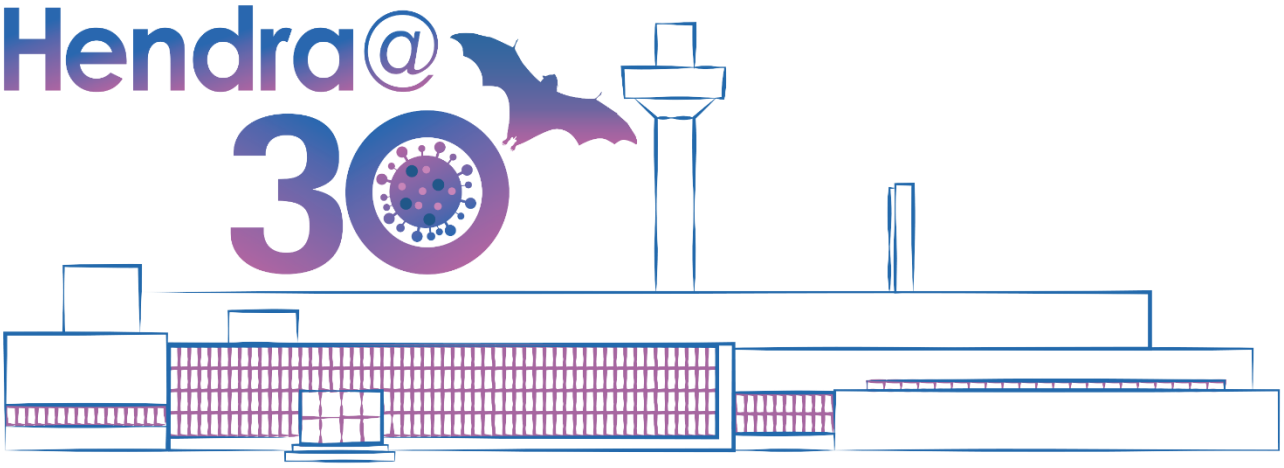


Hendra@30 Henipavirus International Conference

8th – 11th

December 2024

Geelong

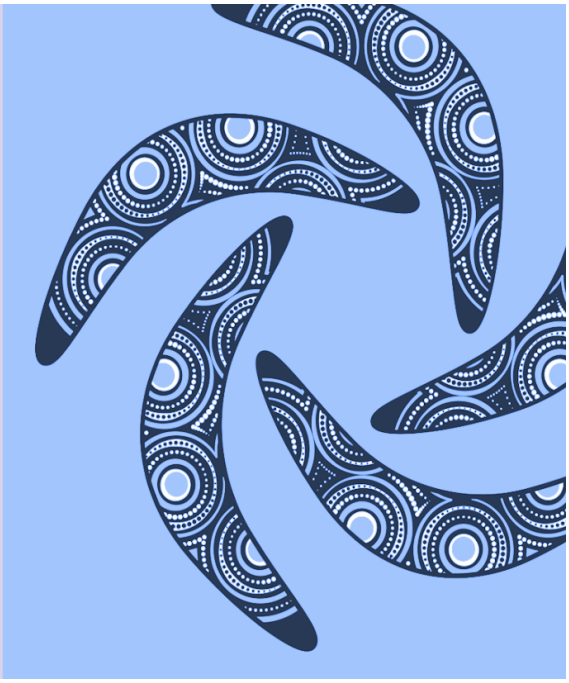
Victoria, Australia

CEPI

CSIRO

We would like to acknowledge the Wadawurrung people as the Traditional Owners of the land that we are meeting on for the Hendra@30 Henipavirus International Conference.

We acknowledge their continuing connection to their culture, and we pay our respects to their Elders both past and present, and recognise them as the First Scientists of this nation.



Contents

Travelling to Geelong

The Gull Bus is the most convenient and simple way to get from Melbourne Airport into Geelong CBD where most hotels will be.

It costs $39 AUD one way or $72 return

Events for Early Career Researchers

(Postdoctoral Scientists and Students)

Sunday 8th of December 7pm (after welcome drinks)

Ice-cream icebreaker - Meet up with ECRs prior to the beginning of the conference with a walk along the Geelong waterfront, grab a sweet treat, chat with other ECR registrants and see the conference venue before the event.

Monday 9th of December - Lunchtime

Trivia session - Get to know your fellow ECRs and test your general knowledge against each other. There will be some Australian themed prizes up for grabs.

Tuesday 10th of December - Lunchtime

Networking session - Have a chance to speak to the well-known experts in the henipavirus field in a welcoming and supportive environment. We will provide some prompts if needed to keep the conversation flowing.

Events outside main conference proceedings

Tuesday 10th of December at 7 AM

Waterfront Run - Get some exercise in and soak up some Australian sunshine with a morning run around the Geelong Waterfront. Meet near the floating Christmas Tree.

Housekeeping

Speakers

Each speaker is given 10-12 minutes to present, plus 3 minutes for questions. Please note that timing will be strict to ensure we keep to schedule. You name and presentation tile will be introduced by you session chair and you will be given a verbal "1 minute left" warning at the 11 minutes mark.

Please make it clear to the audience if you do not want any photos or tweets of your slide/s (we recommend inserting the no photo symbol below on the slide).

Please refer to the program for the timing of your session and ensure you are present at least 15 mins prior to the session starting, to meet your session chairs and for any technical checks.

Chairs

We ask you to join the session in the break prior, at least 15 mins prior to the session starting. This will allow plenty of time for technical checks and for you to meet your co-chair and the speakers.

At the start and end of the session a member of the conference organising committee will remind participants of any housekeeping details and introduce the session chair(s).

During the session we ask that chairs share the roles to:

1) Introduce speakers using the short bio which will be provided, and the name and presentation title of each of the speakers.

2) We will have a timekeeper who will indicate when speakers have 1 minute to go, and when the speaker should finish. Chairs are asked to help ensure that speakers keep to time.

3) Select and moderate questions for the speakers and to keep an eye on time (maximum time per speaker is 15 minutes total).

All participants

We ask you to be respectful at all times. For participants who are sharing/tweeting the conference, we would ask that you do not photograph or tweet any slides or material if the speaker has asked for the material not to be shared.

Program

Day One – Sunday 8th of December

4pm - 5pm	**Optional visit to Geelong bat colony**
5pm - 7pm	**Welcome drinks/canapes at the Royal Geelong Yacht Club**
7pm onwards	**ECR meet and greet**

