamish McCallum[1], Andrew Hoegh[3], Alison J. Peel[1,2]

[4] https://www.biorxiv.org/content/10.1101/2023.09.06.556454v1.full-text

[1]Centre for Planetary Health and Food Security Griffith University, [2]The University of Sydney, [3]Montana State University

Horses are a crucial, yet understudied, component in Hendra virus spillover. Following exposure to the virus, horses amplify the pathogen and act as bridge hosts for transmission to other horses, domestic animals and humans. The high subsequent fatality rates in horses and humans mean that Hendra virus is a significant public health concern. Anthropogenic change and climate have driven annual reports of spillover events across an expanding range. Recent spillovers in temperate zones of New South Wales, including Scone (known as Australia's horse capital), highlight the ongoing risk and the importance of understanding horses' role in Hendra virus spillover.

We collated and generated diverse datasets to quantify the spatiotemporal distribution of domestic horses, equine syndromic surveillance for Hendra virus spillover, and equine susceptibility to Hendra virus in northeast New South Wales and southeast Queensland, Australia, from 2011-2023. We found that domestic horse population density broadly correlated with the distribution of Hendra virus spillovers. However, the highest horse densities in Queensland occurred in highly urbanised suburbs, whereas in NSW, the greatest densities were in suburbs with >70% agriculture land cover and no flying fox roosts. Both Hendra virus surveillance testing and equine vaccination rates have decreased over time.

Domestic horse populations play a central role in the risk of Hendra virus spillover to humans. Declining vaccination rates and decreasing surveillance efforts, alongside an increasing population of susceptible horses, suggest that spillovers are likely going undetected. There is a need for ongoing multidisciplinary and multi-sector engagement to mitigate the risk of infection to horses and humans.

Belinda presented on the role of horses in Hendra virus transmission dynamics. She began by making reference to the ***Pathways to zoonotic spillover***,[5] whereby zoonotic pathogens can often use bridge hosts to amplify its replication. For Hendra, a lot is known about bats as reservoir hosts, but less is known about horses acting as bridge hosts. When the distribution of domestic horses in Australia is mapped, data collation can be patchy and difficult to confirm, but she and colleagues do see higher densities of horses in southeast Queensland (QLD) compared to New South Wales (NSW). When linking this with population growth, the horse population is growing at a finer scale. Simplex plots were drawn to compare three land characteristics (high build, forested and agricultural) between NSW and QLD, showing important differences. The highest horse densities in NSW were in areas with more and agricultural, rural land. However, in QLD, the highest horse densities were in urbanised areas with forest. Reference was made to Eby et al.[6] recognising that pathogen spillover is driven by rapid changes in bat ecology, and mentioning that changes in the roosts of flying foxes intersect with the differences observed in NSW and QLD. In terms of vaccination, several studies have shown that the Hendra vaccine is safe. However, between 2014 and 2023, there has been a clear decrease in the number of horses being vaccinated. In addition, four postcodes with highly dense horse populations in QLD and bottom of NSW are currently under reporting vaccine use. Many horse owners are reluctant to engage

[5] https://www.nature.com/articles/nrmicro.2017.45

[6] https://www.nature.com/articles/s41586-022-05506-2

with a vet, and there is a clear decline in the volume of testing for the exclusion of Hendra. This situation is worrying in terms of risks for spillovers. Belinda ended her presentation emphasising that there is a need to better understand the exposure rates and vaccination rates among horses. She also mentioned that, in terms of transmission from bats to horses and on to humans, it may be important to understand if this is linked to horses being less in the stable (exposure) and subsequently handfed (bridging transmission). When asked about the source of the vaccination data, Belinda said that the data being used are those from Zoetis as well as population data. When asked about the reasons for the lack of update for the vaccine, Belinda mentioned that cost is always an element, but that the need for a vet to administer the vaccine is also a factor.

Abstract O-06

Incredible diversity of Henipaviruses revealed in Australian flying foxes

K9 Jenns, Claude Kwe Yinda, Brent Jones, Karan Kim, Bat One Health team, Raina Plowright, Vincent Munster, Alison Peel, J-S Eden

The University of Sydney, Westmead Institute for Medical Research, Sydney Infectious Diseases Institute, Rocky Mountain Laboratories, Griffith University, Cornell University

In the 30 years since the discovery of Hendra, and then Nipah viruses, only three other bat-associated henipaviruses have been described: Ghana (2008), Cedar (2012) and Angavokely (AngV; 2022). The expansion of rodent-borne Henipa-like viruses, however, has been so great as to precipitate the formation of their own genus, Parahenipavirus. In light of this, the limited number of bat-associated henipaviruses (bHNVs) is peculiar, given the immense viral diversity hosted by bats. Here, we describe the largest survey of paramyxoviruses in Australian flying foxes and reveal 25 novel species of bHNVs. We used unbiased meta transcriptomics, custom PCRs and Nanopore sequencing on urine and faecal samples that were collected from Pteropus alecto individuals and mixed species roosts in southeast Queensland and northeast New South Wales between 2017-2020. Phylogenetic analyses reveal the formation of three Henipavirus clades – Clade I, comprising the canonical HeV, NiV, CeV, and GhV; Clade II, containing AngV and 5 novel viruses; and Clade III, containing 20 novel viruses. These discoveries solidify the evolutionary split between the bat- and rodent-associated genera. Although none of our viruses nested within Clade I, our uncovered diversity might indicate the existence of additional lineages within the Henipavirus genus that may challenge the notion of Nipah and Hendra viruses as evolutionary outliers. Our findings overall suggest a greater diversity of henipa- and paramyxoviruses yet to be uncovered in bats globally. Assessments of the pathogenetic potential of these novel viruses, and their cellular and ecological interactions with each other and co-circulating viruses, like Hendra, are urgently required. Together, these investigations will reframe our understanding of the evolution and ecology of the genus and contextualise the so far unique pathogenicity of HeV and NiV.

K9 Jenns first gave an overview of the taxonomy and history of various henipaviruses and then presented data on the largest survey of bat henipaviruses (bHNVs) from Australian flying foxes. Between 2012 and 2022, there was a significant increase in the knowledge of HNVs due to metagenomics and meta transcriptomics. MojV has recently been separated and confirmed by ICTV. Datasets collected between 2018 and 2021 from sites in Redcliffe and Clunes have advanced our understanding of HNVs. By 2024, there were 24 new viruses with complete or near complete genomes. These can be broken into 3 clades: Clade 1, with HeV and NiV very closely

related based on the L proteins; Clade 2 (5 complete genomes); and Clade 3 (11 complete, 5 near complete and 3 partial l genes). 15 of these 24 new viruses had snippets detected. When asked if any of the new viruses have been isolated, K9 replied that not yet. When asked if any genes were more diverse than others, K9 said the more conserved proteins were L and M. When asked if any bats were coinfected with these new viruses, K9 mentioned the Hendra genotypes. The lab has made a pan PCR for the Hendra genus, and it doesn't pick up rubulaviruses.

Abstract O-07

Co-circulation and co-infection: unravelling viral community dynamic through meta transcriptomics

Brent Jones (1)*, K9 Jenns (2,3,4), Karan Kim (2,3), Bat One Health team, Raina Plowright (5), Hamish McCallum (1), J-S Eden (2,3,4), Nicholas Clark (6), Alison J. Peel (2,4)

1. Griffith University, 2. The University of Sydney, 3. Westmead Institute for Medical Research, 4. Sydney Infectious Diseases Institute, 5. Cornell University, 6. The University of Queensland School of Veterinary Science

Disease ecology explores how ecological factors shape the evolution and transmission of infectious diseases within populations. Through this lens, factors contributing to complex transmission processes—such as those involved in spillover—can be understood and appropriate countermeasures designed. However, the historical focus on host and environmental factors affecting a single infectious agent neglects the complex, real-world relationships among microbial communities. Here, we use a combination of unbiased meta transcriptomics and targeted sequencing to investigate the viral co-circulation and co-infection dynamics of Australian flying foxes within the context of the ecology of Hendra virus.

Urine and faecal samples were collected from Pteropus alecto individuals and mixed species roosts in southeast Queensland and northeast New South Wales between 2017 – 2020. Extracted RNA was screened using Illumina sequencing, and PCR and Nanopore sequencing. Hendra virus and an additional 25 novel henipaviruses were detected (see Jenns et al. Hendra@30 abstract), as well as other mammalian viruses from eight different families. Viral shedding dynamics were modelled using a hierarchical multivariate generalised additive model, chosen for its flexibility in fitting non-linear trends over time series. We found differences in seasonality in the detection of paramyxoviruses and coronaviruses – with broadly synchronous shedding within viral families but asynchronous shifts in shedding between families. Analysis of potential associations between individual viruses is ongoing.

This is the first description of seasonal shedding dynamics of these novel henipaviruses and contributes to existing work demonstrating multi-viral shedding pulses in paramyxoviruses. Investigating potential viral interactions in the context of ecological drivers of paramyxovirus shedding dynamics will improve our understanding of viral persistence, transmission, and evolution in the flying fox reservoir.

Brent started by referencing earlier work by Peel et al. on synchronous shedding of multiple paramyxoviruses.[7] Co-infection of multiple unique viruses in the sample individual is possible, as is co-circulation, co-occurrence of multiple unique viruses in a group of individuals. Hence the aim of the study was to describe co-circulation and co-infection in black flying foxes. To do this, sampling had to be done under roosting bats and with individual sample collection. Meta-transcriptomics and bioinformatics were ran by other team members. They found 25 henipaviruses, orbiviruses and PRB. Brent found that viral richness fluctuated seasonally, with higher detection in the winter and spring season. Peak richness was estimated in springtime. Hence, the co-occurrence model is driven by season. Of the possible 595 pairwise combinations, they chose to exclude 9 viruses from this model. This model can help test if seasonality is the reason, or if random co-occurence is also possible. Phase 2 results suggest viral richness may differ by host age: younger bats have a wider diversity, but the dataset is small. Co-infection of the same individuals increases with richness and prevalence. The key finding here is that seasonal shedding patterns are shared among paramyxoviruses. Further work will elucidate additional variables leading to co-occurrence network. When asked if viruses causing co-infection could be further away from each other, Brent replied that his colleague K9 could look into those phylogenetics. When asked about how one can avoid chimeric reads for pooled samples when looking at coinfection and high throughput screening, Brent said it would be important to ensure the use of individual samples and not the pooled samples for the co-infection analyses.

Abstract O-08

Roosting and feeding ecology of Indian flying fox (Pteropus medius) bats in anthropogenic environment drives the bat-borne pathogens spillover risk in Bangladesh

Monjurul Islam (1)*, A K M Dawlat Khan (1), Emama Amin (1), Pronesh Dutta (1), Shariful Islam (1), Sarah Munro (2), Md Abu Sayeed (1), Abdullah Al Mamun (1), Nabila Nujhat Chowdhury (1), Md Arif Khan (1), Sharmin Sultana (1), Maryska Kaczmarek (2), Tahmina Shirin (1), Shusmita Dutta Choudhury (1), Jonathan H Epstein (2), Ariful Islam (2,3)

1. Institute of Epidemiology, Disease Control & Research (IEDCR), Bangladesh, 2. EcoHealth Alliance, New York, NY, USA; 3. Gulbali Institute, Charles Sturt University, Wagga Wagga, NSW, Australia

Understanding the impact of anthropogenic changes of the Indian flying fox (Pteropus medius) bat ecology is crucial for assessing the spillover risk of bat-borne diseases, particularly Nipah virus. Limited research on roosting and feeding behavior in Bangladesh motivated us to perform this study on Pteropus medius ecology, and human-bat interactions that may facilitate viral spillover. We conducted an ecological and behavioral survey across 13 districts in Bangladesh from 2021 to 2024 to collect data from bat roosts and their community. Additionally, we longitudinally monitored four bat roosts - two in Nipah belt and two in non-Nipah belt, tracking bat population and habitat changes over time. We identified 108 bat roosts, with 87.9% located within 30 meters of human dwellings. Bat roosts were found in 24 tree species, including Mahogany, Siris, and Banyan tree, with an average of 638 bats per roost. We observed scarcity of

[7] https://www.tandfonline.com/doi/full/10.1080/22221751.2019.1661217#abstract

wild fruit trees in community that made bats rely heavily on human cultivated fruits and raw date palm sap, drawing them closer to humans. Community people preferred planting timber trees over fruit trees for economic reasons. Moreover, 25.9% of roosts were disturbed by tree cutting, and bat hunting was reported in community for consumption or sale. Our longitudinal study revealed that 75% of bat roosts were impacted by tree cutting, with an average of 1,700 bats per roost. No suitable alternative roosts were found within a 2-kilometer radius for displaced bats. Such disturbances can stress bats, increasing the risk of virus shedding. Bat populations declined by 70%, 63%, 54%, and 20% at the four sites by end of the study period. The negative binomial model indicated 22.7% more decline in bat populations in Nipah belt. This study highlights the urgent need to protect bat habitats, secure food availability, and support bat ecology to prevent future spillovers and outbreaks, ensuring coexistence with bats without elevating zoonotic disease risks.

Monjurul started his talk describing the population density in Bangladesh, with over 170 million people, and an expected 30% increase in the next 20 years. Deforestation is also increasing and having an impact on bats. *Pteropus medius* are observed in areas with high human population density. His team investigated roosting preferences and feeding behaviours, and monitored habitat change. The longitudinal study was part of the NiV surveillance, focussing on 4 roosts in 4 districts. The team identified 108 bat roosts with 87.9% located within 30 meters of human dwellings. Many roosts dwell in tall timber trees that the community prefer for timber; and cutting of these trees makes it easier for water to be contaminated. 75% of roosts were impacted by tree cutting and had an average of 1700 bats per roost. Bat populations declined by 63%, 70%, 54% and 20% at 4 locations by the end of the study period. Monjurul emphasised key take aways: bat habitats need to be protected; raw date palm sap is not the bat's natural food source, but is available when food is scarce; wild fruits need to be available; human behaviour influences spillover; it is important to secure food availability and support the bat ecologically. When asked if there was a difference in human-bat relationships between Hindus and Muslims and tribal people, Monjurul mentioned that the risk factor in Bangladesh was more about drinking contaminated waters and he did not see any cultural links here. When asked about the reasons for tree cutting, Monjurul said these were both economic and due to a higher demand by the human population rather than feelings about the bats. When asked if there were with bat disturbances, Monjurul replied many roosts were being disturbed but remained, though one roost was cut down in 2023.

Rapid oral session talks

Chairs: Jennifer Barr and Emily Dowling
Monday 9th December, 3 PM – 5 PM

Model-guided henipavirus discovery in museum bat collections

Maya M. Juman, Barbara A. Han, Daniel J. Becker

University of Cambridge, Cary Institute of Ecosystem Studies, University of Oklahoma

Several species of Old World fruit bats (family *Pteropodidae*) are known reservoirs of zoonotic paramyxoviruses, including henipaviruses. However, little is known about how many pteropodid species host paramyxoviruses and which traits are associated with host suitability. We compiled

morphological, ecological, demographic, and evolutionary trait data for 194 pteropodid species from the literature as well as virus occurrence data through a systematic review. We then used boosted regression trees (BRT), a machine learning algorithm, to identify trait profiles of henipavirus-positive pteropodids and predict which additional unsampled or previously negative species are suitable hosts. Our BRT had high predictive capacity (mean test AUC = 97.7%) and suggested that species with larger range areas, greater body lengths, and high sympatry with other bat species were more likely to be PCR positive for henipaviruses. Based on this trait profile, the top four predicted "novel" hosts are Cynopterus sphinx, C. brachyotis, Epomops franqueti, and Rousettus madagascariensis. We are currently empirically testing these predictions by screening specimens in historical museum collections—a promising and underutilized resource for pathogen surveillance and host identification. This involves RNA extraction from frozen tissue samples of predicted host species housed at the Field Museum of Natural History, followed by family-wide RT-PCR targeting the paramyxovirus L gene. Any PCR-positive samples will be sequenced, and novel viral sequences will be published along with the taxonomic, temporal, and geographic metadata of the host specimen. This screening may expand our library of known vertebrate–virus associations and inform future surveillance and spillover prevention efforts. More broadly, this case study lays out a framework for using modern methods like machine learning to unlock pathogen data hidden in historical specimens.

Maya presented on machine learning algorithms applied to the study of bats from historical museum samples. Her team suspects there are other undetected hosts of paramyxoviruses. To predict host suitability, greater body length, larger range area and high sympatry were key parameters. She described the top suspected "novel" hosts listed in the abstract. Current focus will be the study of samples from the Field Museum of Natural History in Chicago, as they have a great archive.

Metabolic consequences of changing ecological conditions in flying foxes: a Hendra virus reservoir host

Avirup Sanyal, Cinthia Pietromonaco, Steve Melvin, and Alison Peel

School of Environment and Sciences, Griffith University, Brisbane, Centre for Planetary Health and Food Security, Griffith University, Brisbane, Australian Centre for Disease Preparedness, CSIRO, Geelong, Victoria, Sydney School of Veterinary Science, The University of Sydney, Camperdown, New South Wales

Flying foxes (Pteropus spp) are nomadic nectarivores and frugivores, such that locally available foraging resources shape patterns of roost occupancy and abundance. Historically, large aggregations of flying foxes roosted nearby areas rich in native flowering eucalypts. However, recent shifts in their foraging habitats have seen increased utilization of urban landscapes, which offer a mix of native and exotic plant species. This variation in availability of diet species in time and space is expected to impact individual flying fox diet, and downstream metabolic pathways. These recent ecological changes—driven by land use change, loss of critical winter habitat and climate-driven changes in food availability (acute food shortages)—have been associated with increased Hendra virus spillover risk. Although current models enable prediction of clusters of Hendra virus spillover clusters through proxies of bat fitness and climate, the mechanistic links between environmental risk factors and physiological outcomes in bats remain unclear. Understanding how flying fox metabolism changes with seasonal and interannual differences in

food availability could be key to linking foraging behaviour with virus shedding dynamics. To address this, we optimised a protocol for under roost sampling from bat populations across four sites, and subsequent metabolomics profiling using NMR. We demonstrate significant changes in the profile of almost fifteen polar metabolites in urine of wild flying fox populations between summer, when an acute food shortage was observed, and the following autumn. We hypothesise that nutritional shifts may affect downstream energy stress pathways or immune resource allocation. This work on wild bat populations builds on earlier metabolomic studies in laboratory settings– in both captive Pteropus and Artibeus spp bats subjected to different diets. It offers a promising approach in understanding the mechanisms of virus shedding in wild bats.

Avirup presented data on changes across the urine metabolome across two seasons. However, the changes were not seen for the fecal metabolome and he's currently figuring out why. Avirup has also looked at the roost differences.

Human-bat competition on cultivated fruit resources promotes bat-borne pathogens spillover to humans and domestic animals in Bangladesh: an exploratory qualitative study

A K M Dawlat Khan (1), Pronesh Dutta (1), Shusmita Dutta Choudhury (1), Sarah Munro (2), Nabila Nujhat Chowdhury (1), Md. Arif Khan (1), Emama Amin (1), Monjurul Islam (1), Abdullah Al-Mamun (1), Sharmin Sultana (1), Maryska Kaczmarek (2), Tahmina Shirin (1), Jonathan H Epstein (2), Ariful Islam (2,3)

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Bats are natural reservoirs of many emerging infectious viruses like Nipah virus (NiV), Ebola virus, and Hendra virus, which cause significant human morbidity and mortality. Due to the rapidly changing ecological conditions, bats increasingly depend on fruits, cultivated for humans. Since 2001, nearly annual NiV outbreaks have occurred in Bangladesh with a fatality rate exceeding 70%, mostly linked to bat-contaminated fruit consumption. Hence, we conducted an exploratory qualitative study to understand how human-bat competition on cultivated fruits promotes pathogens transmission to humans and animals, in four NiV outbreak districts of Bangladesh between 2021 and 2022. Our research included 60 ethnographic interviews and 24 observations with fruit orchard owners, raw date palm sap (RDPS) harvesters, and consumers. Almost all participants reported frequent visits of bats to their cultivated fruit orchards and RDPS trees. They noted wild fruit trees are scarce in their localities due to increasing timber tree plantations, resulting in bats turning to cultivated fruits and RDPS as alternative food sources. Participants also reported consuming bat-bitten dropped fruits and occasionally feeding them to domestic animals. We observed domestic animals eating dropped fruit while grazing in orchards. Orchard owners often use nylon nets to protect their fruits from bats and bats are entangled and die. They are exposed to bats during removal from the nets. Even, some local ethnic people collect the trapped bats for consumption. Although RDPS harvesters use protective measures, bats scratch them and trunks to access and consume sap. The study's findings highlight the significance of increasing human and animal exposure to bat-borne infections through human-bat food competition on cultivated fruits. We recommend future studies on ecological and behavioral interventions to prevent bat-borne pathogen spillover to humans and domestic animals in Bangladesh.

AKM Dawlat presented ecological and human behavioral data combined. Because of wild fruit unavailability, bats are dependant on human cultivated fruits and raw date palm sap (RDPS). RDPS is a winter delicacy for humans, but not a natural food for bats. Fruit growers can have conflicts with bats; for example, orchard owners use nets and sound to scare the bats; this can lead to exposure during removal of dead bats from nets. Some cultures do eat bats.

Henipavirus sero-surveillance in horses and pigs from Northern Nigeria

Andrew Adamu, Lean McNabb, Alex Adikwu, Yakubu Jibril, Idoko Sunday, Aliyu Turaki, Samson Abalaka, Richard Edeh, Godwin Egwu, Mohammed Adah, Kim Halpin

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Hendra virus and Nipah virus are emerging viruses that cause severe zoonotic diseases in humans who have had close contact with horses and pigs in Australia and Asia. Despite large populations of horses and pigs in northern Nigeria, no studies have investigated henipavirus sero-surveillance using the gold standard test-serum neutralization test (SNT). A total of 536 apparently healthy horses and 508 pigs were sampled in northern Nigeria in 2018. Sera were tested for Hendra virus and Nipah virus-specific antibodies using either the Henipavirus Luminex binding assays for horses or the Hendra virus Competitive ELISA and Nipah virus Indirect ELISA for pigs as initial screening tests, followed by the confirmatory Hendra and Nipah virus SNT for both species, according to accredited protocols at the Australian Centre for Disease Preparedness. Although some horse and pig samples cross-reacted or reacted weakly in the screening test, confirmatory SNT proved negative. This study reveals the absence of Hendra and Nipah antibodies in horses and pigs in northern Nigeria, which is consistent with the absence of observable disease in the field. However, there is need for continuous surveillance due to inter and intra-trans-boundary animal movement and trade in Nigeria to safeguard both animal and human health.

Andrew presented data on binding and serum neutralization tests from the horse and pig samples described in the abstract. Only 4 samples tested weakly positive in the Luminex assay and they were from horses. A total of 1044 animals were screened, with low reactivity in the screening Luminex assay and no positive results in the gold standard serum neutralization test (SNT).

Cryptic exposure to henipaviruses in Cambodia and Malaysia

Spencer L. Sterling, Eric D. Laing, Sylvia Daim, Ping-Chin Lee, Phireak Hip, Dolyce H.W. Low, Chian Ho, Lianying Yan, Piseth Ly, Pidor Ouch, Menghou Mao, Nathaniel Christy, Dawn L. Weir, Ahmad Zakuan Kamarudin, Eugene Tan, Christopher Peatey, Brady McPherson, Nicholas Anstey, Jeffry C. Hertz, Ian H. Mendenhall, Giri S Rajahram, Matthew Grigg, Mohd. Arshil bin Moideen, Andrew Letizia, Christopher C. Broder

Department of Microbiology and Immunology Uniformed Services University Bethesda Maryland USA, Henry M Jackson Foundation Bethesda MD USA, Graduate Program in Medical Sciences Faculty of Medicine Chulalongkorn University Bangkok Thailand, Faculty of Medicine and Health Sciences Universiti Malaysia Sabah Kota Kinabalu Sabah Malaysia, Biotechnology Research Institute Universiti Malaysia Sabah Kota Kinabalu Sabah Malaysia, Faculty of Science and Natural Resources Universiti Malaysia Sabah Kota Kinabalu Sabah Malaysia, U.S. Naval Medical Research Unit INDO PACIFIC Detachment Phnom Penh Phnom Penh Cambodia, Programme in Emerging Infectious Diseases Duke-NUS Medical School Singapore, U.S. Naval Medical Research Unit INDO PACIFIC Singapore, Malaysian Armed Forces Malaysia, Australian Defence Force Malaria and Infectious Disease Institute Brisbane Australia, Global and Tropical Health Division Menzies School of Health Research Charles Darwin University Darwin Australia, Infectious Diseases Society of Kota Kinabalu Sabah Malaysia

The recent expansion in the number of species in the genus Henipavirus highlights the current gaps in our understanding of the diversity of this viral genus. The discovery of viruses that impact human health, such as Nipah virus and Langya virus, typically occurs after sustained outbreaks. We investigated two independent cohorts in Cambodia and Malaysia for serological evidence of henipavirus exposure to better understand henipaviral diversity and spillover-risk in Southeast Asia. Serological studies were performed in Cambodia and Malaysia. In Cambodia, serum was collected from individuals presenting to clinics with non-specific acute febrile illness. In Malaysia, cohorts of soldiers were sampled prior to and following deployment. Serum was tested for IgG using soluble henipavirus G glycoproteins with a multiplex microsphere immunoassay and resulting antigen-antibody complexes were analyzed by cluster-based approaches to assess binding profiles.

