Nipah@20

Nipah Virus International Conference

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Meeting report prepared by: Christine Berge and Eirik Torheim

Life Science Team AS, Gaustadalléen 21, N-0349 Oslo
Contact@lifescienceteam.com, +47 905 88 644

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NIH National Institute of
Allergy and
Infectious Diseases

DukeNUS
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List of abbreviations

A	answer
AAHL	Australian Animal Health Laboratory
Ad	adenovirus
AEFI	adverse events following immunisation
AES	acute encephalitis syndrome
Ag	antigen
AGM	African green monkey
AMES	Acute Meningo Encephalitis Surveillance system
APVMA	Australian Pesticides and Veterinary Medicines Authority
ARDS	Acute Respiratory Distress Syndrome
BBB	blood-brain barrier
BSA	baseline situation analysis
BSL	biosafety level
C	comment
CD	cluster of differentiation
CDC	Centers for Disease Control and Prevention
CDSCO	Central Drugs Standard Control Organization
CDV	canine distemper virus
CFIA	Canadian Food Inspection Agency
CFR	case fatality rate
CHO	chinese hamster ovary
CM	cynomolgus monkeys
CNS	central nervous system
CoA	certificates of analysis
CRO	clinical research organisation
CSF	cerebrospinal fluid
CT	computed tomography
CTA	clinical trial agreement
CTM	clinical trial material
DC	dendritic cell
DGDA	Directorate General of Drug Administration (Bangladesh)
DHR	Department of Health Research (India)
DIC	disseminated intravascular coagulation
DPI	days post infection
DRC	Democratic Republic of Congo
DSMB	Data and Safety Monitoring Board
Dx	diagnostics
EBOV	Ebola virus
EGFP	enhanced green fluorescent protein
EID	emerging infectious disease

ELISA	enzyme-linked immunosorbent assay
EMA	European Medicines Agency
ERC	ethical review committee
EUA	emergency use authorisation (FDA)
EUL	emergency use listing (WHO)
FDA	Food and Drug Administration
G	NiV glycoprotein
GAG	glycosaminoglycans
GCM	global coordination mechanism
GLP	good laboratory practice
GHSA	Global Health Security Agenda
GMO	genetically modified organisms
GMP	good manufacturing practice
gp	glycoprotein
F	NiV fusion protein
HCW	health care workers
HeV	Hendra virus
HRC	C-terminal Heptad repeat
HRN	N-terminal Heptad repeat
HRP	horseradish peroxidase
HS	heparan sulphate
HV	herpes virus
icddr,b	International Centre for Diarrhoeal Disease Research, Bangladesh
ICF	informed consent form
ICMR	Indian Council of Medical Research
ICP	infection control practice
ICTV	International Committee on the Taxonomy of Viruses
ICU	intensive care unit
IDRI	Infectious Disease Research Institute (U.S.)
IEDCR	Institute of Epidemiology, Disease Control and Research (Bangladesh)
IFAT	indirect fluorescence antibody test
IFN-γ	interferon gamma
ILI	influenza-like illness
IND	investigational new drug
ip	intraperitoneal
IRB	institutional review board
JE	Japanese encephalitis
km	kilometres
LASV	Lassa virus
LMICs	low- and middle income countries
mAb	monoclonal antibody

List of abbreviations continued

mcsF	molecular clamp-stabilised NiV F
MERS	middle east respiratory syndrome
MERS-CoV	MERS coronavirus
MLV	modified live virus
MoH	Ministry of Health
MRI	magnetic resonance imaging
MTA	material transfer agreement
MV	measles virus
NAT	nucleic acid test
NCFAD	National Centre for Foreign Animal Disease (Canada)
NCID	National Centre for Infectious Diseases (Singapore)
NCPV	National Collection of Pathogenic Viruses
NGS	next-generation sequencing
NHP	non-human primate
NIAID	National Institute of Allergy and Infectious Diseases
NIID	National Institute of Infectious Diseases (Japan)
Nipah@20	Nipah Virus International Conference
NiV	Nipah virus
NiV-B	Bangladesh isolate of NiV
NiV-M	Malaysian isolate of NiV
NIV	National Institute of Virology (India)
NPT	near-patient
OIE	World Organisation for Animal Health
PEG	polyethyleneglycol
PEP	post-exposure prophylaxis
pfu	plaque-forming units
PHEIC	public health emergencies of international concern
POC	point of care / proof of concept
PPE	personal protective equipment
PRIME	PRIority MEdicines
PRNT	plaque reduction neutralization test
PRRSV	porcine reproductive and respiratory syndrome virus
PTT	partial thromboplastin time

Q	question
Q&A	questions and answers
R 0	basic reproduction number
R&D	research and development
RCT	randomised controlled trial FIND – Foundation for Innovative New Diagnostics
RFP	request for proposals
RITM	Research Institute of Tropical Medicine (Philippines)
RNA	ribonucleic acids
RRC	Research Review Committee
RSV	respiratory syncytial virus
RT-PCR	reverse transcriptase polymerase chain reaction
RVF	rift valley fever
rVSV	recombinant VSV
SAG	scientific advisory group
SARS	Severe Acute Respiratory Syndrome
SEAR	WHO South-East Asia Region
SEARO	South-East Asia Regional Office of WHO
sG	soluble G protein
SNT	serum neutralisation test
SOC	standard of care
SOP	standard operating procedure
SPEAC	Safety Platform for Emergency vACcines
SSPE	subacute sclerosing panencephalitis
TNF-α	tumour necrosis factor alpha
TPP	target product profile
Tx	therapeutics
UTMB	University of Texas Medical Branch
VRDL	Virus Research and Diagnostic Laboratory (India)
VSPB	Viral Special Pathogens Branch
VSV	vesicular stomatitis virus
Vx	vaccines
vWF	von Willebrand factor
WHO R&D Blueprint	WHO research and development Blueprint for action to prevent epidemics
ZEBOV	Zaire Ebola virus

EXECUTIVE SUMMARY

The Nipah Virus International Conference of 2019 (Nipah@20) was co-hosted by the Coalition for Epidemic Preparedness Innovations (CEPI), the World Health Organization (WHO), the U.S. National Institute of Allergy and Infectious Diseases (NIH/NIAID) and the Duke-NUS Medical School (Duke-NUS).

The Conference marked the 20th anniversary of the discovery of Nipah virus. Since its first identification in Malaysia in 1998 and Singapore in 1999, the understanding of Nipah disease and its pandemic potential has advanced substantially. The Conference provided a forum to review the history and key scientific findings over the last 20 years, and to understand the current challenges in developing Nipah diagnostics, therapeutics and vaccines.

To foster international collaboration in the context of epidemic preparedness, Nipah@20 brought together 218 scientists and public health professionals working in 21 different countries around the globe. Importantly, all henipavirus-affected countries (Australia, Bangladesh, India, Malaysia, the Philippines and Singapore) were represented in the Conference, with their delegations accounting for 46% of all attendees.

In terms of outcomes, the two-day Conference created a scientific evidence-based framework to: (a) inform discussions between global health stakeholders participating in CEPI's Joint Coordination Group (JCG) on December 11, 2019, (b) discuss the creation of a Nipah-focused regulatory working group to facilitate data sharing and joint review of Nipah vaccine candidates, and (c) identify further multidisciplinary actions needed to respond to the pandemic threat posed by Nipah virus.

This report details the conference proceedings from the two-day event in December 2019.

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OPENING CEREMONY

Co-chairs: Professor Linfa Wang, Duke-NUS / Dr. Raúl Gómez Román, CEPI

A warm welcome to everybody for joining the Nipah Virus International Conference (Nipah@20). This conference is the result of an initiative taken by the Coalition for Epidemic Preparedness Innovations (CEPI) in March 2019, and it was put together in a relatively short time. The conference has 215 participants from 24 countries, which exceeded the organisers' expectations by about 50 %! Impressively, most of the people who were invited to the conference, on relatively short notice, are now present.

As organisers, we are grateful for the contributions made by the scientific community in preparing the conference. Scientists from nine different countries have taken part in the preparations, and the conference programme is the result of their experience and input. We would also like to thank our hosts, organisers, partners, and sponsors, who have entrusted us with the responsibility and privilege of preparing the conference. On behalf of the international organising committee, we would like to wish you a productive and scientifically stimulating Nipah@20.

Message from Ministry of Health, Singapore

SMS Dr. Lam Pin Min, Senior Minister of State for Health and Transport

Nipah@20 marks the 20th anniversary of the discovery of Nipah virus (NiV). There was also a conference marking the 10th anniversary, but hopefully there will not be a need for a 30th anniversary conference – by then, an effective vaccine should ideally have been discovered. It is important to come together at a conference like this, to take stock of the latest developments in global health, and discuss how we may better address emerging infectious diseases (EIDs).

EIDs remain a threat to countries around the world, Singapore included. In addition to Nipah, Singapore has experienced recent outbreaks of Severe Acute Respiratory Syndrome (SARS), endemic Influenza (H1N1), and Zika. We are not able to predict where and when the next EID may emerge, but we are able to prepare for its emergence and mitigate its impact. Our defence against EIDs include three elements: Public health capabilities, cooperation, and commitment to research.

Strong public health capabilities and -systems are important for managing and responding to public health crises. Singapore adopts an integrated and dedicated approach to the management of EIDs, with capability to deliver clinical services as well as public health functions to detect, respond to, and contain EIDs. The National Centre for Infectious Diseases (NCID) was opened this year, to strengthen Singapore's capabilities in public health preparedness and infectious disease management and prevention. In the event of an epidemic, the capabilities of our healthcare professionals are critical. Recognising this, Singapore continues to invest in training for healthcare professionals and public health practitioners.

The responsibility for addressing EIDs does not rest with one institution, one sector, or one country alone. This brings in the aspect of cooperation – or rather, cooperation, collaboration, and coordination. Management of zoonotic diseases, such as Nipah, underscores the need for coordination across sectors. Singapore has adopted an interdisciplinary "one health" approach, integrating human, animal, and environmental health surveillance and response. Robust animal and human surveillance, combined with infrastructure capabilities, are critical in providing early warnings to the animal and human health authorities. The Ministry of Health works closely together with a number of other agencies to develop capabilities and strengthen surveillance.

During the 1998-1999 Malaysia and Singapore Nipah outbreak, international collaboration was instrumental to the identification of pigs as an intermediate amplifying host for NiV infection. Fostering partnerships across regional and national borders is necessary to deal with infectious agents that are not constrained by such borders. In addition, the emergence of new infectious diseases stresses the importance of research investments.

CEPI was established in Davos in 2017, as a private-public partnership with the ambition to accelerate the development of vaccines against EIDs. This conference is yet another example of how different players can come together to support work and research concerning EIDs. Our hope is that the conference may lead to new partnerships and innovations that may boost the fight against Nipah virus.

Message from the Ministry of Health, Malaysia

Datuk Dr. Christopher Lee Kwok Choong, Deputy Director General of Health (Research & Technical Support)

Thank you to the organisers and Singapore for hosting this event. Nipah virus was named after the Malaysian village Sungai Nipah. To many Malaysians, the word Nipah evokes memories of the devastating zoonotic outbreak in 1998, where the pig farming industry was hit hard. A total of 283 cases were confirmed infected, resulting in 110 fatalities. The outbreak also reached Singapore, reminding us that borders are unable to stop infectious diseases.

Since the 1998 NiV outbreak, the ministry of health (MoH) has put in place a number of measures to protect the people of Malaysia from future zoonotic outbreaks. This includes strengthening of the surveillance systems in both human and animal health sectors, and in particular a structured and continuous engagement platform where information may be shared and analysed, and decisions are made collectively. The platform, corresponding to Singapore's "one health" approach, has been in place since 1999.

While the Nipah outbreak in 1998 would ideally have been a one-off event, the last 20 years have proven the opposite. Although Malaysia has not had another outbreak, NiV outbreaks have occurred a number of times in Bangladesh and India. These outbreaks have been traced to food contaminated by fruit bats, which are now known to be the natural host of NiV. Transmission from human-to-human has been confirmed in South India, primarily affecting family members or healthcare personnel. Fatality rates range from 40 to 75 %, yet interest in NiV has been more or less confined to the countries affected.

To this day, there is no cure, vaccine, or specific treatment for Nipah, and affected individuals receive only supportive care. In the 20 years that have passed, WHO estimates that there has been about 600 Nipah cases worldwide, limited to countries in Southeast Asia. There are, however, concerns regarding the devastating zoonotic potential of NiV, as stated by the World Organisation for Animal Health (OIE). The WHO considers the risk of geographical spread of Nipah to be low, yet the migration patterns and distribution of fruit bats are extensive. Evidence from Bangladesh shows that viral spillovers from bats to humans happen regularly, providing opportunities for a more highly transmissible strain to infect and adapt in humans.

For effective countermeasures to Nipah, a broader and more comprehensive approach, with investments in therapeutics, diagnostics, and vaccines as well as surveillance infrastructure, is urgently needed. Furthermore, strengthened capabilities are warranted in rapidly detecting and verifying cases, conducting detailed contact tracing, investigating spillovers, and gaining a better understanding of NiV and its mode(s) of transmission. Equally important is the need for behavioural change, and improved support to local communities for prevention and control measures. The goal is to reduce transmission, especially in the healthcare setting.

Nipah@20 is a timely opportunity for assessing the many gaps that still remain in our understanding of NiV and, hence, our ability to prevent and treat Nipah infection. Malaysia is grateful to be involved in this meeting, and looks forward to learn from the many experts gathered at the event, as well as to engage with the other stakeholders present in the interest of moving towards more effective management of NiV infection.

Message from the WHO

Dr. Michael Ryan, Executive Director, WHO Health Emergencies Programme

Nipah virus and the related henipaviruses are severe emerging diseases with the potential to impact vulnerable populations, generating public health emergencies. Many aspects of the management and treatment of the henipaviral diseases remain unknown, and they have therefore been included on the list of WHO priority diseases, as well as on the WHO research and development Blueprint for action to prevent epidemics (WHO R&D Blueprint). The WHO R&D Blueprint is a global strategy to ensure implementation of critical research findings on highly infectious diseases in a safe, effective and timely way. In collaboration with leading experts, WHO R&D Blueprint allows for fast-tracking of effective medical technologies in the context of an epidemic.

The Nipah virus has a significant epidemic potential. Its bat reservoir has been found in a number of countries, and it has the ability to amplify in livestock, which is a source of exposure to humans. Human infection results in severe neurological disease and respiratory symptoms, causing significant long-term sequelae, and high mortality.

Our current knowledge of the henipaviruses results from remarkable collaborative, long-term efforts of the scientific community, including many of the participants at Nipah@20. WHO R&D Blueprint has contributed to developing the key R&D priorities in the fight against Nipah, by building on these collaborations and bringing together experts from around the world.

During the third Nipah outbreak in India, in May 2018, the WHO R&D Blueprint's coordinated approach led to quick identification of the most promising available therapeutic agent (the monoclonal antibody m102.4), and access to the agent within 48 hours. This case study demonstrates that proactive research planning before an outbreak allows for a quicker outbreak response, allowing for prompt implementation of potentially life-saving medical interventions.

Epidemic risk can only be addressed by accelerating global preparedness, with an integration of research into all of our preparedness and response activities. This conference represents a unique opportunity to share knowledge, and to lay the groundwork for a clear and coordinated research agenda for Nipah, at the regional and national levels.

Message from NIAID

Dr. Cristina Cassetti, Deputy Director, Division of Microbiology and Infectious Diseases, NIAID, NIH

Emerging infectious diseases have been an important research priority at the U.S. National Institute of Allergy and Infectious Diseases (NIAID) for years. The aim is to gain a better understanding of these pathogens and why they emerge, and to develop countermeasures such as diagnostics, therapeutics, and vaccines. With substantial biodefence funding from U.S. Congress, the institute has been able to support Nipah research since the early 2000s, contributing to important advances such as identification of the receptor used by NiV for cellular entry and the development of animal models to be used in the evaluation of vaccines and therapeutics.

NIAID has been involved in the development of therapeutic and vaccine candidates, some of which have now been transferred to CEPI and other partners for further development. The Nipah programme of NIAID remains very active, with about 17 currently ongoing extramural programmes and a number of internal programmes focusing on the development of vaccine candidates, as well as studies on the pathogenesis of NiV using non-human primates (NHP). At Nipah@20, NIAID hopes to reconnect with old friends and partners, as well as to learn about new advances in the field of NiV research.

Welcome Address

Professor Thomas Coffman, Dean of Duke-NUS Medical School

Emerging infectious diseases constitute an important part of the research portfolio of Duke-NUS, and Professor Linfa Wang, who is the co-chair of Nipah@20, runs the Duke-NUS' Emerging Infectious Diseases programme. Duke-NUS is very excited to bring this conference to Singapore, partly because this was where the first Nipah outbreak happened. At the conference venue, there is a storyboard with news clippings from the first outbreak, clearly depicting the terror the disease caused when it first emerged.

Another reason why Singapore is a suitable location for such a conference, is the many travellers that come through its airport, as well as its large volume of shipping. Singapore was very significantly affected by SARS, and emerging diseases represent a topic for concern to authorities as well as the public. Nipah@20 brings together experts from all over the world, and highlights the importance of facilitating global communication between experts from different disciplines such as epidemiology, surveillance, basic science, vaccine development, and clinical treatment. Nothing can replace face-to-face discussions among experts in bringing the field forward.

Inaugural Address

Dr. Richard Hatchett, CEO of CEPI

Nipah virus and the related henipaviruses are some of the world's most frightening causes of disease. WHO has included these as one of 10 pathogens on its list of priority pathogens, since the epidemic potential of henipaviruses poses a grave risk to public health, and because there are no effective countermeasures.

It is highly appropriate that Nipah@20 convenes in Singapore, a beacon for globalism and a city that embodies the modern economic order. When Nipah first emerged in Malaysia, it rapidly spread to Singapore. In the last few decades, other frightening diseases like SARS, Ebola, and MERS have fared similarly, spreading from rural areas to densely populated cities, where the damage they can cause is amplified immensely. No city is more globally interconnected, and thus at greater risk, than Singapore.

Nipah has demonstrated its global potential, with recent outbreaks in Kerala, India – more than 3,000 kilometres (km) from Singapore, and more than 2,000 km from Bangladesh and Eastern India, where other recent outbreaks have occurred. As a result, more than 2 billion people live in areas at risk for Nipah. Unless something is done, Nipah will continue to re-emerge in smaller outbreaks, until it finds the perfect combination of circumstance and opportunity to explode again, as it did some 20 years ago.

CEPI is delighted to co-host Nipah@20 with the Duke-NUS Medical School, WHO, and NIAID. Meetings like this are important, not only because of the opportunity to share knowledge, but because of the momentum created in addressing the threat of Nipah, so that it does not become the next Ebola. In addition to being the 20th anniversary of Nipah, 2019 marks 50 years since the discovery of Lassa fever, and one of the inspirations for Nipah@20 was found in a similar meeting on Lassa fever in Abuja, Nigeria, in January 2019.

Lassa fever has haunted physicians in West Africa for half a century, occasionally flaring up in explosive outbreaks, and likely causing thousands of deaths. The burden of disease has not been known, due to poor or non-existent surveillance systems. In recent years, however, the Nigeria Centre for Disease Control, in collaboration with WHO and other international partners, has been able to draw more attention to the disease and establish Nigeria's research priorities for Lassa fever. Through the Lassa meeting in January 2019, where several hundred physicians, scientists, and public health officials participated, concerted and coordinated action against the disease was established. Subsequently, CEPI and WHO have helped Nigeria and other affected countries establish a network to support important epidemiology studies, which will lead to the design of better and more efficient clinical trials of vaccines. CEPI has also helped move the two first Lassa fever vaccines into clinical trials, with more to follow. The world has woken up to Lassa, and begun to move to take this threat off the table.

CEPI embraces the notion that the countries at greatest risk of the WHO priority diseases should be the ones to set the research agenda for these diseases. Thankfully, more than 100 scientists, public health officials, and policy makers from countries at risk of Nipah have registered for Nipah@20.