Day Two – Monday 9th of December

8am - 9am	**Registration desk open**	
9am - 9.45am	Welcome to country by Traditional Owners at Waterfront location	
9.45 - 10.00	Welcome to Hendra@30	
	Welcome from CSIRO: Brett Sutton & Debbie Eagles	
	Welcome from CEPI: Amy Shurtleff	
10.00 - 11.00	**History of Hendra virus**	**CHAIR: Kim Halpin**
10.00-10.15	Reflections from the Australian Chief Veterinary Officer (CVO)	Beth Cookson
10.15-10.30	The original Hendra virus outbreak in horses	Peter Reid
10.30-10.45	Hendra virus the lab response at CSIRO	Paul Selleck
10.45-11.00	Reflecting on HeV research over the last 30 years	Linfa Wang
11.00 - 11.30	**Morning tea**	
11.30 - 12.00	**Personal Stories**	**CHAIR: Linfa Wang**
11.30-11.45	Personal experience from Natalie Beohm who recovered from HeV	
11.45-12.00	Personal experience from Wee Chee Yap whose family was affected by NiV	
12.00 - 1.00	**Disease Ecology Session 1**	**CHAIRS: Raina Plowright & Belinda Linnegar**
12.00 - 12.15	Ecological perspectives on the emergence of HeV	Peggy Eby
12.15 - 12.30	Thirty years of Hendra Virus Spillover; A synthesis of ecological insights and future directions	Alison Peel
12.30 - 12.45	Hendra virus in Australian bats show a diffuse spatio-temporal structure	C.Kwe Yinda
12.45 - 1.00	Periodic shifts in viral load increase risk of spillover from bats	Tamika Lunn
1.00 - 2.00pm	**Lunch**	
2.00 - 3.00pm	**Disease Ecology Session 2**	**CHAIRS: Alison Peel & Sarah van Tol**
2.00 - 2.15	Incredible diversity of Henipaviruses revealed in Australian flying foxes	K9 Jenns
2.15 - 2.30	Co-circulation and co-infection: unravelling viral community dynamics through metatranscriptomics	Brent Jones
2.30 - 2.45	Bridging the knowledge gap in the HeV: Exploring the role of horses in transmission dynamics	Belinda Linnegar
2.45 - 3.00	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

3.00 - 3.30	**Afternoon tea**	
3.30 - 5.00pm	**Rapid-oral session - 5-minute talks**	**CHAIRS: Jennifer Barr & Emily Dowling**
1	Model-guided henipavirus discovery in museum bat collections	Maya Juman
2	Metabolic consequences of changing ecological conditions in flying foxes: a Hendra virus reservoir host	Avirup Sanyal
3	Human-bat competition on cultivated fruit resources promotes bat-borne pathogens spillover to humans and domestic animals in Bangladesh; an exploratory qualitative study	Abdul Khaleque Md Dawlat Khan
4	Henipavirus sero-surveillance in horses and pigs from Northern Nigeria	Andrew Adamu
5	Cryptic exposure to henipaviruses in Cambodia and Malaysia	Spencer Sterling
6	Serosurveillance of Nipah virus in Malaysia	Vunjia Tiong
7	Development and establishment of diagnostic capability for Nipah virus	Shailendra Mani
8	Investigation of two newly emerged henipaviruses: how the Langya and Angavokely virus matrix proteins interact with nuclear import proteins	Emily Wagon
9	Detection of Cell-Mediated Immune Memory in Nipah virus survivor 25 years post-infection	Puteri Ainaa Syahirah Ibrahim
10	Decoding Henipavirus Latency and Transmission Dynamics; A Deep Learning Multi-Omics Approach Using Single-Cell Transcriptomics and Environmental Data	Rifaldy Fajar
11	Is a Nipah virus-specific vaccine sufficient to prevent the next henipavirus outbreak?	Wee Chee Yap
12	Pathological characterisation of the golden Syrian hamster model of Nipah virus infection	Javier Salguero (on behalf of Ines Ruedas Torres)
13	Targeted immunology gene expression in FFPE samples from Nipah virus infected hamsters	Javier Salguero
14	Nipah mRNA-1215 Vaccine Induces Cross-Reactive Responses Against Henipaviruses and Confers Complete Protection Against Nipah Bangladesh Virus in Old World Monkeys	Aurelie Ploquin
15	Human neutralising monoclonal antibodies against Hendra and Nipah viruses	Wen Shi Lee
16	Observational study on the clinical epidemiology of infectious acute encephalitis syndrome including Nipah virus disease, Bangladesh	Md Zakiul Hassan
5.00pm -7pm	**Poster session plus drinks/grazing tables at the Pier**	

Poster session kindly sponsored by:

Day Three – Tuesday 10th of December

9.00 - 10.30am	**Surveillance**	**CHAIRS: Jon Epstein & Tahmina Shirin**
9.00 - 9.15	Surveillance efforts in Bangladesh	Tahmina Shirin
9.15 - 9.30	Surveillance of NiV in Pteropus medius bats from different states of India, 2022-2024	Sreelekshmy Mohandas
9.30 - 9.45	Serological evidence of emerging henipaviruses and paramyxoviruses in pteropodid bats in the Philippines; Ten years after the 2014 henipaviral disease outbreak in Sultan Kudarat, Philippines	Mary Glazel Noroña-Biocarles
9.45 - 10.00	NiV Seroprevalence among Encephalitis Patients in Thailand 2020 – March 2024	Chayanit Mahasing
10.00 - 10.15	Henipavirus Dynamics in Rousettus Bats from South Africa: Exploration of Serological Patterns and Seasonal Variations	Wanda Markotter
10.15 - 10.30	Mojiang like henipavirus exposure to humans in rural communities in Bangladesh	Ariful Islam
10.30 - 11.00am	**Morning tea**	
11.00 - 11.45am	**Behavioural determinants of henipavirus transmission**	**CHAIRS: Stephen Luby & Ariful Islam**
11.00 - 11.15	Measuring incidence and investigating pathways of henipavirus transmission from bats to livestock and peri-domestic animals in Bangladesh	Emily Gurley
11.15 - 11.30	Assessing the risk of bat-borne pathogen emergence from hunting Indian Flying Fox (Pteropus medius) at high- risk interfaces in Bangladesh	Shusmita Dutta Choudhury
11.30 - 11.45	Risk of Nipah virus transmission through date palm sap trade, Bangladesh	Abdul Khaleque Md Dawlat Khan
11.45 - 12.45pm	**Diagnostics**	**CHAIRS: Liyen Chang & Gervais Habarugira**
11.45 - 12.00	Applying the SCAHLS Recommended Diagnostic Test Validation Pathway: A Novel Approach for Validating Point-of-Care Hendra Virus Assays	Lyndal Hulse
12.00 - 12.15	Development of HeV diagnostic serology assays at ACDP	Leanne McNabb
12.15 -12.30	The HeV nucleocapsid protein as a frontline diagnostic tool to confirm infections	Kalpana Agnihotri
12.30 – 12:45	Viral discovery using agnostic VirCapSeq-VERT platform in patients with unexplained Nipah-like illnesses	Nischay Mishra
12.45 - 1.45pm	**Lunch**	

1.45 – 3.00pm	**Virology and Immunology Session 1**	**CHAIRS: Vincent Munster & K9 Jenns**
1.45 -2.00	Investigating functional diversity of the Hendra virus genotypes	Melanie Tripp
2.00 - 2.15	Structural basis for importin alpha 3 specificity of W proteins in Hendra and Nipah viruses	Jade Forwood
2.15 -2.30	Henipavirus matrix protein employs a non-classical nuclear localization signal binding mechanism	Camilla Donnelly
2.30 -2.45	Constructing Antigenically Diverse Panels of Henipavirus F and G Proteins	Aaron May
2.45 -3.00	Long-term Nipah antibodies and memory B cells in survivors from the 1998 outbreak in Malaysia	Hui Ming Ong
3.00 - 3.30pm	**Afternoon tea**	
3.30 - 4.15pm	**Virology and Immunology Session 2**	**CHAIRS: Sarah Edwards & Melanie Tripp**
3.30 - 3.45	Assessment and characterization of the replication kinetics of Henipaviruses in reconstituted airway epithelia derived from the entire human respiratory tract	Hulda R. Jonsdottir
3.45 – 4.00	Unraveling Henipavirus Infection Biology in Bat Cells Using Functional Genomics	Yaw Shin Ooi
4.00 - 4.15	Salt Gully virus: a novel henipavirus isolated from Australian pteropus bats	Jennifer Barr
4.15 – 5.00pm	**Bat Infection studies**	**CHAIRS: Wanda Markotter & Berta Blanch Lazaro**
4.15 - 4.30	Rousettus aegyptiacus fruit bats as a potential animal model for henipavirus infections in the reservoir host	Anne Balkema-Buschmann
4.30 -4.45	Jamaican fruit bats (Artibeus jamaicensis) effectively control Hendra and Nipah virus infection	Sarah van Tol
4.45 – 5.00	Natural Hendra virus infection of captive flying foxes	Michelle Baker
6.30pm - 9.30pm	**Conference Dinner at the Stadium Captains Room, GMHBA Stadium**	