In Cambodia, 3.8% of individuals were reactive to at least one henipavirus, including one group composed of three individuals with Cedar virus-specific binding and neutralizing antibodies, and a second group possessing cross-reactive antibodies to Ghana virus and Hendra virus, suggesting exposure to a viral most common ancestor. In Malaysia, minimal seroconversion was observed during deployment, however two individuals possessed IgG reactive to the shrew-associated Langya and Gamak viruses. This study presents evidence of multiple henipaviral exposures in humans in two Southeast Asian countries. These findings will guide seroepidemiology models and establish attack rates in populations at-risk for zoonoses. Targeted molecular and clinical surveillance is needed to evaluate pathogenic potential of these unknown viruses. B-cell isolation and monoclonal antibody characterization will help elucidate antigenic relatedness and future diagnostics and medical countermeasure develop.

Spencer mentioned the views he presented were his own. With colleagues, he's trying to model serodynamics over time to inform molecular surveillance. He has screened military personnel to see if there were any infections associated with their deployments.

Serosurveillance of Nipah virus in Malaysia

Vunjia Tiong, Noor Syahida Azizan, Che-Norainon Yaacob, Nur-Hidayana Mahfodz, AsmaAnati CheMatSeri, Mulya-Mustika-Sari Zulkifli, Chee-Sieng Khor, Norhidayu Sahimin, Sazaly AbuBakar

Tropical Infectious Diseases Research and Education Centre (TIDREC), High Institution Centre of Excellence, Universiti Malaya, 50603 Kuala Lumpur, Malaysia

Nipah virus is a zoonotic pathogen that has caused severe outbreaks in Malaysia, India and Bangladesh since first emerging in 1997. Despite being classified as a WHO priority pathogen due to its potential threat to public health, the monitoring and surveillance of NiV and other emerging paramyxoviruses in Malaysia remain inadequate. Here, a One Health approach was employed for serosurveillance of these potential infections among the indigenous population of Malaysia, the Orang Asli, who are at increased risk of contracting the infection due to their living proximity to forest fringe areas, which increases their contact with wildlife. An in-house ELISA-based assay utilising recombinant NiV nucleocapsid was used in the present study to evaluate the presence of NiV IgG. To identify possible risk factors, demographic data and a questionnaire detailing their frequency of contact with animals, and interaction with their surrounding environment were obtained from each participant. A total of 390 samples were tested and 9.7% (38/390) were found to be positive for NiV IgG. No significant risk factors between NiV seropositivity and animal exposure and bites, types of outdoor activities or frequency of entering forests were identified. Confirmation of past infection or exposure to NiV is, however, still needed. This study is currently being expanded to include other at-risk populations and localities. Findings from the study, however, suggest that with the rapid merging of the human-animal-environment fronts, there is a need to expand surveillance programmes to understand the extent of NiV or other potentially novel paramyxovirus infections and their associated risk factors in Malaysia. This study has received IRB approval from the Medical Research & Ethics Committee, Ministry of Health Malaysia (NMRR ID-22-01304-7QQ).

Vunjia started by mentioning that the monitoring and surveillance data coming out from Malaysia has been rather limited. She is focusing on a One Health approach and on looking at a high risk indigenous populations living by the forest fringe. She noted the sample sites are roughly 80 kms from initial outbreaks of Nipah virus. Seropositive rates varied across sites. She also looked at 35 different risk factors, mentioning that the populations are unlikely to report sicknesses; however, if they do report, they will report general respiratory symptoms. The potential source of exposure for the seropositive was unknown. More recently the population lifestyle has changed, as people are moving more into the city. Her team is looking at long-term surveillance for Nipah.

Development and establishment of diagnostic capability for Nipah virus

Banwari Lal, **Shailendra Mani**

Translational Health Science and Technology Institute, Faridabad, India-110021

The Nipah virus (NiV) is a zoonotic pathogen belonging to the Paramyxoviridae family. It was first identified during an outbreak in Malaysia and Singapore in 1998. Since its discovery, Nipah virus has become a major public health concern due to its high mortality rate and potential for human-to-human transmission. Recently, a notable outbreak occurred in the state of Kerala, India. Transmission to humans commonly occurs through intermediate hosts, such as pigs, or directly from bats, which are natural reservoirs of the virus. Infected individuals may present with a range

of clinical symptoms, from asymptomatic cases to severe manifestations including fever, headache, cough, respiratory distress, encephalitis, and convulsions.

Prompt detection and vigilant monitoring of outbreaks are essential for controlling the spread of Nipah virus. Developing effective vaccines and antiviral treatments is a priority to prevent future outbreaks, but this task is challenging due to the high containment requirements necessary for handling the virus. To address these challenges, we have established a pseudovirus system that can be used in a BSL-2 laboratory setting. This system facilitates rapid evaluation of antibody responses, screening of antiviral agents, and assessment of vaccine candidate efficacy. These advancements are crucial for enhancing our understanding of the Nipah virus and improving our ability to combat it, particularly in regions like India where the virus poses a significant threat.

Shailendra noted his work was part of greater efforts on global vaccine development done by the Biotechnology Research and Innovation Council (BRIC) and the Translational Health Science and Technology Institute (THSTI). They are currently planning to add Nipah F and G to the serological assay for antibody responses to SARS-Cov_2. They are also hoping to develop molecular and serological assays for WHO priority pathogens. The aim is to establish a platform for quickly developing vaccines in future contingencies.

Investigation of two newly emerged henipaviruses: How the Langya and Angavokely virus matrix proteins interact with nuclear import proteins

Emily C. Wagon, Camilla M. Donnelly, Gayle F. Petersen, Jade K. Forwood

Charles Sturt University, Gulbali Institute

Langya virus (LayV) and Angavokely virus (AngV) are two novel, emerging pathogens within the Paramyxoviridae family and Henipavirus genus. Currently there are several known henipaviruses including, Hendra, Nipah, Cedar, Mòjiāng, Ghanaian, Gamak and Daeryong viruses. Two zoonotic henipaviruses include the highly pathogenic Hendra and Nipah viruses. Hendra virus (HeV) causes a fatal respiratory illness in equines, while Nipah virus (NiV) causes fatal encephalitis in pigs. Both HeV and NiV infect humans leading to fatality rates between 40 – 90%. Outbreaks of NiV occur annually in Bangladesh and India. As there are no targeted antiviral therapies or FDA approved vaccines for humans, both HeV and NiV are classified as Biosafety Level 4 agents. This project aimed to investigate LayV and AngV, by comparing how the viral matrix proteins bind and interact with host cell nuclear import molecules. Trafficking viral matrix proteins into host cell nuclei has been demonstrated as a vital step in viral replication and budding. Translocation into host cell nuclei occurs due to nuclear localisation signal (NLS) sequences on the viral matrix protein binding to host importin alpha (IMPα) and importin beta (IMPβ) proteins. LayV and AngV were purported to contain two and three NLSs, respectively. Electrophoretic mobility shift assays were used to determine which putative NLS sequences bind to IMP alpha and β isoforms. Fluorescence polarisation assays determined the strength of binding between the functional NLSs and each isoform. Protein crystallography screening of IMP α and β isoforms and LayV and AngV NLS synthetic peptides were used to characterise structures of protein-peptide interactions which may provide targets for future antiviral therapy or vaccine development.

Detection of Cell-Mediated Immune Memory in Nipah virus survivor 25 years post-infection

Puteri Ainaa Syahirah Ibrahim, Hui Ming Ong, Chee Ning Chong, Chong Tin Tan, Jie Ping Schee, Michael Selorm Avumegah, Raúl Gómez Román, Neil George Cherian, Won Fen Wong and Li-Yen Chang

Universiti Malaya

The initial outbreak of the Nipah virus (NiV), a highly pathogenic paramyxovirus, was documented in 1998 in Kampung Sungai Nipah, Negeri Sembilan, Malaysia. Due to the virus's high mortality rate, pandemic potential and lack of viable treatment, it is listed as one of the critical targets that requires urgent research and vaccine development. Crucial information for NiV vaccine development can be obtained from studying the adaptive immune response, particularly the cell-mediated component of immune memory 25 years after NiV infection. This study aims to investigate the cell-mediated immunity specific against NiV infection by evaluating cytokine levels in PBMCs from NiV survivors. PBMCs from four survivors were collected and stimulated with NiV-F and NiV-G overlapping peptides. Memory T cell activation was assessed by detecting IFN-γ and IL-2 cytokines using intracellular cytokine staining (ICS) and T cell ELISPOT. Preliminary ICS testing was performed using samples from two survivors, N11 and N13. These samples were expanded and stimulated with the overlapping peptide minipools. The results showed that N11's CD4+ T cells responded positively to G2 and G3 minipools. In contrast, N13 exhibited mixed responses across minipools, with positive cytokine responses to F2 and G3, but a negative response in IFN-γ production to G2. Further analysis using IL-2 T cell ELISPOT for sample N2 did not show significant activation. However, N13 demonstrated significant positive responses to three minipools: F3, G2 and G9. These findings suggest that long-term immunity against NiV is achievable, supporting the potential for successful vaccine development.

Puteri presented data on cell mediated immune responses in selected NiV survivors from the 1998 outbreak in Malaysia. Peripheral blood mononuclear cells from 4 survivors were stimulated with 15-mer peptide pools. The pools were made from the F protein (9) and the G protein (10). Four peptides in the minipools showed consistent potential to be immunogenic targets for effective vaccines.

Decoding Henipavirus Latency and Transmission Dynamics: A Deep Learning Multi-Omics Approach Using Single-Cell Transcriptomics and Environmental Data

Rifaldy Fajar, Andi Nursanti Andi Ureng, Sahnaz Vivinda Putri, Prihantini

Yogyakarta State University, Andini Persada College of Health Sciences, International University Semen Indonesia, Bandung Institute of Technology

Background and Aim: Henipaviruses, such as Nipah and Hendra, are highly lethal zoonotic pathogens with poorly understood latency, reactivation, and transmission mechanisms. This study uses deep learning on single-cell transcriptomics and environmental data to decode molecular and environmental factors influencing henipavirus latency and transmission, aiming to improve outbreak prediction. Methods: We used a graph neural network (GNN) and recurrent neural network (RNN) to analyze 12,000 single-cell transcriptomes from bat and human immune cells, sourced from GEO and ENCODE. Viral genomes (n=1,200) from Nipah and Hendra were retrieved from GenBank. Environmental data, including temperature, humidity, and deforestation rates, were gathered from NASA's EOSDIS and Global Forest Watch, covering a 10-year period in

Southeast Asia. Transfer learning from human immune models was fine-tuned with bat data to identify reactivation signatures. Unsupervised clustering and attention mechanisms captured the interactions between immune responses and environmental stressors. Model performance was evaluated using accuracy, F1 score, and AUPRC. Results: Our model achieved an accuracy of 89.2% (95% CI: 87.5%-90.8%) and an AUPRC of 0.860 (95% CI: 0.840–0.880) in predicting viral reactivation events. Temperature increases of 1.5°C were associated with a 24.7% (95% CI: 22.3%-27.1%) rise in reactivation within bat populations. A non-coding RNA signature was identified, contributing to 16.8% (95% CI: 14.2%-19.3%) of reactivation cases. Reduced interferon signaling in bats increased the likelihood of viral spillover to livestock by 20.9% (95% CI: 18.2%-23.4%), while environmental stressors, such as deforestation, contributed to 29% of the model's predictive power in identifying high-risk regions. Conclusions: Environmental stressors like deforestation and temperature increases contribute to henipavirus reactivation, reinforcing the need for integrated surveillance and monitoring systems.

According to Rifaldy, temperature increases have the highest predictive value amount the environmental variables tested, to identify high-risk regions for outbreaks.

Is a Nipah virus-specific vaccine sufficient to prevent the next henipavirus outbreak?

Wee Chee Yap, Beng Lee Lim, Madeline Sheng Si Kwek, Wan Ni Chia, Yun Yan Mah, Feng Zhu, Chee Wah Tan

Infectious Disease Translational Research Programme of Department of Microbiology and Immunology under Yong Loo Lin, School of Medicine in National University of Singapore, Programme in Emerging Infectious Disease of Duke-NUS Medical School Singapore

Backgrounds: Surveillance of zoonotic viruses in wildlife has revealed that bats harbor multiple zoonotic pathogens, including the Nipah virus. Paramyxoviruses, often found in bats, pose a high risk of zoonotic spillover due to their use of highly conserved molecules as receptors. Preexisting human adaptive immunity, such as neutralizing antibodies, plays a crucial role in preventing zoonotic spillovers from becoming pandemics. Therefore, active surveillance of population immunity is essential for timely public health interventions. This study aims to establish a high-throughput serological platform for the rapid detection of neutralizing antibodies against henipaviruses, facilitating disease risk assessment for known henipaviruses. Methods :We have established a surrogate virus neutralization assay that allows rapid detection of neutralizing antibodies that block the binding of the henipaviruses glycoprotein to the receptor in a single-tube reaction. With this platform, we perform a disease risk assessment of known henipaviruses by characterizing the cross-reactivity neutralizing antibodies of convalescent serum samples collected from Nipah patients. Besides, we characterize the cross-reactivity neutralizing antibodies of the mouse serum samples collected after Nipah-specific vaccination. Results: Convalescent serum samples collected from Nipah patients possess robust neutralizing antibodies against the Nipah virus but could not neutralize other ephrin-using henipavirus. Furthermore, mice vaccinated against Nipah-specific vaccines induce potent neutralizing antibodies against the Nipah Virus and Hendra Virus but not the Cedar Virus and Ghana Virus. Conclusions: We demonstrated that Nipah-specific infection and vaccination induces high-level neutralizing antibodies against homologous strains, with limited to no cross-reactivity neutralizing antibodies against antigenic distinct henipaviruses, thus highlighting the zoonotic potential of these closely related animal viruses.

We Chee presented data on a high throughput surrogate virus neutralisation test (sVNT). She tested NiV convalescent serum for neutralisation against NiV, Cedar and Ghana, showing that the sera could neutralise the homologous but not the heterologous viruses.

Francisco Javier Salguero presented on behalf of Inés Ruedas-Torres

Pathological characterisation of the golden Syrian hamster model of Nipah virus infection

Inés Ruedas-Torres, Stephen Findlay-Wilson, Stuart Dowall, Emma Kennedy and **Francisco Javier Salguero**

United Kingdom Health Security Agency (UKHSA Porton Down), Salisbury, U.K.

Animal models that can replicate human Nipah virus (NiV) disease are critical for a better understanding of virus pathogenesis and for developing vaccine candidates. We have recently developed a NiV hamster model in the UKHSA, showing many similarities to human infection. The aim of this study is to characterise the lesions and the local inflammatory responses in tissues against NiV infection.

28 juvenile golden Syrian hamsters were infected with NiV Malaysian or Bangladesh strains and culled at different time points (1-21 days post infection). Organs were collected at post-mortem and routinely processed into FFPE blocks for subsequent histopathological evaluation (H&E) and RNAscope in-situ hybridisation (ISH) for NiV RNA detection. Immunohistochemistry (IHC) was used to mark T cells (CD3+), macrophages & microglia (Iba1+) and astrocytes (GFAP+). Multiplexed immunofluorescence (IF) (NiV antigen, CD3, GFAP and Iba1) was also used to study co-localisation of the virus with different cellular populations.

The most severe lesions were found in the lung and brain, consisting of moderate to severe multifocal broncho-interstitial pneumonia accompanied by mild meningoencephalitis, including perivascular cuffing in the brain. NiV RNA was detected in endothelial cells and inflammatory cells within lesions. Abundant macrophages, together with T cells, were detected in areas of pneumonia mainly within perivascular regions. Abundant astrogliosis and microgliosis, with T cells present within perivascular cuffs, were observed in the brain. Mild to moderate hepatitis, splenitis and nephritis was also observed, with the presence of NiV RNA within inflammatory cell infiltrates. These results have helped elucidate the host-pathogen interaction within the hamster animal model of NiV infection that is currently being used in the preclinical testing of antiviral and vaccine strategies.

Francisco showed a fantastic range of images from lung sections of infected animals. In the brain, a range of viruses can be seen in different places, with green marking the presence of NiV. A lot of astro cyctosis was observed. Francisco concluded the hamster model and the methods described are useful models for NiV infection.

Targeted immunology gene expression in FFPE samples from Nipah virus infected hamsters

Inés Ruedas-Torres (1), Stephen Findlay-Wilson (1), Stuart Dowall (1), Emma Kennedy(1), Alison Bird (1), Laura Hunter (1), Simon Waddell (2), Jamie Medley (2) and **Francisco Javier Salguero** (1)

1. United Kingdom Health Security Agency (UKHSA Porton Down), Salisbury, U.K.

2. Brighton and Sussex Medical School, Brighton, U.K.

Formalin-fixed paraffin-embedded (FFPE) samples from infectious disease animal experiments are a valuable source of information about the pathological changes and gene expression within tissue. In this study, we investigated the applicability of NanoString technology in FFPE lung samples from Nipah virus infected and uninfected hamsters using nCounter SPRINT target gene expression.

We have used an immunological panel comprising 547 genes within the murine transcriptome, cross-reactive with hamster RNA, indicative of adaptive and innate immunity, cell signalling, stress response, metabolism, and apoptosis. Moreover, we have used RNAScope in-situ hybridisation (ISH) to study the presence of IL-6 and TNF mRNA in the same FFPE tissues.

Significant differences were found in gene expression between infected and non-infected lungs, with upregulated pathways in the infected group including: Th1 differentiation, type 1 and type 2 interferon signalling, the TNF family and NF-kB signalling, Class I antigen presentation, the innate immune system and lymphocyte activation or T cell receptor signalling.

Using RNAScope ISH, IL-6 mRNA was the most prominent cytokine mRNA expressed in the infected lungs, associated with inflammatory cell infiltrates within areas of broncho-interstitial pneumonia, together with smaller amounts of TNF mRNA.

With these results, we have shown that the quality and quantity of RNA within FFPE samples is good to carry out targeted gene expression analysis using NanoString. We have been able to describe the upregulation of several expression pathways associated to infection and immune responses against Nipah virus, including proinflammatory cytokines, corroborated by ISH. These tools will be beneficial for analysing immunity and correlates of protection for this disease by vaccine candidates in preclinical studies, with the possibility of carrying out retrospective analyses using archived material (FFPE) from previous studies.

Francisco started by saying his team advocates for the 3 Rs in animal experimentation, i.e. replace, reduce, refine. Refinement and reduction includes methods such as the use of formalin-fixed paraffin-embedded (FFPE) samples to get as much information as possible from infected hamsters and controls. His team investigated the applicability of NanoString technology using FFPE samples. RNA extraction from the FFPE samples was vital for the NanoString. The team used a mouse kit for 517 genes that were similar to the hamster and showed this worked well. When conducting an analysis of signaling pathways, the team found many genes for innate immunity and cytokine expression were upregulated. Cytokine mRNA upregulation was also observed in situ (RNAScope).

Nipah mRNA-1215 Vaccine Induces Cross-Reactive Responses Against Henipaviruses and Confers Complete Protection Against Nipah Bangladesh Virus in Old World Monkeys

A. Ploquin (1), L. Loftus (1), A. Heimann (1), N. Storm (2), K. Korzeniwsky (1), C. Dulan (1), M. Seyler-Schmidt (2), S. Andrew (1), H. Staples (2), J. Marquez (1), L. McKay (2), L. Ou (1), I-T. Teng (1), S.S. Sunder (1), R. Du (1), W. Du (4), M. Anderson (4), T. Zhou (1), R.J. Loomis (1), B. Leav (3), K. Foulds (1), A. Griffiths (2), S. Himansu (3), N.J. Sullivan (2), M. Roederer (1), R. Mason (1)

1. Vaccine Research Center, National Institute of Allergy and Infectious Disease, National Institutes of Health, 2. National Emerging Infectious Disease Laboratories, Boston University, 3. Moderna, Inc, 4. Battelle Memorial Institute

Nipah virus (NiV) and Hendra virus (HeV) are considered priority pathogens of concern due to their pandemic potential. While NiV Malaysia (NiVM) strain was responsible for the first and largest outbreak in 1998, Bangladesh (NiVB) and Indian (NiVI) strains are responsible for yearly outbreaks in endemic areas with a high case fatality rate of 40 to 75%. There are currently no vaccines available for humans. The Vaccine Research Center, in collaboration with Moderna, has developed a novel mRNA NiVM vaccine (mRNA-1215) encoding secreted stabilized pre-fusion (Pre-F) protein covalently linked to an attachment glycoprotein (G) through a trimerization domain (Pre-F and 2F;G).

African green monkeys were vaccinated with mRNA-1215 at 2, 10 or 50mcg doses intramuscularly in a prime-boost immunization strategy with either 4-week or 12-week intervals. All animals were challenged six weeks post-boost with NiVB via combined intratracheal and intranasal routes. Immune responses were measured by ELISA against Pre-F and G proteins, pseudovirus neutralization, intracellular cytokine staining and B-cell probe-binding. Robust binding and neutralizing antibody against NiVM were detected with no major differences observed across the doses. While a 4-weeks boost slightly increased the antibody responses, a 12-weeks boost restored the response to post-prime peak levels. Antigen-specific T cell responses and NiVB cross-reactive B-cells were also detected. Importantly, NiVM immune responses were sufficient to confer complete protection against NiVB challenge for all doses and prime-boost interval groups tested. Further, cross-reactive binding and neutralizing antibody responses against HeV were detected through-out the study. mRNA-1215 elicited robust responses against NiVM, NiVB and HeV, protecting animals against NiVB lethal challenge with a dose as low as 2mcg. This vaccine is therefore a suitable candidate to further develop as a prophylactic measure against henipavirus-related disease.

Aurelie presented data on the VRC/Moderna mRNA platform vaccine using the F protein that has been stabilised in prefusion form, and the G protein in which they have forced the timerization domain. A preclinical model is being pursued because there are not a lot of cases of Nipah, so her team anticipates there it will be difficult to test the vaccine through conventional phase 3-efficacy trials. In the non-clinical, non-human primate models, the focus in on the efficacy and minimum dose. In Aurelie's view, what is most important is that they showed immune responses and protection with the clinical dose.

Human neutralising monoclonal antibodies against Hendra and Nipah viruses

Wen Shi Lee (1), Ellie Reilly (1), Robyn Esterbauer (1), Andrew Kelly (1), Danielle E Anderson (2), Glenn Marsh (3), Jennifer Juno (1), Stephen J Kent (1,4), Adam K Wheatley (1).

1. Peter Doherty Institute for Infection and Immunity, University of Melbourne, VIC, Australia, 2. Victorian Infectious Diseases Reference Laboratory, The Peter Doherty Institute for Infection and Immunity, Melbourne, VIC, Australia, 3. Australian Centre for Disease Preparedness, Geelong, VIC, Australia, 4. Melbourne Sexual Health Centre and Department of Infectious Diseases, Alfred Hospital and Central Clinical School, Monash University, Melbourne, Victoria, Australia.

The COVID-19 pandemic illustrated the potential of human monoclonal antibody (mAb) therapeutics as prophylactic and therapeutic agents against pandemic viruses. Current treatment options for Henipavirus infections are limited to repurposed antivirals such as Ribavirin and Remdesivir. Investigational mAb therapies for Nipah have been developed but their efficacy in humans has not been tested. We aimed to isolate neutralising mAbs cross-reactive against both Hendra and Nipah virus entry (G) and fusion (F) glycoproteins for development of potential therapeutic and prophylactic agents.

We recruited a Hendra virus convalescent individual (infected in 2008) and obtained PBMC and plasma samples. We developed a suite of assays to assess antibody and B cell responses against Hendra virus G and F glycoproteins, and characterised cross-reactivity to other henipaviruses (Nipah and Cedar viruses). Using recombinant G and F probes, we single cell sorted antigen-specific memory B cells for B cell receptor (BCR) sequencing and mAb isolation, with recovery of 50 BCR sequences for G and 53 BCR sequences for F. Memory B cell frequencies were generally low (0.02% and 0.1% of IgD- IgG+ memory B cells for G and F respectively), with clonal expansions observed within B cells specific for F but not G. A total of 28 mAbs against G and 47 mAbs against F are being expressed and tested for binding and neutralisation. Neutralising activity will be determined using a lentiviral-based pseudovirus neutralisation assay, with eventual testing of mAbs against live Hendra and Nipah viruses in the PC4 laboratory. Protective and therapeutic efficacies will also be tested in mouse challenge models with live virus.

Henipaviruses continue to be a serious health threat to the Asia-Pacific region, as emphasised by ongoing Nipah outbreaks and the emergence of Langya virus. Our work in developing therapeutic mAbs against henipaviruses will increase pandemic preparedness against future outbreaks.

Wen Shi described work on fully isolated human monoclonal antibodies (mabs) from one human donor who was infected with HeV. Several neutralising mabs were isolated with anti G and anti F specificity and found sequences for the antibodies. Several were cross reactive to NiV. Testing using pseudovirus infection with live virus work to come. . Wen Shi requested collaborators for the HeV neutralising antibody work.