Another inspiration for this conference was the major progress the world has made against Ebola over the last year. While Ebola remains a frightening and disruptive threat, a clinical trial conducted in the Democratic Republic of the Congo for a therapeutic against Ebola was recently terminated early due to positive results. Even more recently, Merck received conditional marketing authorisation from the European Medicines Agency (EMA) for its vaccine. Within the last week before Nipah@20, GAVI announced that they will open a new funding window to establish a global emergency stockpile of Ebola vaccines. Low- and middle income countries will be able to access the vaccine free of charge, and will receive support for the operational costs of vaccination campaigns. As a result, we are approaching the day when Ebola is no longer the terrifying disease that it has been for the last 40 years.

It has taken tremendous effort by countless people to get the results seen with Ebola, but the demonstration that we can move from outbreak response to a global R&D effort that results in a fully accessible stockpile of licensed vaccine is a major victory for global health. This experience provides a roadmap for emerging infectious disease preparedness, where CEPI and the other partners present at Nipah@20 will play a critical role. Beginning today, we can do the same thing for Nipah. We can defeat Nipah, but only if we come together as a community and coordinate our efforts. The triumvirate of capabilities, cooperation, and commitment mentioned by Dr. Lam will be essential to any progress against Nipah. Fostering these was the intention with Nipah@20, and CEPI is confident that the participants will rise to the challenge.
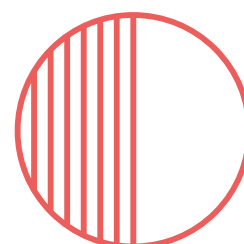
SESSION 1 – HISTORIC REVIEW OF NIPAH OUTBREAKS

Chair: Dr. Marie-Pierre Preziosi, WHO

Overview of outbreaks

Professor John Mackenzie, PathWest & Curtin University, Perth

Outbreaks of henipaviruses, notably Hendra virus (HeV) and NiV, have affected Australia and several countries of Southeast Asia over the past two to three decades (see Figure 1). Outbreaks of HeV have been limited to the east coast of Australia, whereas NiV outbreaks have been found in Malaysia, Singapore, Bangladesh, India (West Bengal and Kerala), and the Philippines. Whereas HeV uses horses as intermediate host, NiV infects pigs. One exception was in the 2014 outbreak in the Philippines, where ten horses were found to be infected by NiV, and culled.
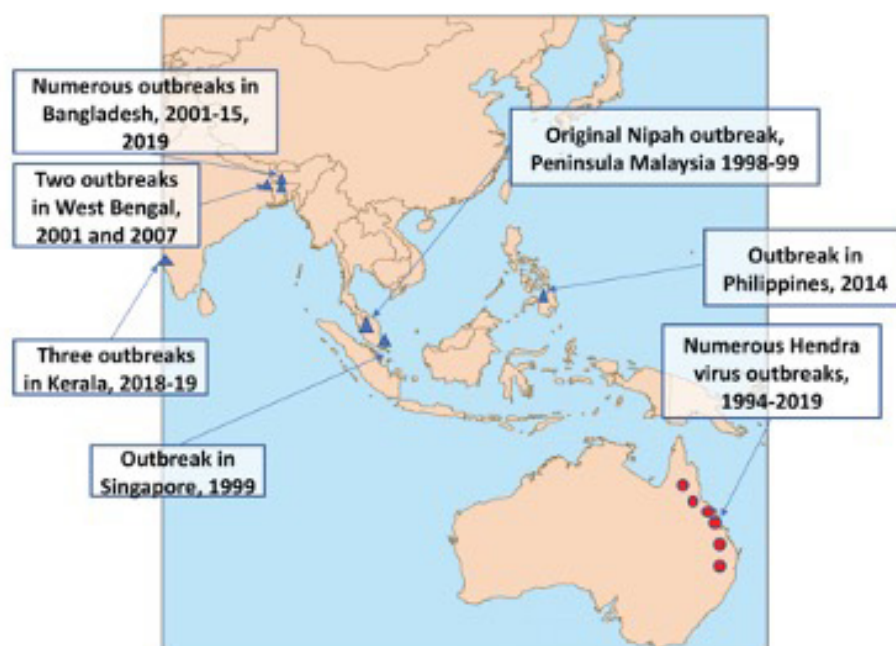
Figure 1. Map showing approximate sites of Nipah and Hendra outbreaks (Prof. John Mackenzie)

The Nipah outbreak in Malaysia and Singapore in 1998-99 was first believed to be caused by Japanese encephalitis. People were vaccinated and premises and surrounding areas fogged, but to no effect. The outbreak first started in Perak state, and later moved about 200 km south to the more intensive pig farm areas of Negeri Sembilan and Selangor, through the movement of infected pigs. During this outbreak, there was no communication between clinicians and veterinarians – an aspect that is now considered very important to the management of zoonotic diseases, as is clear from the widely used One Health approach. Other lessons learned were: 1) a previously unknown pathogen could emerge from a wildlife source at any time, in any place, and without warning, to threaten the health, well-being, and economy of a country – or even globally; 2) there is a clear need for countries to have the capability and capacity to maintain an effective alert and response system to detect and quickly react to outbreaks of international concern, and to share information about such outbreaks rapidly and transparently; 3) responding to novel disease threats of possible international concern requires global cooperation and global participation.

Australia

Dr. Hume Field, Science & Policy Advisor, EcoHealth Alliance & University of Queensland

The Brisbane suburb of Hendra was close to racing tracks, and there are a number of small training stables in the area. In August of 1994, after a trainer brought a sick horse to his stable to better care for it, there was an outbreak of acute respiratory disease, and a cascade of cases that, within a week, saw 13 horses dead, and the trainer and a stable hand in hospital. The trainer died whereas the stable hand recovered. This was an unprecedented event, all the stables were quarantined, and the movement of horses in South East Queensland was brought to a standstill.

Within a week, a novel paramyxovirus was identified by the Australian Animal Health Laboratory. When inoculated into a healthy horse, it caused the same acute, rapidly progressing, fatal infection that was observed in the Hendra stables. While initially named Equine morbillivirus, it was later found that the new virus did not fit into any existing genus. In the subsequent years, there have been nearly 100 equine cases and 7 human cases. The case fatality rate (CFR) for horses approaches 90 %, and in humans 60 %. Both respiratory and neurological presentations are recorded in horses. An effective vaccine has been available for horses for over five years, but is still not widely used due to concerns about cost and exaggerated reports of side effects. Equine cases of Hendra continue to occur almost annually.

Extensive wildlife studies have shown that species of pteropid fruit bats, or "flying foxes", are the natural reservoir of Hendra virus. Infection prevalence fluctuates in these populations, and urine is the primary route of viral excretion. Transmission to horses is likely caused by ingestion of urine-contaminated pasture, or direct inoculation of mucous membranes while horses are grazing under trees where flying foxes are feeding. All human cases have had direct contact with infected horses; direct transmission from bats to humans has not been recorded, and neither has human-to-human transmission.

The multiple similarities between HeV and NiV, and Dr. Field's role in the identification of bats as the natural host of HeV, led to his joining the Nipah outbreak veterinary response team that was assembled by the Malaysian government in early 1999. Epidemiologically, it appeared that pigs were the source of Nipah infection in humans, and the first task of the team was to perform pig necropsies at "hot" farms and collect tissue samples. They were able to isolate the same virus that caused human infection from multiple pigs, at multiple farms. The work then shifted towards gaining a better understanding of the nature of infection and transmission in pigs. It was clear that not all pigs were affected at "case" farms, and they therefore sought out "recovered" farms to serologically survey different classes of pigs, and collect convalescent serum. Parallel to these investigations, the mass culling of pigs was underway. The urgency and logistics of culling meant that the animals were typically killed and buried on the farm, which frequently involved the destruction of pig sheds and other farm infrastructure.

Two other components of the veterinary response were the investigation of infection and/ or disease in non-pig species, and the origin of infection in pigs. Horses, dogs, and cats were identified as incidental hosts. As with Hendra, serological studies identified species of local bats as the likely source of infection in pigs, and the reservoir of the virus in nature. Subsequent studies have confirmed this, and further shown that henipaviruses are widespread in many bat populations, and likely have an evolutionary association with bats. While there may have been deficiencies in the early response to the Nipah outbreak in Malaysia, at this stage the Malaysian government had established collaborative and coordinated efforts to control the outbreak, in a One Health perspective.

Nipah virus outbreak in Malaysia

Dr. Chua Kaw Bing, Sr. Principal Investigator, Temasek Lifesciences Laboratory

One believes that NiV was infecting pigs and humans even prior to the 1998-99 outbreak in Malaysia, though the cases were never picked up on. There were reports, prior to the outbreak, of pigs coming down with encephalitis, which were assumed to be afflicted by "classical swine fever". Once transmitted to humans, public health authorities suspected an outbreak of Japanese encephalitis (JE), which was confirmed by what was later found to be false positive test results (found in about 20 % of the patients) for circulating JE IgM and viral particles. The Malaysian MoH launched an intensive campaign with JE vaccination and fogging of pig farms and surrounding areas to eradicate the supposed JE mosquito vector. When the outbreak persisted, in spite of the authorities' efforts, the possibility of a novel pathogen was finally considered.

The first direct observation of NiV was made on March 15, 1999, by electron microscopy of a sample section made by Dr. Bing, with the help of the U.S. CDC at Fort Collin. At U.S. CDC Atlanta, the same sample preparation later tested positive to Hendra antibodies in an indirect immunofluorescence assay, and it was thus determined that the outbreak was caused by a Hendra-like virus. Final identification of the virus was achieved by molecular characterisation (amplification of a 120 base-pair fragment of the Phosphoprotein gene by reverse transcriptase polymerase chain reaction (RT-PCR), followed by nucleotide sequencing) at U.S. CDC Atlanta. The new virus was named after Kampung Sungai Nipah, the village were it was first discovered.

Already with the first electron micrographs, it became clear that the Malaysia outbreak was not caused by JE, and Malaysian public health authorities were able to implement more appropriate control measures. Malaysia paid dearly for the inaccurate and untimely diagnosis, with 265 patients suffering from acute viral encephalitis, and 105 fatalities. The local swine industry also suffered great losses; about 1 million pigs were culled, and 800 pig farms were demolished.

The 1999 Nipah outbreak in Singapore

Professor Leo Yee Sin, Executive Director, National Centre for Infectious Diseases

The Singapore Nipah outbreak was, in fact, an integrated part of the Malaysia outbreak, yet much more limited in time and space. At the time, there were two abattoirs in Singapore, and the outbreak quickly resolved when the import of live pigs from Malaysia was banned, and the abattoirs closed. The eleven cases of the outbreak, of which there was only one fatal case, were all identified within a period of 9 days in March 1999. All of them had had close contact with live animals. In the Singapore outbreak, there was no human-to-human transmission, and all of the abattoir and healthcare workers screened after the outbreak tested negative for NiV antibodies. It was estimated that about 50 % of the infections were asymptomatic.

In spite of being limited in nature, the Nipah outbreak in Singapore provided several important lessons that have later proven useful, for example in the context of the Singapore SARS outbreak in 2003. Key lessons were to facilitate collaboration across borders, as well as to take a multidisciplinary, One Health approach to infectious disease prevention and control.

Nipah Encephalitis in Bangladesh

Professor Mahmudur Rahman, Consultant, Programme for Emerging Infections, icddr,b

Nipah was first identified as the cause of an outbreak of encephalitis in 2001 in the Meherpur district of Western Bangladesh, though the link to Nipah was discovered three years after the outbreak, in 2004. In 2006, Bangladesh established its Nipah surveillance system and laboratory. Since then, a total of 313 cases have been identified, of which 226 (72 %) have died. Looking only at the outbreaks, and eliminating independent cases, 37 outbreaks have led to 221 cases and 171 deaths, resulting in a very high CFR of 77 %. Many of the outbreaks have been small and localised, often limited to individual households and frequently resulting in 100 % mortality of cases.

Among the 64 districts of Bangladesh, 31 have been affected by Nipah. The affected districts may be said to form a "belt" concentrated in West Bangladesh. The majority of cases are children and young adults. Among the cases, about 60 % report to have consumed date palm sap. The sap is collected overnight, and bats have been found to contaminate the sap by urination.

Nipah outbreak investigations are conducted in a systematic way and with a One Health approach, involving epidemiologists, veterinarians, and anthropologists. In most cases, transmission is successfully traced back to its source, which has often been found to be the ingestion of raw date palm sap. The second most common route of transmission is human-to-human. There is a seasonality to outbreaks, with more outbreaks occurring from December to May. The median incubation period is eight days, and about 35 % of cases are secondary cases.

India

Dr. Nivedita Gupta, Scientist F, Division of Epidemiology & Communicable Diseases, ICMR

Whereas the first Nipah outbreak in Malaysia and Singapore did not involve human-to-human transmission, the outbreaks in India and Bangladesh in 2001 both did. The 2001 outbreak in Siliguri, West Bengal, India, represents the biggest Indian outbreak to date, involving 66 confirmed cases and 45 deaths (CFR: 68 %). Subsequent outbreaks have occured in Nadia, 2007 (5 confirmed cases, 5 deaths – CFR 100 %), Kozhikode, 2018 (19 confirmed cases, 17 deaths – CFR 90 %), and Ernakulum, 2019 (1 single case – CFR 0 %). Surprisingly, the two latter outbreaks were both in Kerala state, at the opposite side of the country as compared to the first two outbreaks.

During the 2001 outbreak, the Indian healthcare system was unable to diagnose Nipah, and instead mistook it for measles infection, since these are related viruses. With the help of U.S. CDC, they were able to confirm cases of NiV infection, but this took a long time. By the time of the 2007 outbreak, diagnosis had been established, with serological and molecular tests provided by the U.S. CDC. At this time biosafety level (BSL)-3 labs were available, yet early detection failed. In 2015, India had one operational BSL-4 laboratory, and capacity for bat surveillance was established. During the 2018 outbreak, early diagnosis and containment was available, though the index case was not correctly diagnosed. Finally, in the 2019 outbreak, the index case was quickly diagnosed and contained, and the outbreak stopped there.

For the two first outbreaks of 2001 and 2007, the origin of infection remains unknown. However, for the Kozhikode outbreak in 2018, *Pteropus* bats have been identified as the source. Since there is no tradition for palm sap consumption in Kerala, one does not know how transmission happens, though the close similarity of viral gene sequences of viruses isolated from cases and local bats clearly suggests that the outbreak originated with the bats.

Philippines

Dr. Fidel Malbas Jr., Research Institute for Tropical Medicine

Following a preliminary investigation into a cluster of deaths in the south of the Philippines, the Philippine government requested technical assistance from the WHO representatives to the Philippines on May 12, 2014. A multidisciplinary team investigated further, and clinical, epidemiological, and laboratory findings indicated an outbreak of a viral infection lasting from March to May. Seventeen cases were identified, displaying symptoms compatible with henipavirus infection. Eleven of the cases had acute encephalitis, and nine of them died.

Epidemiological data suggest that the primary route of human infection was through direct exposure to body fluids from infected horses. At least five cases were considered infected by direct human-to-human transmission, presumably through infected respiratory secretions or contact with other bodily fluids from sick patients. Laboratory results include serological evidence of exposure to henipavirus in recovering or fully recovered patients. Importantly, this is the first time a henipavirus has been detected in humans and domestic animals on the Philippines. The environmental source of infection has not yet been firmly established, but evidence points to the Megabats as a possible reservoir.

Recommendations for further actions, aiming to reduce transmission between bats, horses, and humans, are as follows: 1) Focus on prevention and control of infection in healthcare settings; 2) Use personal protective equipment (PPE) and ensure high levels of hygiene among healthcare workers, butchers, and people dealing with horses; 3) Avoid slaughter and consumption of sick horses; 4) Strengthen surveillance of human and animal cases; 5) Conduct further testing on clinical specimens; 6) Study the bat reservoir host; 7) Develop coordinated response plan between human and animal health agencies; 8) Ensure flow of information from the Department of Health to regional authorities in human and animal health, as well as agriculture.

Session 1 Q&A

Q: It was estimated that 50 % of Nipah cases in the 1999 Singapore outbreak were asymptomatic. Is this not unusual?

A: Thirteen asymptomatic cases were detected as part of the serological screening of abattoir workers. Although they had not had any symptoms, magnetic resonance imaging (MRI) conducted on some of the cases revealed that they had the hyper dense brain lesions typical for Nipah encephalitis. In India, serological surveys among close contacts of Nipah patients from the Kerala outbreak showed seropositivity in only two of about 300 subjects, suggesting a very low proportion of asymptomatic cases. In Bangladesh, four out of 212 subjects exposed to sap and/or bats tested positive for circulating antibodies against NiV. In the first HeV outbreak, 13 horses died, 4 had mild symptoms but were euthanised, and 3 tested positive for circulating HeV antibodies without any preceding signs of disease.

Q: Can asymptomatic cases be a source of infection?

A: There have been no reports of that, so far.

SESSION 2 – WHO NIPAH R&D ROADMAP

Chair: Dr. Christina Spiropoulou, CDC

WHO Nipah R&D Roadmap

Professor Steve Luby, Stanford University

Professor Luby presented the WHO Nipah R&D Roadmap (view draft here) as a member of the taskforce involved in preparing it. The purpose of the Roadmap *was to provide a 5-year framework for identifying the vision, underpinning strategic goals, and prioritising areas and activities for accelerating the collaborative development of* **medical countermeasures** *against Nipah virus infection.* The Roadmap has three big development priorities: 1) Rapid and accurate point-of-care diagnostics; 2) Safe and effective treatment and post-exposure prophylaxis; 3) Safe and effective vaccines.

In the Roadmap, three important issues were identified: 1) The market failure to create medical countermeasures; 2) The centrality of improved Nipah diagnostics; 3) Promise and barriers for Nipah therapeutics. On the positive side, the technologies required for developing effective counter-measures to Nipah are available, and NiV seems to be a fairly easy pathogen to deal with. The reason why effective countermeasures are still not in place, is a lack of incentives for private industry to develop the products: There are few people affected, typically in rural and low-income areas, and when the focus is on prevention of disease, rather than treatment of annual cases, the standard measures of cost-effectiveness are not met.

While the impact of a Nipah epidemic would be high, the probability is low: Over the past two decades, several outbreaks have happened, but sustained human-to-human transmission in a larger geographic area remains to be seen. However, prudent actions should be guided by both probability and impact. Thus, the primary justification for public investment in NiV countermeasures is not the management of the smaller outbreaks that seem to occur on a regular basis, but the prevention of a pandemic where perhaps millions of people could be affected by an infection that has a CFR of 75 %. Importantly, however, cognitive biases discourage attention to low probability events, making them more difficult to prepare for. The low probability of a NiV pandemic creates a setting where the market fails to meet societal needs. This is especially true when obtaining the necessary countermeasures involves expensive research and development, with requirements for licensure, BSL-4 work, and the use of costly animal models.

In the interest of creating a sustainable business model for the development of Nipah medical countermeasures, the Roadmap considers several supportive actions: 1) Public/philanthropic funding for R&D; 2) Clarifying regulatory pathways; 3) Assessing market size. For the

latter, important insights were: 1) Increased surveillance would increase demand for diagnostics; 2) Treatments that can be used to treat several pathogens will have a stronger business case; 3) Demand for treatments and vaccines will come from healthcare and laboratory workers, in addition to patients and people at risk; 4) Rotating stockpile and mass vaccination would result in stronger business cases. Moving forward, one strategic goal of the Roadmap committee is to identify sources of private- and public-sector funding and develop appropriate incentives and competitions to promote R&D of NiV medical countermeasures. They are currently working on a funding plan for moving NiV diagnostics, therapeutics, and vaccines toward clinical evaluation, licensure/approval, acceptance, and sustainable access.

Improved availability and use of Nipah diagnostics can provide insight on the prevalence of human infection. The range of *Pteropus* bats covers areas inhabited by 2-3 billion people, who are potentially at risk of Nipah infection. Given the spread of confirmed human Nipah infections, many human cases are likely to go unrecognised. Better diagnostics would serve to 1) improve response; 2) increase visibility to decision makers; 3) improve risk forecasting. In the low-resource settings where

Nipah typically occurs, the ability to distinguish between Nipah infection and other types of encephalitis (NiV accounts for less than 5 % of cases, even in the "Nipah belt" in Bangladesh) would be essential to guide therapy. However, there are several reasons for why realising widely available Nipah diagnostics is made difficult: 1) NiV is uncommon and occurs in poor communities; 2) Without effective therapy available at the medical facility, there is no clinical justification for testing; 3) The testing of NiV comes with biosafety concerns; 4) There is limited availability of clinical samples for assessing new products. Thus, from a business case perspective, a successful diagnostic requires a successful therapeutic, and vice versa. In order to facilitate further development of NiV diagnostics, the Roadmap committee has defined the following prioritised activities: 1) Create a virtual repository of clinical reference samples for use in R&D; 2) Generate a target product profile (TPP) for NiV diagnostics; 3)

Enhance diagnostic preparedness in areas of known or potential henipavirus spillover risk.