Day Four – Wednesday 11th of December

9.00 - 10.45am	**Pathogenesis**	**CHAIRS: Glenn Marsh & Kerry Goldin**
9.00 - 9.15	Comparative Histopathology of Henipavirus Infection: Insights from Human and African Green Monkey Models	Karla Fenton
9.15 - 9.30	Nipah virus neuropathogenesis in vitro and in vivo	Emmie de Wit
9.30 - 9.45	Discriminating disease outcomes in nonhuman primates exposed to Malaysia or Bangladesh isolates of Nipah virus	Michael Holbrook

9.45 - 10.00	Modelling zoonotic Nipah virus infection in microphysiological systems	Gabriella Worwa
10.00 -10.15	Challenge of African green monkeys with Hendra virus genotype 2 or Hendra virus Australia/Horse/2008/Redlands produces divergent clinical disease phenotypes	Declan Pigeaud
10.15 - 10.30	Long-term detection of Nipah virus replication in IFNAR KO mice by longitudinal in vivo imaging	Katherine Davies
10.30 - 10.45	Targeted transient interferon signalling disruption as alternative mouse model of Nipah virus infection to investigate role of immune responses in disease progression	Jessica Spengler
10.45 -11.15am	**Morning tea**	
11.15 - 1.00pm	**Vaccines**	**CHAIRS: Christina Spiropoulou & Emily Dowling**
11.15 - 11.30	Introduction/overview of henipavirus vaccines and therapeutics	Richard Jarman
11.30 - 11.45	The humoral immune response of foals to HeV vaccination	Kimberley Carey
11.45 - 12.00	Decoding Dose-Dependent Immunity: Insights into Nipah Virus Vaccine Efficacy and Correlates of Protection	Courtney Woolsey
12.00 - 12.15	A Phase 1, Dose-escalation, Open-label Trial of a Structure-based mRNA Vaccine Targeting Nipah Virus, mRNA-1215, Demonstrates Hendra Virus Cross-reactivity	Lesia Dropulic
12.15 - 12.30	Advances in Henipavirus Vaccination Strategies: A Thermostable Needle-Free Nipah virus and Hendra virus Vaccine Confers Broad and Durable Protective Immunity	Christopher Broder
12.30 - 12.45	A replicon RNA vaccine completely protects ferrets and nonhuman primates from lethal challenge with Bangladesh strain of Nipah virus	Robert Cross
12.45 - 1.00	Single-dose replicon particle vaccine rapidly protects African green monkeys against lethal Nipah virus challenge	Stephen Welch
1.00 -2.00pm	**Lunch**	
2.00 - 3.15pm	**Therapeutics**	**CHAIRS: Chris Broder & Dani Anderson**
2.00 - 2.15	Dexamethasone reduces pulmonary pathology but does not alter mortality in Syrian hamsters infected with Nipah virus	Kerry Goldin
2.15 -2.30	Discovery of novel Henipavirus inhibitors	Judith Straimer
2.30 - 2.45	A protective bispecific antibody targets both Nipah virus surface glycoproteins and limits viral escape	Ariel Isaacs

2.45 - 3.00	Development of antiviral therapies against Nipah and Hendra viruses	Olena Shtanko
3.00 - 3.15	Human antibodies with potent cross-neutralizing activity against Hendra and Nipah viruses	Rosemarie Mason
3.15 - 3.45pm	**Afternoon tea**	
3.45 - 4.30	Panel discussion: The WHO Nipah virus Roadmap, and the new WHO Paramyxovirus CORC: how can these initiatives forge progress in the control and prevention of henipavirus outbreaks? Panelists: Stephen Luby, Linfa Wang, Emily Gurley, Emmie de Witt, Tahmina Shirin, Sreelekshmy Mohandas Chairs: Jon Epstein and Kim Halpin	
4.30 - 4.45pm	Awards and close of meeting	

Awards kindly sponsored by

Abstracts

Day 2 – Monday 9th of December

History of Hendra virus

Abstract O-01

Hendra virus: a personal perspective

Paul Selleck

CSIRO Australian Centre for Disease Preparedness

In September, 1994 the director of the Australian Animal Health Laboratory (AAHL) in Geelong, now the Australian Centre for Disease Preparedness, was advised by the Queensland CVO of an outbreak of a potential exotic disease on a horse stable in the Brisbane suburb of Hendra. Samples were collected and submitted to AAHL for testing. This presentation describes the isolation and characterisation of the causative agent, the follow up work to confirm it as the cause of the outbreak, the development of new diagnostic tests and their use in controlling the outbreak and the results of the tests as they relate to the epidemiology. A second outbreak of Hendra occurred in Mackay in August 1995 and the results of tests on the affected people and animals are described. This second outbreak lead to a further investigation and the eventual identification of the host species.

Abstract O-02

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

Alison J. Peel[1], Raina K. Plowright[2], Peggy Eby[3,4], Hamish McCallum[3], Tamika J. Lunn[5,6]

[1]Sydney School of Veterinary Science, University of Sydney, Sydney, NSW, Australia, [2]Department of Public and Ecosystem Health, College of Veterinary Medicine, Cornell University, Ithaca, NY, [3]Centre for Planetary Health and Food Security, Griffith University, Nathan, Qld, Australia, [4]School of Biological Earth and Environmental Sciences, University of New South Wales, Sydney, NSW, Australia, [5]Odum School of Ecology, University of Georgia, Athens, GA, USA, [6]Center for the Ecology of Infectious Diseases, University of Georgia, Athens, GA, USA

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.