Observational study on the clinical epidemiology of infectious acute encephalitis syndrome including Nipah virus disease, Bangladesh

Md Zakiul Hassan, Amanda Rojek, Peter Horby, Piero Olliaro

International Centre for Diarrheal Disease Research, Bangladesh (icddr, b), Dhaka, Bangladesh, Pandemic Sciences Institute, Nuffield Department of Clinical Medicine, University of Oxford, Oxford, UK, International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC), University of Oxford, Oxford, UK

Background: New therapies are currently under development for Nipah virus (NiV), requiring evaluation in trials, but trial design is challenging due to limited understanding of its clinical characteristics. Given the rarity of NiV infections, strategies targeting improved outcomes for broader acute encephalitis syndrome (AES) patients, including those with NiV, are essential for advancing therapeutic research. To address these gaps, we designed a study to describe the

demographics, clinical features, progression, treatment practices, etiologies, and outcomes in AES patients, including NiV, to inform trials.

Methods: This prospective cohort study in Bangladesh, a NiV-endemic country with annual outbreaks, aims to enroll up to 2,000 AES patients across three tertiary care hospitals. With informed consent, study staff will enroll participants and monitor them throughout their hospital stay and for 90 days post-discharge. Data will be collected via patient interviews and medical record reviews at multiple time points: enrollment, day 3, day 7, critical care admission (if applicable), discharge, and 90 days post-discharge. Information will be gathered on demographics, clinical presentations, treatments, complications, outcomes, including 90-day mortality and long-term neurological sequelae. Cerebrospinal fluid (CSF) from each participant will be tested for viral and bacterial pathogens at the icddr,b laboratory. Results and Recruitment Status: Participant recruitment began in March 2024, with 479 enrolled by August. Recruitment continues.

Conclusion: By characterizing AES patients, this study will provide critical data to inform trial design, including frequency of key outcomes (for sample size estimation), predictors of adverse outcomes (for stratification and adjustment of analysis), and the current standard of care (as a comparator). This information will optimize trials for potential interventions to improve outcomes in AES patients, including those with NiV.

Zakiul mentioned that the story from Wee Chi reminded him of a friend who was also a medical doctor in training who passed after 7 days post-infection with Nipah virus after caring for infected individuals in the hospital. This was 15 years ago, but there have been no improvements to the rates of outcomes since- case fatality rates are still high. Currently there is a range of recruitments for several clinical trials, but with little information on the broader acute encephalitis syndrome (AES) symptoms. Zakiul started a centre for these AES patients, excluding those with a clear non-infectious cause. The idea is that integrating NiV infected patients into a broader cohort of AES patients would improve standards of care.

Day 3

Surveillance

Chair: Jon Epstein
Tuesday 10th December, 9 AM – 10.30 AM

Tahmina Shirin

This was a pre-recorded talk. Tahmina presented an update on surveillance of NiV in Bangladesh, where the focus is currently on the response to new detections and the follow-up of survivors. December to April is considered the seasonality of Nipah outbreaks. Surveillance started in 2006. There is currently active surveillance in 12 tertiary care hospitals, passive surveillance at district hospitals, and enhanced surveillance in 500+ health care facilities. Event-based surveillance occurs 24/7. The outbreak response team involves a One Health approach and

includes professionals from the health sector, the wildlife sector and anthropologists. The sequalae in survivors is mostly neurological. This year, there were 14 NiV cases and 3 survivors. NiV was detected in breast milk. Tahmina's team has developed communications material and the national guidelines for detection, prevention and control. This is important because up to 14% of bat roots were positive for NiV. The way forward will focus on diagnostics and surveillance at community level. It will also be important to understand the host and reservoir immune responses.

Abstract O-09

Surveillance of Nipah virus in Pteropus medius bats from different states of India, 2022-2024

Sreelekshmy Mohandas, Pragya Yadav, Anita Shete, Dilip Patil, Basavaraj Mathapati, Rima Sahay, Deepak Patil

ICMR-National Institute of Virology, Pune

Fruit bats of the genus Pteropus are known to be the natural reservoirs for the Nipah virus. India has experienced seven outbreaks of the disease in humans. We conducted a survey for the Nipah virus in the reservoir host, Pteropus medius, in different states of India like West Bengal, Assam, Bihar, and Meghalaya bordering Bangladesh, a country Nipah cases are reported annually and Uttar Pradesh, Goa, Maharashtra, and Madhya Pradesh states, which has not surveyed before and located far from the outbreak reported areas of India. With the requisite approvals, sixteen bat roost sites were identified for the cross-sectional study, and 461 Pteropus medius were sampled between 2022 and 2024. The swab (throat and rectal) samples collected from all the bats tested negative for viral RNA by the real-time RT-PCR, whereas one urogenital swab tested positive. The viral RNA was detected in the kidney and spleen samples of one bat from Binnaguri, West Bengal, and from the spleen sample of one bat from Patna, Bihar. Virus isolation from these samples was not successful. The whole genome could be retrieved from these samples and the sequences showed >97% identity with the NiV-Bangladesh genotype by phylogenetic analysis indicating persistent strain circulation. The bat sera samples were tested using an in-house enzyme-linked immunosorbent assay for IgG antibodies and further confirmed by a live neutralization assay. The seroprevalence estimated by ELISA was 23.5% in Meghalaya, 33.3% in Assam, 62.9% in West Bengal, 65.7% in Bihar, 8.3% in Goa, 25% in Maharashtra, and 46% in Uttar Pradesh. The seroprevalence could be detected in bats from most of the sites surveyed. The virus detection in Uttar Pradesh, West Bengal, and Bihar states and seroprevalence in juveniles indicated active virus circulation in the area. The surveillance suggested a broader geographical area in the country at risk for spillover and a need for strengthening human surveillance in the region.

Sreelekshmy presented data on surveillance of bats in India. The first NiV outbreak was in 2001, and the second in 2007. After nearly 20 years, another outbreak was detected in the south of the country. Initial surveillance data indicated more than 25% of bats surveyed had positive PCR or serology for NiV. A broader, countrywide survey was initiated in 2019-2020; but COVID interrupted the survey, which restarted in 2022. In all, evidence of seropositive bats was seen across 7 states and 7 sites, with two sites being seronegative. Seroprevalence ranged from 8.3 to 65.7%. Viral RNA in urogenital swabs or spleen samples was detected in 3 sites in north/eastern states. Virus isolation was not successful, but the whole genome was nonetheless sequenced

from those 3 samples, including the sequence with GenBank Accession number PP554504.[8] When asked about the age of the bats and especially those in areas with high seroprevalence, Sreelekshmy replied that all details, including age and sex, were recorded. There was a mix of ages, about 10-11 were juveniles from one location. When asked how many spleen samples had been tested, and if they were concordant with other samples, Sreelekshmy replied that she could only detect viral RNA in the samples she described; all others were negative. Only 10 bats were tested in parallel samples. When discussing behavioral determinants and date palm sap consumption, Sreelekshmy said habits are different if different parts of India, with date palm consumption being common in the North but not the South.

Abstract O-10

Serological evidence of emerging henipaviruses and paramyxoviruses in pteropodid bats in the Philippines; Ten (10) years after the 2014 henipaviral disease outbreak in Sultan Kudarat, Philippines

Mary Glazel J. Noroña1, Marana Rekedal2, Jaiure Azel Café1, Catalino Demetria1, Lianying Yan2, McKenna Roe2, Spencer Sterling2, Christopher Broder2, Kirk J. Taray3, Jezryl Jaeger L. Garcia3, Ricardo C. Buenviaje3, Joselito B. Sarmiento3, Wilson B. Bulalacao3, Jayson B. Bulalacao3, Ronnel R. Tongohan1, Jozette Hisu-an1, John Christian Teñoso1, Paulina Alexandra C. Nuñga1, Jester B. Galaraga1, Samantha Lian Magsanoc3, Hazel Ruth Cruz3, Sophie Allison Borthwick4, Dolyce Hong Wen Low4, Liesbeth M. Frias4, Phillip Alviola3, Mary Grace Dacuma3, Fedelino F. Malbas1, Gavin James Smith5, Eric Laing2

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Background: In 2014, outbreak of henipaviral disease occurred in Sultan Kudarat, Philippines. Flying foxes are the presumed wildlife host for NiV and horses as intermediate host,consistent with the transmission chain of the close relative Hendra virus in Australia. Methods: We sampled five species of pteropodid bats, including flying foxes (Pteropus vampyrus and hypomelanus, Acerodon jubatus), rosette bats(Rousettus amplexicaudatus),and dawn bats (Eonycteris speleae, Eonycteris robusta),native to the Luzon Island, Philippines. Monthly collection of sera samples for one year from July 2023 were tested by a multiplex microsphere-based immunoassay for immunoglobulin(Ig)G reactivity against a panel of five henipaviral glycoproteins(Nipah, Hendra, Cedar, Ghana,and Mojiang virus)and three related paramyxoviral receptor binding proteins(Sosuga, Yeppoon, and Grove virus).IgG levels were detected via Luminex xMAP-based technologies, reported as a median fluorescence intensity (MFI).Seroprevalence was estimated using a combination of principal component analysis, k-means clustering, and latent cluster analysis to identify major antigen targets and subsequent MFI cutoff. Results: Serologic evidence of NiV was predominantly detected in flying foxes, and we estimated a total NiV seroprevalence

8 https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2024.1493428/full

of 13.1%(42/320),providing the first indications of NiV circulating in flying fox hosts in the Philippines. Serological evidence of Asiatic paramyxoviruses most closely related to Sosuga, Yeppoon, and Grove virus in flying foxes and the rousette bats. Interestingly, dawn bats did not have significant IgG reactivity against paramyxoviruses in comparison to the other sampled species. . Conclusions: Our findings support the current canon that flying foxes are the primary hosts and likely reservoir for NiV, with history of its presence identified in Philippines' flying foxes. Presence of paramyxoviruses in native pteropodid bats indicates further biosurveillance efforts will need to be conducted in the Philippines.

Mary presented surveillance data on flying foxes in the Philippines. She started by recalling the 2014 henipavirus outbreak, in which the intermediate host was presumed to be a horse linked to horsemeat consumption. There were 17 human infections and 9 deaths. Back then, the initial diagnosis was acute encephalitis. For ten years, no one investigated the outbreak further; but a current collaboration has allowed that outbreak to be further investigated through PHILBATS and the Department of Health Research Institute for Tropical Medicine, the reference laboratory. Various institutions are now collaborating to do bat sampling, human and livestock sampling, nucleic acid and serology, statistical modelling, community engagement, etc. This has been a One Health approach to surveillance with human and livestock data. For the more recent surveillance studies in fruit bats, a MAGPIX platform was created to screen for glycoproteins from 8 henipaviruses: NiV, HeV, CedV, GHVm MojV, SOS, YepV and GroV. The main questions for the study were: Is NiV actively circulating in native flying foxes on luzon island? What other zoonotic paramyxoviruses are present in native frugivorous bats? What are the factors associated with having an anti-NiV antibody response? The sero reactive profile of the bats was collected in the field and only Nipah antibodies have been found thus far in flying foxes. There was a lack of neutralising antibodies in rosesstus species. Mary concluded that NiV is likely circulating only in native flying foxes. Her team will next assess the epidemiological factors associated with seropositive data. When asked if she had observed any cross reactivity to other henipaviruses with the multiplex serological assay using the G protein, Mary said yes; she has observed this and is currently following up with additional testing for confirmation.

Abstract O-11

Nipah Virus Seroprevalence among Encephalitis Patients in Thailand 2020 – March 2024

Chayanit Mahasing, Lilawan Sukkho, Pawinee Doungngern, Spencer Sterling, Eric D. Laing, Sasiprapa Ninwattana, Supaporn Wacharapluesadee

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Background: Nipah virus (NiV) is a zoonotic pathogen known for causing severe encephalitis in humans. While its presence in bat populations in Thailand has been documented, no human cases have been reported to date. This study aims to evaluate Nipah virus serology in encephalitis patients who tested negative for other pathogens by PCR from 2020 to March 2024.

Methods: Samples were obtained through a national laboratory sentinel surveillance that involved 23 hospitals in all regions of Thailand. For serological testing, we selected specimens

with sufficient leftover material and available epidemiological data. These specimens were tested for antibodies against the Paramyxoviridae family, including Nipah virus, using the Henipaviral Multiplex Microsphere Immunoassay (MMIA).

Results: Among 481 samples from 478 encephalitis patients, the median age was 11 years (IQR: 1 year 7 months – 47 years), and most patients were Thai presenting with fever, seizures, and altered consciousness. One patient, a 60-year-old male with no history of bat contact or large bat populations in his village, tested positive for both Nipah virus IgM and IgG. The specimen was collected 12 days after symptom onset, and the patient died 19 days post-onset. Additionally, serology results showed 17.67% positivity for Mojang virus IgM and 24.12% for IgG, as well as 8.73% and 12.06% positivity for Gamak virus IgM and IgG, respectively. One patient was positive for Hendra virus IgG without corresponding IgM positivity.

Conclusion: None of these viruses have previously been associated with human illness in Thailand. Therefore, this study provides new insights into the circulation of these viruses in the region and underscores the need for continuous surveillance and further investigation.

Chayanit presented surveillance data from encephalitis patients in Thailand. No human cases of henipaviruses have been reported before, and the cases are notifiable under the Thailand Communicable Diseases Act. There is also a cerobrospinal fluid (CSF) surveillance system, in which approximately 300-500 samples are sent for PCR testing each year, mostly from patients with acute encephalitis who test negative for other pathogens. For this study, serum samples were collected from January 2020 to March 2024, and screened for IgM and IgG vs henipaviruses and paramyxoviruses, including NiV, HeV, Gamak, Cedar, Mojang. In preliminary findings, out of 481 samples collected form 478 encephalitis patients, four were antibody positive. Data was cross-referenced with epidemiological and clinical data. A 60-year-old male patient who had repeating seizures showed a significant rise in NiV IgM and IgG and no positive results for other paramxyoviruses. Confirmatory PCR did not detect virus in this patient. The patient had had no contact with bats, pigs, or horses; and he had not traveled outside of Thailand. There was also no epidemiological linkage to a 47-year-old female who was also NiV positive. Other pathogens were detected, with a range of positive rates. Chayanit mentioned there was potential cross reactivity between antibodies. She ended her presentation saying the study helps to identify gaps in surveillance, leading to improvements in diagnostic processes, and the contribution to global knowledge. When asked, Chayanit said PCR is included in encephalitis surveillance since 2014 and serum is the main sample. When asked about the cut-off values for seropositive results, Spencer, a co-author of the study, replied they used a cluster approach for cutoffs, as they had no confirmed infected sera to distinguish the negatives from the positives.

Abstract O-12

Henipavirus Dynamics in Rousettus Bats from South Africa: Exploration of Serological Patterns and Seasonal Variations

Marinda Mortlock1, Marike Geldenhuys1, Erika Strydom1, Neil Mittal2, Christopher Broder2, Lianying Yan2,3, Mckenna Roe2, Eric Laing2, **Wanda Markotter**1

1Centre for Viral Zoonoses, Department of Medical Virology, University of Pretoria, RSA; 2Department of Microbiology and Immunology, Uniformed Services University, USA; 3Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., Rockville, MD, USA.

Emerging zoonotic viruses, particularly wildlife-borne henipaviruses, are a significant public health concern due to their pathogenic potential and absence of effective countermeasures. Recent biosurveillance efforts by our group have identified over 18 putative henipa-like viruses in a population of Rousettus aegyptiacus (Egyptian rousette bats) in South Africa, with virus detection occurring during winter and again in spring, suggesting seasonal shedding dynamics. This study incorporated longitudinal serological surveillance to further detect and identify the transmission dynamics of these novel henipaviruses. Using an antigen-based multiplex serological test, we analysed 991 serum samples collected monthly throughout 2022. Overall, 26% of the sampled bats exhibited seropositivity to at least one henipavirus antigen, while 6% demonstrated reactivity to more than one – highlighting the potential for co-circulation of antigenically diverse henipaviruses. Seropositivity remained below 2% during the first four months of the year and increased following the previously reported winter excretion peak. There was also evidence of a Mòjiāng-like virus in 20% of bats exhibiting unique serological dynamics with three distinct peaks throughout the year, ranging between seropositivity of 15% and 30%, as well as seropositivity to some uncharacterised henipaviruses (reactive to Ghana virus and Angavokely virus). These results demonstrate a seasonal pattern of henipavirus presence in Rousettus aegyptiacus. Detection of distinct serological peaks, especially for the more diverse Mòjiāng-like virus, suggests that complex interactions between various (uncharacterized) henipaviruses may be occurring within this bat species. These findings highlight the importance of biosurveillance to better understand the epidemiology of henipaviruses. The observed cross-reactivity raises further questions about virus co-circulation and potential antigenic evolution within bat populations.

Wanda presented bio-surveillance data from Rousettus bats in South Africa. She acknowledged that most of the work being presented was carried out by Marinda Mortlock, who was unable to travel to the conference. Wanda started by pointing out that there may be inherent biases in studying the biggest fruit bats, because it is easier to get samples from them, compared to the smaller frugivorous bats. She also pointed out there are only 2 full genomes of henipaviruses from Africa. In the continent, there is limited evidence for spillover, but it could be that it is not being actively investigated. The African focus has been on the Egyptian fruit bat, Rousettus aegyptiacus, a cave-dwelling fruit bat, well known to be a morbilliviruses vector, but also a vector for paramyxoviruses. For the study, Wanda's team picked a representative site, one where there was a lot of unfenced livestock moving in and around people. One can often see bats that feed in this area, especially around the conservation area and the expansion of human areas. Wanda's research approach was molecular and based on very short, conserved areas of the genome. There is a high diversity of paramyxoviruses and her team has identified 18 putative viral species-Henipavirus and related clades. In South Africa, bats have only one birthing pulse, different from northern Africa, where there are two birthing pulses. Recolonisation of roosts and late gestation happens in early spring. Wanda's question was: Why do we see the peaks at this time of the year? No evidence of parahenipahviruses was found; but this was expected, as parahenipahviruses are associated with rodents. There was a whole load of diversity that was henipah/parahenipah-related viruses, but uncharacterized. Seropositive bats showed unusual cross reactivity with several antigens; it was unexpected that they had this cross-reactivity with Mojiang-like in 20% of bats. It is possible that the correct antigens are not being used, which is impacting the teams' interpretations. The team also saw no serology in the beginning of the year. When overlaying the

data with the aging of bats, the team saw some serology in adults and not in juvenile bats. It is possible that whole diversity is not being picked up in serology. When asked if the team had the diversity data for each cave, Wanda replied that the team works with certain caves in Africa that do have a mix of bats. When asked if there was an opportunity to sequence the full genome, Wanda said yes; they are trying very hard to do this. When asked if the team is trying to look at the patterns of reactivity, Wanda said they have not looked but would want to.

Abstract O-13

Mojiang like henipavirus exposure to humans in rural communities in Bangladesh

Ariful Islam1,2*, Sarah Munro2, Shusmita Dutta Choudhury3, Morgan Kain2, Mohammad Enayet Hossain4, Spencer Sterling5, Abdul Khaleque Md. Dawlat Khan3, Emama Amin3, Monjurul Islam 3, Arif Khan3, Nabila Nujhat Chowdhury3, Sharmin Sultana3, Eric D. Laing5, Maryska Kaczmarek2, Md Ziaur Rahman4, Tahmina Shirin3, and Jonathan H. Epstein2

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The novel Langya henipavirus spillover was reported in humans in China, and the Langya-like virus detected shrews in Bangladesh highlighting the significance of novel henipavirus surveillance. Hence, we conducted serology-based biosurveillance to assess whether, in addition to NiV, other novel henipavirus strains had already spillover to humans in Bangladesh. Serum samples from 1320 humans were tested by a multiplex microsphere-based immunoassay for immunoglobulin (Ig) G reactivity against soluble envelope glycoprotein (GP) ectodomain trimers of henipavirus. Antigen-antibody complexes were detected via Luminex Bio-Plex-based technologies, with IgG levels reported as median fluorescence intensity (MFI). We explore risk factors associated with higher MFI values as a method of investigating higher exposure. A linear mixed-effects model was used to explore risk factors associated with higher MFI values. We selected participant sex, occupation, district, age, and self-reported rodent exposure for inclusion in the linear regression model. In the model, age and occupation were both significantly associated with MFI. On average, for every one-year increase in age, we observe a small increase in Mojiang reactivity. An increase in reactivity by age could indicate l long-lasting immunity to Mojiang virus. Likewise, participants who reported working in an agricultural occupation were associated with higher Mojiang reactivity compared to all other occupational categories. Rodents are identified as putative reservoir for Mojiang virus, so this observation may be a result of increased occupational exposure to rodents or their excreta in agricultural industry. Our findings indicate a substantial exposure to Mojiang-like henipaviruses in the population, with agricultural work and age being key risk factors. The study highlights the need for further targeted surveillance and detect viruses, as well as more refined behavioral risk assessments for henipavirus exposure in Bangladesh.

Ariful presented One Health surveillance data from Bangladesh. In one study, broad-scale surveillance was conducted in shrews and rodents over 4 years (2016-2019), sampling 2 years during wet and dry seasons, at a rate of 200 rodents/year. Novel paramyxoviruses were found. Next, serology-based surveillance was conducted to assess if henipaviruses other than NiV had spilled over to humans. 6 sites were chosen and reactivity to Mojiang-like viruses was found.

Occupation was a factor associated with reactivity, as agriculture stood out as 1,83 times higher odds of having increased reactivity. The team is now looking through archived samples to investigate further. When asked about the size of the sample being surveyed, Ariful replied the total population was 1300 participants, with roughly 300 per site. When asked if he had looked at the densities of the rodent population, Ariful said yes; there is a system to determine this. When asked if Mojiang virus was a significant cause of pneumonia, Ariful said the influenza-like symptoms were the most common.

Behavioural determinants of henipavirus transmission

Chairs: Stephen Luby and Ariful Islam
Tuesday 10th December, 11 AM – 11.45 AM

Abstract O-14

Measuring incidence and investigating pathways of henipavirus transmission from bats to livestock and peri-domestic animals in Bangladesh

Emily Gurley, Ausraful Islam, Clif McKee, Rebeca Sultana, Enayet Hossain, Ziaur Rahman, Eric Laing, Spencer Sterling

Johns Hopkins Bloomberg School of Public Health, icddr,b, Uniformed Services University

Nipah virus can infect a wide variety of new hosts, including humans where outbreaks are amplified by superspreading events. Spillovers into humans are reported almost yearly from Bangladesh and Kerala, India since 2018. Human infections have resulted from consumption of contaminated date palm sap in Bangladesh, and from contact with sick pigs in Malaysia and Singapore, and from contact with sick horses in the Philippines. More spillovers have been reported from Bangladesh than anywhere else in the world, resulting in a case fatality ratio of >70%. Although there is some evidence that other species may also be infected with Nipah in Bangladesh, the frequency and mechanisms of transmission are poorly understood. In January 2023, we began a five-year study to characterize Nipah virus spillovers into livestock and peri-domestic animals in Bangladesh using serologic cohorts. Across our 8 study communities, there are ~6800 residents raising ~4000 cattle, goats and pigs, living around two large Pteropus medius bat roosts. Hundreds of domestic cats and dogs roam the study area. All households reported growing fruit on their household premises, and 25% reported that their animals scavenge for fruit on the ground; 5% reported that their animals scavenge for food underneath bat roosts. Households that feed their animals dropped fruit were more likely to report that their animals were sick in the past month compared to households that did not report this practice (23% vs 18%, <0.031). We will present serologic findings from the baseline cohort and analyses from our sick animal surveillance, as well as other late-breaking results from the study.

Emily presented data on Nipah virus transmission from bats to livestock and peri-domestic animals in Bangladesh. The underlying questions of her research were: how often are domestic and peri-domestic animals being infected with NiV; and how are they being infected? To answer this, she first assembled a multidisciplinary, One Health approach team. Her team was initially looking for sick animals but was not seeing them in Bangladesh and India. A more targeted

approach was for the team to test thousands of animal samples whenever a human case was identified. Such animals included cats, dogs and livestock (i.e. domestic and peri-domestic animals) near 6 bat roosts. The team used the multiplex assays and started enrolling cohorts to move beyond cross sectional studies. In cohort studies, one can look at seroconversion rates over time. Year 1 data presented in Emily's talk was the cross-sectional, basal serology data from the cohort study in humans. The team collected samples from bats as well as samples from under the roost each month. One hypothesis is that animals have either contact with bat urine or are scavenging placentas from bats. I-gotU animal tracking loggers were used to obtain GPS data. Motion red cameras were also used to monitor bats roosting as well as feeding data from domestic and peri-domestic animals. Data revealed that pig herds are moving all around, with the occasional Asian palm civet and jackals being caught in camera. An Epicurve of sick animals revealed that many pigs got swine pox. There was also a cluster of cat deaths last winter. Mojiang virus was also detected. Two goats were found to be positive for NiV antibodies. So this approach is detecting spillovers. In summary, Emily said that scavenging roosts increases the animal's chances to catch NiV. The team is currently conducting a review for all bat viruses. When asked if the age of bats was recorded, Emily said yes; all ages were recorded.