Several promising Nipah therapeutic agents are in development, of which m102.4, fusion inhibitory peptides, favirpiravir, and GS-5734 are but a few. However, patients generally present late in the course of illness, and they often present to facilities with limited capacity, commitment, and practice in diagnostics. Thus, effective treatment requires more than just making a therapeutic agent available. The Roadmap has defined the following strategic goals regarding therapeutics: 1) Enhance preparedness to conduct clinical trials of therapeutic agents during future NiV outbreaks; 2) Develop and evaluate therapeutic agents for the treatment of NiV infection and for post exposure prophylaxis to prevent NiV infection. With CEPI on board, the Roadmap committee has been less concerned with vaccine development. However, the following strategic goals have been

developed for vaccines: 1) Engage national regulatory authorities and WHO to gain guidance on requirements for clinical trials, regulatory pathways, and other considerations that will affect licensure of a vaccine against NiV; 2) Develop and evaluate NiV vaccines for prevention of NiV disease in humans.

These are exciting times, and people are now understanding that the low probability, high impact event of a pandemic NiV outbreak is worth preparing for. Promising technologies are in development for diagnostics, therapeutics, and vaccines against NiV. Currently, the issues that need the most attention are: 1) Development of a sound business case to support product development; 2) Enabling work within the constraints of sporadic cases / outbreaks in settings with limited resources. These are crucial for succeeding with the development of medical countermeasures against Nipah in the next 10 years.

WHO Nipah R&D Roadmap – Monitoring its implementation

Dr. Marie-Pierre Preziosi, WHO R&D Blueprint (on behalf of Dr. Claudia Nannei, WHO Monitoring & Evaluation)

The WHO R&D Blueprint efforts focus on both **research preparedness** and **research during outbreak response**. The aim of the R&D Blueprint is to fast-track the development of effective diagnostics, therapeutics, and vaccines to respond to outbreaks effectively and to prevent them. The R&D Blueprint preparedness activities are organised around three main approaches: 1) Improving coordination and fostering an enabling environment; 2) Accelerating R&D processes; 3) Developing norms and standards tailored to the epidemic context.
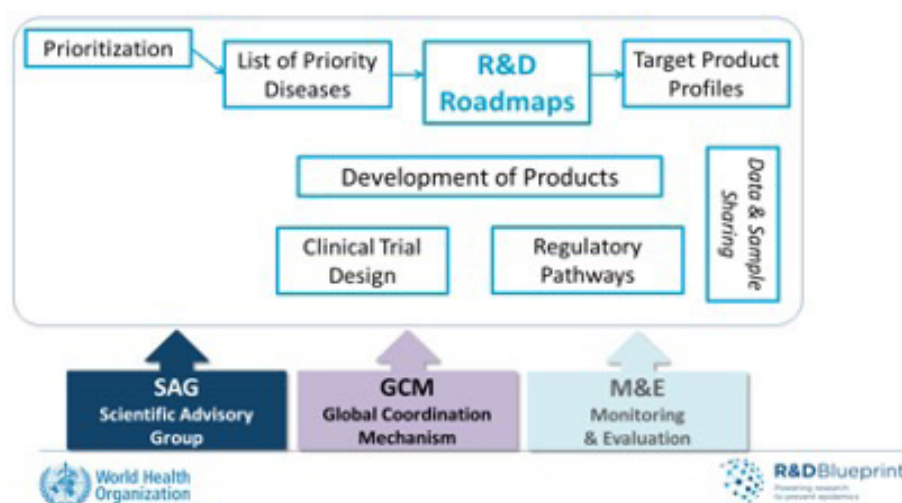

Figure 2. Overview of the WHO R&D Blueprint (WHO)

On an annual basis, WHO R&D Blueprint update their list of prioritised diseases, and for each of the diseases listed, R&D Roadmaps are prepared (see Figure 2). When relevant, TPPs are prepared to guide the development of products. Furthermore, WHO R&D Blueprint take part in developing clinical trial designs for each pathogen, and address the regulatory pathways. They are governed and receive input from a scientific advisory group (SAG), and through a global coordination mechanism (GCM). The monitoring and evaluation (M&E) is important to ensure assessment of progress and observation of any changing circumstance, thus allowing the accomplishment of strategic goals and the realisation of the vision.

The R&D Roadmap needs to be clearly and concisely defined and must, to remain relevant and useful, represent a living document that is monitored and updated regularly. The document itself is not enough – the true measure of success is whether or not the R&D Roadmap is implemented and achieves the desired outcome. To help in this, it is proposed that the R&D Roadmap sets 1) a results chain for each of the strategic goals, implementable over a limited period of time and verifiable in the form of milestones; 2) a framework for monitoring, using performance indicators; 3) regular (mid-term) reviews for assessing risks and assumptions in the critical path towards implementation.

A draft R&D Roadmap has been published for Nipah, with invitations to comment, and the final version is expected to be published shortly – on the WHO website, as well as in a scientific journal.

Session 2 Q&A

Q: In the context of Nipah outbreaks, with such short duration, it is important to strengthen clinical research capacity to the extent where it is available on demand – if outbreaks are to be prevented (described in the "Money & Microbes" report from The World Bank).

A: Response from Prof. Steve Luby: Given the wide spread of NiV, it is hard to predict where the next outbreak is going to be. It will require an investment that may not be efficient, but could be resilient in terms of capturing all emerging cases and allow for data capture to serve the development of diagnostics as well as therapeutics. Definitely an important issue.

Q: Do you have any examples of products that have successfully overcome the low probability high impact context?

A: Response from Prof. Steve Luby: Outside of the U.S. and Milton Friedman's "free market", market failures are real. There is a role for the State in funding projects that lack financial interest from private investors. One example is NASA's tracking of asteroids to prevent asteroids from causing serious damage to the Earth. Solutions exist, even if the private market fails to address them, but involvement of the State is likely to be necessary.

Q: How can we bring scientists together in closer collaboration, in between meetings like this, to be able to address the different market needs more effectively?

A: Response from Prof. Steve Luby: CEPI is a good example, for having put together a large coalition of the different players needed in the development and implementation of new vaccines. They have both public and philanthropic funds available that serve to de-risk the investments of private investors. It is hard to envision a mechanism that addresses the markets in the way asked, if it doesn't share the holistic and strategic approach adopted by CEPI. An integrated approach across affected geographies, as well as across the various components of diagnostics, therapeutics, vaccines, surveillance, and emergency response, is likely to lower overall costs and make the process more efficient.

SESSION 3 – PROGRESS AND CHALLENGES IN SURVEILLANCE OF NIV

Chair: Dr. Peter Daszak, EcoHealth

Southeast Asia is a hotspot for EIDs, as demonstrated in two recent publications. The risk of novel emerging zoonosis is high, and wildlife in the area is brimming with potentially zoonotic viruses. This is why there is such a focus on Southeast Asia and Nipah infection: While NiV is a frightening, lethal virus, other viruses that have not yet emerged may prove worse. For us to be able to prepare for future EIDs, it is vital to discuss surveillance, and a session dedicated to surveillance at Nipah@20 is very welcome.

Nipah surveillance and outbreak response in Bangladesh

Professor Mahmudur Rahman, Programme for Emerging Infections, icddr,b

Following the first Bangladesh Nipah outbreaks in 2001, 2003, 2004, and 2005, the country established its Nipah surveillance system and laboratory in 2006, in a collaboration between Institute of Epidemiology, Disease Control and Research (IEDCR) and the U.S. CDC. So far, Bangladesh is the only country to have implemented dedicated Nipah surveillance. The surveillance system was originally established as a joint JE and Nipah surveillance system (the Acute Meningo Encephalitis Surveillance system, AMES), with 11 sites across the country. Currently, there are five active and two passive sites, with an additional 47 sub-district- and 3 district hospitals added during Nipah season. In addition to surveillance at the sites, IEDCR operates a 24/7 hotline, nationwide media monitoring, and follows up on any informal reports.

IEDCR distinguishes between probable and confirmed cases, where probable cases are linked to deaths in a Nipah-affected community around an outbreak, where sampling was not possible. In total, there have been 313 cases since the surveillance started, and 226 (72 %) deaths. Surveillance case definitions used in Bangladesh are: 1) Axillary temperature > 38.5°C AND evidence of acute brain pathology [e.g., altered mental status, seizures], or 2) Axillary temperature > 38.5°C AND illness < 7 days AND severe shortness of breath [i.e., dyspnoea prevents patient from walking unassisted for 10 steps] AND chest radiograph consistent with diffuse acute respiratory distress syndrome. In addition to fulfilling at least one of the two definitions, an epidemiological link is always sought after. This can be geographic location close to an outbreak, that the time of year is within the Nipah season, or recent consumption of date palm sap. A cluster of Nipah cases is defined as at least two cases within a walking distance of 30 minutes, and with clinical onset within 21 days of each other. All suspected cases are investigated, but clusters of cases receive immediate attention and are prioritised.

The surveillance procedure starts with the reporting of a suspected case or cluster of cases from one of the surveillance sites (see Figure 3), and ends with laboratory-based diagnosis. Some tests are performed by IEDCR and some by the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b). The U.S. CDC, Atlanta, conducts quality controls. Outbreaks are evaluated in a One Health approach, including epidemiological investigations, animal studies, environmental contamination assessment, behavioural studies, and case control studies.

Through Surveillance sites (5 med colleges, Active; 2 sadar hospitals, Passive)
Event Based Surveillance
↓
Identification of a case/cluster of cases
↓
Reporting to IEDCR
↓
Investigation of the outbreak
↓
Laboratory diagnosis   Assessment of risk factors transmission
↓
IEDCR   icddr,b   CDC, Atlanta (Quality Control)
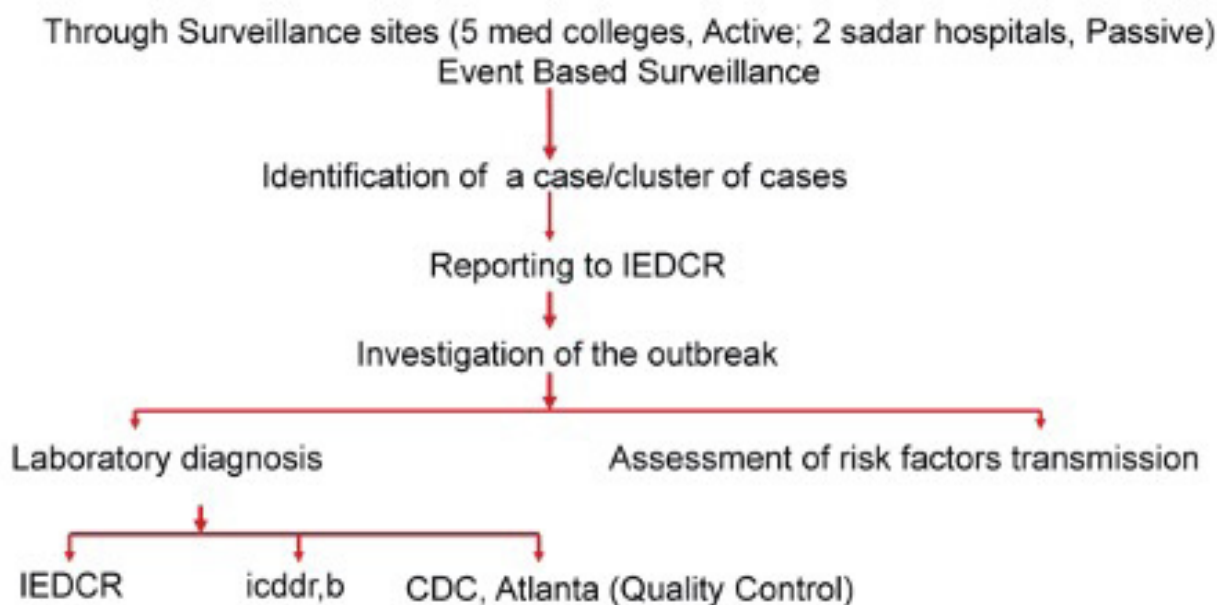
Figure 3. Nipah detection in Bangladesh (Dr. Mahmudur Rahman)

Immediately after a case has been identified, all contacts are listed and accounted for. Symptomatic contacts are subjected to a Nipah IgM test, and all contacts receive weekly follow-up for up to 21 days.

All contacts are interviewed for relevant details and contact history. At the end of the follow-up, contacts are tested for Nipah IgG.

Nipah virus dynamics in bats and implications for zoonotic spillover to humans

Dr. Jon Epstein, EcoHealth Alliance

Part of understanding the probability of outbreaks, is understanding how NiV operates in its natural reservoir, and understanding the risk of spillover. Given the wide distribution of the reservoir host, the *Pteropus* medius bat, why are we not seeing more outbreaks?

We should be prepared for NiV outbreaks anywhere where the bats and virus coexist. As such, the Kerala outbreak should not have been a surprise, in spite of the long distance from other outbreaks. The outbreaks in Bangladesh and Malaysia are linked by the fact that, in both places, the source of infection was a human-provided (anthropogenic) source of food. In Malaysia it was a mango orchard planted next to a pig enclosure, in Bangladesh it was the date palm sap. The bats thrive in the company of humans, and through human harvesting of date palm sap during the winter months, they have been able to access a food source that would otherwise not have been available to them – in a time when food is otherwise scarce. As a result, humans are creating opportunities for the Nipah virus to spill over.

In a study of viral ecology, Dr. Epstein and colleagues are trying to answer two questions: 1) Is there a spatial difference in infection patterns among bats in Bangladesh, between the "Nipah belt" and the rest of the country?; 2) Are there seasonal infection patterns among bats? Results were collected over a period of 8 years, from 2006 to 2014, and a publication is currently in review. Although seroprevalence varied, the team found no difference in seroprevalence inside and outside of the "Nipah belt". Traces of NiV infection were found in all of the eight different bat colonies studied. In Faridpur, one bat colony was followed longitudinally, with sampling every 3 months to follow seroprevalence over time. This resulted in a cyclical pattern, where levels of circulating antibodies waxed and waned. As a rule, circulating antibodies do wane over time, and it is believed that the waxing was in response to active viral transmission in the colony. Modelling suggested that bat epidemics occurred biannually, that it was driven by adults (not births, as has been suggested), and that the population density was an important determining factor.

The Bangladeshi bats are less mobile than the Malaysian bats, but sufficient evidence exists to

suggest that the different colonies are still connected, creating a metapopulation of bats where viral strains may circulate. In fact, there is no genetic distinction between different colonies across Bangladesh. There is even evidence of hybridisation with other bat species, such as *P. lylei* of Myanmar and *P. vampyrus* of Malaysia. Considering the genetic diversity of viral strains within a colony, there seems to be little variation. However, genetic variation between colonies at different geographic locations is considerable. Due to the mobility of the bats, strains typically found in one location may be transferred to another, contributing to the overall genetic diversity of NiV.

A recent study on the evolutionary history of NiV, based on all available genetic sequences, suggests that NiV originally migrated from India to Bangladesh in 1941, spread within Bangladesh, and then migrated back to India, including Kerala. The *P. medius* bat is common throughout the Indian subcontinent, and the Nipah virus travels with it. Nipah or related henipaviruses have also been found in many domesticated animals, including cattle, goats, and pigs.

One mode of transmission between bats and domestic animals could be the feeding of animals with fruit bitten by bats. The mode of transmission causing the Kerala outbreak in humans is still unknown.

Considering the wide distribution of bats and NiV, improved surveillance systems are necessary to better understand where there are potential hotspots for spillovers.

Survey of Pteropus bats during Nipah outbreak in India revealed its association

Dr. Pragya Yadav, National Institute of Virology, ICMR

Only four human outbreaks of Nipah infection have so far occurred in India, yet the Pteropus bats serve as a reservoir for the virus. Cues for the ongoing risk of a new outbreak may be found in the bats, so surveys of the bats are conducted regularly. In the laboratory, samples are handled at the BSL-3 level. In the field, however, some of the security measures that are otherwise found in the lab, such as directional airflow and HEPA filtration, will be missing. Thus, field work involves careful preparations including training, health measures, and risk assessments.

Capacity for bat surveillance in India was first established in 2011, yet a permanent surveillance system still does not exist. The first report to confirm the presence of NiV RNA in *P. giganteus* in India

was published in 2012, following a survey of bats in West Bengal. Dr. Yadav and her team collected samples from five different species of bats, and isolated viral RNA. In addition to NiV, they also found RNA from other viruses, some of which were isolated for the first time. None of the other viruses have so far been detected in humans, but the viral diversity observed in bats underscores their ability to serve as a natural reservoir for pathogens and, potentially, as the source of new EIDs.

During the 2018 outbreak in Kerala, NiV RNA was collected from humans and bats, and sequenced. A large proportion of the bats (23.07 %) proved to be infected with virus that was almost identical to the virus causing the outbreak (99.7-100 % sequence homology),

thereby identifying *Pteropus* bats as the likely source of infection. Similarly, in response to the single transmission of NiV to a 21-year old college student in Kerala in 2019, five sites the patient had recently visited were surveyed for infected bats. Again, some of the bats were found to carry NiV, and 21 % were seropositive. The NiV strains from the 2018 and 2019 Kerala outbreaks were found to be closely related (97.9 % sequence homology) to several Bangladesh strains circulating at the time.

Effective surveillance is necessary for detection, response to, and prevention of Nipah infection. Currently, a nationwide Nipah virus survey in *Pteropus* bats is underway in India, with eight states (out of 28) and two union territories (out of nine) surveyed since January 2019.

Long-term One Health surveillance of Nipah virus in Thailand forewarns risk of outbreak

Dr. Supaporn Wacharapluesadee, Chulalongkorn University, Bangkok

Thailand is an at-risk country for a Nipah outbreak, due to the abundance of *Pteropus* bats carrying the virus, the country's proximity to Malaysia which has previously experienced an outbreak, and the large swine industry (though the pigs have so far tested negative for NiV). Since 2002, Thailand has developed its capacity for Nipah surveillance, as shown in Figure 4.

In a large survey published in 2005, including six species of fruit-eating bats (n = 1,041) and six species of insect-eating bats (n = 263), Nipah viral RNA was found in urine and saliva from *Pteropus lylei* (n = 76, 9.3 %) and saliva of (n = 1). These results were followed by a longitudinal study at 7 sampling sites in central Thailand of the prevalence of NiV in *P. lylei* bats, to see if the viral prevalence was seasonal, as had been demonstrated in Bangladesh. Indeed, pooled urine collected on plastic sheets laid out underneath roosts were found positive for NiV RNA in the months of December through June. Later it was found that *Pteropus hypomelanus* living on the islands in South West Thailand carried Malaysian NiV, whereas the *Pteropus lylei* of central Thailand carried a NiV strain akin to the ones in Bangladesh. Different bat species → different location → different Nipah strain.