Abstract O-03

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

Claude Kwe Yinda[1], John-Sebastian Eden[2], Alison Peel[3], Raina Plowright[4], Bat One Health Team, Vincent Munster[1]

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

Abstract O-04

Periodic shifts in viral load increase risk of spillover from bats

Tamika J. Lunn[1,2], Benny Borremans[3,4], Devin N. Jones[5], Maureen K. Kessler[6], Adrienne S. Dale[7], Claude K. Yinda[8], Manuel Ruiz-Aravena[9], Caylee A Falvo[10], Daniel E. Crowley[10], James O. Lloyd-Smith[11], Vincent J. Munster[8], Peggy Eby[12,13], Hamish McCallum[12], Peter Hudson[14], Olivier Restif[15], Liam P. McGuire[16], Ina L. Smith[17], Bat One Health Team, Raina K. Plowright[10], Alison J. Peel[12,18]

[1]Odum School of Ecology, University of Georgia, Athens, GA, USA, [2]Center for the Ecology of Infectious Diseases University of Georgia, Athens, GA, USA, [3]Wildlife Health Ecology Research Organization, San Diego, USA, [4]Evolutionary Ecology Group, University of Antwerp, Antwerp, Belgium, [5]Department of Microbiology & Cell Biology, Montana State University, Bozeman, MT, USA, [6]Department of Ecology, Montana State University, Bozeman, MT, US, [7]Department of Biological Sciences, Texas Tech University, Lubbock, TX, USA, [8]Laboratory of Virology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT, USA, [9]Department of Wildlife, Fisheries and Aquaculture, Mississippi State University, Starkville, MS, USA, [10]Department of Public and Ecosystem Health, College of Veterinary Medicine, Cornell University, Ithaca, NY, [11]Department of Ecology and Evolutionary Biology, UCLA, Los Angeles, CA, USA, [12]Sydney School of Veterinary Science, University of Sydney, Sydney, NSW, [13]School of Biological Earth and Environmental Sciences, University of NSW, Sydney, NSW, Australia, [14]Center for Infectious Disease Dynamics, Pennsylvania State University, State College, Pennsylvania, PA, USA, [15]Department of Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom, [16]Department of Biology, University of Waterloo, Waterloo, ON Canada, [17]Health and Biosecurity Business Unit, Commonwealth Scientific and Industrial Research Organisation (CSIRO), Canberra, ACT, Australia, [18]Sydney School of Veterinary Science, University of Sydney, Sydney, NSW.

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.

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]

[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.

Abstract O-06

Incredible diversity of Henipaviruses revealed in Australian flying foxes

K9 Jenns[1,2,3], Claude Kwe Yinda[4], Brent Jones[5], Karan Kim[1,2], Bat One Health team, Raina Plowright[6], Vincent Munster[4], Alison Peel[1,3], J-S Eden[1,2,3]

[1]The University of Sydney, [2]Westmead Institute for Medical Research, [3]Sydney Infectious Diseases Institute, [4]Rocky Mountain Laboratories, [5]Griffith University, [6]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 metatranscriptomics, 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.

Abstract O-07

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

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 metatranscriptomics 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.

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

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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 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.

Rapid oral session talks

Abstract RO-01(Poster P-01)

Model-guided henipavirus discovery in museum bat collections

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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.

Abstract RO-02 (Poster P-02)

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

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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.

Abstract RO-03 (Poster P-03)

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

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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 (58/60) 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.

Abstract RO-04 (Poster P-04)

Henipavirus sero-surveillance in horses and pigs from Northern Nigeria

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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.

Abstract RO-05 (Poster P-05)

Cryptic exposure to henipaviruses in Cambodia and Malaysia

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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% (57/1469) 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.

Abstract RO-06 (Poster P-06)

Serosurveillance of Nipah virus in Malaysia

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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).

Abstract RO-07 (Poster P-07)

Development and establishment of diagnostic capability for Nipah virus

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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.

Abstract RO-08 (Poster P-08)

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

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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/NiV are classified as Biosafety Level 4 agents. This project aimed to investigate LayV/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 IMPα/β isoforms. Fluorescence polarisation assays determined the strength of binding between the functional NLSs and each IMPα/β isoform. Protein crystallography screening of IMPα/β isoforms and LayV/AngV NLS synthetic peptides were used to characterise structures of protein-peptide interactions which may provide targets for future antiviral therapy or vaccine development.

Abstract RO-09 (Poster P-09)

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

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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 ($p<;0.05$), G2 ($p<0.001$) and G9 ($p<0.05$). These findings suggest that long-term immunity against NiV is achievable, supporting the potential for successful vaccine development.

Abstract RO-10 (Poster P-10)

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

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Background andAim: 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.

Abstract RO-11 (Poster P-11)

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

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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/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.

Abstract RO-12 (Poster P-12)

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

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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.

Abstract RO-13 (Poster P-13)

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

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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/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.

Abstract RO-14 (Poster P-14)

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

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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/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.

Abstract RO-15 (Poster P-15)

Human neutralising monoclonal antibodies against Hendra and Nipah viruses

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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.

Abstract RO-16 (Poster P-16)

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

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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 enrol up to 2,000 AES patients across three tertiary care hospitals. With informed consent, study staff will enrol 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: enrolment, 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/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.

Poster Session

Surveillance

Poster P-17

Two different Nipah virus strains circulating in Pteropus lylei colony in Chonburi province, Thailand

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Nipah virus (NiV) caused outbreaks in Malaysia, Bangladesh, and India. Pteropus bats are the natural reservoir. From 2002 to 2017, NiV was monitored in a P. lylei (PL) colony in Chonburi, Thailand. Bangladesh strain NiV (NiV-B) has been detected in bat urine, saliva, and blood; no human or animal infections have been reported in Thailand. In 2017, we sequenced the NiV-B genome from bat urine, which showed 99.2% identity with NiV from Bangladeshi patients. In May 2024, pooled bat urine samples were collected from PL colony in Chonburi. NiV heminested RT-PCR targeting the N gene was conducted. 7/54 (12.96%) tested positive for NiV. Phylogenetic analysis of a 357 bp partial nucleocapsid fragment identified two distinct NiV strains: the NiV-B (n=2) and the Malaysia strain (NiV-M) (n=5). Two positive samples were selected for whole-genome sequencing (WGS) using Illumina RNA Prep with Enrichment. NiV-M genome (18,246 bp, 98.7% complete) shared 98.7%, 98.7%, and 97.5% identity to NiV from a Malaysian patient in 1999, P. hypomelanus in Malaysia in 1999 (60% coverage), and PL in Cambodia in 2003, respectively. NiV-B genome (18,238 bp, 96.7% complete) demonstrates a 99.3% identity to NiV-B WGS from 2017, with no significant mutations found over the 7-year period. In a previous surveillance study in Thailand, NiV-B was the dominant strain in the PL population, while NiV-M was detected in P. hypomelanus in the southern region. The NiV-M found in this study may have been shed by PL, as seen in Cambodia, or by P. hypomelanus populations in a shared habitat within the PL colony. However, the lack of individual bat samples hinders species identification. This underscores the need for future research to focus on species identification, which could provide valuable insights into the dynamics of Nipah virus transmission. This discovery also raises a red flag about a potential NiV outbreak in Thailand, underscoring the critical need for immediate and thorough surveillance.

Poster P-18

Detection of a Hendra virus genotype 1 variant in a flying fox, Australia

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The detection of a second Hendra virus genotype in horses as well as multiple flying foxes reinforces the importance of surveillance for henipaviruses in general. Veterinary diagnostic laboratories have a unique opportunity to undertake this passive surveillance on samples submitted for disease investigation. At the Biosecurity Sciences Laboratory we have developed a real-time PCR capable of sensitive detection of Hendra virus genotype 1 (HeV-g1), Hendra virus genotype 2 (HeV-g2) and Nipah virus. This real-time PCR has been used to extensively screen horse samples submitted for Hendra virus exclusion as well as flying fox tissue samples from animals submitted for exclusion of Australian bat lyssavirus. A novel HeV-g1 variant sequence has been detected in the spleen and kidney of a black flying fox as part of this screening that is not able to be detected by the HeV-g1 or HeV-g2 real-time PCR assays utilised by the State veterinary diagnostic laboratories. Further characterisation of this virus was conducted using Next-generation Sequencing (NGS) at CSIRO Australian Centre for Disease Preparedness, and approximately 90% of Hendra virus genome was discovered. Sequence analysis demonstrates that the virus has 94% nucleotide identity to HeV-g1, and 86% to HeV-g2. The findings indicate that this virus is a new variant of HeV-g1.