Abstract O-15

Assessing the risk of bat-borne pathogen emergence from hunting Indian Flying Fox (Pteropus medius) at high- risk interfaces in Bangladesh

Shusmita Dutta Choudhury1,3, Ariful Islam1,2, Shahanaj Shano1,3, Emily Hagan1, Melinda K Rostal1, Sarah Munro1, Ava Sullivan1, Tahmina Shirin3, Leilani Francisco1, Meerjady Sabrina Flora3, Peter Daszak1, Jonathan H Epstein1

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Bats are known as the main reservoir of various emerging zoonotic viruses. Close contact with bats, including shared food resources, hunting, butchering, trading, and meat consumption can lead to disease transmission in humans. There are limited studies on bat hunting, trading, and consumption patterns at the community level in Bangladesh. Hence, this qualitative study aimed to understand hunting behavior, wildlife consumption patterns, and wildlife value chain structures assessing the risk of zoonotic disease transmission in different communities of Bangladesh.

Between 2015- 2018, we conducted participant observations and 44 ethnographic interviews among wildlife hunters, transporters, vendors, and consumers in three districts of Bangladesh. Interview data were coded using computational data analysis software (MaxQDA), and emergent themes were identified using a modified grounded theory approach.

Hunting wild animals is a traditional practice, a key protein source, traditional medicine, alternative livelihood, and pest control in these communities. Hunters are mostly illiterate and unaware of zoonotic disease risks. None of the hunters use protective equipment. Men were involved in hunting whereas women were primarily involved in butchering. Sometimes children handled and played with hunted bats. Communities with religious prohibitions were reported to be involved in hunting and consumption of bats. Participants were observed to use bat bones to

remedy joint pain and asthma. Hunters reported declining local bat populations due to over hunting and deforestation.

Hunting and interactions with bats may represent a previously unrecognized pathway for zoonotic virus emergence in Bangladesh. Unprotected hunting practices and limited or no hygiene measures can yield a greater risk of zoonotic disease spillover. However, successful mitigation and reduction in hunting strategies in communities need sustainable alternative livelihood opportunities and protein sources.

Shusmita presented data on observations and ethnographic interviews related to the hunting of flying foxes in Bangladesh. She mentioned that most of the interview participants have a range of other occupations, as bat hunting is seasonal. Bat hunters use bare hands and club the head. Hunting is mostly done in winter to avoid hunting pregnant and newborn bats. A key question was why they were hunting the bats. Answers varied, from it being a traditional activity passed on from their ancestors, to selling the bats as income, to using the bats for medicinal purposes (cure asthma, arthritis and rheumatic fever), to do pest control, to consume their meat, to trade them, etc. Observational data revealed that: children are exposed to the bats, playing and in market settings; dogs eat improperly disposed bat meat remnants; if people get a bat bite, they are not likely to go get treated. Whilst more health education is taking place across communities, deforestation keeps affecting bat populations. In summary, Shusmita said the described exposure routes were not as common previously; therefore, behavioural determinants are changing and this is being explored further. When asked if certain communities were more exposed than others, Shusmita said yes; the communities exposed are those with more hunting. When asked if there was an opportunity for hunters to wear PPE and/or if it was accessible, Shusmita said the PPE is available but the hunters choose not to wear it.

Risk of Nipah virus transmission through date palm sap trade, Bangladesh

A K M Dawlat Khan1*, Ariful Islam2,3, Sarah Munro2, Pronesh Dutta1, Shusmita Dutta Choudhury1, Md. Zulqarnine Ibne Noman1,4, Nabila Nujhat Chowdhury1, Maryska Kaczmarek2, Tahmina Shirin1, Jonathan H. Epstein2

1Institute of Epidemiology, Disease Control and Research (IEDCR), Bangladesh; 2EcoHealth Alliance, New York, NY, United States; 3Biosecurity Research Program and Training Centre, Gulbali Institute, Charles Sturt University, Wagga Wagga, NSW-2678, Australia; 4Infectious Diseases Division (IDD), icddr,b, 68, Shaheed Tajuddin Ahmed Sarani, Mohakhali, Dhaka 1212, Bangladesh

Nipah virus (NiV), an emerging zoonotic virus poses a significant pandemic threat to humans, primarily transmitted through consumption of bat-contaminated raw date palm sap (RDPS). Despite its regional threat in South and Southeast Asia, there is limited research on RDPS harvesting and trading practices in Bangladesh, where nearly annual NiV outbreaks have been occurring since 2001. A qualitative ethnographic study was conducted in two NiV recurrent outbreak districts in Bangladesh to understand RDPS harvesting, trade, and the risk of NiV spillover at the community level. We conducted participant observations (n=14) and ethnographic interviews (n=31) with RDPS collectors (gachis) to gather data in February 2021 and March 2022. Data analysis followed a grounded theory approach, identifying themes related to RDPS harvesting and trading. Gachis predominantly sell RDPS to local communities but also distribute to non-harvesting areas through middlemen or social media, highlighting the economic

incentives driving RDPS trade beyond simply a local cottage industry. Our observations and participant reports revealed that fruit bats and rodents visited the trees and drank and contaminated RDPS with their saliva, urine, and feces. A few gachis reported knowledge of NiV and used protection specifically to prevent exposure to NiV from drinking raw sap. Gachis prefer to use non-conventional protective apparatuses like jute bags, plastic bags, polythene, and nylon nets due to the time and resource constraints to protect bats' and rodents' access to RDPS trees. The study underscores the importance of understanding RDPS distribution and trading networks in reducing the risk of NiV transmission. A culture-sensitive intervention with educational outreach programs and alternative economic supports for RDPS collectors, and practicing safe RDPS harvesting and efficacy tests of protective measures, can aid in preventing spillover of NiV and other bat-borne emerging viruses in Bangladesh.

AKM Dawlat presented data from observations and ethnographic interviews with *gachis*, the Bangladeshi traditional collectors of raw date palm sap (RDPS). He noted that electronic commerce is increasing; so the trading is starting to go further by social media and the product may be frozen before distribution. Dawlat also mentioned that many *gachis* do not know about NiV. There are several ways to get the RDPS and protect it from bats, like using nets covering the collection container, etc. *Gachis* do get exposed when collecting the liquid, and prefer to use plastic bags to store it and transport it. The government has implemented signage campaigns to warn people; but in Dawlat's opinion, they aren't working well.

Diagnostics

Chairs: Liyen Chang and Gervais Habarugira
Tuesday 10th December, 11.45 AM – 12.45 PM

Abstract O-17

Applying the SCAHLS Recommended Diagnostic Test Validation Pathway: A Novel Approach for Validating Point-of-Care Hendra Virus Assays

Lyndal Hulse, Leonard Izzard, Nagendrakumar Singanallur Balasubramanian, Darren Underwood, Luke Driver, David Williams, Axel Colling, Ben Ahern

School of Veterinary Science, The University of Queensland, Gatton QLD, Australian Centre for Disease Preparedness, CSIRO, East Geelong VIC, Biosecurity Sciences Laboratory, Department of Agriculture and Fisheries, Coopers Plains, QLD, World Organization for Animal Health Collaborating Centre for Test Validation Science in the Asia-Pacific Region

Equine veterinarians and stakeholders face significant risks from potential Hendra virus (HeV) infection when in close contact with horses. HeV is a highly lethal zoonotic pathogen, making rapid, reliable point-of-care (POC) diagnostic tools essential for infection control or exclusion during clinical evaluations. Currently, the standard detection method is laboratory-based RT-PCR, requiring trained molecular professionals and regulated sample transport, often delaying results by days. This delay can impact welfare, as rapid diagnoses are critical in low-risk cases. POC platforms such as LAMP or portable RT-PCR could address this need.

This study pilots a diagnostic assay validation template developed by the Australian Centre for Disease Preparedness (ACDP) and Agriculture Victoria. Using the SCAHLS-recommended validation pathway, we evaluated two POC platforms for HeV detection: DARQ RT-LAMP and real-time RT-PCR, focusing on analytical sensitivity, specificity, and practical field application.

The HUDSON sample preparation method (heating to inactivate nucleases) was optimized to inactivate the virus while preserving RNA integrity, reducing exposure risk. DARQ RT-LAMP demonstrated rapid detection but lacked sufficient sensitivity (1,000 copies/µL) for clinical use, while real-time RT-PCR showed superior sensitivity (1 copy/µL) and diagnostic accuracy, making it more suitable for field diagnostics.

This study provides equine veterinarians a critical resource for prompt HeV diagnosis, reducing exposure risk to both horses and humans. Future work will seek regulatory approval and industry support for widespread adoption, enhancing biosecurity and protecting the health of animals and humans alike.

Lyndal presented data on two assays and on their potential feasibility as POC diagnostics tools. She started by mentioning that vaccination rates in horses have gone down about 17% since 2017. Approximately 500,000 potential cases are examined each year, with roughly 1,000 exclusions tests being performed annually at BSL-4 facilities in Queensland. Many of these are from the Vet School, meaning the potential ex (?). Due to delays and the complexity and availability of testing, most potential cases are not being excluded. This results in horse welfare issues for unvaccinated horses because vets must wait for the lab to test for Hendra to be excluded before releasing horses from quarantine. In some remote areas, testing may take up to a week. Hence the need for Hendra POC to reduce turn around times, aiding equine vets in screening and improving horse welfare. The Loop-mediated isothermal Amplification (LAMP) method was compared against the RT-PCR method. While the former usually yields results in 30 minutes, the latter may take a few hours. Large collaborations were necessary to get the assays up and running. Test samples were processed through the HUDSON (heating unextracted diagnostics samples to obliterate nucleases) sample preparation method that can potentially be done in the field. Assay validation was done using Vero cells; the range of samples swabs and viral transport media. Samples were run either in a Genie III (real time PCR) or a CART RT LAMP. Whilst the the sensitivity of the real time PCR was good, the DART RT LAMP assay was not as sensitive as desired and was deemed unfit for purpose.

Abstract O-18

Development of Hendra virus diagnostic serology assays at ACDP

Leanne McNabb, Antonio Di Rubbo, Jennifer Barr, Amy McMahon, Ezana Woube, Tim Bowden, Kalpana Agnihotri, Axel Colling, Nagendrakumar Singanallur Balasubramanian, Kim Halpin.

CSIRO Australian Centre for Disease Preparedness

The CSIRO ACDP routinely receives specimens for Hendra virus (HeV) exclusion and diagnosis. Clotted blood/serum, cerebrospinal fluid (CSF), swabs and tissue samples from horses or other species are typically submitted for agent and antibody detection using molecular methods, serological assays and virus isolation. HeV serology is the prevalent activity undertaken for surveillance, compliance with the certification requirements for the international movement of horses and confirming their vaccination status. The initial development of an indirect ELISA using crude HeV antigen resulted in many non-specific reactions that required confirmatory testing using the virus neutralisation assay conducted at biosafety level 4 containment. With the production of a recombinant HeV soluble G (sG) protein, an indirect sG ELISA was developed, which is part of the LEADDR program and used by the State Laboratories around Australia. To

improve the sensitivity and specificity of HeV antibody detection, a multispecies competition ELISA, which uses a monoclonal antibody directed to the sG protein, was developed and validated at ACDP. Additionally, Luminex-based indirect antibody assays using HeV sG and Nipah virus (NiV) sG proteins coupled to magnetic beads were developed for antibody detection and differentiation of henipavirus infections. To assist in the acute phase of an infection, a HeV IgM ELISA was developed using a recombinant N protein for early detection of antibodies. With the release of the commercial HeV Equivac vaccine (Zoetis), which contained the same HeV sG protein as used in all serology tests, this significantly complicated the result interpretation. Thus, using the sG protein in combination with the N protein in a single assay, a HeV DIVA ELISA was established and has recently been validated at ACDP. This HeV DIVA ELISA can serologically differentiate between HeV-infected and vaccinated animals and is a major development which fills an important gap in HeV serology.

Leanne gave an overview of HeV serology assays and presented the rationale behind the need for a DIVA assay. She began by mentioning that HeV virus neutralisation assays require live virus and PC4 facilities. In addition, the assay can present readability issues due to toxicity of the virus on Vero cells. Hendra indirect ELISAs can be performed in PC3 conditions, but must have a long set up time, and a number of non-specific antigens that need PC4 VNT validations. Alternatively, Henipavirus Luminex microsphere immunoassays can be done in a single well and in PC3 facilities, published in 2014.[9] Further, a Hendra soluble G indirect ELISA was rolled out within LEADDR program and State labs around Australia, but can only be used for horses. In collaboration with a Lithuanian lab, a Hendra IgM MAC ELISA using the HeV N protein has also been developed. Because the Equivac vaccine also contains the soluble G protein used in serology tests, the design of a DIVA assay became necessary after the vaccine became available. The Hendra DIVA ELISA is a competitive ELISA that uses both the recombinant sG and N proteins and two different monoclonal antibodies. This is especially useful when vaccination status is either not readily available or unknown. Analytical specificity and sensitivity is good- the G protein is 32 times stronger than the N protein when testing serum from Hendra vaccinated horses. The DIVA assay is being further validated with 1138 Hendra samples from truly unvaccinated horses. When asked, Leanne clarified the DIVA test is 2 ELISAS on one plate. When asked if the assay is fully validated, Leanne said that it was and that it will be rolled out soon. A member of the audience commented this is highly appreciated by the vet field.

Abstract O-19

The Hendra virus nucleocapsid protein as a frontline diagnostic tool to confirm infections

Kalpana Agnihotri, Amy McMahon, Leanne McNabb, Jianning Wang, Brenda van der Heide, Anthony Keyburn, Kim Halpin

Australian Centre for Disease Preparedness (ACDP), Commonwealth Scientific and Industrial Research Organisation (CSIRO), Geelong, Australia

9 https://www.sciencedirect.com/science/article/abs/pii/S0166093414000202?via%3Dihub

Numerous molecular and serological assays are routinely employed in laboratory investigations to confirm Hendra virus (HeV) infections in horses. While serology tests exhibit broad cross-reactivity and are less sensitive to genetic variations, the kinetics of antibodies for henipavirus infections remain incompletely understood. Over recent years, the sporadic horse cases reported from eastern Queensland (QLD) to eastern New South Wales (NSW) have gradually extended the southern boundary of detections. These incidents correlate with temporal and spatial distribution of the flying-fox species emphasizing the geographical expansion of bat-to-horse infections. Early detection of HeV infection is critical in the non-endemic areas of Australia. The confirmation of HeV infection in a horse from Cardiff Heights, NSW stands as the most recent southernmost infection confirmed through a combination of various diagnostic tests. This incident has spurred further assessment of the nucleocapsid (N)-based HeV IgM and DIVA ELISAs in sera from experimentally infected and vaccinated horses. The early antibody response to HeV N protein in infected horses holds promise as a frontline diagnostic tool for detecting HeV infections.

Kalpana presented a recap of Hendra incidents in 2008, 2011 and 2022. Henipavirus nucleoprotein/nucleocapsid (N)-based serology has a diagnostic sensitivity of 100%. A case study of an unvaccinated 24-year-old male horse from Cardiff Heights NSW was presented. The horse had confirmed HeV genotype 1, about 30 Ct value; the whole HeV genome was sequenced. There is a need for ongoing validations with N-based serology.

Abstract O-20

Viral discovery using agnostic VirCapSeq-VERT platform in patients with unexplained Nipah-like illnesses

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One hundred thirty-six patients with Nipah-like symptoms were enrolled through national Nipah surveillance platform in Bangladesh between late-December 2022 and early-April 2023 but tested negative in lab for Nipah virus (NiV) exposure. Throat-swabs from these patients were used for viral discovery using VirCapSeq-VERT (VCS), an agnostic capture sequencing platform for detection of vertebrate viruses. VCS also provides a 100-1000 folds increase in sensitivity than unbiased metagenomic sequencing, at a lower cost, in a shorter time and with a higher genomic coverage. VCS analyses revealed presence of wide range of common human respiratory viruses

including; Adenoviruses, Coronaviruses, Rhinoviruses, Enteroviruses, Metapneumoviruses, Herpesviruses, and Parvoviruses etc.

However, in 5 of 136 patients Pteropine orthoreovirus (PRV) was identified. The PRV positive throat-swabs were also used for virus culture in MDCK and passaged in Vero-E6 cell-lines. PRVs were cultured from 3 throat-swabs, and 2 throat-swabs failed in culture due to low viral-load. All segments from 3 PRVs showed 93.7%-100% nucleotide identity with each other. Phylogeny revealed different segments of Bangladesh PRVs are clustered with different bat and human PRVs from South East Asia and Africa. All patients lived within 25-250 kilometers radius of central Bangladesh and had no known contact with each other. All five had consumed date-palm sap 3-14 days before developing acute symptoms and recovered after 2-3 weeks of hospitalization. During a follow-up in November 2024, one 56-year-old male died in October 2024 with lingering neurological implications, and a 65-year-old male reported persistent fatigue, and difficulty in walking. The other three recovered without apparent sequalae.

PRVs are emerging bat-borne viruses previously linked to sporadic acute respiratory infections in humans, especially in Southeast Asia. PRV infections may present with symptoms similar to those observed with NiV infection and PRV infections may be linked to consumption of date-palm sap contaminated with bat-excreta. VCS platform can be used for pan-viral surveillance and differential diagnosis of respiratory illnesses with encephalitis, and other unexplained febrile illnesses in areas where date-palm sap is routinely consumed.

Nischay presented data on the VirCapSeq platform to test for unknown viruses in human samples. He mentioned issues using next gen sequencing in the clinic or field, plus the complexity of bioinformatics and poor sensitivity due to background. There have been improvements to sequencing methods by introducing the design of probes. The VirCapSeq-VERT platform uses 2,100,000 probes, now cut down to 900,000 with reduced diversity. This results in 100-1000 fold reads increase compared to traditional metagenomics methods. Studies in the clinic have been completed using this platform to detect a range of viruses, including: rotaviruses, coronaviruss, astroviruses, JEV, enteroviruses, Herpes viruses, etc. and even for bacterial capture sequencing. Nischay described a pilot project for viral discovery among patients with unexplained acute respiratory cases that are NiV-like symptoms. As expected, he found a range of respiratory viruses described in the abstract, including PRVs, which stood out. Most viruses were segmented viruses and he could get all segments for those investigated. Looking further into the phylogeny, some viruses were closer to bat virus sequences than to human virus sequences. There were also unstable constellations. Work in progress includes the design of high throughput serology using printed peptides like the microarrays for dengue. When asked if the objective was to establish causality, Nischay mentioned that 2 or 3 viruses are usually found through a combination of sequencing and serology. When asked if it was unexpected to see some viruses, he said it was more of a clinical question.

Virology and Immunology Session 1

Chairs: Vincent Munster and K9 Jenns
Tuesday 10th December, 1.45 PM – 3 PM

Abstract O-21

Investigating functional diversity of the Hendra virus genotypes

Melanie Tripp1,2, Sarah J. Edwards1, Cassandra David2, Stephen Rawlinson2, Kim Halpin1, Glenn A. Marsh1, Gregory W. Moseley2

1CSIRO-Australian Centre for Disease Preparedness; 2Monash University

HeV comprises at least two genotypes (HeV genotype 1 and the recently defined HeV genotype 2) (Wang et al., 2021). Amongst the viral proteins, P, V and W show the greatest sequence divergence, suggestive of potential functional differences. Along with P being critical in viral replication, P, V and W have various innate immune evasion mechanisms including inhibition of the Type 1 interferon (IFN) response which is considered a key pathogenesis factor (Audsley & Moseley, 2013). Analysis and comparisons of the Hendra genotypes in these areas, which may have significant impact on viral infection and disease, is lacking.

Here, we examined the capacity of P, V and W to antagonize IFN induction and signalling pathways and associated subcellular trafficking of P/V/W. Using protein expression and viral infection assays, we found that the capacity to antagonize immune signalling is broadly conserved, but significant differences in the extent of antagonism of IFN production differs. Intriguingly, this correlated with altered capacity for nucleocytoplasmic trafficking. Furthermore, viral growth kinetics also appear to differ. Together these data suggest that pathogenesis may vary between the HeV genotypes.

These data provide the first indications that sequence differences in the P gene of genotypes of a henipavirus result in altered function, including in immune evasion/replication. Ongoing research is addressing the potential relationship of these functional differences, which is important to understanding risks associated with outbreaks of different genotypes. Additionally, this work allows examination of potential conserved functions which is useful for identifying potential drug targets.

This was a recorded talk in which Melanie presented data on the effect of P, V and W Hendra proteins on IFN induction and cell signalling pathways. The cell lines used were Vero, HeLa and PaKi. For the cell signalling inhibition assays, she found that STAT1/2 and STAT4 inhibition was conserved, with no significant differences between the genotypes. MDA5 induced activation was also conserved across genotypes. For the IFN induction, however, HeV-g1 proteins showed consistently stronger inhibition than HeV-g2, with associated changes in the proteins themselves. When proteins were tagged with GFP, proteins from HeV genotype 1 were more cytoplasmic, whilst those from genotype 2 were more localised in the nucleus. Overall, the data match observations of genotype 1 having increased replicative capacity than genotype 2. Ongoing experiments include: the design of V protein chimeras to test for protein trafficking and MDA5 inhibtion, and investigation of the small basic (SB) protein using mass spectrometry.

Abstract O-22

Structural basis for importin alpha 3 specificity of W proteins in Hendra and Nipah viruses

Kate M Smith1, Sofiya Tsimbalyuk1, Megan R Edwards2, Emily M Cross1, Jyoti Batra2, Tatiana P Soares da Costa3, David Aragão4, Christopher F Basler5, **Jade K Forwood**6

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Seven human isoforms of importin α mediate nuclear import of cargo in a tissue- and isoform-specific manner. How nuclear import adaptors differentially interact with cargo harbouring the same nuclear localisation signal (NLS) remains poorly understood, as the NLS recognition region is highly conserved. Here, we provide a structural basis for the nuclear import specificity of W proteins in Hendra and Nipah viruses. We determine the structural interfaces of these cargo bound to importin α1 and α3, identifying a 2.4-fold more extensive interface and >50-fold higher binding affinity for importin α3. Through the design of importin α1 and α3 chimeric and mutant proteins, together with structures of cargo-free importin α1 and α3 isoforms, we establish that the molecular basis of specificity resides in the differential positioning of the armadillo repeats 7 and 8. Overall, our study provides mechanistic insights into a range of important

Jade opened his talk by posing the question: Why are RNA viruses sending proteins to the nucleus, if they don't need to? They do for highly pathogenic viruses, such as: Nipah, Hendra, ZIKA, SARS-CoV, Dengue, etc. It is possible that proteins are being sent to the nucleus to inhibit immune signalling pathways, potentially targeting Pathogen-Associated Molecular Patterns (PAMPs), Pattern Recognition Receptors (PRR), adaptor kinases, and other transcription factors. Humans as hosts have importin alphas (IMPa), which can affect the interferon regulatory factor (IRF) signalling pathway. Jade and colleagues have the highest IMP alpha resolution ever solved, which facilitated mapping of protein-protein interactions. Hendra W proteins are made from the P gene and have a role in interferon suppression. Jade presented data confirming that this was due to direct suppression through high affinity interactions. The internal motifs responsible for binding specificity are the armadillo repeats from arms 2 to 4, across arm 3. Jade reminded the audience the data are based on structural differences, rather than sequence data. The team has created various conformations of the proteins and is looking at sequence-based differences, which reside in arms 7 and 8. When asked, Jade confirmed that the data are from human, not bat, IMP alpha proteins. When asked if he has looked at Cedar viruses, Jade replied those viruses have no W protein.

Abstract O-23

Henipavirus matrix protein employs a non-classical nuclear localization signal binding mechanism

Camilla M Donnelly, Olivia A Vogel, Megan R Edwards, Paige E Taylor, Justin A Roby, Jade K Forwood, Christopher F Basler

Training Hub promoting Regional Industry and Innovation in Virology and Epidemiology (THRIIVE), Gulbali Institute Charles Sturt University Wagga Wagga NSW Australia,

Nipah virus (NiV) and Hendra virus (HeV) are highly pathogenic species from the Henipavirus genus within the paramyxovirus family and are harbored by Pteropus Flying Fox species. Henipaviruses cause severe respiratory disease, neural symptoms, and encephalitis in various animals and humans, with human mortality rates exceeding 70% in some NiV outbreaks. The henipavirus matrix protein (M), which drives viral assembly and budding of the virion, also performs non-structural functions as a type I interferon antagonist. Interestingly, M also undergoes nuclear trafficking that mediates critical monoubiquitination for downstream cell sorting, membrane association, and budding processes. Based on the NiV and HeV M X-ray crystal structures and cell-based assays, M possesses a putative monopartite nuclear localization signal (NLS) (residues 82KRKKIR87; NLS1 HeV), positioned on an exposed flexible loop and typical of how many NLSs bind importin alpha (IMPα), and a putative bipartite NLS (244RR-10X-KRK258; NLS2 HeV), positioned within an α-helix that is far less typical. Here, we employed X-ray crystallography to determine the binding interface of these M NLSs and IMPα. The interaction of both NLS peptides with IMPα was established, with NLS1 binding the IMPα major binding site, and NLS2 binding as a non-classical NLS to the minor site. Co-immunoprecipitation (co-IP) and immunofluorescence assays (IFA) confirm the critical role of NLS2, and specifically K258. Additionally, localization studies demonstrated a supportive role for NLS1 in M nuclear localization. These studies provide additional insight into the critical mechanisms of M nucleocytoplasmic transport, the study of which can provide a greater understanding of viral pathogenesis and uncover a potential target for novel therapeutics for henipaviral diseases.