A longitudinal study in *Pteropus lylei*, lasting 17 years from 2002-2019, demonstrated the genetic stability of NiV: Among 119 NiV-positive pooled urine specimens collected throughout the study, sequence homology in a partial coding sequence of the
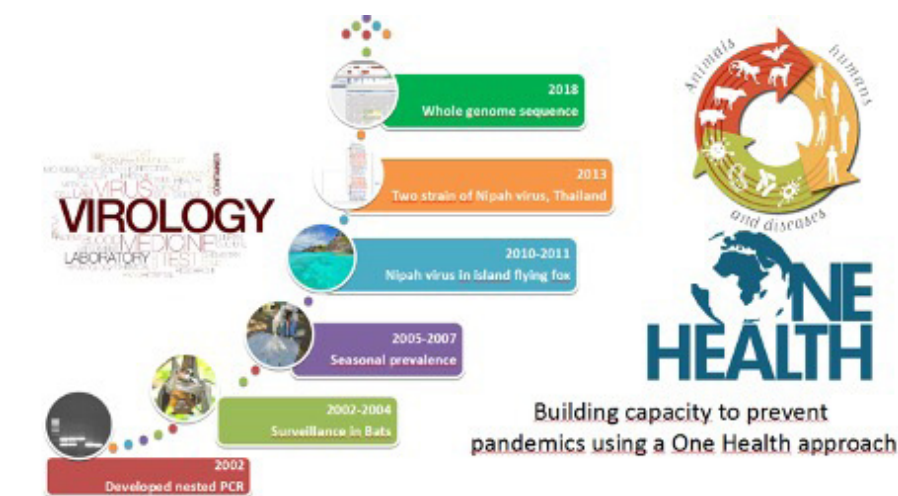
Figure 4. Thailand: Building capacity to prevent pandemics using a One Health approach (Dr. Wacharapluesadee)

nucleocapsid gene was 99.44-100 %. Whole genome sequencing of a NiV-positive Thailand bat revealed 99 % sequence homology to NiV isolated from a patient in Bangladesh in 2004, and 98 % sequence homology to NiV isolated from one of the patients from the 2018 outbreak in India.

In a One Health surveillance study of NiV in Thailand in 2012, bats (n = 374), pigs (n = 248), and humans (n = 418) were tested for NiV RNA and circulating NiV IgG. Among the bats, 2 % tested positive for NiV RNA and 10 % were seropositive. The pigs and humans were all seronegative. A public awareness campaign was conducted in relation to the study.

GPS tracking of *Pteropus lylei* showed that maximum linear distances between day roosts and foraging areas varied greatly between individuals, and ranged from 2.2 to 23.6 km. With this

information, Dr. Prateep Duengkae has been able to draw Nipah risk maps for central Thailand, based on the estimated density and activity of bats.

Long-term One Health Surveillance of Nipah virus in Thailand forewarns risk of outbreak, for two main reasons: 1) *P. lylei* bats have tested positive at the same location for 18 years, indicating that *P. lylei* is a natural reservoir for NiV; 2) High sequence homology between NiV carried by Thai bats and the 2004 Bangladeshi patient indicates that virus circulating in Thailand is capable of infecting humans.

Henipavirus biosurveillance in South African bats –A research overview

Professor Wanda Markotter – Centre for Viral Zoonoses, University of Pretoria, South Africa

Nipah and Hendra viruses are not present in Africa, and neither are the reservoir *Pteropus* bats. However, one related virus was recently identified in Ghana and added to the henipavirus family under the name of *Ghanaian henipavirus* (see Figure 5). This virus is known to infect bats, notably fruit bats, and since fruit bats of different species are found all over Africa outside of Sahara, the virus is expected to be widespread. So far, henipavirus RNA has been detected in South Africa and several Central African countries.

Figure 5. Phylogeny of paramyxoviruses (ICTV, 2019)

There is very little evidence to support spillover of African henipaviruses, though one study has found antibodies to henipa-like viruses in domestic pigs in Ghana, and another found evidence of spillover into human populations. No outbreaks have been reported, and longitudinal studies are non-existent.

At a study site in Limpopo, South Africa, Prof. Markotter and her team study bats roosting in a local cave, with primary attention to the Egyptian fruit bat, *Rousettus aegyptiacus*. This is a cave-dwelling fruit bat species found throughout Africa. In pooled urine collected from the bats, a number of different viruses have been identified (by RT-PCR and next-generation sequencing [NGS]), of which some are henipaviruses and some are henipa-like viruses. Through monthly sampling over 14 months, it was found that virus excretion varied over time, with a characteristic peak in July on two consecutive years. This peak coincided with the breeding season of *R. aegyptiacus*, as well as the dry winter period (nutritional stress?) and the waning of maternal antibodies in juveniles (susceptible individuals). Other factors may also influence the observed excretion pattern.

In conclusion, the *Rousettus aegyptiacus* population in South Africa hosts a diversity of paramyxoviruses including henipaviruses. The detected viruses group closely with ones previously described from other African countries, either due to co-evolution or the forming of meta-populations. The first study reporting on the paramyxoviral excretion dynamics in *R. aegyptiacus* identified the dry winter as a high-risk period for potential paramyxovirus transmission and spillover.

Session 3 Q&A

Q: Prof. Markotter showed very nice peaks of excretion, up to 60 %. It seems this is very high in terms of nucleic acid detection?

A: Yes, in the peak seasons, 60-70 % of the collected samples can be positive. Keep in mind that these are pooled urine samples. Assuming the observed frequencies reflect the true prevalence, one explanation could be the high density of R. aegyptiacus, which lives in larger populations than the Pteropus species.

Q: Have you been able to look at other virus species through NGS?

A: Not on these samples, but we have also studied other virus families, including the other paramyxoviruses, coronaviruses, and rubulaviruses. Corona- and rubulaviruses have different excretion patterns, where coronaviruses seem to be present at all times, whereas rubulaviruses have a second peak during the pregnancy season of the bats.

Q: While Nipah and Hendra represent pressing needs of Southeast Asia, one should keep in mind the diversity in viruses found there and elsewhere. Polyclonal, neutralising antibodies made for NiV and HeV are often unable to cross-neutralise even closely related viruses, such as Ghanaian henipavirus, and this needs to be considered in developing therapeutics and vaccines. There is also the question of how pathogenic these related viruses can be, if they were to spill over into humans.

Q: The longitudinal data were interesting to see, as were the data illustrating differences between the different countries, concerning bats and virus shedding. Notably, there seemed to be an inverse relationship between viral shedding and the frequency of outbreaks. For example, Thailand has a high prevalence of NiV in bats, but no outbreak. How could this be?

A: A lot of the high shedding rates were from pooled urine samples from whole colonies, and these may not be comparable to individual samples. There may also be large variation between colonies, so that the low shedding documented in one colony may not reflect the overall shedding in the area, especially if the colonies are out of sync in terms of viral prevalence. Studies in Bangladesh have observed elevated shedding over a period of time around spillover events, which seems to contradict the assumption made in the question. Another factor that affects transmission rates is human behaviour, as exemplified in outbreaks caused by the drinking of date palm sap. Public awareness on safe practices is key to prevent outbreaks.

Chair: We are all extremely grateful for everything WHO, NIAID, and CEPI are doing to push the agenda for improved surveillance, in spite of criticisms from other scientists. What we have seen here, is that the Nipah vaccine initiative is likely to be only the beginning, with many more initiatives following, due to the wide diversity of circulating viruses. Imagine what spillovers we would catch if we had the same level of surveillance everywhere, as around hospitals in Thailand and Bangladesh. The challenge is to cover all high-risk interfaces, not only in Southeast Asia, but in Africa, too. Hopefully, this sort of initiative will go down in history as the beginning of the end of pandemic risk.

SESSION 4 – PROGRESS AND CHALLENGES IN DIAGNOSTICS

Chair: Dr. Joel Montgomery, CDC

Overview of Nipah diagnostics

Dr. Jillian Sacks, FIND

The Foundation for Innovative New Diagnostics (FIND) is a global non-profit organisation driving innovation in the development and delivery of diagnostics to combat major diseases affecting the world's poorest populations. With diversified public and private funding, they partner to develop and deliver field-adapted diagnostic solutions to low- and middle income countries (LMICs). Their pandemic preparedness programme, which covers many of WHO's priority pathogens, has been crafted on the premise that

diagnostics are fundamental to the identification, containment, and resolution of outbreaks. They partner with WHO and others, and perform pathogen, technology, and partner landscape assessments, to identify critical needs and gaps in diagnostics – and address these through requests for proposals (RFPs) and funding of prioritised R&D initiatives.

Concerning Nipah, a TPP has already been developed by WHO, and is currently open for comments. Some diagnostic tests

are already available, such as nucleic acid tests (NAT), plaque reduction neutralization tests (PRNT), and enzyme-linked immunosorbent assays (ELISA), for use in centralised labs (see Figure 6). There are also tests available for veterinary diagnostics (not shown). What is still missing, is NAT, rapid diagnostic test (RDT) or panels to be used in decentralised settings. We also lack a standard, which would be required for the development of new diagnostics and to ensure proficiency of testing in existing laboratories.

	Target Use Setting						
	Facility-based, recommended diagnostic technology						**Non-facility based testing**
Health Care Level	4. National Reference Lab	3. Regional/ Provincial Lab	2. District Hospital			1. Health Center	0. Health Post/ Community
Type of Technology	NAT, PRINT, ELISA	NAT, ELISA	POC NAT	ELISA	RDT	RDT	RDT
Outbreak Setting							
Diagnosis/confirmation	PCR, virus isolation	PCR, lgM ELISA LDT	None	lgM ELISA LDT	None	None	None
Non-outbreak setting							
Confirmation of pathogen	PCR, virus isolation	PCR	None	lgM or Ag ELISA LDT	None	None	None
Clincial setting – pathogen detection in syndromic case management (syndromic diagnostics)	LDT PCR Panel	LDT PCR Panel	None	ELISA panel LDT	None	None	None
Support syndromic surveillance systems (adding specificity through diagnostic testing)	PCR, sequencing, ELISA LDT	PCR, ELISA LDT	Not Cost Effective	ELISA, LDTs, LDT panel	None	None	None

Legend:
- ■ Tests available
- ■ Lab and/or field trials ongoing
- ■ Late-stage development
- ■ Needed-or needs improvement
- ■ Not needed/ useful

Figure 6. Nipah diagnostics heat map of available test technology for a given target use setting (FIND)

Since the symptoms of Nipah are easily confused with other conditions, it is useful to have testing panels, covering multiple pathogens, that can be used in a non-outbreak setting for syndromic case management. These are available for centralised labs, but not in a decentralised setting. Some of the tests that can be used to confirm a Nipah diagnosis, such as virus isolation and neutralisation assays, require a BSL-4 lab, which serves to drastically limit the availability of these tests. Other tests that are suitable for detecting circulating virus are based on the detection of RNA, antibodies, or viral antigens. For these, a number of laboratory-developed tests (LDTs) exist, typically intended for local use, but they are not validated or commercially available (see Figure 7). Though appropriate for select countries, LDTs are generally not easily implementable or scalable for response to new outbreaks. NATs for use at the point of care (POC) have not been developed, and neither has RDTs. FIND strongly advocates for the development of commercially available tests to ensure quality and facilitate comparison across testing sites.

Biomarker	Test Format	Availability		Application
Viral Particle	Virus isolation			Confirm Active infection
				Exclude infection
RNA	NAT – Laboratory	> 5 LDTs; limited commercial		Confirm Active infection
	NAT – POC			Exclude infection
Antibodies	Print	LDT		Confirm Active infection
				Exclude infection
lgM	ELISA / IFA	> 1 LDT; commercial reagents only		Diagnose Active Infection
	RDT			Exclude infection
lgG	ELISA / IFA	> 1 LDT; commercial reagents only		Immune Status, exposure
	RDT			Surveillance
Viral Antigens	ELISA / IFA	> 5 LDT; commercial reagents only		Confirm Active infection
	RDT			Exclude infection

Legend:
- Validated, commercial assay available
- LDT or commercial assay with no independent data
- Under development
- Does not exist

Figure 7. Nipah diagnostic test availability (FIND)

There are important diagnostic gaps for rapid identification and response to Nipah outbreaks: 1) Limited availability of commercialised or standardised kits with regulatory approval; 2) Lack of an international reference standard, and a sample repository to aid in development of new diagnostics; 3) Lack of tests suitable for POC or community settings; 4) Lack of tests that can be used on minimally invasive samples, to minimise biosafety requirements. Better understanding of Nipah epidemiology and viral kinetics will facilitate the development of appropriate NiV diagnostic tests to serve both outbreak and non-outbreak intended uses.

Note: A list of in-house NAT and LDT serology tests for NiV is provided in the presentation Annex and in Mazzola LT and Kelly-Cirino C. BMJ Global Health 2019;4:e001118.

The WHO Nipah diagnostics target product profile (TPP)

Dr. Laura Mazzola, WHO

Following the WHO R&D Blueprint, published in 2016, a 5-year framework for Nipah in the form of the Nipah R&D Roadmap was drafted in May 2018. The R&D Roadmap addresses current primary challenges, key needs, and knowledge gaps for diagnostics, therapeutics, and vaccines; sets strategic goals for all of these; and defines priority areas and activities for obtaining the goals. From the needs and knowledge gaps described in the Roadmap, use cases and TPPs for Nipah diagnostics were developed and recently published for consultation on WHO's website. In this document, key priority actions needed to drive new NiV diagnostic test development were identified, with the following two use cases in mind: 1) Screening for active NiV infection; 2) Confirmation of active NiV infection. Screening is typically done in the field, in a POC or near-patient (NPT) setting, where laboratory infrastructure is limited or non-existent. Confirmation is typically done at a centralised reference laboratory, though often without BSL-3/4 capacity. Nipah being a BSL-4 pathogen, some sort of on-site or immediate inactivation procedure would be desirable.

For the priority use case, detection of active NiV at a peripheral health centre or hospital, a set of requirements were established as shown in Figure 8. These laid the basis for the development of TPPs for rapid screening and confirmation, respectively.

Clinical Impact	Rapid detection of active NiV infection to support early outbreak detection and case management, and to ensure early implementation of infection control measures Confirmation should identify NiV from other diseases including henipaviruses
Use Setting	Primary care facility, near-patient hospital laboratory, community clinic Resources may be limited: benchtop, microcentrifuge, transfer pipets, refrigerator
Target Population	Patient meeting the clinical definition if suspect NiV, presenting to health care facility
Test Demand (max)	Up to 50 specimens per day at peak outbreak for screening
Test Operator	Laboratory technician (1–2 year certificate); doctor, nurse, healthcare worker
Test Complexity	Lab tech can reliably process moderate text complexity (≤ 3 steps) but preferably minimal (sample addition only) processing Minimal to no-capacity for manual sample preparation; preferably BSL-1 containment
Turnaround Time	Same-day or next-day test results(can be while-you-wait test)
Appropriate Diagnostic Options	**Screening tests: RDT, ELISA, NPT/POC NAT** **Confimatory tests: NPT/POC NAT**

Figure 8. Use case: Rapid detection (screening & confirmation) for suspected NiV infection at peripheral setting during an outbreak (WHO)

The TPPs were made to describe the requirements for a rapid screening and a rapid confirmation diagnostic test, respectively, for human use in an outbreak setting. They specify minimal and optimal requirements – a newly developed test is expected to at least meet the minimal requirements, whereas being able to meet the optimal requirements will yield a better test. A screening test is 1) intended for the detection of NiV-specific IgM and/or NiV antigen (Ag); 2) an ELISA or RDT kit; 3) validated for at least NiV-B and NiV-M strains; 4) quicker than 4 hours to get results; 5) used on at least serum; 6) appropriate for biosafe collection and includes inactivation protocol; 7) sensitive (> 90-95 %); 8) specific (> 80-90 %); 9) minimally cross-reactive with other pathogens, may have some cross-reactivity to other henipaviruses. A confirmation test is 1) intended for the detection of NiV ribonucleic acids (RNA) by RT-PCR; 2) semi-automated or fully automated; 3) validated for at least NiV-B and NiV-M strains; 4) quicker than 6 hours to get results; 5) used on at least plasma and serum; 6) conducted in three or fewer steps, including inactivation (sample preparation); 7) detecting

down to 1,000 copies/ml; 8) sensitive (> 95-98 %); 8) specific (> 95-98 %); 9) not cross-reactive with other pathogens, including other henipaviruses.

There are other issues related to the development of new Nipah diagnostics, besides the diagnostics themselves: 1) For clinical validation, access to specimens is critical, and specimen repositories that are representative for endemic and at-risk regions should be established; 2) International reference standards need to be developed for calibration, comparison, and quality control; 3) Criteria for test performance and use need to be established; 4) Harmonisation of regulatory requirements is desirable; 5) For biosafety, a safe and simple method of sample inactivation needs to be devised that does not interfere with diagnosis. Then there are also a number of issues related to the procurement, implementation, training, proficiency testing, and post-market surveillance of new diagnostics. Finally, there needs to be put in place appropriate geographic deployment strategies for outbreak preparedness.

By 2021, WHO hopes to have completed preclinical evaluation of at least two POC or NPT diagnostics that align with the TPP. By 2022, the aim is to complete field studies for these.

Role of early diagnosis during Nipah virus outbreak in India

Dr. Pragya Yadav – National Institute of Virology, ICMR

In the initial phase of the Kerala outbreak in 2018, the logistics of sample analysis and clinical management proved somewhat chaotic. Systems were not in place for safe handling of samples and clinical cases. Additionally, as Nipah is a frightening disease, many wanted to be tested, and the resulting rapid increase in workload put severe strains on the available diagnostic capacity. Two major infrastructural requirements were identified during the outbreak: 1) Laboratory facilities for quick diagnosis; 2) Isolation wards for confirmed and suspected cases. In spite of the difficulties, Indian healthcare workers eventually succeeded in containing the outbreak. The lessons learned from 2018, and the resulting awareness campaign, led to rapid response and the successful containment of the ensuing 2019 outbreak, which affected only one person, who survived.

There is a need for NiV diagnostics that can ensure rapid and reliable diagnosis in the event of an outbreak, to achieve early implementation of infection control measures. Affordable POC diagnostics are a top priority. The ability to detect and confirm infection on-site allows not only for proper case management, but also for early public health interventions such as contact tracing, isolation, and prophylactic measures. It is important that POC diagnostics are easy and safe to use, with minimal training.

The National Institute of Virology has developed a number of diagnostic tools to be used in India: 1) Anti-Nipah human IgM ELISA; 2) Anti-Nipah bat IgG ELISA; 3) POC assay for NiV detection. These are being validated by IEDCR, Bangladesh. A mobile diagnostic system developed in collaboration with MolBio, as private industry partner, has received approval for clinical use by the Indian Central Drugs Standard Control Organization. The system includes an automated RNA extraction machine and a real-time PCR device tailored to detecting NiV. It is portable and battery-operated, uses stabilised, disposable consumables, and requires minimal handling and, thus, training. The lysis buffer included in the kit has been shown to inactivate NiV, allowing for POC use. Suitable sample materials include throat swabs, serum/plasma, urine, whole blood, other body fluids, and tissue materials (including bat tissues).

The mobile diagnostic system is especially suited for outbreak response in remote areas, and offers easily deployable state-of-the-art technology for effective field diagnosis. However, it does not replace the need for centrally located containment laboratories, more of which are still needed in India. Effective surveillance is key to effectively managing future outbreaks of NiV.

Nipah virus diagnostics at CDC – current strategies

Dr. John Klena – Viral Special Pathogens Branch, U.S. CDC

The Viral Special Pathogens Branch (VSPB) of the U.S. CDC provides support to outbreaks around the world. They have a BSL-4 high-containment laboratory, which is used for high hazard virus diagnostics and research: They also contribute to rapid detection and control during outbreaks through epidemiology support, provision of mobile labs, and health communications. Finally, they conduct ecological investigations to detect the source of zoonotic outbreaks (most EIDs are zoonotic RNA viruses), and devise strategies for disease prevention and control. In the context of Nipah, CDC is working to improve assays for both serological detection and nucleic-acid based detection. While they do not seek commercialisation of their own assays, they are happy to support others who are working to commercialise new diagnostic tests. CDC willingly shares serological and PCR assay components with partners, but this has become increasingly difficult in recent years, due to stricter regulations on shipping of biological materials – something that is especially challenging with the shipping of positive viral controls.

The ELISA-based serological assay of CDC has been known to be difficult to work with, and some changes have been implemented over the past couple of years to improve the assay's sensitivity. One major change has been to replace the historically used goat anti-human IgM capture antibody with goat IgM F(ab')2 (Thermo Scientific A24490). This change will be implemented in the testing in Bangladesh this season. Furthermore, the use of chinese hamster ovary (CHO) cell slurry with recombinant antigen (N-protein), rather than Vero E6 cell slurry with infected cells, seems to improve the background signal, but comes with a loss of sensitivity of the NiV IgM assay. When testing survivors for IgG, serology results remain positive for more than 5 years. Still, more systematic testing of survivor serum is needed to evaluate the longevity and protection of the IgG response.