Poster P-19

Behavioral and ecological drivers of geographic variation for Nipah virus spillover risk in Bangladesh

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Nipah virus (NiV) spillover from bats into human populations is spatially clustered in Bangladesh, primarily occurring in the Western region (Nipah Belt). However, NiV has been detected in P. medius bats found throughout the country. It is therefore unclear what drives the particular pattern of NiV occurrence in human populations. To begin to answer this question, we designed the human study with the underlying goal of determining how behavioral and ecological drivers contribute to spatial differences in exposure and outbreak risk. We aimed to administer human behavioral questionnaires and collect blood samples from 2400 individuals comprising 400 per site among six sites (3 Nipah Belt and 3 Eastern regions, outside of the "Nipah Belt). Sampling was conducted at household level, and participating households were selected through a randomization process within communities. The study revealed significantly ($p<0.001$) higher proportion of households in the Nipah Belt that consume raw date palm sap (RDPS) (55.5% vs 16.8%), harvest RDPS (13.4% vs 1.6%), own date palm trees (28.6% vs 7.2%), grazing domestic animal under bat roost (43.5% vs 15.3%), and eating bat bitten fruits (43.2% vs 29%) compared to households outside of the Nipah Belt. Using a generalized linear mixed effects model (GLM), we found that gender, education, income, harvesting RDPS, and owning a date palm tree significantly influenced the consumption of RDPS. Consumption of half-eaten fruits has not been linked to human infection, but it was the putative route of transmission from pteropid bats to pigs in Malaysia, and it might plausibly route of exposure for livestock and humans in Bangladesh. The higher consumption of RDPS in western regions in Bangladesh could conceivably drive a spatial discrepancy in human outbreaks. However, it does not appear to explain the relationship in its entirety, given that RDPS consumption does occur outside of the Nipah belt, albeit to a lesser extent.

Poster P-20

Bat sampling using traditional methods and new technologies

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Bats play a crucial role in the emergence of zoonotic disease outbreaks due to their well-documented ability to harbor viruses of human and animal concern, such as henipaviruses, coronaviruses and filoviruses. Regular surveillance of bat colonies is crucial for detecting and monitoring both known and unknown circulating viruses and assessing the potential risk of these viruses spilling over to humans and or animals. Scientists at ACDP have extensive experience in bat surveillance, employing both passive methods (such as collecting urine and faeces from under roost colonies) and active sampling methods (such as catching and handling bats for sample collection). Previous projects looking at samples collected from Australian Pteropus bats have led to the successful detection and isolation of known viruses like Hendra virus and Menangle virus and the discovery of new henipaviruses such as Cedar virus and Salt Gully virus as well as multiple novel rubulaviruses. Current bat surveillance projects continue to focus on passive under-roost urine and faeces collection to detect and monitor viral pathogens, while also comparing detection methods like commercially available air sampling units. Identified novel viruses will be evaluated for their pathogenic and zoonotic potential in humans and animals using advanced cell culture models, in vivo studies and genetic analysis. The outcomes of this research can be used to inform relevant government authorities of the potential zoonotic risks posed by circulating bat-borne viruses.

Poster P-21

Implication of a bat reovirus in an outbreak of respiratory disease and encephalitis in Bangladesh

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Pteropine orthoreoviruses (PRVs) are emerging bat-borne viruses previously linked to sporadic acute respiratory infections in humans, especially in Southeast Asia. Here, we report the first outbreak of PRVs in Bangladesh. Five 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 patients were used for viral discovery using VirCapSeq-VERT (VCS), an agnostic capture sequencing platform for detection of vertebrate viruses. The PRV positive throat-swabs were also used for virus culture in MDCK and passaged in Vero-E6 cell-lines.

VCS data showed reads for PRVs in all patients. 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.

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. Pan-viral surveillance and differential diagnosis of respiratory illnesses with encephalitis, and other unexplained febrile illnesses is warranted in areas where date-palm sap is routinely consumed.

Poster P-22

Just another Friday!

Sue Lowther, Paul Selleck

CSIRO Australian Centre for Disease Preparedness

In 1994, late on a Friday, we were alerted to the arrival of samples from several racehorses exhibiting pneumonia-like symptoms. Initially, poisoning was considered the most likely cause, but with the racehorse owner also showing signs of illness, the AAHL team shifted focus toward a viral etiology. Lung tissue samples were cultured on Vero cells, and by Monday morning, cytopathic effects (CPE) were evident, confirming the presence of an infectious agent. Multiple teams mobilized together to address the growing list of critical questions: What is it? Will it cause disease? Which other species are at risk? Where did it come from? These are the events that took place starting on that fateful Friday.

Poster P-23

Characterising the P gene products of Australian henipaviruses and their role in pathogenesis

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Henipaviruses are zoonotic, single-stranded, non-segmented, negative-sense RNA viruses of the Paramyxoviridae family. Hendra virus (HeV) and Nipah virus (NiV) are prototypic henipaviruses, with a lethality rate of approximately 70 percent. Other henipaviruses found in Australia include Cedar virus (CedV) that has failed to cause disease in experimentally challenged animals and recently isolated Salt Gully virus (SGV) with unknown pathogenicity. Henipaviruses have three non-structural proteins derived from the phosphoprotein, including the C protein, encoded by an alternative open reading frame, as well as V and W from additional guanine residues within the mRNA editing site that causes frameshift mutations. These proteins differ in host cell localisation where V is cytoplasmic and W is nuclear, causing varied interactions of the innate immune system. The C protein is the most unexplored regarding its function in pathogenesis and evasion of the immune system. The aim of this project is to understand the role of the non-structural proteins in disease outcomes of henipaviruses and define the differences between viruses within the same genus that may or may not cause clinical manifestations. This research will investigate whether CedV and SGV have pathogenic potential and the ability to cause editing as observed with HeV and determine the role that the non-structural proteins play in this process. The significance of this research will aid in defining molecular determinants of henipavirus disease, thereby identifying any potential threat that emerging Australian henipaviruses pose to animal and human health.

Poster P-24

Batting for structure: the shrewd secrets of Henipavirus fusion proteins

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The genus Henipavirus (HNV) includes highly pathogenic zoonotic viruses originating from bats, such as Nipah virus (NiV) and Hendra virus (HeV), for which no vaccines exist for humans. Moreover, a recent outbreak caused by novel shrew-borne HNV like Langya virus (LayV) in humans has highlighted the spillover of these viruses. Recently, two shrew-borne HNVs, Daeryong (DarV) and Gamak virus (GmkV), along with a bat-borne HNV, Angavokely virus (AngV), have been described. Notably, GakV has been found capable of infecting mammalian cell lines, but the potential for these viruses to cause disease in humans is uncertain due to limited characterisation. HNVs enter host cells by fusing to host cells, mediated by receptor binding protein (RBP) and fusion (F) glycoproteins, which are also the main targets of humoral immunity. Here, we present the prefusion F protein structure of HNVs determined by cryogenic transmission electron microscopy. Despite sharing only approximately 40% sequence identity with prototypical HNV like NiV and HeV, they adopt a largely similar overall architecture. Furthermore, well-characterised anti-NiV and HeV neutralising F antibodies do not react to AngV, DarV or GakV F proteins. These findings suggest that newly described bat and shrew-borne HNV are antigenically divergent from the prototypical HNVs. By determining the structure of divergent HNV F proteins, this work provides a foundation for the development of new vaccines and therapeutics and enables pandemic preparedness against potential outbreaks of novel HNVs in the future.