Camilla presented data on the henipavirus M protein. Whilst in the nucleus, M interacts with various proteins. Camilla's team has worked out the key amino acids driving import of the M protein through the classical nuclear import pathway. The structure for the HeV and NiV M proteins has been solved. The Nuclear Localisation Signal 1 (NLS1) on the henipavirus M binds to the host importin alpah (IMPa) major binding site and is a region for nuclear import. NLS2 is a putative partite signal that binds to the minor site of IMP alpha. Protein-protein interactions have been confirmed when looking at M proteins in their native state and in gel shift assays, showing that NLS1 and NLS2 can bind the IMP alpha 1, 3 and 5 importins. X-ray crystallography, fluorescence, co-immunoprecipitation studies have resolved structural data further. However, Camilla's team still does not know if M binds to the IMP alphas as a monomer or dimer. When asked, Camilla said there didn't seem to be a preference of M for IMP alpha 1, 3 or 5. Interactions between IMP alpha 1, 6 and 7 and W and M proteins have been reported, but Camilla's team has not yet seen this.

Abstract O-24

Constructing Antigenically Diverse Panels of Henipavirus F and G Proteins

Aaron J May, Alex Berkman, Muralikrishna Lella, Kijun Song, Victor Ilevbare, Salam Sammour, Chan Soo Park, Radha Devkota, Priyanka Devkota, Katarzyna Janowska, Yanshun Liu, Priyamvada Acharya

Duke Human Vaccine Institute (DHVI), Duke University Department of Biochemistry, Duke University Division of Surgery

Recent discoveries of several new Henipavirus (HNV) species, including the zoonotic Langya virus, have revealed much higher antigenic diversity of HNVs than currently characterized. To facilitate the development of pan-HNV vaccines and therapeutics, here we aim to construct an expanded, antigenically diverse panel of HNV fusion (F) and attachment (G) glycoproteins to

better reflect the global HNV diversity than is currently covered. We identified available genetic sequences, ranging from previously identified Nipah and Hendra virus strains to newly discovered species including those hosted by shrew species. We expressed and purified the soluble ectodomains of the F and G glycoproteins from these strains and characterized their biochemical, biophysical and structural properties. We constructed a phylogenetic tree comprised of 56 unique HNV strains, most having no data available in the literature on the antigenicity of their F and G proteins. Of the proteins expressed, all the G protein head domains were purified to yields ranging from 2.45-35.3 mg/L, whereas the F proteins yielded between ~0.1 and 5.7 mg/L, with several too low to recover. Differences in the stability and conformational mechanisms of HNV F and G proteins were revealed by their thermal unfolding profiles measured by Differential Scanning Fluorimetry (DSF). Visualization by single particle electron microscopy and analysis of the DSF profiles revealed pre- and post-fusion forms for all F proteins purified with a few showing high proportions of pre-fusion F. Of these was one of the most divergent members of the panel, Angevokely virus (AngV), where soluble AngV-F proteins showed single F particles as well as self-assembling into a "ring of six" lattice. These studies add foundational data to better understand the rapidly expanding Henipavirus genus and begin to draw new antigenic boundaries that can be used to understand the limits of vaccine efficacy when targeted to specific HNV species.

Aaron presented data on many different F and G proteins derived from various henipaviruses. His team used the NIH Genbank database to generate these, but focussing on complete genomes and completed F/G protein sequences, as they did not want to assign an arbitrary sequence during protein expression and production. Soluble proteins were made: 33F, 34G and 34 G-head (non-redundant). The studies focussed on the F and G-head. Depending on how trees were made, the team went mostly with the classic clade names. The shrew clade was interesting. The metastability of the F protein was tested using Differential Scanning Fluorimetry (DSF). Good grouping was observed for F proteins that had a low temperature vs others that were relatively unchanged. Langivirus could show some pre fusion characteristics, but the rest showed no evidence of change. The Angavokely Virus F protein stood out from the rest and seemed a variety of antigenically distinct DSF profile, including an oligomeric state suggestive of a hexametric lattice. The protein has an expanded internal cavity relative to other HNV F proteins. Antibody binding studies show cross reactivity with shrew virus proteins. The crystal structure has been done, and Aaron announced the preprint was going live the day of his presentation.[10]

Abstract O-25

Long-term Nipah antibodies and memory B cells in survivors from the 1998 outbreak in Malaysia

Hui Ming Ong, Puteri Ainaa S. Ibrahim, Chee Ning Chong, Chong Tin Tan, Jie Ping Schee, Michael Selorm Avumegah, Raúl Gómez Román, Neil George Cherian, Won Fen Wong, Li-Yen Chang

Department of Medical Microbiology Faculty of Medicine Universiti Malaya, Division of Neurology Department of Medicine Faculty of Medicine Universiti Malaya, Coalition for Epidemic Preparedness Innovation (CEPI), International Cooperation Embassy of Mexico in Norway

[10] https://www.biorxiv.org/content/10.1101/2024.12.11.627382v1

Nipah virus (NiV) is a highly pathogenic paramyxovirus that causes severe respiratory and neurological disease in humans. It was first isolated from a patient during the 1998 outbreak in Malaysia. This study analyzed the long-term humoral immune response to NiV within a cohort of 25 survivors from this outbreak. Among the survivors, 20 (80%) were male, and 5 (20%) were female. Nearly all survivors (96%) reported NiV-associated symptoms during the outbreak, and 84% had contact with sick pigs. The survivors' serum IgG antibody response to NiV nucleocapsid (N), fusion glycoprotein (F) and attachment glycoprotein (G), was evaluated using indirect ELISAs. Among the survivors, 56% had detectable levels of anti-NiV-F antibodies and and 60% showed detection of anti-NiV-G at 1:100 dilution, while only 20% showed specific reactivity to rNiV-N at the same dilution. All the samples that were tested positive for NiV-F and NiV-G at 1:100 dilution also exhibited neutralizing antibodies against live NiV, emphasizing the specificity and reliability of these indirect ELISAs. The live virus neutralization assay showed that sera from 72% of the survivors contained detectable antibodies with neutralizing effects against NiV at varying titers, indicating enduring immune memory. Furthermore, memory B cell responses against NiV-F and NiV-G were identified in six randomly selected survivors, suggesting the potential for persistent immunological memory. Despite the small sample size and lack of recent NiV cases for comparison, this study provides crucial insights into the lasting immune response against NiV. The results support the application of NiV-F and NiV-G as reliable markers for detecting NiV exposure and highlight the crucial need for continuous surveillance and research to guide vaccine development and improve readiness for future NiV outbreaks.

Hui Ming presented data on long-term follow-up of 25 Nipah survivors from the 1998 outbreak in Malaysia. Data focussed on NiV-specific antibodies at various dilutions. The study was under review at the time of presentation and has now been published in the *Journal of Infection*, available in open-access.[11] Because of the small sample size and reduced statistical testing, no significant correlations were found between the antibodies present and the tested epidemiological factors. However, there was good evidence of long-lived memory B cells specific to NiV-F and NiV-G.

Virology and Immunology Session 2

Chair: Sarah Edwards
Tuesday 10th December, 3.30 PM – 4.15 PM

Abstract O-26

Assessment and characterization of the replication kinetics of Henipaviruses in reconstituted airway epithelia derived from the entire human respiratory tract

Denise Siegrist, Daniel Zysset, Adrian Zürbrugg, Damian Jandrasits, Roland Züst, Olivier B. Engler, **Hulda R. Jonsdottir**
Spiez Laboratory, Federal Office for Civil Protection, Spiez, Switzerland

The usage of reconstituted airway epithelia to study viruses with respiratory tropism provides a physiologically relevant in vitro model to assess replication, pathogenesis, and innate epithelial immunity.

[11] https://www.journalofinfection.com/article/S0163-4453(24)00333-5/fulltext

We have assessed the replication of HeV, NiV-B, and NiV-M in reconstituted airway epithelia representing four separate areas of the human respiratory tract, i.e., nasal, bronchial, bronchiolar, and alveolar epithelia. Differential replication kinetics are observed between the four different epithelia as well as the three viruses. Similarities are observed between the two NiV strains, Bangladesh and Malaysia, while the replication of HeV in the upper airway, is impaired in comparison. For both HeV and NiV-M, replication in the nasal epithelia 7 days post infection is markedly lower compared to the nasal replication of NiV-B. All three viruses replicate to similar titers in epithelia derived from the lower respiratory tract.
Due to their pandemic potential, discovery and development of antivirals against Henipaviruses has become a public health priority. By utilizing these epithelial models, we intend to develop and validate a standardized testing protocol for antiviral drugs against Henipaviruses in a physiologically relevant cell culture model, thereby bridging the gap between high-throughput compound screenings in cell lines and antiviral drug testing in laboratory animals.

Hulda presented data on reconstituted airway epithelia as a model for the study of viral replication and pathogenesis. She mentioned many studies have attempted to use primary epithelial cells to emulate the human lung. She is using the air liquid interface (AIL) from airway cultures (cell lines or organoids). The AILs will be from the upper or lower airway depending on the initial cultures. These can be obtained from Epithelix (labclinics.com), a Swiss company that has cultures which span the whole respiratory tract (nasal, bronchial, small air/bronchiolar, or alveolar). Hulda emphasised that replication kinetics and studies on innate immunities are examples that you do not get from regular tissue lines. Whilst this is not novel for HeV, her team works on standardised protocol inputs and outputs. The methods are open-access to increase reproducibility globally. She first found that the multiplicity of infections (MOIs) range greatly across the cell types, with bronchiolar and alveolar being better. In terms of Standard Operating Procedures (SOP) for infection parameters for HeV, NiV-B and Niv-M, she said infection studies should aim for 1 week. They can go up to day 11, but after 5 days there is seepage. 7 days will be ideal to test drugs. RT-qPCR looks good. Optimisation was performed in the 4 different tissues. The nasal tissue is a good option to test NiV but not HeV. Hulda ended her presentation by stating she would like to be able to offer their services for any labs that need it. When asked about the origin of the tissue and the variability between donors, Hulda mentioned work thus far involves 1 donor and she's checking variability. She would like to have 3 individual donors and run them in triplicate, i.e. take the more simplified parameters and run with that to look at innate immunity etc. When asked how her data could be reconciled with the observation that only one in ten Nipah patients transmits to everyone else, Hulda mentioned that sometimes we see a lot of virus but that it is not necessarily all infectious, as in the case of SARS. When asked f the input virus was well characterised and if the facilites were under a quality system, Hulda replied that the NiV was from Malaysia and the Hendra from CSIRO, and that the facilities were not yet accredited.

Abstract O-27

Unravelling Henipavirus Infection Biology in Bat Cells Using Functional Genomics

Kam Leng Aw-Yong1, Joseph A. Printz2, Biaoguo Yan1, Meisam Yousefi1, Cythia Lingli Yong1, Felic Fanusi1, Yung-Juin Chen1, Zhiru Huangfu1, Moushimi Amaya3, Danielle E. Anderson4, Christopher C. Broder3, Lin-Fa Wang1, So Young Kim2 and **Yaw Shin Ooi**1

1Program in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore; 2Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC, USA;

3Department of Microbiology and Immunology, Uniformed Services University, Bethesda, MD, USA; 4Victorian Infectious Diseases Reference Laboratory, The Peter Doherty Institute for Infection and Immunity, Melbourne, Australia.

Bats are reservoir hosts for numerous zoonotic viruses with pandemic potential, including coronaviruses and henipaviruses, which pose serious threats to livestock and human populations. Unlike humans, bats can often tolerate viral infections without severe illness, highlighting the importance of understanding virus-bat interactions to inform viral spillover and transmission dynamics. Such insights may provide novel strategies for preventing zoonotic virus transmission and averting future pandemics. In this study, we engineered a genome-scale CRISPR knockout library, named the Gotham library, targeting the genome of Pteropus alecto (black flying fox) to facilitate genetic dissection of henipavirus-bat cell interactions. Using recombinant Cedar virus (rCedV)—a biosafety level 2 (BSL-2), non-pathogenic bat-borne henipavirus—we conducted a pilot genetic screen to validate the utility of the Gotham Library in discovering cellular factors essential for henipavirus infection in bat cells. Our screening identified Ephrin B1 (EFNB1) as the top host dependency factor required for rCedV infection in P. alecto kidney (PaKi) cells. We observed that rCedV failed to infect isogenic clonal EFNB1-deficient PaKi cells. Ectopic expression of the EFNB1 cDNA in the knockout cells effectively restored henipavirus infection, ruling out off-target effects from genome editing. These preliminary findings functionally validate the Gotham Library as a valuable tool for unbiased discovery of cellular factors critical for henipavirus infection in bat cells, advancing our understanding of the cell biology underlying henipavirus infection.

Yaw Shin presented data on a bat genome CRISPR knockout library aiming to dissect cellular host factors involved in cell-virus interactions. This technology has been useful for a range of viral genra recently. Studies were conducted with different recombinants of Cedar virus, as Hendra virus research is difficult to conduct in Singapore. The proof of concept was conducted in human cells, yielding three factors involved in Cedar virus-host interactions: eHap, IGROV-1, U87-MG. It also took two years to build GeneRaMeN: Gene Rank Meta analyser; this algorithm is a bioinformatic tool to combine multiple gentetic screning hit lists and applies ranking also. From the 3 datasets they had high confidence in the Cedar virus host factors. Next, they applied this to studies on Pteropus alecto host factors, using the Cedar virus recombinants and the CRISPR knockout library described in the abstract (Gotham Library). This could uncover 99.98% of the sgRNAs. Ephrin B1 (EFMB1) came up repeatedly as the top hit, confirming the validity of the model. Ectopic expression of EFMB1 in EFMB knock out cells restored Cedar virus infection and this was confirmed in plaque and luciferase assays, and with the use of PaKi cells, which express EFNB1. When asked how to control for knock outs that may be killing cells, Yaw Shin replied that one often checks that cells remain growing and use that as reference.

Abstract O-28

Salt Gully virus: a novel henipavirus isolated from Australian pteropus bats

Jennifer Barr1, Sarah Caruso1, Sarah J. Edwards1, Shawn Todd1, Ina Smith2, Mary Tachedjian1, Gary Crameri1, Lin-Fa Wang3 and Glenn A. Marsh1

1CSIRO, Health and Biosecurity, Australian Centre for Disease Preparedness, Geelong, Australia; 2CSIRO, Health and Biosecurity, Canberra, Australia; 3DUKE-NUS, Singapore

Henipaviruses are single-stranded negative-sense RNA viruses belonging to the family Paramyxoviridae and display broad host tropism. The original virus members, Hendra virus (HeV) and Nipah virus (NiV), are highly pathogenic and lethal to humans. In 2012, the first non-pathogenic henipavirus, Cedar virus (CedV), was isolated from Australian bat urine and characterised. Recently, new henipavirus-like viruses with unknown pathogenicity have been discovered in bats, shrews, and rodents in other countries and have been classified in a new species Parahenipavirus. Here, we isolated a novel henipavirus designated Salt Gully virus (SGV) from Australian pteropus bat urine and characterised in vitro. Full length genome analysis was performed, and sequence identity compared to HeV and CedV. Furthermore, unlike the classical henipaviruses, we found SGV does not utilise either ephrin-B2 or ephrin-B3 as a receptor for host cell entry and was unable to grow in pig or primary horse cell lines. The risk of disease in animals and humans remains unknown and further studies in relevant animal models will be vital to understand disease-causing potential of this novel henipavirus.

Jennifer presented data on the newly isolated and characterised Salt Gully virus (SGV). Initial characterisation focused on comparison to the other Australian henipaviruses. It was initially difficult to grow, but kidney cell lines (PaKi) were key to the growth. The virus was named after the bat colony from which the urine was collected. Getting the ends of the virus was a challenge, but the full-length genome was finally obtained containing 19,884 nucleotides (rule of 6). There were 6 open reading frames (ORFs) and an alternative start codon in the P gene, indicating the 7th ORF (C protein). Overall, the P, C and G nuclear sequences were more conserved than the corresponding amino acid sequence. The virus infected several cell lines, including Vero, PaKi, HeLa, but not the pig or horse cell lines EFK or PK. SGV does not use the ephrin receptor; so the virus must be using a different host cell receptor. Jennifer's team found no evidence of virus neutralising antibodies and they will next attempt to test the virus in an animal model. SGV is currently being handled at PC4. When asked if the virus has coding regions for V and W, Jennifer said yes, and that Emily Dowling, the PhD candidate in the lab, had a poster presentation on this.

Bat Infection Studies

Chairs: Wanda Markotter and Berta Blanch Lazaro
Tuesday 10th December, 4.15 PM – 5 PM

Abstract O-29

Rousettus aegyptiacus fruit bats as a potential animal model for henipavirus infections in the reservoir host

Anne Balkema-Buschmann1, Stefan Finke2, Björn-Patrick Mohl1, Kerstin Fischer1, Sandra Diederich1

1Institute of Novel and Emerging Infectious Diseases, Friedrich-Loeffler-Institut, Greifswald - Insel Riems, Germany; 2Institute of Molecular Virology, Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany

Introduction: Cedar henipavirus (CedV), which was isolated from the urine of pteropodid bats in Australia, is closely related to Hendra virus (HeV) and Nipah virus (NiV) that have been classified into the highest biosafety level (BSL4). Meanwhile, CedV is apathogenic for humans and animals,

and is often used as a model virus for the highly pathogenic henipaviruses HeV and NiV. In this study, we therefore challenged eight Rousettus aegyptiacus fruit bats of different age groups with CedV in order to assess their age dependent susceptibility to a CedV infection.

Methods: Eight bats (four adult females with their unweaned pups) were intranasally inoculated with 8 x 104 pfu per animal in a 150 µL volume. Two mock-inoculated juvenile bats served as controls. Intraperitoneally implanted data loggers monitored the body temperature and locomotion activity of two infected and both mock-infected animals. Clinical score, body weight, oral and anal swabs as well as nasal lavage samples were gathered throughout the study. Necropsies of infected adult bats and their pups were performed at 2-, 6- and 14-days post infection (dpi).

Results: None of the animals developed clinical signs, and only trace amounts of viral RNA were detectable at 2 dpi in the upper respiratory tract, the kidney as well as oral and anal swab samples. Monitoring of the body temperature and locomotion activity of four animals however indicated minor alterations in the challenged animals which would have remained unnoticed otherwise.

Conclusions: Results of this study will be discussed in the light of published NiV challenge experiments in the same species and in the reported reservoir host, Pteropus alecto. Overall, the challenge route will play a crucial role, as shown by our recent intracranial CedV inoculation of hamsters which lead to a virus dissemination throughout the brain and into other tissues. Comparative studies using different inoculation protocols in bats are therefore in preparation.

Anne presented data on her work with Egyptian fruit bats and their susceptibility to henipaviruses. She started by reminding the audience that there are a lot of fruit bats in close contact with humans in the African continent, citing Ghana as an example. She also gave an overview of Cedar virus research, and made reference to Mortlock et al, who found henipavirus-related paramyxoviruses in Egyptian bats.[12] Reference was also made to Marsh et al., who isolated Cedar virus from Australian bats and showed that usage of ephrin-B2 as the cell entry receptor.[13] Schountz et al. have shown disparate *in vivo* pathogenesis of NiV and Cedar virus in hamsters.[14] Lastly, Anne presented some data from her recent paper, where she showed that Egyptian fruit bats did not support productive replication of Cedar virus upon experimental challenge.[15] In brief, the bats were necropsied on days 2, 6 and 14-post infection. Her team did not see very much; there was no seroconversion. Circadian rhythm was nicely maintained but dataloggers revealed minor changes were seen in some animals. Anne's team did not expect severe disease in these animals, but the lack of seroconversion was intriguing when compared to studies with Ebola virus, where all animals seroconvert even though only 1 out 13 shed virus. What were they missing? Was this the wrong combination of bat and virus? Was it not the correct model? Should different infection routes have been used? Was the infecton dose too low? Did they not wait

[12] https://www.nature.com/articles/s41598-021-03641-w

[13] https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1002836

[14] https://www.mdpi.com/1999-4915/11/3/291

[15] https://www.mdpi.com/1999-4915/16/9/1359

enough for symptoms to appear? Many of these unknowns have been addressed in the case of Hendra virus and *Pteropus alecto*. In a different study, Syrian hamsters were challenged intracranially with fluorescent Cedar virus and showed evidence of virus dissemination to CNS and non-CNS tissues. When asked if she had made a cell line from the Egyptian fruit bats, Anne said she had and was already following up on that. When asked if she had tried to starve the animals a bit to trigger stress and virus replication, she said this was possible but also limited by animal ethics.

Abstract O-30

Jamaican fruit bats (Artibeus jamaicensis) effectively control Hendra and Nipah virus infection

Sarah van Tol, Vincent Munster

Laboratory of Virology, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT, USA

Henipaviruses Hendra (HeV) and Nipah (NiV) cause severe pulmonary and neurological disease in humans and other mammals. Bats in the genus Pteropus naturally host HeV and NiV. Understanding bat-henipavirus interactions that regulate shedding and replication could enable improved mitigation of spillover events. Pteropid bats have a low reproduction rate, are difficult to obtain outside of their natural range, and may be endangered or vulnerable, rendering in vivo experimental infection studies largely not feasible. Here, we assess the suitability of the Jamaican fruit bat (Artibeus jamaicensis) to model HeV and NiV infection. Bats were inoculated with 2x104 TCID50 of HeV (n=8) or NiV (n=8) strain Malaysia through a combination of intranasal and oral routes and monitored for 7 days to evaluate viral shedding and dissemination. HeV RNA was detected in the oral swabs of all challenged bats with peak shedding at 2 days post-infection (DPI). Only one of the NiV-challenged bats was viral RNA positive at 2 DPI. No infectious virus was recovered from the oral swabs of either HeV- or NiV-challenged bats. At the day 7 necropsy, HeV RNA was detected in the bladder and kidney of one bat and the liver of another, but all other samples were negative. No NiV-challenged bats tissues were viral RNA positive. In both NiV and HeV inoculated bats, circulating monocytes increased at both 3 and 7 DPI compared to baseline suggesting a pro-inflammatory response. HeV-challenged bats had increased expression of interferon stimulated genes in the spleen at 3 DPI and the lung at 3 and 7 DPI. In vitro, HeV and both NiV strains Malaysia and Bangladesh replicate to high titres on multiple primary Jamaican fruit bat cell lines. Overall, these results support that Jamaican fruit bats are permissive to both viruses, but replication is quenched rapidly in vivo. Future studies will optimize the in vivo model to leverage the Jamaican fruit bat to further our understanding of bat-henipavirus interactions.

Sarah presented data on the susceptibility of Jamaican fruit bats to henipavirus infections. She stated there are concerns with working with endangered and threatened species, such as the Pteropus bat species. Jamaican fruit bat cells support HeV and NiV replication and induce a mild, late-onset innate antiviral response. No changes in IL6 are seen over time. In one study, kidney cell lines from eight different Jamaican fruit bat donors were infected with HeV, NiV-Bangladesh and NiV-Malaysia. The cell lines varied in their robustness for henipavirus replication. Despite expression of ephrins, one cell line did not support the replication of any other the henipaviruses. Next, experimental infection of bats was done with HeV-g1 or NiV-Malaysia. Blood, swabs, weights and temps were taken. Sarah saw limited clinical signs like some hypothermia, but no

significant changes in body weight. Infections mostly resolved by day 7. There was HeV shedding at low levels, peaking at day 2 and all bats had at least one positive sample. NiV was detected only in one positive oral swab, and it is possible this was due to a localised infection such as the nasal turbinates. Hendra virus infection induces interferon-stimulated genes (ISGs) in the lungs and spleen, as seen by a 2-3 fold induction. When asked if interferon was constitutively expressed in these bats, Sarah said they didn't have as high of a basal transcript level as the other bats and cell lines she had worked with in the past suggesting they do not. She sees constitutive elevated ISG transcripts in horse cells, suggesting this is not a bat-specific phenomenon. When asked if the bats have henipavirus-specific antiviral factors, Sarah replied she wasn't sure.

Abstract O-31

Natural Hendra virus infection of captive flying foxes

Victoria Boyd, Jianning Wang, Anjana Karawita, Shawn Todd, Rachel Layton, Sarah Riddell, Grace Taylor, Sarah Caruso, Christopher Broder, Richard Ploeg, Gough Au, Anthony W Purcell, **Michelle L. Baker.**

CSIRO Australian Centre for Disease Preparedness

In this study, we describe natural Hendra virus (HeV) infections in Australian black flying foxes (Pteropus alecto) that were transferred from captivity in Queensland (QLD) to the Australian Centre for Disease Preparedness (ACDP) in Victoria. Twenty flying foxes that were PCR-negative for known zoonotic viruses (HeV, Menangle virus, and Australian bat lyssavirus) and either HeV seronegative (12 flying foxes) or with low to medium HeV antibody titres (8 flying foxes) were maintained in captivity in QLD and monitored for four months prior to transport. Serological testing three weeks before transport showed no changes in serostatus. However, upon arrival at ACDP, 11 flying foxes had seroconverted, indicating exposure to an active HeV infection during the three-week period before transport. Furthermore, two male flying foxes that had seroconverted began shedding HeV at one and eight days post-arrival, despite the presence of high levels of neutralizing antibodies. PCR and immunohistochemistry provided further insights into the nature of natural HeV infections. These findings offer valuable data on the infection dynamics and viral shedding in P. alecto.