Nucleic acid testing, using real-time RT-PCR, seems very sensitive and suitable for use in the field when testing for NiV. CDC has historically run these tests against the nucleocapsid (N) protein, and are looking to improve their assay. With sequences from the 21 Nipah whole genomes sequenced by CDC so far, one new domain in the N gene has been identified that has no mismatches among the 21 different viruses. Therefore, this seems a promising target for a new and improved RT-PCR-based diagnostic test, and a set of primer and probe is currently being used to screen samples collected from Bangladesh in the seasons 2017-2018 and 2018-2019. As new, full-length genome sequences are being made available, further revisions to the test will be made.

Hendra DIVA assay for the detection of antibodies in vaccinated horses

Dr. Kim Halpin – Australian Animal Health Laboratory (AAHL)

Horses are highly susceptible to HeV infection, and will often die. However, it was infection in humans that led to the development of a vaccine against Hendra. The Equivac® HeV vaccine by Zoetis is a subunit vaccine containing the soluble G protein (sG), which is a HeV glycoprotein responsible for attachment of HeV to target cells. It was released late 2012 upon approval by the Australian Pesticides and Veterinary Medicines Authority (APVMA) for minor use, and fully registered in 2015. The vaccine requires three doses initially, and a yearly booster to remain effective.

The Hendra DIVA test is a serological test to assess whether a horse has been infected by or vaccinated against HeV. It is an ELISA-based assay, in which the plates are coated with HeV antigen (HeV sG or recombinant N protein, in separate wells) and detected by a specific mouse monoclonal antibody (mAb; anti-sG or anti-N, as appropriate). The secondary antibody is anti-mouse IgG, conjugated with the enzyme responsible for colorimetric reaction. When first incubating with serum from infected horses, binding of the mAb is blocked, resulting in a "blank" colour reaction for both sG and N antigen wells. For vaccinated horses, only the sG wells will be "blank", since this is the only protein used in the vaccine. Serum from previously unexposed horses will yield colorimetric results for both sG and N wells.

The test is being validated in 1,000 unvaccinated and 1,000 vaccinated horses, so that it can be used for healthy horses that need to be transported internationally. HeV vaccination is entered in a national database in Australia, which allows for assessment of the number of vaccines given to a particular horse, and see the time elapsed since the last vaccination. In the current validation, where about 250 horses have so far been included, most horses appear to achieve protective antibody titres upon the third vaccination, as evaluated by serum neutralisation tests (SNTs). In these horses, the protective antibody titres seem to last for at least 20 months after the last vaccination. The vaccine thus appears to be effective, and the hope is that documenting efficacy of the vaccine will counter some of the negative attention the vaccine has received from certain circles in the general population.

Today, the Australian Animal Health Laboratory (AAHL) is the only OIE Henipavirus Reference Laboratory. Within the OIE network, with their priority diseases, reference laboratories are assigned to support other laboratories with training, technology transfer, and technology support. As the only reference laboratory on henipaviruses, the synergies that would otherwise be present in a larger network of henipavirus laboratories are missing. Thus, AAHL is interested in talking to other laboratories with an interest in joining an informal network of laboratories performing henipavirus diagnostics. Network activities could for example include sharing of protocols, reagents, and samples.

Serological assays for henipavirus diagnostics

Dr. Anne Balkema-Buschmann – Friedrich-Loeffler-Institut (FLI)

Presented on Day 2 of the conference

Friedrich-Loeffler-Institut (FLI) is developing diagnostic assays for henipaviruses in Europe, far from the endemic areas, in the interest of preparing for potential epidemic and bioterrorism threats. Testing European animals that may have been exposed to henipaviruses requires previous validation of the diagnostic assays against indigenous livestock breeds. First, an indirect henipavirus ELISA assay was developed with NiV N protein expressed in E. coli, and the cut-off value was determined against negative samples from Canadian pigs. Furthermore, the establishment of indirect ELISAs for NiV G and HeV G, using proteins expressed in L. tarentolae (which closely mimics the protein glycosylation patterns seen in humans), allowed for differentiation between NiV and HeV infection: While there is still cross-reactivity between NiV and HeV in these two latter assays, the homologous virus yields a higher response. Finally, Western blot is used to confirm positive samples against plasmid-derived NiV G, using a monoclonal antibody for NiV G as positive control. The assay has now been validated for North American and European serum samples, and is available for screening and diagnostic purposes.

Working to establish a Hendra diagnostic for horse samples, an indirect ELISA was developed based on HeV G expressed in L. tarentolae and HeV N expressed in baculovirus-infected insect cells. Screening of 288 German negative horse sera yielded the cut-off, and assay validation was conducted in Geelong using samples with confirmed diagnosis (135 negative, 95 vaccinated, and 21 infected horse sera). The German ELISA assay compared very well with the Australian assay, having very similar cut-off values and high, comparable levels of sensitivity and specificity.

There is serological evidence of henipavirus spillover into human populations in Africa, and although the clinical relevance is unclear (there have been no detectable cases), there is a desire to establish strain-specific tools for serosurveillance. Positive sera were generated immunising fruit bats (Rousettus aegyptiacus and Eidolon helvum) hosted at the FLI with different recombinant viral proteins. In preparing for these infection studies, the circadian temperature baseline had already been established (body temperatures in the bats range from 34 to 41 °C depending on time of day and activity levels). Sera from vaccinated bats, when tested with ELISA or the corresponding multiplex setup (on Luminex®), again demonstrate cross-reactivity, but show the strongest response with the homologous test. FLI now continues to work on establishing multiplex assays for henipavirus differentiation in pigs, horses, and bats, as well as detecting antibodies against other relevant antigens in bats and pigs.

Establishment of an immunofluorescence assay to detect IgM antibodies to Nipah virus using HeLa cells expressing recombinant nucleoprotein

Dr. Yoshihiro Kaku – National Institute of Infectious Diseases, Tokyo

The National Institute of Infectious Diseases (NIID) started working on Nipah in response to the 2014 outbreak on the Philippines, partnering with Research Institute of Tropical Medicine (RITM), WHO, and AAHL. NIID was involved in performing serological and nucleic acid testing on the samples from three of the seventeen cases detected in the outbreak.

The ambition of NIID is to develop diagnostic methods for NiV that are available under BSL-2 conditions and use inexpensive equipment. At the time, NIID did not have an IgM detection system in place, due to the lack of positive control human serum. Instead, they embarked on developing a positive control through the immunisation of macaques (*Macaca fascicularis*), since IgM and IgG from macaques are typically cross-reactive to human IgM and IgG. The macaques were immunised with 1 mg NiV N-protein purified from baculoviruses, followed by 0.2 mg booster doses on days 15, 28, 42, and 56. Serum was collected on days 5, 7, 9, 11, 13, 15 (30 ml), 53, and 63 (whole blood), where serum from day 15 was used as positive control for IgM, and whole blood from day 63 served as positive control for IgG.

An IgM capture ELISA was designed as follows: Wells were coated with goat anti-human IgM, to which samples and positive controls containing human and macaque IgM, respectively, were added. Half of the wells were incubated with NiV-N antigen corresponding to the antigen to which the macaques had been immunised, and the other half with irrelevant antigen from baculovirus. Captured antigen was detected by rabbit anti-NiV-N serum and goat anti-rabbit IgG linked to horseradish peroxidase (HRP). The IgM assay was tested against serum collected from two immunised macaques on days 5-15, demonstrating seroconversion for one of the macaques around day 9 (which could then be used as positive control). Macaque number two failed to elicit a significant immune response to NiV-N.

An IgM indirect fluorescence antibody test (IFAT) was developed by (stably or transiently) transfecting HeLa-229 cells with plasmids containing NiV-N cDNA and blastcidin as reporter. For transient expression, the HeLa cells were trypsinised on day 4, seeded onto 14-well glass slides and fixed with acetone. For stable expression, blastcidin-expressing cells were selected, trypsinised, and mixed with mock-transfected cells in a 1:3 ratio prior to fixation, since this yields a setup better suited for distinguishing between false positive and true positive results. The IFAT was conducted by adding test serum, and subsequently Alexa Fluor 488-conjugated goat anti-human IgM, to the fixed HeLa cells. If the test serum contains anti-NiV-N IgM, the NiV-N-expressing HeLa cells will emit fluorescence.

The IgM capture ELISA proved to have a lower detection limit for anti-NiV-N IgM than the IFAT assay, yet the three patient samples received by NIID from the 2014 Philippines outbreak were all confirmed NiV positive by both assays. When testing the IgM IFAT assay against normal (uninfected) sera from The Malaysian Cohort (TMC) project, all samples were negative at a 1:100 serum dilution. The IFAT assay was also shown to be able to detect anti-NiV-N IgG, and to be cross-reactive for anti-HeV-N IgG. The IFAT antigen slides are fairly stable, and can be stored at -80 °C for several years awaiting future use. It is possible to use them directly after thawing, which makes them suitable for rapid diagnostic response in a laboratory setting.

NIID has also developed a pseudotyped VSV neutralisation assay, which is more sensitive than neutralisation assays with live NiV. The assay is suitable for both screening (in a high-throughput setting) and confirmation of infection status, and could potentially limit the need for confirmation with a second assay. NIID are happy to share the technique and reagents with other laboratories.

Session 4 Q&A

Q: Are there plans for using the mobile diagnostic system developed in India as part of prospective surveillance? Can you bring the system on a flight? What is the cost?

A: The system can be used both prospectively and in an outbreak setting. Yes, the system can be brought on the plane, but may sometimes be rejected by staff, for example due to safety concerns. The cost per test is reasonable, about 800 Indian rupies.

Q: What more can be said about the vocal groups opposing the Hendra vaccine?

A: One challenge is social media like Facebook, where people hesitant to the use of vaccines come together and share stories about horses getting sick from vaccination, and claims that the manufacturer is pushing the vaccine in regions where HeV has never been seen. A recent spillover event close to Sydney may serve to counter this view, and has caused all thoroughbred stables in the area to vaccinate their horses. A recent pro-bono legal class action has caused a number of people to come forward saying that their horse had a reaction to the vaccine. This certainly detracts from our cause of trying to save the lives of people and horses, but we have to keep offering up all the good information about the vaccine and ensure continued use.

Q: Can the HeV vaccine be used in humans?

A: The approach in Australia has been to vaccinate the horses, rather than people, and protect everyone – since the horses are the amplifying host (humans have never gotten Hendra directly from a bat). From a One Health point of view it makes sense to vaccinate the horses.

Q: How is the use of the HeV vaccine being monitored, in terms of side effects?

A: The monitoring is ensured by the APVMA, and all reports of adverse events are sent to them. The manufacturer has a role in facilitating this, and in following up on the reports. According to the vaccine company, adverse events linked to the use of vaccine have been minimal.

Q: Annual booster doses of the HeV vaccine seems a bit excessive. Is there any evidence to support this?

A: With the documentation available, 12-month boosters are still recommended. With more data, one may be able to reduce the number and frequency of boosters.

SESSION 5 – PATHOGENESIS AND ANIMAL MODELS

Chair: Dr. Amy Shurtleff, CEPI

Development of a nonhuman primate model for Nipah virus infection that accurately reflects human disease

Dr. Mike Holbrook – NIAID, NIH

NIAID works to develop a non-human primate model that accurately recapitulates human disease to aid in the development of medical countermeasures to Nipah virus infection. While, historically, such an animal model would have been designed to be uniformly lethal, NIAID seeks to develop a model that is more true to NiV infection in humans. Specifically, they wish to replicate the clinical hallmarks of Nipah disease: 1) Acute Respiratory Distress Syndrome (ARDS), as evidenced by "ground glass" opacities of lung computed tomography (CT) scan or X-ray; 2) late-onset encephalitis, manifesting as focal hyper-intense lesions or confluent cortical involvement on brain MRI scans; 3) widespread vasculitis, especially of highly vascular organs such as the spleen. At the NIAID integrated research facility, one has been able to study the impact of exposure to different-sized particle aerosols with NiV in animals, using clinical imaging to assess the disease progression in lungs and brain, and immunological tests to evaluate immune responses. These are the only facilities in the world to allow for advanced clinical imaging of live animals kept at BSL-4.

Currently, NiV-M and NiV-B particles about 12 µm in size are used to infect wild-caught Caribbean origin African green monkeys (AGM) at a dose of about 500 plaque-forming units (pfu) per monkey. Surprisingly, infecting with NiV-M or NiV-B results in widely different disease progression, where NiV-M causes extended disease with about 30 % survival, and NiV-B leads to rapidly progressing disease with about 50 % survival. Quantification of RNA in blood, nasal swabs, and cerebrospinal fluid (CSF) demonstrates viral shedding during the acute phase of disease, as well as penetration of NiV-M across the blood-brain barrier (BBB) into the central nervous system (CNS). Seroconversion is detected in only some of the animals, starting around day 10 post infection. Neither seroconversion nor the presence of neutralising antibodies seem to be reliably protective. While antibodies may limit respiratory disease, no effect has been found on the progression of neurological disease.

A number of different coagulation assays point to a link between acute-phase disease progression and the development of

haemorrhagic disorders: 1) Reduced platelet levels, as well as increased partial thromboplastin time (PTT) and thrombocytopenia suggest onset of disseminated intravascular coagulation (DIC); 2) Elevated fibrinogen suggests severe DIC; 3) Elevated von Willebrand factor (vWF) is indictive of vasculitis. Brain imaging is conducted regularly on live, infected animals (CT throughout infection, MRI from day 15 since it requires anaesthesia), and later guides the acquisition of brain biopsies that allow for confirmation of encephalitis. Detection of circulating cytokine levels may serve to corroborate the inflammatory status of the animal, though clinically predictive patterns have not yet been identified. Lung infiltration of NiV-B has been demonstrated to severely affect lung capacity.

The AGM model results in the development of neurological disease several weeks after exposure, and requires survival of the acute respiratory phase of the disease. The animals respond differently to infection, as would be expected in the human population: Some show no clinical evidence, some are infected with clinical

manifestations but survive, and some succumb to the infection. When delivered to the oropharynx and upper respiratory tract, NiV-M causes less respiratory disease than NiV-B. Haemorrhage and coagulopathy seems to be a critical aspect of Nipah disease. Neurological disease results from vasculitis (microinfarcts) or inflammation (encephalitis) rather than virus specifically targeting neural tissue, and this occurs even in the presence of NiV neutralising antibodies.

Some points to consider: 1) NiV infection may initially present as a mild to moderate respiratory infection, progressing to neurologic disease in some cases if unrecognised or untreated; 2) Serological and nucleic-acid based diagnostic tools complement each other, and should be used in combination, with whole blood and nasal swabs as suitable sources of viral RNA; 3) Therapeutic approaches focusing on vasculitis/ DIC should be developed; 4) Continued development of the

NHP model for NiV infection will aid in understanding the disease and in developing clinical countermeasures.

Advances in non-human primate models of henipavirus disease: refinement of routes and the importance of species selection

Dr. Robert Cross, University of Texas Medical Branch (UTMB)

In the AGM model, NiV infection by the intratracheal or intratracheal + oral route results in disease characterised by thrombocytopenia, leukopenia, neutrophilia, generalised vasculitis (lung and brain), hypoalbuminemia, pulmonary distress, and (often) neurological symptoms. NiV-B has proven more potent than NiV-M, and consistently kills the monkey in 9-10 days. Respiratory disease has a rapid onset around day 8-10, showing up on X-ray shortly before the animal needs to be euthanised. Neuronal and endothelial deposition of viral antigens causes the characteristic brain lesions. Circulating viral genome may be detected from day 7 post infection in this model.

Whereas the intratracheal route may have its uses, it is labour-intensive and cumbersome to use. Therefore, an intranasal NiV exposure AGM model has been developed using the mucosal atomization device MAD Nasal™

from LMA®. Using doses of 2,000 and 20,000 pfu, the intranasal model was uniformly lethal, with time to death of about 9-10 days. Vasculitis and viral depositions in the spleen may be one reason why a protective immune response to the infection is not established and the animals succumb. Nipah is a vascular disease, and wherever endothelial cells are present, viral replication may occur. Thus, viral RNA is found in blood as well as in most tissues examined. Cytokine profiling indicates no systemic inflammation until late in the disease.

While the AGM model is uniformly lethal, another model has been developed using cynomolgus monkeys (CM) and administration of NiV-M, NiV-B, or HeV intratrachially (5.0×10^5 pfu). This model has pathologies consistent with human infection and other animal models, yet the animal survives. While circulating virus may be detected from day 5 or 6 – with peak viremia around day

7 – spleen, lung, and brain tissues are minimally affected. One reason for this may be stronger immune responses to henipaviruses in CM, as detected by considerable titres of serum neutralising antibodies and high circulating levels of the chemokines MCP-1 and IL-6. A differential gene expression microarray analysis showed characteristic differences between AGM (fatal infection) and CM, and this is hoped to help clarify the mechanisms underlying CM survival. Furthermore, while reagents for phenotyping of immune cells are lacking for use in AGM, digital cell quantitation based on results from the microarray analysis has allowed the in silico identification of B cells and Th1 cells as possible key cell populations in survival of AGM infected by NiV. In contrast, mast cells and NK cells may play a detrimental role in the pathogenesis of Nipah infection.

# Oronasal challenge of ferrets with henipaviruses

Dr. Glenn Marsh, CSIRO, Australian Animal Health Laboratory (AAHL)

Over the past 15 years, AAHL has developed a ferret model for Nipah infection that closely mimics the human disease. When infected with NiV-B, the ferrets experience predominantly respiratory disease, whereas with NiV-M the disease has more of a neurological character. Clinical endpoints are typically reached day 6-9. As with the non-human primate models, terminal animals experience a drastic drop in platelets (causing haemorrhaging) and circulating lymphocytes. The model has been extensively used for the assessment of clinical countermeasures like the HeV sG equine vaccine, monoclonal antibody m102.4, peptides, and repurposed medications.

Significant differences are seen between NiV-B and NiV-M, in animal models as well as in humans. NiV-M is the result of a single spillover event with bat-to-pig and pig-to-human transmission, yet no human-to-human transmission. NiV-B, on the other hand, has seen multiple spillovers over the last years, with bat-to-human and human-to-human transmission. The question is whether the epidemiological differences are a result of distinct viral properties, or if environmental and host factors also play a role. Notably, different NiV-B isolates used in animal testing may have different properties, as well. The strain used by AAHL was isolated from an outbreak in Rajbiri in 2004, where human-to-human transmission was not a major source of infection (other strains have not been available to them).

Comparing NiV-B and NiV-M in the ferret model, NiV-B was demonstrated to be more infectious by a factor of about 100 (5 pfu needed to infect two out of two animals, whereas the corresponding dose for NiV-M was 500 pfu). The pathology of infection, however, is comparable for the two strains, with onset of symptoms day 5-6 and euthanasia day 7-9. As in other models, gross pathology of NiV infection in ferrets involves visible haemorrhaging on the surface of the lungs and other organs. One difference between NiV-B and NiV-M in ferrets is the higher viral load and, hence, higher oral and nasal shedding, of virus in NiV-B infection. However, this shedding is not sufficient to cause transmission of virus to other animals through regular contact, suggesting that transmission requires direct transfer of bodily fluids, and that aerosol transmission occurs rarely if at all. This suggests that preventing close contact with infected individuals is likely to be an effective measure against human-to-human transmission.
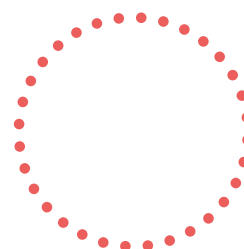
# Nipah virus pathogenesis in experimentally infected swine

Dr. Brad Pickering – Canadian Food Inspection Agency (CFIA), Winnipeg

During the first Nipah outbreak in Malaysia, close to a million pigs were culled, and the financial repercussions led to a high interest in studying NiV infection in pigs. There has since not been a single outbreak, outside of the Philippines, involving pigs as an intermediary host. Pigs remain, however, an interesting model for the study of NiV infection – especially given their potential to cause new epidemics. Although clinical signs of NiV infection in pigs include acute febrile illness with respiratory and neurological symptoms, as well as increased salivation and nasal discharge, the infection is often asymptomatic and may result in sudden death. During the Malaysia outbreak, the incidence of infection within affected farms was 100 %, with 1-5 % mortality. The disease would typically last for about 2 weeks on each farm, providing ample opportunity for spillover into humans.