Poster P-25

Equivac® HeV vaccine for horses is cross protective against HeVg2 variant, as demonstrated by a recombinant Cedar Virus surrogate neutralisation assay

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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.

Poster P-26

Serological Dynamics in Captive Flying Foxes: Insights from Natural and Experimental Hendra Virus Infections

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This study investigated the Hendra virus (HeV) serological dynamics of a captive cohort of black flying foxes (Pteropus alecto) held in captivity in Queensland (QLD) followed by transport to ACDP in Victoria for the purpose of performing an experimental HeV infection. Twenty flying foxes that had come into the care of bat rehabilitators due to injuries but were otherwise healthy were recruited for this study and were monitored over four months in QLD. This included 12 individuals that were seronegative to HeV and 8 with low to medium levels of HeV antibodies. Samples were collected on recruitment to the study and again at three weeks prior to transport to ACDP and provided evidence for stable HeV serostatus in all 20 individuals. Serological analysis of samples collected one day after arrival at ACDP revealed that 11 flying foxes had seroconverted within the three-week period between their final testing in QLD and their arrival at ACDP, indicating that an active HeV infection was circulating prior to transport. Additionally, two individuals developed natural HeV infections following their arrival. Experimental infection of 11 flying foxes with HeV provided further insight into HeV antibody dynamics following infection. This study provides insight into the nature of the antibody response in relation to viral infection in P. alecto.

Poster P-27

New tools to characterise bat antigen presentation and their anti-viral response.

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Bats are associated with the transmission of deadly viruses to humans, including Hendra virus and other members of the henipavirus genus. Despite being reservoirs for these viruses, bats often remain asymptomatic, suggesting a strong evolutionary adaptation to these pathogens. While considerable research has been carried out on the innate immune system of bats, the understanding of their adaptive immune system has been hindered by a lack of specific tools and reagents. We have generated a series of next generation reagents to dissect bat adaptive immune responses. For example, we have examined the nature of peptide antigens bound to different P.alecto (Australian black flying fox) major histocompatibility complex (MHC) I allotypes isolated from the surface of bat cell lines and tissues. We show that P.alecto MHC class I α1 domain can have unique 3- to 5- amino acid insertions that allow for the accommodation of longer peptides, up to 15 amino acids in length, highlighting novel aspects of the bat adaptive immune response. Using these reagents, we have probed the presentation of Hendra virus derived peptides in experimentally infected bats, gaining the first insights into antigen presentation of Hendra virus in vivo. Understanding the ability of bats to control infection compared to other mammalian hosts will be fundamentally important for the development of effective anti-viral strategies to prevent future bat mediated transmission of Hendra and other zoonotic pathogens.

Poster P-28

Establishment of the hamster Nipah virus infection model at the UK Health Security Agency (UKHSA)

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United Kingdom Health Security Agency (UKHSA), Medicines and Healthcare products Regulatory Agency (MHRA)

With the increasing interest and investment of vaccines against pathogens with epidemic potential, such as Nipah virus, the capability to assess the efficacy of such interventions is required. Evaluation in animal model systems is a critical part of the vaccine development pathway, with the Golden Syrian hamster being the leading small animal model for Nipah virus infection.

Due to variations in model clinical endpoint parameters between different laboratories, when establishing the Nipah virus hamster model we conducted dose determination studies to obtain the relevant challenge dose for subsequent efficacy studies. In addition, two routes of challenge were assessed: intranasal to resemble natural human infection and intraperitoneal which has been widely reported in laboratory studies as generating a consistent disease progression. The model was established for both the Malaysian and Bangladesh strains of Nipah virus.

Our results show that the dose that uniformly causes severe disease in hamsters is 10 and 1000 TCID50 for the intranasal and intraperitoneal challenge routes, respectively. In our studies, we found no difference in the challenge doses leading to humane clinical endpoints between the two virus strains. With higher challenge virus doses, a predisposition to respiratory clinical signs were observed whereas with lower doses neurological disease sequalae were regularly recorded.

The establishment of the Nipah virus animal model in the UK expands the network of laboratories with the capability for conducting protection studies against live virus challenge. It is hoped that this will strengthen the response in the assessment and prioritisation of vaccine candidates against this important priority pathogen.

Poster P-29

Aggregate data analyses to characterize the effects of strain and inoculation route on clinical course and outcome in the Syrian Hamster Model

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Centre for Disease Control

The two characterized strains of Nipah virus (NiV), NiV-Malaysia and NiV-Bangladesh, share approximately 92% sequence similarity but exhibit diverse clinical presentations in both human and in animal disease models. Developing effective medical countermeasures, such as vaccines and antiviral therapies, requires the use of well-defined and meticulously characterized animal models of both NiV-strains. The Syrian hamster model effectively captures the respiratory and neurological presentations observed in human cases making it a valuable tool for developing and evaluating vaccines and therapeutics. Previous studies have demonstrated infection outcomes – such as clinical course, presentation, and mortality – vary based on experimental variables such as strain-, dose- and route of infection. However, these outcomes have not yet been extensively characterized. In this study, we analyze aggregate data from unvaccinated and/or untreated control hamsters from 18 independent NiV-hamster studies conducted since 2017 at CDC, Atlanta. We focus on exploring how key outcomes including weight, survival, clinical score, body temperature, and viral tissue loads were influenced by experimental variables. Here, we describe strain- and route-associated differences in mortality rates, and incidence of respiratory and neurological signs. This analysis will be used to enhance experimental study design and provide insights for translating findings to human disease.

Poster P-30

Henipavirus infection of a human airway epithelium model

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Henipaviruses, genus Henipavirus within the family Paramyxoviridae, are deadly zoonotic viruses that can cause respiratory illness and encephalitis in humans. Despite their mortality rates exceeding 70%, no vaccines or therapeutics are licensed for human use. In this study, the human airway epithelium model was determined if it is a suitable non animal model to assess the zoonotic potential of henipaviruses and to screen therapeutics. Primary normal human bronchial epithelial (NHBE) cells were grown and cultured in Transwells at air liquid interface (ALI) for 6-8 weeks to form a pseudostratified airway epithelium consisting of basal, ciliated and goblet cells. These physiologically relevant ALI cultures mimic the key features present in the human lung in vivo by producing mucus, exhibiting ciliary beating and maintaining strong barrier function. The human airway epithelium was infected with a range of henipaviruses, both pathogenic and non pathogenic, and infectivity was assessed.

Poster P-31

Health Impact Modelling for New Vaccines against Nipah Virus Disease and Nipah Viral Disease-like Disease X: Epidemic Preparedness and Response Modelling

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Nipah virus (NiV) is an emerging zoonotic pathogen with a high mortality rate and recurrent outbreaks in South Asia, more regularly in Bangladesh and India, posing a global health threat. Despite its urgency, no vaccines or drugs are available for prevention or treatment. My PhD research seeks to address this gap by understanding the transmission dynamic of NiV disease (NiVD) and vaccine impact modelling through simulation-based projection.