Michelle presented data on Hendra virus infection in captive Australian black flying foxes. There currently are no captive colonies of these bats, nor do they fly as far down as Victoria. Researches must travel to Queensland to source bats trapped in the wild from wildlife rehabilitators. Setting up a colony is challenging, as the viral infection history of these animals is unknown. The team sourced a cohort of animals and started sampling and testing them as described in the astract. In December 2022, all serology and PCR results were negative. This was confirmed three months later, in March 2023; and three weeks later, in April 2023, the bats were sent from QLD to ACDP. Although the PCR were negative, there was now a mix of serostatus to HeV. Animals were handed over to animal studies team. 12 went into a room that can be elevated to PC4 and 8 went into a BSL3 room. By the end of April, one animal got a PCR positive Ct 33 in the BSL3 study room, and therefore all animals had to be euthanized. The other animals were kept going in the PC4 room. But then one animal in PC4 tested PCR positive for HeV in urine (Ct 25.7) and the team decided to keep going 1 more week and study pathogenesis. Detection of viral RNA and antigen was confirmed in Bat 4 following natural HeV infection. What had happened? Was this a cryptic infection that was triggered due to the impact of stress from travel? The team looked at the serology for 9 of the animals, pre and post travel, and saw no change. But the other 11 bats had

undergone seroconversion after infection in Queensland. It is possible that they came in contact with expression from wild bats or a reconnaissance event. Michelle highlighted the two PCR positive bats, with a lot of Luminex data. Bat R1, the one that was positive on arrival, seroconverted from low to high seropositive. It is possible that the other bat, Bat4, went under. When asked if there were any clinical signs for any of the animals, Michelle said there weren't any symptoms in any of them. When asked about why seroconversion was so high, reminiscent of an anamnestic response, Michelle concurred this could be due to a prior infection and that T cells could be looked into. Linfa Wang commented he had seen this in his studies too. When asked if she had been tempted to move the animals closer together to see what would happen, Michelle said yes; she was tempted but one cannot do that.

Day 4 – Wednesday 11th of December

Pathogenesis

Chairs: Glenn Marsh and Kerry Goldin
Wednesday 11th December, 9 AM – 10.45 AM

Abstract O-32

Comparative Histopathology of Henipavirus Infection: Insights from Human and African Green Monkey Models

Karla A Fenton1,2, Natalie S Dobias1,2, Thomas W Geisbert1,2

1Galveston National Laboratory, University of Texas Medical Branch, Galveston, TX, USA; 2Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX, USA.

Nipah virus (NiV), a zoonotic paramyxovirus in the genus Henipavirus, is known to cause a spectrum of clinical manifestations in humans ranging from asymptomatic infections to severe acute respiratory illness and encephalitis. Accurate characterization and validation of animal models are crucial for advancing medical countermeasures against reemerging and novel Henipaviruses. This study systematically compares the histopathologic and immunolabeling characteristics of human tissues affected by Nipah virus with those of the African green monkey (AGM) model, which is established as the gold standard for Henipavirus research. The comparative analysis highlights extensive vasculitis with endothelial syncytial cell formation in small caliber arteries across the lung, central nervous system (CNS), heart, and kidney, accompanied by immunolabeling for anti-Nipah N protein. The CNS showed the most severe parenchymal necrosis and hemorrhage adjacent to inflamed vessels, with positive immunolabeling of neurons, indicating a primary endothelial infection and potential breach of the blood-brain barrier. The olfactory bulb was also implicated as a potential route for CNS infection in some Henipavirus cases. Additionally, significant lesions were observed in non-CNS tissues, including lymphoid depletion, necrosis, and the presence of multinucleated giant cells in lymphoid organs. While the histopathology for Nipah virus has been described in humans and AGMs, other Henipaviruses described here exhibited similar but slightly varied lesions in the CNS. Histopathologic evaluation remains a key endpoint in translational research for these models, offering insights into early disease stages and long-term sequelae. A comprehensive

understanding of pathologic findings in AGMs throughout all stages of infection is essential for elucidating disease mechanisms and evaluating therapeutic interventions.

Karla presented histopathology data on Afican Green Monkeys (AGM). She started by stating that the henipavirus associated pathology described for humans is the basis of pathogenesis to help develop an animal model. Her presentation focused on the effects of Nipah virus infection on the respiratory track and CNS. AGM is a very good model for this disease and the question is: How does the virus get to the CNS? It could be vascular, crossing of the blood brain barrier, but it could also be olfactory too. The latter just has not been described well. For Hendra, there is one well-described paper with human lesions in CNS and for AGM also; again, it could be due to vascular degeneration. For HeVg1 Redlands and Nipah virus, we know that the lung gets thickened and this obliterates the alveoli, possibly allowing virus to get through to the CNS. Virus is seen in inclusion bodies and in Inferior Olivary Complex (IOC) positive neurons. A high percentage of these have positive olfactory nerves; therefore the team thinks the olfactory bulb does play a role. Virus is also seen in kidney and spleen, where it hits hard and where IOC positivity is observed within the white poles. For the pathologist, it is difficult to tell which cell type is positive, so multiplex assays are done to see which co-localise with the antigen. CK7, TTF1, VWF, CD50, DAPI co-localised with Hendra in all cell types expressing the receptor and also in alveolar macrophages. When asked if she observed any nerve-to-nerve spread in AGMs, Karla said she gets the animals at full disease so difficult to assess. When asked if she has looked at the olfactory epithelium, Karla said she has tried, but that it is difficult to do that work in BSL4 and also difficult for the monkeys. She has asked around, but many pathologists find it very difficult to do without creating sharps and risks at BSL4.

Abstract O-33

Nipah virus neuropathogenesis in vitro and in vivo

Manmeet Singh, Kerry Goldin, Brandi Williamson, Tessa Lutterman, Meaghan Flagg, Cathryn Haigh, and **Emmie de Wit**

National Institute of Allergy and Infectious Diseases

In humans, neurological disease is a main clinical manifestation of NiV. Yet, animal models currently in use display uniformly lethal respiratory disease with overt neurological signs of disease not consistently observed. This has resulted in a lack of understanding of NiV neuropathogenesis further exacerbated by a dearth of human clinical data. To fill this gap, we are exploring new in vitro and in vivo models that enable us to study NiV neuropathogenesis.

For our in vitro studies, we use human cerebral organoids (COs): three-dimensional, self-organizing tissue-like structures derived from human induced pluripotent stem cells. We compared NiV infection in both 2mo and 6mo CO over a 14-day period. Supernatant was analyzed daily for the presence of viral RNA, infectious virus, and cytotoxicity and whole COs were collected regularly for host gene expression and histologic analysis. We found that NiV replicated continuously over a 14-day period in both 2mo and 6mo COs, with replication slightly more efficient in the 6mo COs. Interestingly, only the 6mo NiV-infected COs mounted an effective interferon response.

For our in vivo studies, we are trying to develop a hamster model that consistently displays neurological disease and neuropathology. One approach was to perform intracranial NiV

inoculation. This resulted in a rapid progression towards severe neurological disease requiring euthanasia. High viral loads were detected in the brains, and NiV spread from the CNS to the lungs. Histopathologic examination of the brain showed ischemic necrosis, often accompanied by marked edema and hemorrhage. These histological lesions were different from the typical lesions observed in NiV-infected humans. An alternative approach is to combine the existing hamster models with inadequate remdesivir treatment at a dose that prevents severe respiratory disease but does not prevent neurological disease.

This work was supported by the Intramural Research Program of NIAID, NIH.

Emmie presented studies exploring *in vitro* and *in vivo* models for NiV neuropathogenesis. In humans, >50% people infected with NiV develop neurological disease. However the existing animal models mainly display severe respiratory disease, with neurological signs observed only sporadically. In a retrospective analysis of brains of African green monkeys inoculated with Nipah virus only 14/48 brains contained lesions; 4/14 showed mild neurological signs; 7/14 had neurological signs at time of euthanasia. During low dose inoculation in Syrian hamsters, Emmie has previously shown that hamsters develop weight loss by day 4, acute respiratory disease by days 5-8; and in a portion of surviving animals, neurological signs develop by days 9-12. Therefore, the virus is in the brain early enough, but neurological disease is preceded by often-fatal respiratory disease. Intracranial inoculations of hamsters with Nipah virus resulted in neurological disease requiring euthanasia in 2 days. With doses below 1 TCID50, animals survive a day or two longer, but some do not get infected. When looking at the brains of these animals, the pathology does not look like what we see in humans and other animals; so this is not a useful model to study Nipah virus neuropathogenesis. In separate studies in AGM, remdesivir treatment starting at 1 day post infection protected monkeys from lethal Nipah virus Bangladesh challenge. But when the remdesivir treatment is given on day 3, monkeys are only partially protected and develop neurological disease. Therefore, they hypothesized it may be possible to treat hamsters with remdesivir so that they survive the respiratory disease and subsequently allow for neuropathology analyses. In such studies, a high dose of remdesivir increased survival and time to death. High viral loads were seen in the brains of animals with or without remdesivir treatment; so Emmie's team looked for lesions and presence of viral antigen. She observed those lesions in 6/6 animals, which is much closer to what we see in humans. The team is next looking into cerebral organoids; and Kerry Goldin has done this work. The aim is to develop a model for the study of CNS diseases in human organoids at the molecular level.

Abstract O-34

Discriminating disease outcomes in nonhuman primates exposed to Malaysia or Bangladesh isolates of Nipah virus

Yu Cong, Bapi Pahar, Claudia Calcagno, Jeffrey Solomon, Saurabh Dixit, Sanae Lembirik, Shawn Hirsch, Lirong Peng, Venkatesh Mani, Monika Mehta, Vincent Munster, and **Michael R. Holbrook**

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Previous work in our laboratory demonstrated that large-particle aerosol exposure to the Malaysia strain of Nipah virus (NiV-M) led to an extended disease course and varied outcomes in a green monkey model. We also showed that similar exposure to the Bangladesh strain of Nipah

virus (NiV-B) led to varied outcomes. However, animals that succumbed to NiV-B infection had a predominate and rapidly progressing pulmonary disease, while NiV-M-exposed animals had a prolonged disease course, with some developing neurologic signs. To better understand differences between the disease courses from these virus strains, we further evaluated peripheral immune responses and lung tissue transcriptome profiles. Nine of 12 animals in the NiV-B group succumbed by Day 12 post-exposure, and survivors showed no clinical signs of disease. Consequently, the interpretation of adaptive responses is limited. However, data indicate an expansion of CD4+ T cells, IgM+ B cells, and CD206+ monocytes at the terminal phase of disease. Eight of 12 animals exposed to NiV-M survived, but several did not seroconvert. We observed a clear expansion of B cells at 12–18 d and an expansion of CD8+ T cells peaking on Day 18. CD4+ T cell populations were generally unchanged over the course of NiV-M infection, but the ratio of individual cell populations changed, with a peak of CXCR3+ T cells on Day 14. Analysis of immune cell populations in the brain of NiV-M-exposed animals suggests an influx of macrophages and CD8+ T cells. Transcriptomic data from terminal lung samples clearly separates survivors from non-survivors and NiV-M non-survivors from NiV-B non-survivors. Upregulated signaling pathways are largely related to the antiviral response, with some upregulated to a greater extent in lung tissues collected from NiV-B-exposed animals. This result is unsurprising given that pulmonary disease was more significant in NiV-B-exposed animals. Additional analysis of brain tissue is ongoing.

Michael presented data on nonhuman primates exposed to aerosolised NiV, ranging from small to large particle exposures, in order to more closely mimic the actual deposition route in people. The lab has clinical imaging equipment to help observations of infected monkeys. Studies were done 4 years apart due to the COVID-19 pandemic, but the design was the same for both the Malaysian and Bangladesh strains of NiV. Target doses were around 500 plaque-forming units (pfu), which got down to at least 60 pfu with a clog of the aerosol instrument. Clinical disease is observed between 7 – 14 (acute period) post aerosol challenge. For monkeys exposed to NiV-M, those that did not succumb to disease were not infected; for those exposed to NiV-B, all the ones that succumbed were infected. Haemorrhagic disease was documented for both groups. In terms of virus distribution, low levels were seen in blood of survivors versus higher levels in those that succumbed. Albeit at lower levels, virus was also seen in nasal and oral swabs of acutely infected animals. While there was no evidence of virus or lesions in the brains of NiV-M infected monkeys, virus was diffused in various areas of the brain of those infected with NiV-B. In-house assays for IgG and IgM were developed and the team was able to detect an antibody response to NiV-M exposure. Phenotyping by flow cytometry revealed a decline in B and T cells, but not in animals that did not succumb; so the team will explore this observation further. In the lymphoid tissues in the spleen of infected animals, there was a decrease in CD4+ cells in the lymphoid tissues in the spleen, as well as an expansion of monocyte populations. Neuroimaging (CT scans conducted 3-6 days post infection) was valuable in evaluating disease, as the team was able to see one animal with encephalitis. When looking at lung transcriptomics, animals grouped very well, with the cellular pathways looking as expected. Examination of lung tissue revealed consolidation of oedema (progression of pulmonary disease) among non-survivors. In summary, large particle aerosol exposure provided a model that closely replicated different courses of human disease for both isolates of the virus. Non-aerosol challenges may not be ideal for vaccines or antivirals; further Nipah is not specifically neurotropic, according to the aerosol challenge data. When asked if small particles were also tested in aerosol challenge, Michael replied this was done for NiV-B.

Abstract O-35

Modeling zoonotic Nipah virus infection in microphysiological systems

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Nipah virus (NiV) causes a severe zoonosis in humans and domesticated pigs. The World Health Organization has prioritized research on therapeutic candidates against NiV due to its high pandemic potential and the absence of medical countermeasures. As a Risk Group 4 virus, handling of infectious NiV requires substantial resources and is limited to a few maximum containment facilities. Development of therapeutics is additionally hindered by the limited translatability of data gained from frequently underpowered animal studies. Alternatives to animal experiments, such as microphysiological systems, could help refine, reduce, and potentially even replace animal experimentation, provide an innovative path toward therapeutic licensure, and offer unexpected insight into NiV pathogenesis in animals of different species.

Establishment of advanced organ–chip technology in our maximum containment facility enabled us to test whether pulmonary NiV infection could be modeled in vitro. Using a microfluidic human small-airway chip, seeded with microvascular endothelial and primary bronchial cells subjected to an air-liquid interface, we found that NiV replication in both cell types affected permeability of the chip membrane, thereby emulating the physiology of the alveolar–capillary barrier. A similar porcine alveolus chip recapitulated these results and enabled us to measure a proinflammatory immune response. As a proof of concept, we next evaluated the activity of two small-molecule antivirals, remdesivir and zotatifin, in infected human and porcine lung chips. Both antivirals inhibited NiV replication, albeit resulting in varying cytokine production (presumably due their distinct mechanisms of action and cell donor variability).

Our experience and preliminary findings provide an encouraging stepping stone for the integration of microphysiological systems into maximum containment laboratories and henipavirus research community activities.

Gabriella presented data on advanced organ-on-a chip technology as a model for the study of NiV pathogenesis in a microphysiological system. MPS (microphysiological system) is a complex in vitro, microfluidic, multicellular, organoid on a chip system. A range of organoid chips is available. Gabriella's team aims to emulate the lung *in vivo* physiology. The chip has two channels: the top is the "alveoli" and the bottom is the "capillary." Media can be collected from either channel. A semi permeable membrane separates the airflow on top and the blood/media flow in the bottom. Considering the role of pigs in the transmission of NiV historically, the team developed a pig lung chip at BSL2. Once in BSL4, the workflow is a series of events: exposure, dynamic and static inoculation, effluent collected at 24 and 48 hours for endpoint assay, and fixation and lysis of the cells at 48 hours. Gabriella's team first phenotyped the cells in the system and confirmed the presence of ephrin b2 (EFNB2) receptor in lung cells for both human and pig organoid chips. NiV infection was done by exposing the top air layer. A quantitative assessment of virus replication was done by testing the effluent from each channel, at 24 and 48 hours, by RTqPCR. There were minimal donor-to-donor differences and communication between the two

channels was seen. The team ran mock and untreated chips, and this was similar between the two donors. Next, to test the lung chips, an evaluation of small molecule antivirals against NiV was done using either remdesivir or Zotatifin. Gabriella showed both porcine and human plaque assay data, with a reduction of NiV in the antiviral-treated lung chips. The antivirals could also reduce leakage by limiting the flow/movement of neutrophils from human donors through the vascular channel. Cytokine responses were also examined and the team saw more variability in a donor whose cytokines went up. The introduction of neutrophiles promoted infection by increased expression of proinflammatory cytokines mimicking infection in humans. An advantage of the lung-on-a-chip system is that it allows for very well defined experimental conditions and concentrations of molecules to be tested. Two disadvantages of the system are: they are highly complex and need to be performed in BSL4; and they are very expensive, reaching a cost of approximately one thousand US dollars per chip.

Abstract O-36

Challenge of African green monkeys with Hendra virus genotype 2 or Hendra virus Australia/Horse/2008/Redlands produces divergent clinical disease phenotypes

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Hendra virus (HeV) is a medically important, zoonotic paramyxovirus which emerged thirty years ago in Hendra, Australia and causes severe, often fatal disease in humans and horses. HeV is classified as a NIAID Category C priority pathogen and currently has no approved vaccines or medical countermeasures to prevent or treat human disease. Humans can become infected by HeV through direct contact with bodily fluids and/or respiratory secretions of acutely ill or deceased horses. Until recently, all known human and/or equine cases were attributed to isolates belonging to the prototype HeV genotype. However, the continued re-emergence of HeV resulted in several genetically distinct isolates being identified, which correlated to slight variations in pathogenesis and disease phenotype. One such isolate Australia/Horse/2008/Redlands (HeV-R) was found to produce a neurologic-skewed disease in experimentally infected horses. More recently, a variant Hendra virus, HeV genotype 2 (HeV-g2) was identified to be responsible for a fatal equine case in 2021, and also retrospectively in a case from 2015; however, no human infections have been reported. To assess the pathogenicity of the contemporary Australia/Horse/2008/Redlands HeV isolate, as well as that of divergent HeV genotype 2, we performed challenge experiments in the African green monkey (AGM) model. The AGM model faithfully recapitulates the primary features of prototype HeV infection in humans, including acute respiratory distress and neurological disease. Ten adult AGMs were experimentally infected with 5.0E5 PFU of HeV-g2 or HeV-R via the combined intratracheal/intranasal route of exposure and monitored for clinical signs of disease. Four of the five AGMs challenged with HeV-g2 survived

until the study endpoint while all five AGMs infected with HeV-R met clinical criteria for euthanasia. Here, we present the findings of these two studies including the transcriptional and pathological differences identified.

Declan presented data comparing the pathology of HeV-g2 versus HeV-Redlands in African Green Monkeys (AGM). He began by making a retrospective description of HeV-g2 vs HeV-g1, 83 and their similarity. The attachment and fusion proteins have 92% sequence identity. For the AGM model challenge study and design, two groups of 5 each were infected intranasally or intratracheally with either HeV-g2 2015 Gimpie horse isolate, or HeV-R. All but one of the AGM challenged with HeVg2 survived until the study endpoint (35 days), and the one that met the euthanasia criteria was euthanized earlier, at ten day post-infection. In contrast, none of the AGM challenged with HeV-R survived to the study endpoint. An increase in respiration rate was observed for all animals, but was much higher in the HeV-R group. The team also observed severe neurological disease only in the HeV-R group. In terms of blood chemistry and heamatology, all animals presented increases in C-Reactive Protein (CRP), creatinine (CRE), total protein (TP) and glucose (GLU), as well as reductions in platelets and lymphocytes. Immunohistochemistry revealed characteristic pneumonia in the lungs, as well as infected neurons with moderate vacuolation in HeV-R group. Lungs from HeV-g2 survivors had neither multifocal lesions nor HeV antigens. Targeted trascriptomics sampling was done and analysed by uniform manifold approximation and projection (UMAP). This revealed that whilst both groups were largely becoming sick and had the same initial alterations in signalling pathways, the animals in the HeV-2 goup came back to baseline parameters. In summary, HeV-R was 100% lethal and caused severe respiratory and neurological diseases, whilst HeV-g2 was 20% fatal and caused a mild respiratory disease with no neurological disease. When asked if there was work looking for the presence of antigen in the survivors, Declan mentioned the team had looked at the RNA levels, detecting with qPCR. When asked if any differences were seen in neutralising antibodies between the two groups, Declan said none that were statistically significant.

Abstract O-37

Long-term detection of Nipah virus replication in IFNAR KO mice by longitudinal in vivo imaging

Katherine A Davies, Stephen R Welch, Teresa E Sorvillo, JoAnn Coleman-McCray, Micheal K Lo, César G Albariño, Christina F Spiropoulou, Jessica R Spengler

Centre for Disease Control

Small rodent models are critical in Henipavirus research as they facilitate larger experimental groups to better reflect the spectrum of human disease in pathogenesis and therapeutic studies. To advance their utility, we further characterized Nipah virus (NiV) infection in IFNAR knockout (ko) mice, detailing viral tropism, clinical course, and outcome. We demonstrate that NiV-infected IFNAR ko mice exhibit both respiratory and neurological signs, supporting their use as disease models. Consistent with our studies in the hamster model of NiV infection, we observe strain- and route-associated differences in disease progression and mortality rates. Recombinant NiV strains were engineered to express an innovative reporter-protein construct utilizing bioluminescent resonant energy transfer which yields enhanced signal compared to traditional bioluminescent reporters. Using these reporter viruses and non-invasive in vivo imaging techniques we tracked sites of viral replication over the course of infection in IFNAR ko

mice infected with either the Malaysian or Bangladesh strains. We observed differences in replication kinetics between intranasal and intraperitoneal routes of infection and between strains. Notably, we detected NiV replication in the absence of clinical signs, and in convalescent mice up to 6 weeks post-infection. Currently, there are no models to investigate long-term infection. These data highlight the potential of this model to identify sites of viral persistence. Overall, our work indicates novel applications of the mouse model to further explore clinical implications of infection, including late-onset disease and recrudescence.

Katherine presented data on the use of IFNAR knock-out mice to study NiV pathogenesis. Her team has relied on in-house characterisation and refinement of the mouse model, where disease is not as severe as that observed in hamsters. The team has also designed *in vivo* imaging methods that allow observation of the whole animal across each time point. For this, there is a need for more specific reagents and reverse genetics. They use a Spectrum Ct in the lab, as well as a BREP-labelled NiV-B construct expressing a Bioluminescent Red protein that can be visualised at the whole-body imaging level by shaving the animal and by injecting its substrate. NiV-B or NiB-M (10E6) were administered intraperitoneally and the mice were observed at 6 time points. NiV-M and NiV-B were seen in the chest and head up to day 16 and 28 post infection, respectively. A very strong chest and abdominal signal up to 28 post infection was seen for NiV-M infected mice, whereas those infected with NiV-B lit up mostly in the forebrain. There was a signal also in the nose, head, throat, chest and abdomen. It was also possible to use the mice to look at late onset, as a model for relapse, which we know can occur in humans. The virus could be detected in IFNAR ko mice up to 6 weeks post intranasal infection with NiV-B but not with NiV-M. When infection was done intraperitoneally, virus could be seen up to 6 weeks post challenge in both groups. In some cases, there was oscillation of signal in the animals. Katherine mentioned that there was a difference between the two NiV strains: NiV-B was from a direct spillover, whilst NiV-M was derived from a clinically sick individual after several passages. When asked if she had looked for any changes in terms of clinical symptoms or any other features, Katherine mentioned she was still looking through the data and nothing seemed outstanding thus far. She was also cautious that these were small group sized and further work would need to be done. When asked if there were any differences between the reporter virus and the wild type, Katherine said she had seen no differences in the replication kinetics. When asked if the mice could stay for a long time in the cells, Katherins said she was not sure but thought the bioluminescence had a half-life of 11 days.

Abstract O-38

Targeted transient interferon signaling disruption as alternative mouse model of Nipah virus infection to investigate role of immune responses in disease progression

Teresa E. Sorvillo, Katherine A. Davies, Stephen R. Welch, JoAnn Coleman-McCray, Jessica R. Harmon, Micheal K. Lo, Christina F. Spiropoulou, **Jessica R. Spengler**

Viral Special Pathogens Branch, Division of High-Consequence Pathogens and Pathology, Centers for Disease Control and Prevention, Atlanta, GA, USA

Several small animal models of NiV infection have been reported, including hamsters, ferrets, and mice. Mice offer advantages in disease modeling, including reagent availability, ease of handling, and improved logistical considerations for BSL-4 studies. However, current mouse models of NiV disease are limited to immunosuppressed strains (e.g., IFNAR-/-). An alternative

to IFNAR-/- mice is immunosuppression using a monoclonal antibody (mAb 5A3) that targets the IFNAR-1 subunit of the IFN-alpha/beta receptor. This approach transiently immunosuppresses (IS) type I IFN responses, making otherwise immunocompetent animals susceptible to lethal viral infection. Critically, the IS approach also permits use knockout mouse strains with other targeted disruptions to investigate viral pathogenesis or immune correlates of protection. Here, we investigated whether an IS model could be developed for NiV. C57BL/6J mice received 2.5 mg of mAb 5A3 on one of the following schedules: 0, 0/+4, or 0/+4/+7 days post infection (dpi). IS mice and IFNAR-/- mice (for comparison) were challenged intraperitoneally with NiV Malaysia (NiV-M; 1.0 × 107 TCID50). Mice were euthanized serially at 4 or 6 dpi, when meeting euthanasia criteria, or at study end (28 dpi). NiV was 75% and 62.5% lethal in IFNAR-/- and IS mice (both 0 and 0/+4 dpi cohorts), respectively. Lethality decreased in IS mice given mAb 5A3 at 0/+4/+7 dpi (12.5%). Both mouse models resulted in respiratory and neurological signs consistent with human disease. Tissues (liver, spleen, kidney, heart, lung, eye, and brain) and mucosal swabs were collected for viral load quantification from all animals. Plasma and tissue were collected for immunologic analyses, including investigation of lung- and brain-specific innate immune signaling. Here, we establish a new transient immunosuppression mouse model of NiV infection for use in medical countermeasure studies and to further investigate host factors associated with disease outcome.