A NiV-M infection model in swine used at CFIA involves the oronasal or nasal inoculation of 105 pfu per animal, resulting in relatively mild infection with some clinical signs such as laboured breathing and sometimes CNS involvement. Viral shedding peaks between days 3 and 5, allowing for detection in oral and nasal swabs. Through a nasal lavage it is possible to detect viral shedding as early as 2 days post infection (DPI). In spite of few symptoms, the animal's organs may be severely affected by the infection, with enlargement and bleeding. Oronasal infection typically spreads through local lymph nodes, lungs, and nose, and into the brain via cranial nerves (specific to pigs).

It is important to understand whether NiV-B may also infect pigs, as a potential mode of spillover into humans. Thus, Canada's National Centre for Foreign Animal Disease (NCFAD) has obtained a recombinant NiV-B (rNiV-B) isolate for comparisons with NiV-M in vitro and in vivo. The kinetics of virus replication was tested in three different cell lines and the two viral strains were found to be comparable in all cell lines. When used to infect piglets (who were indeed susceptible), at 2.5×105 pfu, rNiV-B caused oral shedding from day 2 post infection (as assessed on a community basis by cotton ropes hanging in the pen, which the pigs would chew on). One difference between NiV-M and rNiV-B is that the latter also sheds rectally at detectable levels, from 1 to 10 DPI. Furthermore, only low-level antibody responses were induced after oronasal infection with rNiV-B, and titres of neutralising antibodies were low. Thus, detection of circulating antibodies may not be an option for diagnosis of potential NiV-B infection in swine, and emphasis should be put on active surveillance and ensuring diagnostic capability of veterinary laboratories in at-risk regions.
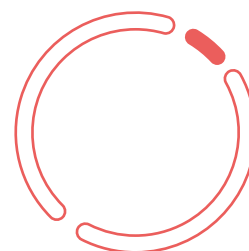
Session 5 Q&A

Q: The differences in pathology seen between NiV-B and NiV-M infections are intriguing. Has the same been seen in humans?

A: More data is needed to assess this.

Q: In the NiV-B AGM model, the lungs of the animals were gravely affected in the acute phase (example shown from diseased animal at day 8). Was this consistent for all animals, and did respiratory disease occur at a later stage with NiV-M?

A: It was pretty consistent in the animals infected with NiV-B that succumbed to the infection, and typically occurred around day 8-10. NiV-M infection given by inhalation of large particles did not result in respiratory disease.

Q: Were the infected ferrets displaying respiratory symptoms, such as coughing, sneezing?

A: Yes, coughing and sneezing did occur in some of the animals. In others, the neurological symptoms were more prominent.

Q: Were cohabiting ferrets able to interact physically?

A: Yes, the ferrets are typically housed in pairs, and for transmission studies three ferrets are housed in the same cage. They will often curl up in a corner of the cage, and thus enjoy close interactions. This is true even for sick animals, in general. With other pathogens than NiV, transmission is frequently seen under these conditions.

Q: Could airflow between cages in the BSL-4 facilities contribute to transmission?

A: Yes, with easily transmissible pathogens, transmission has been observed across cages due to the forced airflow of BSL-4 facilities. One tends to try and avoid this through careful positioning of the cages.

Q: Will the swine model be used for transmission studies?

A: Yes, once the plans for expanding the facilities have been completed, transmission studies will be conducted.

Q: The swine model is one of few non-fatal NiV animal models. Could this be used to investigate a potential role of "immune amnesia" in NiV infection, akin to what has been described for measles?

A: Some work is being done on the actual immune cells, including infected cells. This will be published soon.

Q: Could the lack of neutralising antibodies induced by rNiV-B be linked to the age of the animals (piglets) used?

A: Yes, age is definitely a factor regarding the development of immunity in pigs. Piglets are used in the interest of ease of handling in a BSL-4 context. The use of a recombinant strain could potentially be a factor, though not necessarily.

Q: Have you looked at blood gas oxygenation as an endpoint in the pig model?

A: No, but more chemistry and blood work will be included in future studies.

SESSION 6 – TRANSMISSION/ CASE MANAGEMENT

Chair: Dr. Pierre Formenty, WHO

Person-to-person transmission of Nipah virus

Dr. Emily Gurley, Johns Hopkins Bloomberg School of Public Health

The first studies of human-to-human transmission of NiV were published in 2001 and 2002, with inconclusive results (data from healthcare workers in Malaysia and Singapore). In 2004, the first study to conclude with human-to-human NiV transmission, with data from two outbreaks in Bangladesh, was published. The conclusion was further corroborated by studies in India and Bangladesh (published in 2006 and 2007, respectively). In the latter studies, so-called superspreaders were identified, which served as the source of infection for a large number of individuals. Importantly, the 2007 publication observed that contacts who washed their hands were at reduced risk of infection. In contrast, a publication from 2010 suggested that transmission of NiV could also happen in the absence of close contact. Moreover, a case-report from 2012 of a woman who had presented with late-onset Nipah encephalitis 11 years after the initial outbreak in Malaysia, questioned the original conclusion that the Malaysia outbreak did not involve human-to-human transmission. A 2013 report also demonstrated transmission from a dead person. With the publication in 2015 of the first henipavirus outbreak in the Philippines, one began to realise that the "emergence" of Nipah and related

diseases was linked to human-to-human transmission, which causes outbreaks big enough to be detected even in the absence of active surveillance. The outbreak in Kerala in 2018 was a surprise, with Nipah emerging far from previous outbreaks, yet again the outbreak was caused by a hospitalised superspreader.

Key questions concerning human-to-human transmission are: 1) When and where does transmission occur?; 2) Which patients are more likely to transmit to someone else?; 3) Which contacts are most likely to be infected?; 4) What mechanisms are the most important drivers of transmission? Models trying to describe human-to-human transmission consider 1) the availability of infected (and infectious) individuals; 2) the availability of susceptible individuals; and 3) the ways these individuals interact. Whether a patient is infectious depends on the level of viral shedding, clinical symptoms that may help spread the virus (such as coughing and sneezing), and the infectiousness of the virus, which can vary from strain to strain. When it comes to NiV susceptibility, all humans are susceptible to infection. The nature of human interactions, which are often affected by the disease itself in terms of seeking healthcare and

receiving visitors, is an important driver for transmission. Population density and duration of illness are other factors influencing the risk of transmission through contact.

Importantly, the route of transmission affects the progression of Nipah disease. Patients infected through date palm sap have more rapid onset of disease and a higher mortality (~90 % vs. ~50 %) than patients infected through human-to-human contact. The latter are also more likely to be diagnosed before death. This has broad-reaching implications for identification, containment, and resolution of outbreaks.

The basic reproduction number (R_0) of Nipah infection, defined as the average number of people one infected person will infect in a susceptible population over the course of infection, has been as low as about 0.2 during the last decade. For a larger outbreak to occur, an R_0 close to or higher than 1 is required. There is substantial variation in the viral strains spilling over from bats to humans every year, and a low R_0 indicates that the spillover viruses have been less suitable for human-to-human transmission. A case-contact study in Bangladesh (published 2019) identified male adults with breathing difficulties

to be the primary source of human-to-human transmission of NiV. This is an important clue for safe case management. In total, 9 % of cases (22 out of 248) were shown to transmit NiV to contacts. Highest at risk for human-to-human transmission were family caregivers, individuals with prolonged exposure (> 12 hours) to a case, and individuals who came into contact with a patient's body fluids. All of the transmitters died from the infection, and the number of contacts (physical or fluid) went up in the later stages of the disease, increasing the risk of transmission. Notably, only 4 of the 22 transmitters were admitted to a surveillance hospital, representing a serious limitation for surveillance.

A systematic review of 133 henipavirus transmission studies (52 human and 81 animal studies) revealed that the proportions of transmitters were comparable in Bangladesh, India, and the Philippines (9 %, 10 %, and 12 %, respectively). The patterns of human-to-human transmission were consistent across countries; adult males with respiratory symptoms being the primary source of infection, and family members being primarily affected. While representing only a tenth of the individuals infected, transmitters were shown to be an important determinant for the size of an outbreak. Published data on viral shedding was only available for 37 patients, in which shedding was documented in oral/

nasal swabs as well as in urine, but only during acute infection. Viral shedding in semen was documented in one Indian case, for at least 25 days after the onset of disease. Not a single human study has so far documented circulating viral loads.

Patient management & infection control practices

Dr. Chandni Sajeevan – Government Medical College Kerala

The Kerala outbreak in 2018 seemed to start on May 17 with a 32-year old female presenting to a private hospital with ARDS and myocarditis. The day after, lab results showed the presence of a potentially dangerous virus, and on May 20 the Indian National Institute of Virology (NIV) officially declared the NiV outbreak. Later on, it became clear that there had been another case in Kerala of viral encephalitis with ARDS on May 5, a 26-year old male. He died the same day as he arrived at the hospital. The clinical profile of NiV in the Kerala outbreak often included myocarditis in addition to encephalitis and ARDS, an interesting new observation.

The response to the outbreak was immediate, both on the hospital and public health levels. Outbreaks are not unusual in Kerala, and plans for action in the face of an outbreak already existed. Seven

steps were deemed critical for controlling the NiV outbreak: 1) Maintain strict infection control practices (ICPs); 2) Ensure proper use of PPE; 3) Follow appropriate house-keeping practices; 4) Monitor staff health (no sick people at work); 5) Implement strict visitor policy; 6) Ensure safe collection and handling of samples; 7) Strict adherence to protocol for handling deceased individuals. A successful response requires preparations based on continuous learning and committing to the necessary practice changes during an outbreak.

During an outbreak, individuals presenting to the hospital with fever are triaged and, if matching the Nipah case definition, are put in isolation. Seriously ill patients are managed by the intensive care unit (ICU). Suspected NiV cases are individuals from an area known to be affected by an outbreak, who has

encephalitis, ARDS, or myocarditis. A probable case is a suspected, now deceased (before diagnosis could be made) individual who had been in contact with what is assumed (or confirmed) to be an infected individual. A confirmed case is a suspected case with laboratory confirmation of NiV infection, by way of real-time RT-PCR.

In the 2018 Kerala outbreak, there was one index case and 18 confirmed cases (12 male, 7 female), as well as 4 probable cases (3 male, 1 female). Of the former, two patients survived, one who had typical symptoms and one with only mild disease (flu-like symptoms). All cases had fever, and in two instances the decision to isolate the patient was based solely on the fever and contact history. Altered sensorium was observed in 15 of the 19 cases at presentation (see Figure 9 for more details).

Symptom/sign/Lab parameter	No	Present	%
Fever	19	19	100.0
Altered sensorium	19	15	78.9
Headache	19	12	63.1
Vomiting	19	11	57.8
Convulsions	19	7	36.8
Breathlessness	19	6	31.5
Cough	19	4	21
Crepitations	19	16	84.2
Tachypnoea	19	12	63.1
Developed hypotension	19	12	63.1
Developed bradycardia	19	8	42.1
Infiltrates in chest X-ray	17	14	82.3
Hypoxia in ABG analysis	19	15	78.9
Platelet count < 140X10³/µl	18	9	50
Platelet count < 100x10³/µl	18	4	22.2
LV Hypokinesia in Echo	13	6	46.1

Figure 9: Symptoms of Nipah cases at time of presentation (Kerala, 2018; Dr. Sajeevan)

After the 2018 outbreak, a number of challenges were identified and addressed, including the need for 1) early diagnosis of suspected cases; 2) contact management; 3) uniform standard of care; 4) safe and dignified burials; 5) resolving psychosocial problems; 6) long-term follow-up of survivors. When the second spillover in Kerala happened in May 2019, response plans were implemented within 24-48 hours, perhaps as a result of the experiences from 2018. Implementation included training, setting up an isolation facility, contact tracing, and establishing a point of care laboratory.

A case-control study of henipavirus outbreak, Philippines, 2014

Dr. Paola Katrina Ching, Dr. Jose N. Rodriguez Memorial Hospital, Philippines

The 2014 henipavirus outbreak in the Philippines was the first in this country and affected two villages in the southern province of Sultan Kudarat. It was first reported as a possible case of food-borne illness from the consumption of horse meat, on April 1. Among 128 residents who had eaten horse meat, initially four individuals were reported to have gotten sick with CNS symptoms and died. Later in April it became clear that two potentially related syndromes had been identified: 1) Food poisoning; 2) Febrile illness with CNS symptoms. These were both linked to the consumption of horse meat from sick, injured, or deceased animals. The Philippines Field Epidemiology Training Program (FETP) conducted an investigation, and identified an outbreak of a human, fatal encephalitis syndrome that had resulted in 79 cases, causing 9 deaths. The causative agent was not identified, but JE, Chikungunya, and dengue were suspected. As a precautionary measure, consumption of meat from sick or dead horses was banned, and animal handlers and health care workers (HCW) were advised to follow protocols on infection control. On May 12, the Philippine Government requested WHO's assistance for further outbreak investigation.

In a joint mission starting May 22, Philippine health officials and WHO conducted a detailed outbreak investigation, with the objectives of confirming the aetiology of the outbreak and ensuring containment. Cases were now serologically confirmed, and defined as any person from Sultan Kudarat positive for henipavirus antibodies. Hence, there were 17 confirmed henipavirus cases in the outbreak (16 males, 1 female), of which 11 had acute encephalitis syndrome (AES). The rest had milder disease characterised by meningitis (n = 1) or influenza-like illness (ILI; n = 5). All of the cases had headache and fever, plus a range of other classical symptoms including difficulty of breathing, coughing, vomiting, and altered sensorium. Case onset lasted from March 10 to May 23, 2014. The incubation period ranged from 3 to 27 days (median for AES: 15 days; median for ILI/meningitis: 10 days). CFR was 83 % among cases with AES. For ten of the cases, transmission was linked to consumption of horse meat, whereas five cases had been in contact with sick humans. For two of the cases, transmission was likely to have been caused by contact with a sick horse and/or a sick human. The two AES survivors had long-term sequelae, whereas the other survivors did not experience sequelae.

In the two villages, 11 horses had died, and four cats had died after eating horse meat. It was found that horses were often tethered day and night, in forests where bats roosted. Potential food sources for flying foxes were available. Eighty percent of the horses had neurological symptoms, and four died of AES within 12 hours after initial onset of symptoms. In a case-control study, infected individuals were 20 times more likely than controls to have taken care of a sick horse, and 64 times more likely to have taken care of a sick person. NiV RNA found in the Philippine outbreak had 99 % sequence homology to NiV isolates from Malaysia. However, it was not possible to isolate the replicating virus. While flying foxes were not formally confirmed to serve as reservoir in this outbreak, it remains likely that flying foxes infected the horses, which in turn infected humans that later resulted in human-to-human transmission. Since the outbreak, measures have been taken to improve nationwide readiness and surveillance of AES, yet there have been no new cases.

Lessons learned from pathology and disease course: Malaysia

Professor Kum Thong Wong, University of Malaya

Acute henipavirus infection is a multi-organ infection caused by dual pathogenetic mechanisms; 1) vasculopathy (endothelial infection, vasculitis, thrombosis, microinfarction); and 2) parenchymal cell infection (infection of organ cells). Viremia is thought to result in vascular infection, which causes vasculitis leading to thrombosis. Thrombosis, in turn, leads to vascular occlusion and microinfarction, damaging the endothelium and allowing the virus to escape and infect the parenchymal cells of the CNS, lungs, kidneys, and other organs.

Relapsing and late-onset henipavirus infections seem to be confined to the CNS, and are not accompanied by the vasculopathy of acute-phase infection. Both NiV and HeV have been demonstrated to cause relapsing or late-onset infection, resulting in inflammation and confluent (geographical) lesions of the brain – as opposed to the necrotic plaques seen in acute infection. In late-onset henipavirus infection (seen to affect 3.4 % of cases), the patient does not experience encephalitis in the acute phase – instead, encephalitis occurs weeks, months, or years later. In relapsing henipavirus infection (observed in 7.5 % of cases), the encephalitis experienced during the acute phase recurs at a later time.

Viremia has never been documented for henipavirus infection, though it is believed that viremia is a prerequisite to account for the ability of the virus to affect multiple organs. One hypothesis explaining the differences seen between acute and relapsing/ late-onset encephalitis, is that the viral foci observed in acute encephalitis are disseminated by viremia, whereas relapsing/ late-onset encephalitis involves the recurrence of infection from residual foci. In some cases, the confluent lesions typical for relapsing/late-onset infection is seen very early, even in the acute phase. More clinical and animal model data (ideally in an animal model for relapsing/late-onset infection, which has not yet been developed) is necessary to address the mechanisms behind the observed differences in pathogenesis between cases. Possible factors influencing the development of relapsing/late-onset encephalitis include 1) viral dose; 2) pathogen virulence and mutations during the infection (as seen with measles, which along with NiV and HeV is a paramyxovirus); 3) host immunosuppression (also seen with measles).

Session 6 Q&A

Q: Could the reduced mortality of NiV upon human-to-human transmission indicate that the virus is attenuated upon replication in humans?

A: There is no data to support this. Another relevant factor is the dose of virus given through the different routes of transmission.

Q: Is there any evidence of "barking coughing" in superspreaders of NiV, as there has been for superspreaders of measles virus?

A: Not too much is known about the superspreaders of NiV, who often do not present themselves at hospitals, though all of the transmitters have respiratory symptoms. The superspreader from the Kerala outbreak did have a prolonged cough.

Q: What protective measures were available for HCW during the first Kerala outbreak?

A: The HCW had access to gloves and masks, but it is not clear to what extent the measures were adhered to. Two HCW got infected, of which one died (the nurse who cared for the index case, who vomited and was very ill).

Q: In the Philippine henipavirus outbreak, there were five dogs identified with antibodies against NiV. Were you able to eliminate the possibility of anyone being infected by these dogs, who had been infected?

A: All the identified cases had been in contact either with a sick horse or a sick person, as revealed through interviews. Contact with dogs did not come up as a likely source of transmission.

Q: What have been the measures implemented for bat surveillance on the Philippines, and what has been found, so far?

A: In 2014, 144 bats were sampled, of which six were positive for NiV antibodies. Whether subsequent surveys in bats have been conducted is not clear.

C: Prof. Wong mentioned a lack of data in animal models concerning variations in CNS tropism among the different NiV strains. In ferrets, there are no apparent differences between NiV-B and NiV-M. However, some differences have been observed between wild-type and W- or CW knockout animals.

A: Thanks. To get the full picture, it is recommended to do pathology on complete sections of the brain, not just fragments.

C: Autopsies have so far not been available in Bangladesh, but could be instructive for improving our understanding of CNS-involvement in Nipah disease, as well as for the development of therapeutics.

A: Yes.

Q: Are there any pathological similarities between the rare subacute sclerosing panencephalitis (SSPE) following measles infection and relapsing/late-onset encephalitis in henipavirus infection?

A: SSPE typically occurs 6-7 years after measles infection, in a slow process where the brain shrinks and the patient eventually dies. In contrast, cases with relapsing/late-onset encephalitis typically survive. Neurons are the target for both viruses, but the progress is slower in SSPE.

SESSION 7 – EPIDEMIOLOGICAL NEEDS FOR CLINICAL TRIALS

Chair: Dr. Jakob Cramer, CEPI

Approaches to demonstrate the effectiveness of Nipah vaccines

Dr. Marie-Pierre Preziosi, WHO R&D Blueprint (on behalf of Ana María Restrepo, WHO R&D Blueprint)

There are several pathways for bringing a new vaccine to licensure: 1) Traditional approval (the gold standard), based on a randomised controlled trial (RCT) with clinical endpoint or accepted correlate of protection; 2) Accelerated approval for therapeutics with considerable benefit over existing therapies (breakthrough status, based on preliminary clinical data) that are also considered PRIority MEdicines (PRIME); 3) Animal rule (only U.S. Food and Drug Administration [FDA]), or the somewhat corresponding Exceptional circumstances (EMA), where approval is based on animal studies due to inability to conduct clinical trials for serious or life-threatening conditions; 4) WHO emergency use listing (EUL), which is a time-limited approval for use during public health emergencies of international concern (PHEIC), based on basic clinical safety data and/or immunogenicity data. In addition, there is a separate procedure for immune markers, provided they are reasonably likely to predict protection against disease. As indicated, the amount of evidence required for each pathway varies, and data missing at the time of licensure must be provided in a timely fashion post licensure.