The primary objective is to reconstruct the NiV transmission dynamic model to understand the current outbreak patterns and probability of a major outbreak using the branching process model. Furthermore, the research will conduct vaccine impact modelling to estimate the potential health impact of future NiV vaccines under various vaccination strategies and epidemiological scenarios. The base-case vaccine characteristics will employ the draft Target Product Profile for NiV vaccines published by the WHO in 2017. These assessments will estimate the number of cases, deaths, and disability-adjusted life years associated with different vaccination strategies, aiding policy decisions and resource allocation.

Additionally, the study will extend its findings to NiVD-like Disease X by conducting a scoping review of other zoonotic infectious diseases to understand clinical characteristics and outbreak patterns. Insights from this review will inform the evaluation of health impacts and benefit-risk assessments for hypothetical vaccines targeting NiVD-like Disease X.

This research has the potential to inform vaccine development decision-making, enhance epidemic preparedness, and facilitate timely responses to emerging infectious diseases. Despite limitations such as data incompleteness and uncertainties in vaccine characteristics, the study aims to address these challenges through extensive sensitivity analyses and contribute to epidemic preparedness against emerging infectious diseases.

Poster P-32

Cross-reactivity of henipavirus vaccines and assessment of protective efficacy

Stuart Dowall, Nazia Thakur, Stephen Findlay-Wilson, Lucy Flett, Linda Easterbrook, Franscisco J. Salguero, Ines Ruedas-Torres, Susan Fotheringham, Emma Kennedy, Teresa Lambe and Dalan Bailey

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Soluble glycoprotein vaccine candidates against Nipah virus (NiV) and Hendra virus (HeV) were produced at the Pirbright Institute, UK. In an initial study, the cross-protective activities of NiV and HeV protein vaccines were assessed as part of establishing the hamster model of NiV disease at UKHSA. Results demonstrated protection with both vaccines, providing further evidence for effective pan-henipavirus vaccines.

To establish further evidence, a Ghanaian bat henipavirus (GHNV) soluble glycoprotein vaccine candidate was produced for comparison with the NiV candidate. Immunogenicity studies performed in mice demonstrated cross-recognition of antibodies to both NiV and GHNV proteins after immunisation with either protein. In functional antibody assays, NiV-immunised mice effectively neutralised NiV pseudoparticles, but not their GHNV counterparts. In contrast, sera from GHNV-immunised mice effectively neutralised both NiV and GHNV pseudotyped viruses, indicating functional cross-reactivity with this approach. To test whether these responses translated to protection, a further efficacy study was conducted in the NiV hamster model. Whilst the NiV vaccine afforded complete protection against a dose where all control animals met endpoint criteria, the GHNV vaccine did not provide protection, with just 1 animal from the group of 6 surviving.

Our results demonstrate that cross-recognition of antigens from divergent henipaviruses does not always correlate with protective efficacy. However, further investigation of the immune responses induced by the GHNV vaccine and increasing progress on correlates of protection has the potential to further develop this approach in pursuit of a pan-henipavirus vaccine.

Poster P-33

Immunization of wildlife by mosquito-delivered vaccine

Dan Wen, Hongyue, Li, Fei Yuan, **Aihua Zheng**

Institute of Zoology, Chinese Academy of Sciences, China

Most zoonotic viruses, such as the Zika, Nipah and rabies viruses, are mainly maintained in vertebrates or between vertebrates and arthropod vectors. Zoonotic viruses pose great threat to wildlife and sporadically spill over into human populations, resulting in endemics or pandemics. Targeted immunization of wild animal reservoirs would be a promising approach, not only for control of zoonotic diseases infecting domestic animals and humans, but also for protection of endangered wildlife. Here, we developed a proof-of-concept mosquito-delivered vaccine to control the Zika virus (ZIKV) within inaccessible wildlife hosts using an insect specific flavivirus, Chaoyang virus (CYV) as the vector. The vaccine is constructed by replacing the pre-membrane and envelope (prME) proteins of CYV with those of ZIKV, assigned as CYV-ZIKV. CYV-ZIKV replicates efficiently in Aedes mosquitoes and disseminates to the saliva, with no venereal or transovarial transmission observed. To reduce the risk of CYV-ZIKV leaking into the environment, mosquitoes are X-ray irradiated to ensure 100% infertility, which does not affect the titer of CYV-ZIKV in the saliva. Immunization of mice via CYV-ZIKV carrying mosquito bites elicits robust and persistent ZIKV-specific immune responses and confers complete protection against ZIKV challenge. Correspondingly, the immunized mice could no longer transmit the challenged ZIKV to naïve mosquitoes. Therefore, immunization with an ISF-vectored vaccine via mosquito bites is feasible to induce herd immunity in wildlife hosts of ZIKV. Recently, we further developed mosquito-delivered vaccines against bat-borne rabies and Nipah viruses. Using White-bellied Tube-nosed bat (Murina leucogaster) as a model, protective immunity was elicited by eating or being biting by vaccine-carrying mosquitos. Our study provides a future avenue for developing a mosquito-delivered vaccine to control zoonotic viruses in the sylvatic cycle.

Poster P-34

The Equivac® HeV Hendra Virus Vaccine is Highly Effective in Inducing Neutralising Antibody Titres in Horses: a Field Study

Kim Halpin, Kerryne Graham, **Gervais Habarugira,** Peter Durr

CSIRO – Australian Centre for Disease Preparedness

Hendra virus (HeV) is one of the zoonotic paramyxoviruses posing a significant threat to humans and animals. Since it was discovered in 1994, more than 100 horse cases and seven human cases of Hendra virus infection have been reported with a 70% case fatality rate in humans. A Hendra virus vaccine, the Equivac® HeV Hendra virus vaccine, became available for horses in late 2012. This study aimed to collate post-vaccination seroconversion data from vaccinated horses under field conditions. Three hundred and thirty-two sera samples were tested using serum neutralising assay (SNT). The SNT results were analysed, together with age, dates of vaccinations, date of sampling and location.

Provided horses received at least three vaccinations (as prescribed by the manufacturer and consisting of two doses 3–6 weeks apart, with a third dose six months later), horses had high neutralising titres (median titre for three or more vaccinations was 2048), and none tested negative. In this study, three horses tested negative, but all had only received two vaccines, and the time between last vaccination and sampling date ranged from 1327 to 2393 days, or approximately 3.6 to 6.6 years.

This study shows that the Equivac® HeV Hendra virus vaccine induces significant neutralising antibody titres following the completion of the priming schedule and annual boosters. While the humoral immune response has been studied, the cellular immune response to the HeV vaccine is yet to be elucidated., There is also a need to develop a surrogate assay to substitute the traditional SNT that requires BSL4 laboratories and has inherent biological variability.

Poster P-35

Development of Machine Learning Models for the Discovery of Novel Antiviral Candidates Targeting Henipaviruses (NiV and HeV) Fusion Inhibition

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We evaluated the feasibility of developing machine-learning capabilities for the discovery of new NiV and HeV antiviral small molecules and identifying novel antiviral candidates targeting NiV and HeV fusion to the host cell for further development. The project leverages current knowledge of inhibitors for related paramyxoviruses, namely the measles virus (MeV), to predict HeV/NiV antivirals through a machine learning algorithm using the Rank Support Vector Machine (RankSVM) and logistic regression. From a virtual screening of 30 million compounds, three distinct chemotypes were identified, and from those, thirty compounds were purchased for direct testing in a BSL-4 setting. Of the thirty compounds, ten reduced HeV virus infectivity in HeLa cells by 50% or more at a concentration of 50 micromolar without exhibiting cytotoxicity at that level.