Jessica presented data on an immunosuppressed (IS) mouse model for NiV infection. She started by pointing out that there is a range of animal models used in her team and by giving an overview of the mouse models. While immunocompetent mice can be infected and support virus replication, they do not succumb to the disease. Immunocompromised mice, on the other hand, do succumb to disease. Both respiratory and neurological disease can be observed in susceptible mice. However, it can be difficult to work with immunocompromised mice during vaccine studies and other options should be made available. Mouse monoclonal antibodies specific for mouse interferon alpha and beta can be used to immunosupress. One can use several background mouse strains but must minimize the use of sharps in BSL4 (reduce the number of injections). Mab 5A3 was chosen as the anti-IFN mab to induce transient immunosuppression. One or two injections of 5A3 resulted in 62.5% lethality, whilst three injections lowered the lethality, which was difficult to explain. Higher levels of virus replication were seen in immune suppressed animals. Virus was detected in discrete tissues, including tissues of surviving animals. Histopathology results are pending. When asked is she had looked in other tissues, like bone marrow or heart, for tissue damage or viral presence, Jessica said work was still ongoing with forlmalin fixed samples, in addition to PCR.

Vaccines

Chairs: Christina Spiropoulou and Emily Dowling
Wednesday 11th December, 11.15 AM – 1 PM

Richard Jarman CEPI

Rich is the Nipah Programme Leader at CEPI. He gave an introductory overview of Henipavirus vaccines and therapeutics. There are several reasons for Nipah medical countermeasures (MCMs). In addition to preparing for the pandemic potential of the pathogen, MCMs can mitigate biosecurity risks (bioterrorism potential) as well as the economic impact and community fear associated with an outbreak. Nipah disease poses several epidemiological challenges because outbreaks are sporadic and unpredictable, making conventional efficacy trials unlikely with

today's epidemiology. Limited funding, unclear business model and vaccine markets and policy issues are additional challenges to the development of henipavirus MCMs. In terms of use cases, henipavirus MCMs could be used for: prevention, pre-exposure prophylaxis, post-exposure prophylaxis, and treatment of disease (encephalitis). In the current landscape, there are neither vaccines, nor monoclonal antibodies, nor small molecules licensed for human use. Although there are several vaccines in development, mAb product development is limited. In terms of small molecules, Ribavirin was useful in Malaysia outbreak. BSL-4 requirements hamper vaccines and therapeutics development. Vaccine progression beyond preclinical trials can be difficult because of BSL4 requirements. When asked, Rich said there could be a role for mRNA technology in therapeutics.

Abstract O-39

The humoral immune response of foals to HeV vaccination

Kimberley J. Carey1, Alka Jays2,3, Prajit Dharmavaratha2,3, Jennifer Barr4, Sarah Caruso4, Gough G. Au4, Ina Smith5, Carol A. Hartley1, Kirsten E. Bailey1, Wendy Perriam6, Christopher C. Broder2, Moushimi Amaya2, James R. Gilkerson1

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Hendra Virus (HeV) causes fatal disease in horses and humans. The Equivac® HeV vaccine has been shown to protect adult horses from infection, although further studies regarding foal vaccination and the efficacy against the recently identified HeV variant (HeVg2) remains largely unexplored. However, the requirement for BSL-4 containment facilities when using infectious HeV limits the scope of studies investigating HeV specific neutralising antibodies. Here, using a large series of horse serum samples, we first correlated the traditional authentic HeV neutralisation assay with a recombinant Cedar virus (rCedV)-based assay that uses a GFP-encoding rCedV that express the F and G glycoproteins of HeV (Redlands isolate) (rCedV-HeV-GFP), which can be performed at BSL-2 laboratory containment as a quantitative fluorescence reduction neutralisation test (FRNT). The rCedV-based assay was then used to assess the efficacy of the current vaccine to HeVg2 using a rCedV-HeVg2-GFP virus. Serum from vaccinated mares and their foals were collected from a thoroughbred horse farm. A total of 100 samples were tested using the traditional HeV neutralisation test (HeV-VNT) and the rCedV-HeV-GFP FRNT assay. A Spearman's rank correlation test was used to correlate the titres of both tests. A subset of serum samples (n=28) that demonstrated protective neutralising titres against rCedV-HeV-GFP were also tested using the rCedV-HeVg2-GFP FRNT assay. With a conservative cut-off (1:100 titer), 26 of those 28 samples analysed showed protective rCedV-HeVg2-GFP titres (1 mare and 1 foal had a titer of 1:80), demonstrating that the Equivac® HeV vaccine offers cross-protection against the newly described HeVg2. There was a very strong correlation (correlation coefficient = 0.94) between the traditional HeV-VNT and the rCedV-based FRNT assays demonstrating its utility as a reliable method to detect HeV specific neutralising antibodies without the need for authentic HeV and BSL-4 containment.

Kimberley presented an update of her data from a recently published paper. As background, she pointed out that there is no evidence of plancental transfer antibodies from the mother to the foal. Instead, foals acquire maternally-derived antibodies (MDA) from their dams following ingestion of collostrum. In the case of Equivac HeV Vaccination, it is important to find out if sufficient HeV-specific antibodies can be maternally derived and acquired through collustrum, or if it is preferrable to vaccinate foals to reach good antibody titers. In a first study (Farm 1, New South Wales), just prior to the 6th month booster of the vaccine, most mares had shown a drop in antibody titers; therefore it was important to boost at 6 months and yearly after that. Under these conditions, foals acquired high levels of antibodies through ingestion of colostrum. However, titers dropped below the protective threshold by 3-6 months of age, indicating both the need for a booster after waning, as well as the potential interference of MDA in the establishment of active immunity. This raises the question about what would be the best timing and foal age to adminster the booster. In a second study (Farm 2, Victoria), 18 foals and 17 mares were followed. There was a low to moderate antibody titre after the first and second vaccine doses and an increase after the third dose. Although older foals had a peak in antibody levels, there were no statistically significant differences between the age groups (4, 5 and 6 months). Data suggests that the 6th month booster should be useful for foals as well. Further work is needed to determine the optimal age for boosting and the potential interfering role of MDA. Field studies on working farms can be limited. When asked, Kimberley said there are currently no data on neutralising antibodies over time in the absence of any booster dose.

Abstract O-40

Decoding Dose-Dependent Immunity: Insights into Nipah Virus Vaccine Efficacy and Correlates of Protection

Courtney Woolsey1,2, Alyssa Fears1,2, Jacquelyn Turcinovic1,2, Daniel J. Deer1,2, Viktoriya Borisevich1,2, Krystle N. Agans1,2, Jasmine Martinez1,2, Mack B. Harrison1,2, Natalie S. Dobias1,2, Abhishek N. Prasad1,2, Karla A. Fenton1,2, Robert W. Cross1,2, Thomas W. Geisbert1,2

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Nipah virus (NiV) is a highly pathogenic zoonotic virus with significant public health implications, necessitating the development of effective medical countermeasures. The identification of correlates of protection is critical for advancing vaccine and therapeutic strategies. Previously, we showed that a single-cycle, recombinant vesicular stomatitis virus-based vaccine expressing the NiV glycoprotein (rVSVΔG-NiV-G) provided both rapid (≤ 7 days) and durable protection (>1 year) in an African green monkey (AGM) model. In this study, we conducted a dose down experiment in AGMs immunized with 10^2 (N=4), 10^4 (N=8), 10^5 (N=8), or 10^6 (N=4) plaque-forming units (pfu) of rVSVΔG-NiV-G to establish the minimum protective dose. AGMs were subsequently exposed to NiV-Bangladesh via mucosal atomization (intranasal instillation; 40,000 pfu) or a combined intranasal/intratracheal route (500,000 pfu). Our results demonstrated incomplete protection across different vaccine doses, with breakthrough lethality observed in all groups except the 10^6 pfu group. These breakthrough cases present a unique opportunity to identify and characterize immunological correlates of protection via transcriptomics and

proteomics, thereby providing essential insights into the mechanisms of vaccine-induced immunity. This research will inform the development and optimization of vaccines and therapeutics against NiV, ultimately contributing to enhanced preparedness and response strategies for future outbreaks.

Courtney Woolsley delivered her presentation through a pre-recorded session. She began by outlining several advantages of the VSVdeltaG platform, including its prior and current use against Ebola virus disease using the Ervebo vaccine (Merck). The platform is also suitable for outbreak settings due to its single injection modality, the ability to elicit rapid and durable protection, and the possibility of heterologous protection. In this case, the vaccine contains the NiV glycoprotein as the immunogen, with the possibility of cross protection against Hendra. African Green Monkeys (AGM) were immunised and challenged as outlined in the abstract, and most succumbed at 7-9 days post infection. The only survivors were AGM that had been immunised with the highest vaccine dose (10^6) and subsequently exposed to NiV-Bangladesh via mucosal atomization (intranasal instillation; 40,000 pfu) or a combined intranasal/intratracheal route (500,000 pfu). EliSpots showed minimal cell mediated immunity, whilst IgG antibody titres increased from day 10 to 35 but only in the high dose group. IgG remained relatively low across study groups. Survival was associated with a predicted increase in B cells and a lower abundance of neutrophils. Vaccine-mediated survival was associated with immunoregulation, i.e. lower pro-inflammatory cytokines and chemokines in vaccinated survivors.

Abstract O-41

A Phase 1, Dose-escalation, Open-label Trial of a Structure-based mRNA Vaccine Targeting Nipah Virus, mRNA-1215, Demonstrates Hendra Virus Cross-reactivity

Dropulic L, Ploquin A, Mason R, Widge A, Holman L, Novik L, Happe M, Gordon I, Yamshchikov G, Coates E, Ortega-Villa A, Foulds K, Heimann A, Loftus L, Korzeniwsky K, Andrew S, Sunder S.S., Ou L, Teng I-T, Zhou T, Strom L, Norris M, Loomis R, Himansu S, Leav B, Beigel J, Bok K, Sullivan N, Roederer M and the VRC322 Study Team

Vaccine Research Center, Biostatistics Research Branch, Division of Clinical Research, and Division of Microbiology and Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, U.S.A, Moderna, Inc., Cambridge, Massachusettes, U.S.A.

Introduction: Hendra virus (HeV) and Nipah virus (NiV) are highly pathogenic members of the genus Henipavirus with 79% and 88% amino acid sequence homology between their attachment (G) and fusion (F) glycoproteins, respectively. HeV and NiV cause severe respiratory illness and encephalitis. No approved vaccines for humans are available.

Methods: We conducted a phase 1, first-in-human, open-label, dose-escalation trial (NCT05398796) of a lipid nanoparticle mRNA vaccine encoding chimeric pre-F and G NiV Malaysia proteins. The study evaluated four doses,10, 25, 50, and 100 mcg, of mRNA-1215 administered intramuscularly on Days 0 and 28 to ten healthy adults (22-59 years-old) per dose group. The primary endpoint was safety; secondary endpoint was NiV-specific antibody (Ab) responses 2 weeks (wks) post boost; exploratory endpoints were cross-binding and neutralization of HeV.

Results: mRNA-1215 was safe and well tolerated. Mild pain and tenderness were the main solicited local reactogenicity symptoms (n=33; 82%); the most frequent systemic symptoms were mild malaise (n=16; 40%), headache (n=14; 35%), and myalgia (n=12; 30%). No serious adverse events (AEs) occurred. Ten unsolicited AEs related to vaccination resolved without sequelae. mRNA-1215 elicited robust binding Ab (pre-F and G) and neutralizing titers to NiV Malaysia two wks post-prime. There was a significant increase in binding Ab and neutralizing titers two wks post-boost ($p>0.0001$, all dose groups), with no dose-dependent differences. All groups had detectable cross-reactive HeV binding Ab titers by 4 wks post-prime and HeV neutralizing titers by 2 wks post-boost. Post-boost titers were elevated through the final reported timepoint: 56 wks for the 25, 50, 100 mcg groups and 24 wks for the 10 mcg group.

Conclusion: The favorable safety and immunogenicity profile of mRNA-1215 and its ability to elicit cross-reactive HeV-specific Ab responses make it a promising candidate for advanced development.

Lesia Dropulic shared data from the Phase 1 clinical trial described in the abstract. ******She requested that photos and specific summaries of the data <u>not</u> be shared.****** This summary only shares notes that were deemed sharable by note-takers attending her talk. Reference was made to the structure-based design of Nipah vaccines paper published by Rebecca Loomis in 2020.[16] Importantly, all antibody data presented by Lesia was normalised to the WHO International standard for anti-NiV antibodies (International Units versus virus neutralising titres vs antibody binding titres). When asked, Lesia also mentioned that the VRC will be testing for breath of immune responses and that monoclonal antibodies have also been isolated from vaccinees.

Abstract O-42

Advances in Henipavirus Vaccination Strategies: A Thermostable Needle-Free Nipah virus and Hendra virus Vaccine Confers Broad and Durable Protective Immunity

Saniya Mahendiratta1,#, Stephen C. Balmert1,#, Robert W. Cross2,3,#, Moushimi Amaya4, Viktoriya Borisevich2,3, Cara Donahue Carey1, Sanpreet Singh1, Ashish Dhayani1, Alkay Jays4,5, Declan D. Pigeaud2,3, Rachel O'Toole2,3, Alexander Marin6, Raman Hlushko6, Lacin Cevhertas1, Jiying Zhang1, Tina L. Sumpter1,7, Victor H. Leyva-Grado8, Stefan Hamm8, Alexander K. Adrianov6, Anthony S. Dimitrov4,5, Louis D. Falo Jr.1,9, Thomas W. Geisbert2,3, Emrullah Korkmaz1,9*, **Christopher C. Broder**4*

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16 https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2020.00842/full

Medicine, Pittsburgh, PA, USA; 8Auro Vaccines, LLC., Pearl River, NY, USA; 9Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA, USA,

#Contributed equally

Hendra virus (HeV) and Nipah virus (NiV) are global-priority pathogens, with no licensed medical countermeasures for human use. Here, the first thermostable needle-free henipavirus (HNV) vaccine that confers broad and durable protective immunity is described. This HNV vaccine is enabled by integrating a recombinant soluble HeV G glycoprotein (HeV-sG) immunogen into a polyphosphazene (PPZ) adjuvanted dissolvable microneedle patch (MNP). Skin immunization with PPZ MNP-HeV-sG induces potent humoral and cellular responses in mice, which are superior to those obtained by intramuscular injection (IM) of HeV-sG in Alhydrogel®, the current gold-standard HeV-sG vaccination method. PPZ MNP-HeV-sG retains its immunogenicity during exposure to major stress factors, such as long-term thermal loads (up to at least a year) and gamma irradiation. PPZ MNP-HeV-sG-elicited antibodies are cross-neutralizing (HeV and NiV-Bangladesh (NiV-B)), and long-lived (up to at least 18 months), highlighting broad and durable immunity. PPZ MNP-HeV-sG-evoked cellular responses are cross-reactive (HeV and NiV-specific CD4+ and CD8+ T-cell responses), polyfunctional (antigen-specific T cells that produce IFN-γ, TNF-α, and IL-2), and multifaceted (systemic and pulmonary T-cells), revealing the additional dimension of PPZ-MNP-HeV-sG-mediated immunity. PPZ-MNP-HeV-sG also favorably conditions the human skin microenvironment, generating immunostimulatory skin migratory antigen-presenting cells, supporting the use of PPZ MNP-HeV-sG as a vaccine for people. Further, the robust and cross-reactive adaptive responses, elicited by skin vaccination with PPZ MNP-HeV-sG, in a prime only or prime-boost fashion, conferred complete protection in ferrets against a lethal challenge with NiV-B. Together, these results provide compelling evidence for the continued development of this broadly effective and globally accessible PPZ MNP-HNV vaccine for equitable human use to protect against HNV infection and disease.

Chris first gave an overview of the henipavirus medical countermeasures he has worked on over the years. In the case of monoclonal antibodies, 18 people have received m102.4 (HeV G-specific) as post-exposure prophylaxis and before seeing clinical signs. Reference was also made to the cross-reactive, F-specific mabs, hu1F5 and hu12B2, described by Larry Zeitlin in 2024.[17] hu1F5 is human but modified and it is being produced and stockpiled. CEPI will support Phase I trials in India and Bangladesh so that the mabs can be used in the event of an outbreak. In the case of vaccines, he recounted how his work started in 2002 and how his data has consistently shown that Hendra sG elicits more cross-reactive responses than Nipah sG, therefore being suitable as a potential pan-henipavirus vaccine. The Equivac vaccine has been administered to more than 200,000 horses (over 1.1 million vaccine doses). The human vaccine, adjuvanted in alhydrogel, is currently in clinical trials, with the potential to be used as a single-dose modality.

For the vaccine described in the abstract (Polyphosphazene (PPZ) adjuvanted micro-needle patch (MNP) vaccines), Chris mentioned the MNP platform was initially used for cancer treatments. The patches are like a cast/mould, with ultra sharp, little needles that are dissolvable. They have been applied *in vivo* and ex vivo in a variety of animals and humans.

[17] https://www.science.org/doi/10.1126/scitranslmed.adl2055

Patches are thermostable, with microneedles lasting for 1.5 years (can be stored at room temperature for over a year, or at 40C for 3 months). They can be self-administered and have the potential for a single use modality. They elicit robust, virus-specific systemic cellular immune responses. The aim is to have a thermostable platform that can circumvent the cold chain issues of conventional vaccines. When asked, Chris mentioned that the patches have elasticity to be applied to the skin and that he cannot wait to apply it to himself.

Abstract O-43

A replicon RNA vaccine completely protects ferrets and nonhuman primates from lethal challenge with Bangladesh strain of Nipah virus.

Robert W. Cross1, Nikole L. Warner2, Viktoriya Borisevich1, Krystle Agans1, Daniel Deer1, Mack Harrison1, Declan Pigeaud1, Rachel O'Toole1, Jasmine Martinez1, Natalie Dobias1, Alyssa Fears1, Stephanie Park2, Troy Hinkley2, Amit Khandhar2, Peter Berglund2, Karla Fenton1, Courtney Woolsey1, Jesse H. Erasmus2, Thomas Geisbert1

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Nearly 20 years ago Nipah virus (NiV) emerged and was shown to be a previously unknown paramyxovirus, now classified along with Hendra virus (HeV) within the Henipavirus genus. NiV causes febrile encephalitis and severe respiratory disease in humans with a fatality rate up to 100% in some outbreaks. In addition to causing morbidity and mortality as a naturally acquired infection, NiV is also categorized as a Category C priority pathogen by several US government agencies because of the concern for deliberate misuse. In addition, NiV was recently included on the World Health Organization's (WHO) 2018 List of Priority Pathogens. Currently, there are no vaccines licensed for the prevention of NiV disease and one that could be deployed during outbreaks or to endemic regions is urgently needed. HDT Bio has developed a self-amplifying replicon RNA (repRNA) vaccine platform delivered by a cationic nanocarrier, called LION™ that has achieved emergency use authorization in India following a successful phase II/III clinical trial of a COVID-19 vaccine based on the platform. In contrast to lipid nanoparticle-formulated RNA vaccines that elicit dose-limiting reactogenicity following systemic biodistribution of drug product, LION retains delivery of RNA to the injection site, limiting systemic reactogenicity. We have made both repRNA and LION drug products under current good manufacturing practice (cGMP) regulations and, having demonstrated safety and immunogenicity in humans, we have laid the necessary groundwork to facilitate rapid translation of vaccine candidates for other indications into the clinic, such as for the prevention of NIV. Here we will present the development of several LION candidate vaccines expressing various permutations of known NIV immunogens and their evaluation in ferrets and nonhuman primates where uniform protection with several of the tested vaccine approaches was achieved.

Robert presented data on self-amplifying, replicon RNA Nipah vaccines, delivered in a Lipid InOrganic Nanoparticles (LION) platform. He made reference to the potential reactogenicity of mRNA vaccines and the systemic malaise some may experience during the second dose. This is, in his view, partly explained by the systemic biodistribution of mRNA vaccines. Biodistribution of

antigen expressed by LION-repRNA vaccines, however, is limited to the injection site, as evidenced by animal toxicity data. Vaccines against Marburg, Zika, and even cancer antigens are being developed using the LION platform. In the case of Nipah, LION-delivered vaccines were developed with repRNA encoding for the Nipah Glycoprotein (repG-LION), the Fusion protein (repF-LION)), and Nucleoprotein (repN-LION). repG and repF LION vaccines elicited robust B and T cell responses in mice. repF LION also elicited neutralising antibodies. The vaccines were tested in the ferret model using a prime, boost regimen, followed by intranasal challenge. All vaccine candidates protected ferrets uniformly and no animals presented symptoms. Unlike the control animals immunised with mRNA vaccines, antigen expression was not detected in the blood. Vaccine efficacy was subsequently tested in non-human primates (NHPs). Immunization with multiple antigens combined was protective, as was immunization with repG-LION and repF-LION, whereas immunization with NiV-N (repN-LION) alone was not. Breakthrough infections give the opportunity to examine immune correlates of protection, which is ongoing. When asked further about the delivery of LION nanoparticles, Robert confirmed that the nanoparticles do not go into the organs.

Abstract O-44

Single-dose replicon particle vaccine rapidly protects African green monkeys against lethal Nipah virus challenge

Stephen R. Welch, Yu Cong, Jessica R. Spengler, Saurabh Dixit, Scott M. Anthony, Bapi Pahar, Jeffrey Soloman, Erin Kollins, Philip Sayre, Joseph Laux, Kurt Cooper, David Drawbaugh, Sanae Lembirik, Connie S. Schmaljohn, Michael R. Holbrook, Christina F. Spiropoulou

Viral Special Pathogens Branch, Division of High Consequence Pathogens and Pathology, Centers for Disease Control and Prevention, Atlanta, GA, USA, NIAID Integrated Research Facility, Ft. Detrick, Frederick, MD, USA.

Currently, there are no licensed vaccines for prevention of Nipah virus (NiV) disease. Previously, we developed a novel NiV viral replicon particle (VRP) vaccine based on a recombinant NiV lacking the fusion (F) protein gene (termed NiVΔF). This design restricts NiVΔF to authentic transcriptional and translational processes in the initial host cell entered only, preventing egress and further spread of the VRP. Extensive evaluations of NiVΔF in four highly sensitive animal models have revealed no evidence of disease or pathology, confirming its safety profile. Previous studies demonstrated 100% protection in two rodent models of disease, Syrian hamsters and Ifnar-/- mice. Building on these results, we evaluated both intramuscular (IM) and intranasal (IN) vaccine administration in an African green monkey (AGM) model of disease. In the uniformly lethal intratracheal/intrabronchial AGM challenge model, single-dose IM delivery conferred 100% protection in as little as 7 days post vaccination. All clinical parameters remained within normal limits, and no abnormalities were noted when using both CT and MRI to assess changes in the lung and brain, respectively. Using the novel IN vaccination route in the same model, 66% of animals survived with minimal clinical signs observed. As our previous data in small animal models indicated that protection can be achieved in the absence of neutralizing antibodies, we performed detailed immunological analyses of humoral and cell-mediated responses in the AGMs to investigate non-neutralizing mechanisms of protection. Here, we present data on vaccine safety, efficacy, and immunological correlates of protection, advancing knowledge of NiV infection and supporting continued pre-clinical evaluation of the NiV VRP vaccine.

Stephen presented data on the Nipah viral replicon vaccine (VRP) lacking the fusion protein (NiVdelta F). The VRP doesn't fit into any box like other vaccines. It is based on a very good reverse genetics system for Nipah whereby the F protein is exclusively and constitutevly expressed in the cell line used to make the full particle. Once in the host cells, however, there are no F proteins, so the virus is not able to move beyond the cells and replicate. Inoculation of NiVdeltaF in three highly sensitive animal models demonstrates high level of safety and efficacy. In the Syrian Golden Hamster (SGH) model, intranasal innoculation of NiVdeltaF VRP resulted in no damage or spread in the lungs compared with wild type NiV infection. Further, immunisation with NiVdeltaF VRP protected hamsters against intranasal and intraperitoneal challenges. In the African Green Monkey (AGM) model, animals were immunised intramuscularly (IM) with NiVdeltaF VRP and subsequently challenged via the intratracheal and intrabroncheal (IT/IB) route on day 28. All immunised AGM were protected. Protection was also seen when animals were challenged earlier, on day 7 or 14 after immunisation. CT scans of the lungs and MRI of the brains show no damage in vaccinated animals compared to controls. IgG and IgM titres do increase and cell mediated immunity is being examined.