WHO R&D Blueprint works to ensure a systematic approach to accelerated development of vaccines (Vx), therapeutics (Tx), and diagnostics (Dx), including the development of a baseline situation analysis (BSA), an R&D roadmap, TPPs, as well as trial designs for therapeutics and vaccines. For Nipah, this work is well underway, with trial designs already established. The main challenge for a Nipah vaccine trial is sample size, since outbreaks occur irregularly – as is true for all EIDs. The advantage of Nipah is that outbreaks do occur relatively frequently, with some regularity for example in Bangladesh. The TPP for a NiV vaccine includes two distinct use cases: 1) Preventing disease in a non-emergency setting through vaccination of populations living in endemic areas, as well as HCW; 2) Protection of individuals at risk in the area of an ongoing outbreak. In a population, everyone should be considered for vaccination, including pregnant and lactating women. End points to be considered for a vaccine trial include laboratory-confirmed Nipah clinical illness as the primary end point, as well as death and infection as secondary end points. Individual randomisation should be done within each study

site. All study participants should be screened for seropositivity before vaccination, to allow for exclusion or stratification of seropositive individuals. If included and stratified, differences between groups of individuals with or without a history of NiV infection could serve to inform the design of an immune correlate of risk and/or surrogate of protection. The development of a correlate of protection, as noted in the TPP, requires availability of validated and standardised immunogenicity assays. Both neutralising antibodies and cell-mediated immunity appear to have a role in preventing NiV infection. Correlates of protection may vary across vaccine platforms.

In the absence of RCTs, observational studies such as case-control studies can provide valuable evidence. Suitable controls could be test negative (without circulating evidence of Nipah infection) or standard, matched controls from the same communities. This requires a population with high but partial vaccine coverage (< 90 %). An alternative approach would be to design a one arm study with a prospective cohort of unvaccinated individuals. Such a design involves challenges in terms of selecting comparator, choosing statistical methods, as well as adjusting for key confounders, but could provide additional evidence. Obviously, a highly effective vaccine will yield more robust results, regardless of the design. Furthermore, one powerful approach could be to conduct studies (observational studies or RCTs) across several outbreaks with a standardised protocol and blinding until the data monitoring committee deems the results of the trial to be conclusive and motions to end the trial for safety, efficacy, or futility reasons. In such a setting, care must be taken with α−spending (the overall probability of error), blinding for analysis, and comparability of risk in clusters as more are added to the trial.

Resolving epidemiological uncertainties to inform development of Nipah countermeasures

Professor Steve Luby, Stanford University

Some epidemiologic uncertainties remain for Nipah, which must be addressed before we can progress to licensure of therapeutics and vaccines. There are about a dozen promising therapeutics in the pipeline for treating NiV infection, but to test just one of them will require a clinical RCT with 132 Nipah patients (based on the assumptions of improving the survival seen in India and Bangladesh from 25 % to 50 %, as well as having 80 % power and 95 % two-sided confidence). Over the last 18 years, India and Bangladesh have seen 377 Nipah cases. In order to reach 128 study participants, all of the cases in the past eight years would need to be included, with no dropouts. Thus, it will only be feasible to test one therapeutic or one package of therapeutics for Nipah, and the question is how to select the most promising candidate(s) for testing.

Or, perhaps, are there undetected Nipah cases that could improve the situation? In Bangladesh, most of the cases from 2007 to 2014 were detected near the surveillance hospitals, which are typically situated in urban areas where people do not consume date palm sap. Correcting for health care utilisation among patients with symptoms of meningo-encephalitis, it was estimated that 48 % of the outbreaks were left undetected. Resolving this epidemiological uncertainty, through improved surveillance and access to rapid diagnostics, could help improve the power of Nipah clinical trials. Another prerequisite for clinical testing is clinical trial capacity in endemic, under-resourced settings.

How much can we shorten the time between outbreak detection and enrolment? This will be a critical parameter in terms of identifying cases. One challenge (in Bangladesh, which probably has the best NiV surveillance system in the world) is that epidemiologists are usually called in only at the end of an outbreak, when most of the cases have already died or recovered. This precludes enrolment in a clinical trial. Multiple strategies for early detection and enrolment could be tested.

Is it possible to test Nipah vaccine efficacy in humans? Applying the ring vaccination trial design that was successfully used for testing a vaccine against Ebola, with immediate and delayed vaccination of contacts of cases, would require 516 years with the NiV incidence observed over the past 15 years. Thus, in the absence of a large NiV outbreak, this strategy will not work for Nipah. Using a cluster randomised RCT design measuring prevention of spillover infections and onwards transmission will reduce time and allow for vaccination beforehand, yet a study including the entire population of the Faridpur and Rajbari districts of Bangladesh (who see the most Nipah cases; 3 million people) would still take 81 years to accumulate a sufficient number of cases and reach conclusive results. In contrast, a case-control study with vaccination of the same population and recruitment of 10 matched controls whenever a case is identified would require only 7 years to be completed (provided 70 % vaccine coverage). Such a study is within the realm of the possible, and would yield data on the effectiveness of a vaccine (case-control efficacy estimates of other vaccines have generally aligned with RCTs). Notably, a study like this is easier to implement with a single dose vaccine, and increasing case identification would improve power.

Some years have shown higher spillover rates than others.

Understanding the reasons for this could improve our ability to model future outbreaks and design successful clinical trials for therapeutics and vaccines. Variations in viral shedding from bats, temperature, and ecological stress have all been suggested to impact spillover rates. Another epidemiological uncertainty that is worth resolving, is the apparent decrease in spillovers in Bangladesh during the past few years, correlating with fewer people in Bangladesh consuming date palm sap (in accordance with guidelines from the public health authorities). Is the number of cases actually declining, and how will this affect future clinical trials? The conclusion is that it should be possible to clinically test one therapeutic regimen and one vaccine against Nipah, though the international community will need to come together to decide on which candidates to advance towards clinical evaluation.

Practical issues in advancing human testing of Nipah vaccines in Bangladesh

Professor Steve Luby, Stanford University

With its five study sites across Bangladesh, icddr,b has been involved in about 85 clinical trials, of which many have been vaccine trials (phases I-IV). The first trial involving a Nipah vaccine could conceivably be designed as a randomised phase I/II placebo-controlled trial, assessing the safety and immunogenicity of the vaccine in healthy adults and children at different doses/dosing schedules, and potentially providing some preliminary information on vaccine efficacy. Such a trial could involve about 100 participants and last for about 36 months (provided enough cases).

The Directorate General of Drug Administration (DGDA) is the national regulatory body responsible for approving new therapies for use in Bangladesh. Their objective is to ensure easy access to safe, effective, and good-quality therapeutics/vaccines at affordable prices, as well as rational and safe use of these. Moreover, they have an interest in facilitating domestic production and export, and of implementing effective surveillance systems for post-licensure follow-up. A Nipah vaccine trial at icddr,b will require approval from the icddr,b institutional review board (IRB), consisting of the Research Review Committee (RRC) and Ethical Review Committee (ERC). The study will also have to be approved by DGDA, based on the provision of 1) project summary including a summary of formerly obtained preclinical and clinical data; 2) ERC-approved study protocol; 3) IRB approval; 4) the investigator's brochure, compiling information about the vaccine from preclinical and other clinical trials; 5) informed consent form (ICF); 6) sponsor agreement; 7) good manufacturing practice (GMP) certificate for the manufacturer. Vaccine import will require the presentation of a proforma invoice, as well as the DGDA approval letter, the investigator's brochure, and the IRB approval letter.

Issues that need to be considered in relation to the development and testing of a new vaccine include: 1) Establishing preclinical and clinical study protocols; 2) Selecting study sites; 3) Implementing a Data and Safety Monitoring Board (DSMB); 4) Selecting a clinical research organisation (CRO, of which there are about 8 in Bangladesh); 5) Obtaining a clinical trial agreement (CTA) as well as material transfer agreements (MTAs); 6) Maintaining cold chain for vaccine- and sample shipping and storage; 7) Obtaining permission from the Ministry of Commerce for vaccine import. Other challenges pertaining to the conduct of a clinical trial include 1) rapid identification and enrolment of eligible participants; 2) completion of follow-up on a highly mobile study population; 3) effective management of adverse events.

Epidemiologic preparedness for Nipah vaccine/therapeutic trials

Dr. Tarun Bhatnagar, National Institute of Epidemiology, ICMR

Improving epidemiologic preparedness for Nipah trials involves 1) defining the geographic area where transmissions may occur; 2) defining the population at risk; 3) gaining a better understanding of the risk factors involved in Nipah transmission; 4) improving surveillance for rapid detection of cases; 5) understanding the natural history of disease. Important parameters for assessing the risk of Nipah transmission and guiding surveillance include 1) the distribution of fruit bats; 2) evidence of NiV infection in bats; 3) previous outbreaks of NiV; 4) high-risk behaviour in humans. Fruit bats are found all over India, and Nipah outbreaks have been seen in the North East (West Bengal) and in the South West (Kerala). Thus, a systematic, nationwide surveillance system could be necessary to be able to rapidly detect the next outbreak. With risk modelling (using information about the habitats and movements of the known NiV reservoir bats), some areas have been defined that have a higher risk of spillover, and such models may serve to guide surveillance and ensure more efficient use of available resources. A strategy for identifying Nipah outbreaks that have previously gone undetected could be the testing of archived samples from AES patients with unknown aetiology. Identifying previously unknown outbreaks would help to raise awareness about the need for improved surveillance, and could shed more light on the natural history of Nipah.

Consumption of contaminated date palm sap is a known risk factor for NiV infection, and a proposed survey plans to find the frequency of date palm sap consumption among rural residents of the six West Bengal districts bordering Bangladesh. In addition, the number of *Pteropus medius* bat roosts near rural residents will be determined. Understanding the different behaviours that may influence the risk of Nipah transmission in different geographic regions is of importance. Outbreaks may occur in clinical settings (like in the 2001 Siliguri outbreak where 66 individuals were infected) or in family settings (like in Kerala, 2018, with 12 infected individuals). The clinical features of infection may vary from outbreak to outbreak. By the time an outbreak is detected, human-to-human transmission to secondary cases have often occurred, and it may be too late for clinical intervention with therapeutics or vaccines.

Surveillance could be facility-based or community-based, targeting different populations. Event-based surveillance for identifying Nipah cases often targets events of AES (primarily due to JE), but (in Bangladesh and India, at least) overlooks ARDS. In addition, at least two suspected cases are required to warrant further investigation. This means that many cases are likely to be left undetected. One future approach for India could be to establish sentinel sites for NiV detection in high-risk areas, and detect outbreaks through clustering of cases of AES or ARDS that occur in close geographical proximity and within a timespan of 21 days, where an algorithm serves to select samples from possible NiV cases for laboratory testing. The testing can be conducted by the > 75 laboratories in the Department of Health Research (DHR) / Indian Council of Medical Research (ICMR) Virus Research and Diagnostic Laboratory (VRDL) network.

Respiratory illness surveillance was set up in India in 2016-2018 as part of the Global Health Security Agenda (GHSA), with nine participating laboratories across the country. This system could potentially be used for identification of Nipah cases among the many patients with respiratory symptoms who are never properly diagnosed (50-80 %, depending on age group). In addition to the public healthcare system, private institutions and hospitals should be involved in the work of identifying cases and clusters, sharing the same protocols for surveillance and sample referral. Based on learnings from Bangladesh, contacts are now classified into two categories: 1) Has had contact with body fluid (blood, urine, saliva) from confirmed/probable case; 2) Has spent time in close proximity of a confirmed/probable case for ≥ 12 hrs. This allows for active surveillance and management (including isolation) of high-risk contacts, and passive surveillance of low-risk contacts. Screening contacts for NiV may identify individuals with subclinical infections: 3 of 279 close contacts from the 2018 Kerala outbreak had subclinical infection, though this has not been seen in Bangladesh.

# Current epidemiological status of Nipah virus encephalitis in Malaysia in the context of vaccine/therapeutic trials

Professor Tan Chong Tin, University of Malaya

The 1998-1999 Nipah outbreak in Malaysia lasted for 8 months, spreading from the Perak district to Negeri Sembilan and Selangor districts by way of transportation of sick pigs between pig farms. The pigs were the primary source of transmission to humans, but at least one patient had been infected by his dog. Cats, horses, and goats were also infected, and could potentially have served as a source of infection to humans (it has later been shown that horses were the source of infection in the 2014 Nipah outbreak in the Philippines). Since 1999, there has not been another outbreak involving pig farms in Malaysia, partly due to increased awareness by pig farmers not to have fruit trees in close proximity to the pig sties. However, the swine industry in Asia is huge, and another outbreak may just be waiting to happen. Other livestock, such as goat, may also pose a risk. Goats represent a major Asian industry, are susceptible to NiV infection, and are herbivorous scavengers. Whether the infection of goats involves the lungs remains unknown – this could aid transmission to humans. Consumption of date palm sap is known to be a risk factor of NiV infection, and this cultural habit is found in and around Bangladesh.

The risk of direct NiV infection from bats to human is low. However, in a seroprevalence study in Malaysia, more than 10 % of the participants tested positive for antibodies against NiV. Among the 110 members of the Malaysian households affected by the 1998-1999 outbreak, 27 % had symptomatic infection and 8 % had subclinical infection with positive serology. Of the asymptomatic subjects, 16 % were found to have brain abnormalities by MRI. Relapsing encephalitis was seen in 9 % of the cases. In total, there are more than 100 Nipah survivors in Bangladesh, and the survivors experiencing relapsing disease could be suitable for participation in clinical trials for Nipah therapeutics.

Despite the absence of reported Nipah outbreaks in Malaysia for the past 20 years, transmissions may have occurred that were not detected. Cases of NiV encephalitis may have gone undetected, in Malaysia as well as in other countries of Southeast Asia. Populations at risk are those exposed to bats and other susceptible animals, as well as those consuming date palm sap. Ribavirin and other therapeutics may have a role in the management of Nipah disease. In a clinical trial with 190 patients, the 140 who received ribavirin had a 36 % reduced mortality compared to controls.
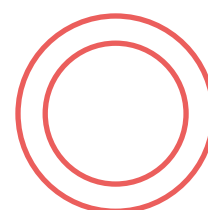
Session 7 Q&A

Q: Has the WHO R&D Blueprint considered recommending certain data formats in accumulated clinical trials to allow for aggregation of data across studies?

A: Yes, this is certainly part of the master protocol. There should be standard operating procedures (SOPs) in place to allow for similar collection and processing of data across sites.

Q: A master protocol will be extremely challenging to implement, as it involves many sites. What would you say are the ethical aspects of conducting long-term RCTs for Nipah?

A: This applies to all of the EIDs, and the best guarantee of ethical conduct is a well-designed clinical trial. A solid data monitoring committee and governance structure, with well-defined plans for study reviews (including triggers), is a prerequisite – as is the involvement of national authorities and close collaboration across sites. One good example was the testing of therapeutics against Ebola.

Q: Does WHO support each of the pathways outlined, in terms of prequalification?

A: Discussions with regulators in WHO R&D Blueprint indicate that the traditional pathway to licensure, which includes an individual RCT, is the preferred pathway. WHO believes that this is possible, also for therapies targeting EIDs.

Q: Bangladesh has a very high vaccine coverage in general. If the vaccine coverage of a NiV vaccine for clinical testing exceeds 90 %, will the clinical trial still succeed?

A: No, for a case-control study of a NiV vaccine to succeed, vaccine coverage needs to be below 90 %. However, mass vaccinating people of all ages in a clinical trial is expected to yield less coverage than the ordinary child vaccination programmes.

Q: What would happen if the NiV vaccine had 70 % vaccine efficacy instead of 90 %?

A: A poorer vaccine efficacy would require a larger trial for evaluating the vaccine, and in planning the trial you could play around with a number of factors like this. However, the main point was to discuss the feasibility of the different study designs, where the case-control design is perhaps the only one that will yield sufficient data within a feasible time frame.

C: In Bangladesh, people are willing to take a vaccine once they have seen evidence of the disease and/or effects of the vaccine.

A: There is not a simple technical response to this, but we need to understand how an outbreak is experienced by the population, in terms of fear and other emotions, and consider how this affects vaccination rates. Reaching out to the community in a good way is what will enable us to reach 70 % vaccine coverage in an outbreak setting.

C: Perhaps one should not be so pessimistic in terms of sample size, consider the success we had with bringing an Ebola vaccine to licensure. Focusing on the alternative licensure pathways, with licensure based on immunogenicity data and limited clinical data, could be more constructive.

A: This was not to pre-empt the choice of licensure pathway. Large-scale vaccination trials would provide protection, insights, and safety data, even if being part of the post-marketing follow-up.

C: Comment from U.S. FDA: Seeking licensure through the animal rule (probably more straightforward than the accelerated rule), while planning for a large-scale clinical study as part of post-marketing follow-up would be recommended.

A: That would be brilliant. There could also be a conversation between innovators and authorities on a way forward.

C: New data could change the presumptions used in sample-size modelling, so far.

A: The modelling is indicative of a potential for realising larger sample sizes, but requires field trials.

C: You never know how an outbreak develops, as seen with Ebola. It would be good to plan for alternative scenarios, where a trial spans several outbreaks given that a single one of them is not large enough to reach a conclusion. Also, experience with Ebola has taught us that improving the overall standard of care is important for acceptance in the communities affected by the outbreak and the trial. A Nipah trial should also include measures to improve the standard of care.

A: Yes, there is a need for trust in the communities, and people need to feel that when they seek healthcare they benefit from it.

Q: To FDA: If doing the alternative pathway and relying on safety data for licensure, what sample size would be required?

A: Safety data will depend on a variety of factors. There will need to be safety data from the population where a vaccine or therapeutic will be used, but these could be substantiated by global data. In general, a considerable amount of safety data is required, for licensure as well as for informing confirmatory studies post licensure.

C: Dr. Zaman's presentation on vaccine testing in Bangladesh did not mention the need for permission to use genetically modified organisms (GMOs). There may be different implications for using replication competent versus replication incompetent organisms.

A: Ok, more information about this is welcome.

Q: Phase II efficacy testing of a NiV vaccine will have to be conducted in Bangladesh or another Nipah endemic location. Are there any preferences for the location of a phase I safety trial, which could also be conducted in the country of manufacturing of the vaccine since it only involves healthy volunteers?

A: Either way works, no strong preferences.

Q: In terms of feasibility, a phase II trial would be the only opportunity for a randomised Nipah vaccine trial, though there may be ethical issues with giving placebo to at-risk individuals. Is it worth spending time on considering randomisation just for phase II?

A: Yes, randomisation is valuable since it is not known how effective the vaccine will be. Also, ethical considerations are less poignant while the efficacy of the vaccine is unknown.

Q: Were anthropologists involved in the investigations of the 2018 Kerala outbreak? If so, at what stage? What role may anthropologists have in Nipah surveillance?

A: The epidemiologists were called in late in the outbreak, after the last case had been identified. In a subsequent case-control study, an anthropologist was engaged to talk to family members and neighbours to gain a better understanding of the index case, who had been to the jungle, and his pet rabbit and dog were dead. He also enjoyed eating fruits, etc. These are valuable insights. In a comprehensive surveillance system including risk assessments, anthropological support may be included, but the plans for such a system in India have not yet been finalised.

Q: Prof. Tin, given your experience and the fact that we may only have one shot at a trial for a Nipah intervention, do you have a preference for a vaccine or a therapeutic?

A: There are pros and cons to both, therapeutics having the disadvantage that onset of therapy may be delayed, and too late to save the patient, while mass vaccination may be difficult to achieve in real life. Vaccination of targeted high-risk groups could be one way of mitigating the issue, but I would rather not conclude one way or another.

SESSION 8 – PROGRESS AND CHALLENGES IN VACCINE DEVELOPMENT

Chair: Melanie Saville, CEPI

Nipah vaccines: Overview

Dr. Eun-Chung Park, NIAID/NIH

Vaccines against NiV typically target glycoprotein (G) or fusion protein (F), found on the surface of the virus, which are both involved in cellular entry. Viral strains used in vaccine research include HeV, NiV-M, and NiV-B. Vaccine evaluation is conducted in a wide range of NiV-susceptible animals, including mice, hamsters, ferrets, cats, pigs, ponies, and AGM. Different vaccine platforms exist, including subunit vaccines (mostly variants of sG), nucleic acid-based vaccines, passive transfer (monoclonal or polyclonal antibodies), and numerous viral vectors. In addition to the human vaccines, there have also been made vaccines for immunisation of intermediate host animals, primarily tested in pigs.