Poster P-36

Optimization of Bangladesh and Malaysian genotype recombinant reporter Nipah viruses for in vitro antiviral screening and in vivo disease modeling

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Nipah virus (NiV) causes near-annual outbreaks of fatal encephalitis and respiratory disease in South Asia with a high mortality rate (~70%). Since there are no approved therapeutics for NiV disease in humans, the WHO has designated NiV and henipaviral diseases priority pathogens for research and development. We generated a new recombinant green fluorescent reporter NiV of the circulating Bangladesh genotype (rNiV-B-ZsG) and optimized it alongside our previously generated Malaysian genotype reporter counterpart (rNiV-M-ZsG) for antiviral screening in primary-like human respiratory cell types. Validating our platform for rNiV-B-ZsG with a synthetic compound library directed against viral RNA-dependent RNA polymerases, we identified a hit compound and confirmed its sub-micromolar activity against wild-type NiV, green fluorescent reporter, and the newly constructed bioluminescent red fluorescent double reporter (rNiV-B-BREP) NiV. We furthermore demonstrated that rNiV-B-ZsG and rNiV-B-BREP viruses showed pathogenicity comparable to wild-type NiV-B in the Syrian golden hamster model of disease, supporting additional use of these tools for both pathogenesis and advanced pre-clinical studies in vivo.

Poster P-37

Unveiling the Structures of N0-P Complex in High-Risk Paramyxoviruses and Development of Small Inhibitors against Paramyxoviral Infections

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Paramyxoviral (PMV) infections, caused by a group of highly pathogenic viruses such as Nipah Virus (NiV), Hendra Virus (HeV), Langya Virus (LayV), Newcastle Disease Virus (NDV) pose a significant threat to global human and animal health. These viruses are known for their zoonotic potential, high mortality rates, and devastating economic impacts on agriculture and livestock. Despite the severity of these infections, there are currently no approved therapeutics or vaccines for many of these pathogens, emphasizing the urgent need for innovative therapeutic strategies. The PMV genome is composed of negative-sense single-stranded RNA and encodes six structural proteins. Among them, Nucleoprotein (N) binds to RNA genome to form ribonucleoprotein complex (RNP) for viral genome wrapping. So, for replication and transcription, the viruses require a continuous supply of unassembled, replication-competent N (N0) monomers which is tightly regulated by the interaction of N0 with its viral chaperone, the Phosphoprotein (P). Disruption of this interaction could inhibit viral replication, making the N0-P interface a promising target for antiviral drug development.

This research aims to characterize the structural details of the N0-P complexes in high-risk PMVs through a combination of recombinant protein expression and X-ray crystallography. By resolving the structures of N0-P complexes, this study will focus on the molecular mechanisms underlying their formation. Particular attention will be given to identifying conserved residues and motifs critical for the interaction, as these could serve as universal targets for therapeutic intervention across multiple PMVs. The study has also broader aims to design inhibitors. Insights gained from structural analysis will enable the design of broad-spectrum antivirals against those N0-P complexes to block viral replication.

Ultimately, this research offers viable solutions for improving the response to emerging paramyxoviral infections.

Poster P-38

One Health Approach in Practice - Hendra virus response in Queensland

Jenny Thomlinson

Queensland Health

The Centre for Disease Control and Prevention Atlanta (CDC) describes the One Health approach as a collaborative, multisectoral and transdisciplinary approach with a goal of achieving health outcomes through recognising the interconnection between people, animals, plants and their shared environment. Queensland has adopted this approach for over a decade with the catalyst being the emergence of Hendra virus.

Hendra virus is a zoonotic disease transmitted from flying foxes (natural revisor) to horses (amplifying host) to humans. Both human and animal infection can have catastrophic outcomes. Since its emergence in 1994 there have been seven human infections with four of these people dying and the remainder left with long term health issues.

It was recognised that a collaborative, cross agency approach was required to identify and manage transmission risks, focussing on understanding the bat ecology, animal and human health exposure risks. Further to this, this collaborative approach extended to working at the national and international level with medical and veterinary research institutes to develop and trial monoclonal antibodies to protect humans from infection following exposure, and a Hendra virus vaccine for horses.

This poster presentation will pictorially describe how this approach was put in practice in Queensland to manage Hendra virus incidents and how it has successfully evolved as an integral model for the management of all zoonotic threats and incidents in Queensland.

Day 3 – Tuesday 10th of December

Surveillance

Abstract O-09

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

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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.

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. Noroña[1], Marana Rekedal[2], Jaiure Azel Café[1], Catalino Demetria[1], Lianying Yan[2], McKenna Roe[2], Spencer Sterling[2], Christopher Broder[2], Kirk J. Taray[3], Jezryl Jaeger L. Garcia[3], Ricardo C. Buenviaje[3], Joselito B. Sarmiento[3], Wilson B. Bulalacao[3], Jayson B. Bulalacao[3], Ronnel R. Tongohan[1], Jozette Hisu-an[1], John Christian Teñoso[1], Paulina Alexandra C. Nuňga[1], Jester B. Galaraga[1], Samantha Lian Magsanoc[3], Hazel Ruth Cruz[3], Sophie Allison Borthwick[4], Dolyce Hong Wen Low[4], Liesbeth M. Frias[4], Phillip Alviola[3], Mary Grace Dacuma[3], Fedelino F. Malbas[1], Gavin James Smith[5], Eric Laing[2]

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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 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.

Abstract O-11

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

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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.

Abstract O-12

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

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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.

Abstract O-13

Mojiang like henipavirus exposure to humans in rural communities in Bangladesh

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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.

Behavioural determinants of henipavirus transmission

Abstract O-14

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

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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.

Abstract O-15

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

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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.

Abstract O-16

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

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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.

Diagnostics

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

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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.

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 and 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.

Abstract O-19

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

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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.

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.

Abstract O-21

Investigating functional diversity of the Hendra virus genotypes

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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.

Abstract O-22

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

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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

Abstract O-23

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

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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.

Abstract O-24

Constructing Antigenically Diverse Panels of Henipavirus F and G Proteins

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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.

Abstract O-25

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

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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.

Abstract O-26

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

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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.

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.

Abstract O-27

Unravelling Henipavirus Infection Biology in Bat Cells Using Functional Genomics

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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.

Abstract O-28

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

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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.

Abstract O-29

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

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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.

Abstract O-30

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

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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 titers 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.

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.

Day 4 – Wednesday 11th of December

Pathogenesis

Abstract O-32

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

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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.

Abstract O-33

Nipah virus neuropathogenesis in vitro and in vivo

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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.

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Abstract O-34

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

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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.

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.

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.

Abstract O-37

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

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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.

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

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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.

Vaccines

Abstract O-39

The humoral immune response of foals to HeV vaccination

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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.

Abstract O-40

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

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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.

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

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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.

Abstract O-42

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

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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.

Abstract O-43

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

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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.

Abstract O-44

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

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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.

Abstract O-45

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

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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.

Abstract O-46

Discovery of novel Henipavirus inhibitors

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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.

Abstract O-47

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

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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.

Abstract O-48

Development of antiviral therapies against Nipah and Hendra viruses

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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.

Abstract O-49

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

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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.

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