Therapeutics

Chairs: Chris Broder and Dani Anderson
Wednesday 11th December, 2 PM – 3.15 PM

Abstract O-45

Dexamethasone reduces pulmonary pathology but does not alter mortality in Syrian hamsters infected with Nipah virus

Kerry Goldin, Tessa Lutterman, Brandi Williamson, Manmeet Singh, Emmie de Wit

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Nipah virus (NiV) is a zoonotic pathogen that causes severe respiratory and neurologic disease in humans, and mortality is typically very high (~70%). In cases of severe COVID-19, treatment with dexamethasone at an advanced state of disease improved clinical outcomes. In this study, we determined the effect of dexamethasone treatment on Syrian hamsters infected with NiV. Syrian hamsters were treated with an anti-inflammatory dose of dexamethasone for 10 days, starting at 4 days post infection. Dexamethasone treatment did not result in differences in virus shedding from the nose or throat, viral loads in tissues, or cytokine levels in the lungs. However, animals treated with dexamethasone had marked reduction in pulmonary pathology, compared to animals in the PBS-treated groups. In the dexamethasone treated animals, the lungs either lacked any pathologic findings entirely, or mild, non-specific changes were observed, such as alveolar histiocytosis. In PBS-treated animals histologic lesions in the lungs resembled those described in African green monkeys, including marked pulmonary edema, hemorrhage, interstitial lymphohistiocytic pneumonia, and vasculitis with fibrin thrombi formation and rare endothelial syncytia. Surprisingly, the reduction in pulmonary pathology observed with dexamethasone treatment did not result in changes in disease onset, nor in a reduced mortality rate. Lesions were observed in the brains of both groups, but were more frequent in the PBS-treated animals. The most common finding in the brains of both groups was non-suppurative meningoencephalitis. In human cases of NiV infection, where supportive care can be administered, the absence of pulmonary lesions may be of clinical benefit. Next, we will combine

dexamethasone with the antiviral drug, remdesivir, to determine if combined treatment improves outcome compared to treatment with either drug on its own. This work was supported by the Intramural Research Program of NIAID, NIH. 96

Kerry presented data on the effect of dexamethasone (DEX) in the Syrian Golden Hamster model of NiV infection. In brief, DEX treatment of NiV infected animals resulted in a drop in pulmonary lesions but had no impact on survival. Kerry mentioned this was a pilot study that may not have been well powered, so her team pursued further investigations. In a separate study, her team extended DEX treatment; the drop in pulmonary lesions continued but the mortality was not altered. In yet another study, to address whether DEX could reduce pulmonary lesions when combined with other drugs, Kerry combined remdesivir and DEX. They saw little difference in shedding kinetics in any of the treatment groups. A subgroup of animals was euthanized and necropsied at 6 days post inoculation, and viral loads were similar between all the groups. It is possible DEX treatment reduces pulmonary pathology but negates the benefits of remdesivir. This is new data and there is work in progress looking at cytokine panels. Kerry Goldin, on the effect of dexamethasone (DEX) in the Syrian Golden Hamster model of NiV infection. In brief, DEX treatment of NiV infected animals resulted in a drop in pulmonary lesions but had no impact on survival. The hamsters did develop neurological disease and the decline was rapid. Emmie mentioned this was a pilot study that may not have been well powered, so her team pursued further investigations. In a separate study, her team extended DEX treatment; the drop in pulmonary lesions continued, but there was no alteration in the brain lesions now. When looking at lung tissue by hematoxylin and eosin (H&E) staining, it was evident why animals succumbed to respiratory disease. There was reduced pathology, but the mortality was not altered. In yet another study, to address whether DEX could reduce pulmonary lesions when combined with other drugs, Emmie's team combined remdesivir and DEX. They saw little difference in shedding kinetics in any of the treatment groups. Animals were necropsied at 6 days post infection, and viral loads were similar between all the groups. It is possible DEX treatment reduces pulmonary pathology but negates the benefits of remdesivir. This is new data and there is work in progress looking at cytokine panels. When asked whether these treatments would be beneficial to humans, who could potentially survive a limited respiratory disease, but who could be left with the neurological sequelae, Emmie concurred that it would be an interesting and difficult choice for clinicians to make. When asked about the potential mechanisms for DEX to inhibit remdesivir, Emmie said this is a peek behind the curtain and the data require further analysis. When asked about the timing of DEX treatment, and whether it may have been too soon to administer (i.e. before disease onset), they said it would be counterintuitive to administer after disease onset, but that the experiment would need to be performed.

Abstract O-46

Discovery of novel Henipavirus inhibitors

Judith Straimer1, James R. Manning1, Johanna Jansen1, Richard T. Eastman1, Ryan Chan1, Fred King2, Yvonne Wang2, Ahmed Rohaim1, Tiffany Tsang1, Colin Deniston2, Cosmo Buffalo2, Darlene Chen1, Atul Sathe1, Shreeya Hegde1, Jeanne Dudley1, Debapriya Sengupta1, Debjani Patra1, Katherine Chan1, Zhenhang Chen1, Alka Jays3, Moushimi Amaya3, Rachel O'Toole4,

Viktoriya Borisevich4, Olivier Escaffre4, Robert Cross4, Bo Liang5, Alexander Freiberg4, Christopher Broder3, Thomas Geisbert4, Nadine Jarrousse1

1Biomedical Research, Novartis, Emeryville, CA, 94608, USA; 2Biomedical Research, Novartis, San Diego, CA, 92121, USA; 3Uniformed Services University, Bethesda, MB, 20814; 4University of Texas Medical Branch, Galveston, TX, 77551, USA; 5Emory University, Atlanta, GA, 30322, USA

There are no currently approved vaccines or therapeutics to prevent or treat NiV or HeV infection for human use. Work with authentic NiV requires BSL-4 containment which represents a substantial challenge to drug discovery efforts. Here we report the successful application of recombinant Cedar henipavirus encoding nano-Luciferase (rCedV-nanoLuc), a BSL-2 approved surrogate system, to screen for compounds that inhibit NiV and HeV replication. We screened 1.7 million compounds and processed hits through a hit-finding flow chart which includes secondary assays such as a novel BSL-2 NiV, HeV and CedV minigenome reporter and two-high throughput biochemical assays probing the polymerase activity of the NiV L-protein. Counter-screens were also included to filter out non-selective hits and hits that may prevent viral replication through inhibition of host factors. Compounds that passed all filters were subsequently tested for inhibitory activity against authentic NiV in BSL-4. We have identified several series of interest and have binned them into two categories: phenotypic hits with unknown mode of action and compounds that inhibit the NiV polymerase activity in a biochemical assay. Exploratory chemistry has been initiated to further evaluate the different series, and target identification efforts are underway to confirm direct antiviral activity of the phenotypic hits.

Finally, in order to further accelerate henipavirus antiviral drug discovery and enable structure-based approaches, we solved the cryo-EM structure of NiV L polymerase in complex with the P protein. Here, we present a comprehensive approach that uses a combination of cell-based NiV surrogate systems, high-throughput biochemical assays and structural biology analyses to identify direct-acting antiviral candidates for the treatment of highly pathogenic henipaviruses.

Judith presented work funded by the US National Institutes of Health in the non-profit part of Novartis. She presented data after having screened 1.2 million small molecules for antihenipaviral activity using the assays described in the abstract. The first assay is a phenotypic high throughput system (HTS) based on a BSL-2 Cedar virus and minigenome methods. The minigenome-based assay first gets rid of compounds that are not desirable and the remaining 67% are confirmed with Cedar virus neutralisation assays. It is based on a 384-well format. A lot of compounds were effective against Dengue virus; so the minigenome assay was not specific and was not very useful filtering out. A T7 polymerase counter screen assay was developed to test for compounds that may also inhibit NiV polymerase activity. This latter biochemical assay produced data with a nice dose response. Combined, the assays have yielded two chemical series of interest with inhibitory activity confirmed with live virus assays in BSL4. Compounds in Series 1 have an unknown mode of action, whilst compounds in Series 2 have NiV RNA-dependent RNA-polymerase (RdRp) inhibitor activity. When asked, Judith confirmed that the compound library she used was an in-house (Novartis) library that is very vast. When asked if the library was based on Lipinski's Rule of 5, she said that they library is what Novartis uses for all drug discovery programs.

Abstract O-47

A protective bispecific antibody targets both Nipah virus surface glycoproteins and limits viral escape

Ariel Isaacs, Guillermo Valenzuela Nieto, Xinghai Zhang, Naphak Modhiran, Jennifer Barr, Nazia Thakur, Yu Shang Low, Rhys H Parry, James B Barnes, Ronald Jara, Johanna Himelreichs, Yanfeng Yao, Camila Deride, Barbara Barthou-Gatica, Constanza Salinas-Rebolledo, Ehrenfeld Pamela, Jun Jet Hen, Noah Hayes, Devina Paramitha, Mahali S Morgan, Christopher LD McMillan, Martina L Jones, Trent Munro, Alexander A Khromykh, Patrick C Reading, Paul R Young, Keith J Chappell, Yi Shi, Dalan Bailey, Glenn Marsh, Sandra Chiu, Alejandro Rojas-Fernandez, Daniel Watterson

School of Chemistry & Molecular Biosciences, The University of Queensland QLD Australia, Universidad Austral de Chile Valdivia Chile, Wuhan Institute of Virology Wuhan China, CSIRO Geelong Victoria Australia, The Pirbright Institute Woking United Kingdom, WHO Collaborating Centre for Reference and Research on Influenza Victoria Australia, Australian Infectious Disease Research Centre QLD Australia, The Australian Institute for Biotechnology and Nanotechnology, The University of Queensland QLD Australia, CAS Key Laboratory of Pathogen Microbiology and Immunology Beijing China, University of Science and Technology of China Heifei China

Nipah virus (NiV) and Hendra virus (HeV) are highly pathogenic henipaviruses (HNVs) with case fatality rates between 50-100%. Currently, there are no approved human vaccines or antiviral treatments. Both NiV and HeV make use of a receptor binding protein (RBP) and fusion glycoprotein (F) to mediate entry into host cells. As such, both RBP and F are major targets in both vaccine designs and therapeutic development. Here, we report on a first-in-class camelid nanobody, DS90, that engages a unique, conserved site within prefusion F of NiV and HeV. Dimeric DS90 provided ultrapotent neutralization of both NiV and HeV and complete protection from NiV disease. Through cryogenic electron microscopy, we demonstrated that DS90 binds a quaternary pocket within NiV F, and blocks a site previously shown to be involved in F dimer-of-trimer assembly that is necessary for viral membrane fusion. As RNA viruses, HNVs are prone to immune escape under selection pressure. To address this, we combined DS90 with an anti-RBP antibody, m102.4, to deliver a dual-targeting biologic that is resistant to viral escape. Bispecific engineering of DS90 with m102.4 resulted in synergistic neutralization, elimination of viral escape and superior protection from NiV disease compared to leading monovalent approaches. Together, our findings provide proof-of-concept for the use of nanobodies to treat HNVs. Moreover, our results carry implications for the development of cross-neutralizing immunotherapies that limit the emergence of henipaviral escape mutants.

Ariel presented data on the DS90 nanobody and asked the audience **not** to take any photos of the results. Here we summarise some of his more general findings with Ariel's consent. Nanobodies are small compared to antibodies, with an antibody-binding domain of roughly 15 kda. To obtain them, an alpaca was vaccinated to make an immune library, and bacteria display was used to pull down the nanobodies. Three candidates were studied further and DS90 was chosen because it was very potent in both BSL2 pseudoviral and BSL4 live virus neutralisation assays. DS90 can be expressed as a dimer and monomer, with the former being very potent in the neutralisation assay. Early data using cryo-electron microscopy reveals that the CDR3 engages a unique quaternary pocket that is sequestered in the NiV prefusion F protein. This may explain why the dimers neutralise more potently than the monomers. To further avoid any potential immune escape, a bispecific antibody was engineered combining DS90 and m102.4. This bispecific biomolecule is highly neutralising *in vitro* and was passaged with Nipah virus in sub-neutralising

conditions before sequencing by nanopore for escape mutations. In collaboration with a team in a China, the bispecific antibody was shown to be highly efficacious in the hamster Nipah challenge. When asked if he has considered making a dimer between two F epitopes rather than F and G epitopes, Ariel mentioned that he has and he would like to try it, although he has seen earlier work showing that targeting two F epitopes is less effective than targeting one

Abstract O-48

Development of antiviral therapies against Nipah and Hendra viruses

Olena Shtanko, Nadean M. Gutierrez, Marija A. Djurkovic, Carson G. Leavitt, Vanesa N. Estevez, Jenny Hsieh, Stanton McHardy, Jonathan A. Bohmann

Texas Biomedical Research Institute, Southwest Research Institute, University of Texas San Antonio

Nipah virus (NiV) and Hendra virus (HeV) belong to a rapidly growing group of recently identified henipaviruses and can cause fatal encephalis and respiratory distress in affected human populations and livestock. No FDA-approved therapies to prevent or treat henipavirus disease are currently available. The 2018 annual review of the WHO has included NiV in the list of priority pathogens due to pandemic potential and recommended accelerated research and development for this virus. Henipaviruses initiate infection by attaching to the host receptors, ephrin-B2/B3, in a process mediated by viral glycoprotein, G, making the G/F ephrin interaction an attractive target for antiviral development. Our proprietary machine learning platform, Rhodium™, has used HeV G protein/ephrin-B2 complex crystal structure to rapidly identify several structurally distinct chemotypes of potential inhibitors targeting viral G protein. Importantly, among 35 analogs synthesized across all series, several specifically blocked authentic NiV infection in cell-based assays, including clinically relevant primary human endothelial cells, with selectivity indexes >10. Notably, our most potent lead compound significantly blocked NiV, but not Ebola virus, rapid spread from the initial sites of infection in a 3D cortical organoid model of human cerebral cortex, demonstrating specificity and low toxicity of this treatment. Our data highlight differences in individual virus-host interactions during infection, making realistic 3D human organoids an integral part of investigations into viral pathogenesis and validating them as an essential test model during antiviral discovery programs, leading to more accurate predictions of the course of infection during efficacy studies in vivo.

Olena presented data on *in silico* design of antiviral compounds, followed by screening them in *in vitro* assays, and subsequently confirming activity of selected compounds in a brain organoid system. She began by making reference to other medical countermeasures like m102.4, which though effective, may not be suitable for mass use during a pandemic due to costs and administration challenges. Several Rhodium-designed antivirals were predicted to disrupt binding of HeV to Ephrin-B2. These compounds were then synthesised and screened for inhibitory activity using cell-based assays relying on recombinant henipaviruses expressing GFP and automated Nikon imaging. Screens were run in triplicate, usually in 96 well or 384 well plates. Binding inhibition curves were shown, and the preliminary screen yielded several inhibitors; so Olena went further and did the more careful study of two compounds which confirmed inhibition results through dose-dependant analyses. Next, she tested the compounds in the 3D brain tissue organoid model described in the abstract. She showed immunofluorescence, RNA sequencing and electroshock data of the organoid at 3 days post infection with NiV Bangladesh. When asked about the binding site of the compounds, Olena

said more in-depth characterization is needed. A member from the audience also commented that the compounds are promising as they are likely blocking protein-protein interactions.

Abstract O-49

Human antibodies with potent cross-neutralizing activity against Hendra and Nipah viruses

Ashley Heimann1, Katia Korzeniwsky1, Renguang Du1, Aurélie Ploquin1, Danielle Wagner1, Amelia Thompson1, Mary McDonald1, Lorin Loftus1, Lesia Dropulic1, Laura Novik1, David Ambrozak1, Daniela Ischiu Gutierrez1, Brett Leav2, Sunny Himansu2, Li Ou1, I-Ting Teng1, Rebecca J. Loomis1, Tongqing Zhou1, Mario Roederer1, **Rosemarie Mason**1

1. Vaccine Research Center, National Institute of Allergy and Infectious Disease, National Institutes of Health, 2. Moderna, Inc.

Background: Hendra virus (HeV) and Nipah virus (NiV) are paramyxoviruses that cause mild to fatal respiratory illness or encephalitis. There are no licensed therapeutics or vaccines for either disease.

Methods: We screened plasma from 40 healthy donors vaccinated with NiV mRNA-1215 expressing chimeric glycoprotein (G) and pre-fusion (F) proteins of NiV Malaysia in a phase 1 clinical trial (NCT05398796, 2 dose regimen, 4-week interval) for cross-neutralizion of HeV.

Results: Most donors had high neutralizing activity against HeV Redlands 2 weeks post-boost. Two donors with the highest neutralizion ID50 titers against HeV were selected for sorting of preF- and G-specific memory B cells from peripheral blood mononuclear cells. Approximately 2,500 individually sorted B cells (positive for pre-F or G probe binding) per donor were expanded. B cell culture supernatants were tested for neutralization of NiV Malaysia, and 39 and 70 wells scored positive for donors 1 and 2, respectively. Immunoglobulin genes were amplified from neutralization-positive B cells, and 27 and 30 mAbs have been cloned and expressed for donors 1 and 2, respectively, (roughly 2:1 ratio of G- to pre-F-specific mAbs isolated for each donor). So far, 12 mAbs from donor 1, and 3 mAbs from donor 2, have been tested for neutralization against HeV Redlands, and NiV Malaysia, India and Bangladesh. Nine of fifteen mAbs neutralized all 4 viruses, with 2 mAbs (1 each pre-F and G-specific) exhibiting higher potency against each virus tested than the well-characterized HeV G-specific m102.4 mAb (ACTRN12615000395538). In-depth epitope mapping and structural analyses of the cross-neutralizing mAbs is ongoing as is testing of additional mAbs, and production of pseudotyped viruses to test cross-neutralization of other HeV and NiV strains.

Conclusion: We have isolated human-derived cross-neutralizing mAbs from NiV mRNA-1215-vaccinated donors with broad prophylactic and therapeutic potential against HeV and NiV.

Rosemarie presented data on Antibody secreting cells (ASC) isolated from human donors vaccinated with NiV mRNA-1215. The vaccine elicits robust neutralising antibody responses, which made it possible to isolate NiV-specific B cells trough flow cytometry cell-sorting *in vitro* using 384-well plates. Cells were sorted by gating using the G trimer and the preF trimer. Roughly 10% of the gated cells were ASC. The sorted ASC were expanded and screened further for secretion of IgG, IgA and neutralising activity. Immunoglobulins were recovered and sent for gene synthesis. In terms of subclasses, some were IgG3 and one was IgG2. The majority of the synthesised IgG3 antibodies were neutralising and potent, but not the IgG2. They cross-neutralised the two HeV genotypes, and the three NiV strains indicated in the abstract.

Panel discussion

The last session of the conference was a panel discussion on the WHO Nipah Virus Roadmap and the new WHO Paramyxovirus Collaborative Open Research Consortium (CORC) initiative. The main question was: how can these initiatives forge progress in the control and prevention of henipavirus outbreaks? The panelists and their affiliations are indicated in the Programme. Here we summarise parts of the discussion.

The WHO Nipah Roadmap

Emmie de Witt started by thanking all the local organisers and by commending the participants for a great conference. She made a disclaimer stating that she was not a WHO employee, and therefore her opinions were not those of the organisation. In her understanding, WHO created the Nipah Roadmap as a result of the following series of events milestones: WHO first made a list of priority pathogens; they then incorporated those pathogens as part of the WHO blueprint; the focus was preparedness for epidemics and pandemics; the tool to prepare was the Roadmap; they created a group of experts invited by other experts; they focused on four areas (diagnostics, therapeutics, vaccines, cross cutting issues) and set ambitious milestones; the experts needed to think about how to convince funding bodies to resource and fund medical countermeasure R&D; experts also thought about how to tackle access and implementation challenges once a vaccine was made available, etc. Policy was also part of the process: for example, the group of experts were also asking: what are the regulatory issues that could come up in the face of an outbreak? The original objective of the Roadmap was to have effective countermeasures and diagnostics by 2030. Emmie was one of the experts in the process; she thought the expert group was passionate and wanted to get the process done under ambitious timelines. However, as soon as the Roadmap was written, WHO changed their strategy.

Here, Kim Halpin pointed out that the change of strategy was that the WHO wanted to go focus on viral families rather than on individual viruses, hence the creation of a paramyxovirus family CROC now incorporating the henipaviruses. The Roadmap was nonetheless published as an independent manuscript.

The Paramyxovirus CORC

Sreelekshmy Mohandas then described how the Paramyxovirus CORC will be running from India. She backtracked by stating that WHO will focus on 12 viral families and the Paramyxoviridae will be one of these. In principle, the CORC would like to invite all researchers across the group to come under one umbrella to facilitate more research under the initiative. It will attempt to identify research gaps, knowledge gaps and challenges to ensure availability, equity and access to medical countermeasures. It will also aim to build and strengthen trust from communities. On behalf of the Indian Council of Medical Resarch, ICMR, and WHO, Sreelekshmy invited all to participate in the CORC.

The discussion then moved to a more informal structure.

Linfa Wang started by noting that the concept of the CORC is so new and complex that even the CORC leaders are unsure about what to do. For the Coronavirus CORC, he thinks the group is at 50% understanding. In his view, Roadmaps were key for the WHO because they were conceived as something that would have been important to have when Ebola outbreaks hit. After COVID-19, the focus in now on being prepared for Disease X. Focus is also on preparedness, perhaps not so much in prevention, with broad acceptance to two concepts: no individual organisation can manage preparedness, and WHO can help coordinate. In terms of the CORCs, there are now priority pathogens for bacteria and viruses; however, key to the new strategy is to focus on a prototype virus, hopefully representative of the virus family. For paramyxoviridae, it might be something like Cedar virus, as illustrated in this conference. Everyone knows Cedar virus is not a priority pathogen because it is not going to provoke a pandemic, but it is still a prototype virus with which one can prepare model systems (platforms, assays, etc.) against other henipaviruses. The next step will be to create a library of prototype vaccines. For example, for the coronaviruses CORC for which Linfa is the lead, he has to be prepared with 10 coronaviruses vaccines. The hope is that this will also balance globally for roadmaps at the family level. For example, one CORC will be run from India, another from Singapore, another from the UK and so on. For the orthomyxoviruses, WHO is still waiting for that assignment. The hosting government must support the CORC. In his view, there are three ways to be part of this CROC process: you must have the funding to start the work; you must already do work in this area; or if you don't have the funding yet, it will go to the country or government that does. Linfa noted that one potential gap is social and behavioral research; that needs to be put in the CORCs. In summary, Linfa thought the whole process was exciting but still complex.

Emily Gurley started by pointing out that she liked what had heard about this process, particularly focus on research and development. However, she expressed being worried that the CORCs were quite different from the Roadmap in several aspects. In the Roadmap, the priorities were set, the responsibilities were assigned, the targets were ambitious, and there was a "bell" to keep everyone on track. She also expressed concern about how long it would take to get funding, noting that there is a risk of losing the momentum built from the Roadmap. One option she laid out was waiting and seeing what went on with the CORC. Emily also expressed that even if the WHO is not very interested in the Roadmap, there is no reason why the henipavirus community can't still proceed with parts of it. It is possible that the Roadmap and the CORC could be complementary.

Stephen Luby recalled that many of the participants at Hendra@30 had also been in Singapore for the Nipah@20 meeting. He noted he was very impressed with how much progress had been made within the henipavirus research and development community within 5 years. He also said holding these meetings every 4 or 5 years would be very important. In his view, people that really understand henipahviruses are getting together again and it is key that those people come together again whether or not they are part of a CORC.

For the Q&A session, members of the audience expressed several views and concerns. One of them was that One Health is usually thrown out of the equation and is used very lightly in comparison to Public Health. Panellists thought this would be an area where CORC leaders could make improvements, i.e. by involving One Health more into pandemic preparedness. Other concerns were related to CORCs taking roughly 10 years to do their work, citing CEPI as a benchmark for this. From CEPI's perspective, the henipavirus vaccine pipeline was the most important aspect to consider in the paramyxovirus family CORC; nonetheless, all CORCs must be ready if Disease X is in their family group. Another view is that the henipavirus community should focus on the roadmap and get the funders to come forward for the CORCs, but also for prevention of spillovers, outbreaks and pandemics. Questions were also raised about the location of the headquarters for the Paramyxovirus CORC, with the Indian delegates noting that the CORC will be run from Delhi. Another question was related to setting research priorities, ie. Who are the CORCs for: local, regional, global stakeholders? Panellists thought the global priorities are to prevent a pandemic or a public health emergency of concern. Still another concern was related to preparedness and feasibility; for example, if there is a very good countermeasure but people might not use it. Other concerns related to being included in the CORC, i.e. "the amazing part of the CORC is if you could be part of the CORC." Data sharing, open-access platforms, and keeping local and regional stakeholders will be key, according to the panellists. There was wide consensus that a henipavirus meeting should be held again in 5 years.

Closing remarks

Kim Halpin gave a few words on how the conference came together. One precedent was the 2019 Nipah Conference in Singapore. For the Hendra Conference, Kim was able to apply for CSIRO seed funding and CEPI contributed further. The International organising committee was pulled together, and so was the local and the ECR committee, all members who were amazing. In terms of participants, 16 countries were represented and coming all the way to Australia, and the local hosts feel a sense of community.

Kim mentioned she and colleagues were hoping for NipahM@30 to be held in Malaysia in 2029, and/or a Bangladesh conference (possibly NipahB@20 in 2026).

Award Winners: student and early career research prizes provided by Zoetis

Two Best PhD Student Posters: Kimberley Carey and Avirup Sanyal

Best PhD Student Talk: Ariel Isaac

Best ECR Poster: Wee Chee Yap

Best ECR Talk: Declan Pigeaud