Protection with a single dose of vaccine is very important for the ability to curb an outbreak. A number of vaccines have been tested pre-clinically with a single dose, with good results in different animal models. The degree of variability in viral sequence may be of importance to the vaccine's efficacy, since challenge with a homologous viral strain (the same NiV strain as was used in the vaccine) yields the highest level of protection.

Two important challenges in NiV vaccine development are 1) difficulty of comparing the vaccines with respect to efficacy, since results are obtained using different study protocols (dose, schedule, route of administration, route of challenge), different strains of challenge virus (NiV-B, NiV-M, HeV), and different types of animals (e.g., hamster, ferret, AGM); 2) correlates of protection remain unknown. Important gaps include 1) lack of a therapeutic vaccine; 2) little is known about the T-cell response; 3) lack of vaccine targeting T-cell immunity; 4) unknown durability of protection; 5) lack of tailored adjuvants; 6) incomplete cold chain (alternatively, stability issues in the absence of cold chain).

Ending with a question to the audience: Should a bat vaccine be considered to control NiV transmission? Bats constitute the reservoir, not only of NiV but also other viruses causing disease in humans. Preventing viral shedding from bats could prevent human disease.

# A subunit vaccine (HeV-sG-V) to protect against Nipah and Hendra disease

Dr. Rong Xu, Profectus BioSciences

The Profectus subunit vaccine (HeV-sG-V) is a HeV sG vaccine adjuvanted with alum. It has been marketed in Australia as a vaccine for horses (Zoetis Equivac® HeV) since 2012, and a human vaccine has now completed pre-clinical development. The pre-clinical studies that have been conducted include three rabbit immunogenicity studies, four proof-of-concept (POC) challenge studies in ferrets (one study) and AGM (three studies), and one good laboratory practice (GLP) rabbit toxicology study. Based on the data from these studies, CEPI awarded $25 million to Profectus BioSciences, Emergent BioSolutions, and PATH in May 2018, for the development of a NiV vaccine within 5 years. This work shall include 1) manufacture of the vaccine and evaluation in phase I and II clinical trials; 2) conduct of non-clinical correlate of protection studies to support licensure under the animal rule; 3) stockpiling of up to 100,000 vaccine doses for emergency use.

The vaccine substance is to be manufactured by Emergent BioSolutions, whereas the vaccine product will be manufactured by the Infectious Disease Research Institute (IDRI). GMP manufacture of vaccine substance and product for the clinical trials were completed in June and October 2019, respectively. Certificates of analysis (CoA) for the clinical trial material (CTM) to be used in the investigational new drug (IND) submission were obtained in December 2019.

Three clinical trials have been planned: 1) Phase I adult trial (U.S., n = 192) to assess safety and tolerability (primary endpoint), as well as immunogenicity (secondary endpoint) of the vaccine, at one and two doses of escalating dosages (10, 30, and 100 μg); 2) Phase II adult trial (endemic area, n = 600) to confirm safety and immunogenicity of the dose and dosing regimen selected after phase I, as well as assessing the value and timing of a booster dose; 3) Phase I/II paediatric trial (endemic area, n = 300) to assess safety and immunogenicity in children (2-17 years). Assays for clinical testing, including ELISA assays as well as pseudotyped vesicular stomatitis virus (VSV) neutralisation assays, for both NiV-B and NiV-M, have already been developed and verified. Interim reference standard serum from NiV-B sG + NiV-M sG immunised rhesus macaques has also been generated and characterised, as have serum pools for normal controls (NC) and low- and high-titre quality controls (QC-L and QC-H, respectively).

Profectus will now pursue the animal rule regulatory pathway, with the HeV-sG-V IND. A request for fast-track was submitted to the FDA on December 13, 2019. The phase I study is likely to commence in February 2020.
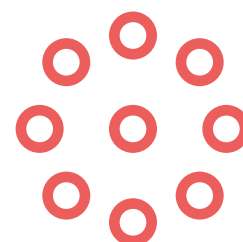
ChAdOx1 Nipah vaccine

Professor Sarah Gilbert, Jenner Institute, University of Oxford

In spite of being replication deficient, Ad used in vaccines is still able to elicit strong immune responses: Not only does the virus present the antigen, but it also elicits danger signals in the host that are powerful immune activators. This leads to a potent immune response with good immunological memory in the form of memory B- and T cells. Replication deficiency is an important safety measure to avoid dissemination in immunocompromised individuals. Using simian adenoviruses, which do not naturally infect humans, has the advantage of avoiding neutralisation of the vaccine vector upon vaccination into a patient who has already experienced human Ad infection (this has been seen for the human Ad5 vector). The antigen is expressed by double-stranded DNA fragments contained within the vector, and is not a structural part of the virion. This means that vaccines using the same strain Ad are structurally identical, streamlining the GMP production of Ad vaccines against a variety of different antigens, and eliminating the need for optimisation of each individual vaccine vector. Furthermore, the expression of antigen can be controlled by a repressor (halting expression during manufacture) and/or a mammalian promoter (inducing antigen production once injected into a human/mammalian recipient).

ChAdOx1- and ChAdOx2-vectored vaccines have been developed for a wide range of EIDs, including MERS, Lassa, Nipah, and Ebola. For each of the pathogens, a vaccine construct has already been made. The subsequent development involves 1) testing immunogenicity and capacity for inducing neutralising antibodies; 2) efficacy studies in animals; 3) obtaining funding for clinical trials; 4) phase I/II clinical testing. The Jenner Institute already has a number of vaccines in clinical development, including vaccines against pandemic flu, MERS, Zika, and Chikungunya. More than 250 volunteers have so far received a dose of ChAdOx1-vectored vaccine.

One big advantage of the ChAdOx vaccines, is that they can be thermostabilised by mixing with sugar and storing on a dry membrane. This solves the issue of maintaining a cold chain in an outbreak situation, and vaccines may be stored for months at an elevated temperature without loosing efficacy (as demonstrated for the ChAdOx1 rift valley fever [RVF] vaccine). Storing at ambient temperature will also greatly reduce the cost of stockpiling.

The ChAdOx1 NiV vaccine, expressing NiV G protein, proved to result in complete protection against NiV-B and NiV-M challenge in a study involving hamsters. A booster dose yielded higher titres of neutralising antibodies, but even one dose was protective. The vaccine was well tolerated by the hamsters, and circulating virus levels remained below the limit of detection throughout the study. The vaccine also resulted in partial protection against HeV after a single dose. Next steps for this vaccine include 1) testing in AGM 2) preparing seed stock for GMP manufacturing; 3) phase I trial in UK; 4) further studies in Bangladesh, most likely.

NiV is a pathogen for which it is really easy to demonstrate vaccine efficacy. Thus, other points are more important to consider: 1) Rapid onset of immunity; 2) One (ideally) versus two doses; 3) Duration of immunity; 4) Thermostability (long-term for stockpile, short-term for transport to clinic); 5) Safety for the whole population, including the very young, elderly, and pregnant women; 6) Plans for vaccine licensure, where immunobridging could help mitigate the issue of not being able to conduct a full-blown phase III clinical trial. In parallel to the development of vaccines, it is also important to 1) ensure the development of standardised assays (preferably cross-species); 2) make sure that clinical trial protocols are ready and available for whenever the next major Nipah outbreak occurs; 3) develop plans for vaccine licensure.

Development of measles virus-vector vaccine for Nipah virus infection

Professor Chieko Kai, University of Tokyo

Vaccines vectored by morbilliviruses such as measles virus (MV) and canine distemper virus (CDV) have been used world-wide for more than 50 years, and demonstrated excellent safety and stability, as well as good efficacy yielding life-long immunity. The MV and CDV vectors both have the capacity to express several immunogenic genes simultaneously, and are therefore attractive vector candidates for multivalent vaccines.

Using reverse genetics, the Edmonston MV strain was genetically modified to incorporate the NiV G protein and create a recombinant NiV vaccine strain (rMV-Ed-NiVG). In a golden hamster model, this vaccine (given twice, on day 0 and day 21) protected against intraperitoneal (ip) challenge with homologous rNiV (day 28), which was comparable to the results seen with rMV-HL-NiVG, a NiV G protein vector vaccine based on the wild-type MV HL strain. Other vaccine candidates using NiV G protein, but based on other MV vector strains, did not perform as well. The rMV-Ed-NiVG vaccine also protected against NiV infection in AGM, using a similar protocol.

Some concern has been raised regarding the chance of pre-existing antibodies against MV in humans affecting vaccine efficacy, but results from studies in hamster show that pre-immunisation with plain MV-Ed did not impair the ability of rMV-Ed-NiVG to elicit an antibody response. With funding from CEPI, the development of an MV-based vector vaccine against NiV will continue.

Development of a vesicular stomatitis virus-based vaccine to prevent Nipah virus disease

Dr. Gray Heppner, Public Health Vaccines LLC

The objective of Crozet BioPharma is to develop a safe, single-dose vaccine with a rapid onset of protective immunity against all Nipah strains. This work is based on experiences with the VSV Ebola vaccine, which had a rapid onset of protection of about 10 days when used in the Ebola outbreaks of West Africa and the Democratic Republic of Congo (DRC), caused by the Zaire Ebola virus (ZEBOV). The rVSV-Nipah vaccine candidate (rVSVΔG/ZEBOVgp/NiVG) is a live, attenuated recombinant VSV (rVSV) expressing NiV G protein, as well as glycoprotein (gp) from Ebola virus (EBOV), and is similar to Merck's now licensed rVSV-ZEBOV (Ervebo™) vaccine. The EBOV gp moiety is retained to maintain receptor-mediated viral entry of the vaccine, and support immunogenicity. A single dose of the rVSV-Nipah vaccine protects hamsters as well as AGM against NiV challenge. Furthermore, passive immunisation (in hamsters) with serum from rVSV-Nipah-immunised hamsters was protective, suggesting a role for neutralising antibodies in protection against Nipah disease.

The TPP of rVSV-Nipah include the following criteria for a successful vaccine: 1) Capable of controlling a NiV outbreak and prevent disease in high-risk groups; 2) Can be used on individuals of all ages; 3) Has a safety profile on par with WHO recommended vaccines; 4) Has > 90 % efficacy, with rapid onset of < 14 days; 5) One dose is sufficient for protection; 6) Protective for at least 6 months when used in an outbreak setting; 7) Intramuscular or subcutaneous administration; 8) Covers all circulating NiV strains (NiV-B, NiV-M); 9) Is stable for 5 years at 2-8 °C (for stockpiling); 10) Presented in 1-dose vials (liquid frozen or lyophilised); 11) Suitable for emergency use authorisation (EUA)/EUL; 12) Appropriate for WHO prequalification.

The VSV vaccine platform has certain advantages: 1) Naturally attenuated due to host range restriction (self-limited disease in horses, pigs, and cattle, but generally asymptomatic in humans); 2) Low prevalence of immunity to the vector; 3) VSV RNA does not integrate; 4) Allows for expression of large, foreign transgenes (multigenic potential); 5) Readily pseudotyped with heterologous viral glycoproteins; 6) Complete VSV G protein deletion results in attenuated phenotype; 7) VSV G protein does not elicit neutralising antibody responses targeting the vector; 8) Recombination has not been reported; 9) Strong documentation already exists for the potency of the vaccine platform (in a phase Ib trial, rVSV-ZEBOV induced rapid and long-lasting seroconversion

upon a single immunisation with a wide range of vaccine doses [n = 512]; in the phase III trial in Guinea, 100 % efficacy was demonstrated in humans [n = 3,537]).

With funding from CEPI, pre-clinical and clinical development of the rVSV-Nipah vaccine will continue.

Key activities include 1) production of CTM; 2) animal toxicology and immunogenicity/ efficacy testing; 3) assay development for serology testing and virus detection/ quantification; 4) defining surrogates of protection in NHPs; 5) clinical testing in phase I (U.S.) and phase II (Bangladesh) clinical trials.

F vs G Nipah vaccines: Stabilising the pre-fusion conformation of F for a fair comparison

Dr. Keith Chappell, University of Queensland

Most of the Nipah vaccines currently in development target the NiV G protein, but the F protein may also be a viable target. Before viral fusion to target cells, the F protein exists in a highly unstable pre-fusion conformation. For a fair comparison of vaccines based on NiV G versus NiV F, it is vital that the F protein is stabilised in its pre-fusion conformation. The importance of fusion proteins was first seen with respiratory syncytial virus (RSV), where antibodies targeting the pre-fusion form of the fusion protein were shown to be the ones responsible for neutralisation. These results were published in 2012.

The University of Queensland is developing a "molecular clamp" platform technology, which enables trimerisation of viral surface proteins, thereby exposing the surface protein's epitope that is primarily responsible for inducing neutralising antibodies. Trimerisation is achieved through the incorporation of a highly stable trimerisation domain into class I and III viral fusion proteins (which are trimers in their natural form). Importantly, there are a number of class I and III envelope proteins of human and veterinary importance, including MERS coronavirus (MERS-CoV), Lassa virus (LASV), and ZEBOV. The molecular clamp is a trimerisation domain based on the six-helix bundle of human immunodeficiency virus (HIV) gp41, providing very high stability and a melting temperature of about 90 °C.

The manufacture of antigen is conducted in CHO cells, which are well known to the industry, scalable, and fairly straightforward from a regulatory perspective. Protein produced in the endoplasmic reticulum naturally trimerises, are stabilised by the molecular clamp, and secreted. Monoclonal antibodies for the molecular clamp are used for purification, resulting in a high yield of soluble, pure, and stable recombinant protein. Trimerisation is verified by size exclusion chromatography and electron microscopy, and the antigens are tested against available clones of mAbs binding the neutralising epitope. With the molecular clamp platform, The University of Queensland aims to supply CEPI with highly stable reference antigens, which can be subjected to multiple freeze-thaw cycles without loosing their binding properties.

The question now is, which antigen will produce the better vaccine; sG or molecular clamp-stabilised F (mcsF)? This is addressed in the following talk.

Developing a 'One Health' Nipah virus vaccine to protect animal and public health

Dr. Simon Graham, The Pirbright Institute

Pigs played a really important role in the outbreak in Malaysia and Singapore. The damage that was done to the Malaysian pig industry in eradicating the virus is still felt today. Nipah infection in pigs results in a less severe disease, yet poses a threat to human health. In a One Health perspective, a Nipah vaccine for pigs could reduce the severe economic consequences of an outbreak, as well as the risk for human disease.

Eight vaccine candidates for NiV were selected and compared (see Figure 10), all of which had been tested for immunogenicity in pigs, and three of which had been tested for efficacy. Six of the candidates targeted the NiV G protein, whereas two targeted the F protein. The antigens used included mRNA, protein, and viral vectors.
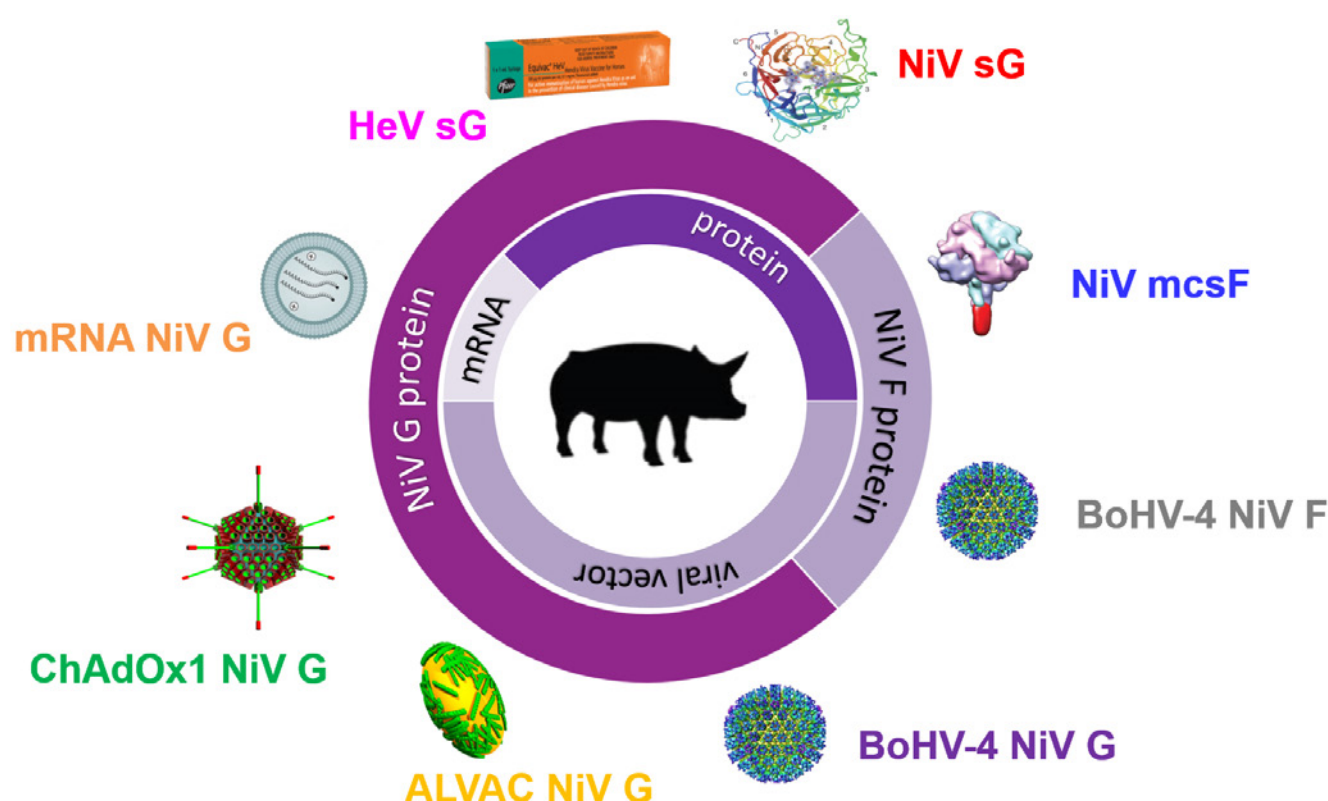

Figure 10: Nipah virus vaccine candidates (Pirbright Institute)

ALVAC-NiV G was the only one to have demonstrated protection against NiV in pigs in experimental studies. Herpes viruses (HVs) naturally stimulate very strong T-cell responses, and this property is being exploited to develop HV vector vaccines with the potential to elicit strong cell-mediated immunity. All vaccines were tested in the same regimen, with intramuscular immunisations at day 0 and day 21, and antibody titres were tested. All of the vaccines elicited NiV antigen-specific antibodies and neutralising antibodies (ALVAC-NiV G only after the second immunisation), where the NiV sG vaccine yielded the highest short-term titres. NiV sG, mcsF, and bovine HV NiV G (BoHV-NiV G) provided the highest titres of NiV cell-to-cell fusion inhibiting antibodies. The HV vector vaccines yielded the strongest T-cell responses, as measured by intracellular interferon gamma (IFN-γ) and tumour necrosis factor alpha (TNF-α) double-positive cluster of differentiation (CD) 4 and CD8 T cells on flow cytometry.

Efficacy was also tested, though only for three of the vaccine candidates (NiV sG, NiV mcsF, ChAdOx1 NiV G), with the same vaccination protocol. All three vaccines resulted in strongly reduced titres of circulating virus, as well as reduced levels of virus found in tissue biopsies, indicating a satisfactory level of protection. With immunogenicity and efficacy tests conducted, next steps include 1) testing single-shot efficacy; 2) selecting a prototype vaccine; 3) studying duration of immunogenicity and, finally, safety and immunogenicity in pigs under field conditions in Bangladesh and Malaysia.

The Pirbright Institute is also tracking NiV G-specific plasma B-cell responses, and using cell sorting to isolate NiV sG tetramer-labelled B cells. These, in turn, are cloned, and the resulting mAbs are tested for NiV G specificity. In an early attempt, one of the isolated mAbs was found to be cross-reactive to NiV sG and HeV sG, as well as neutralising in a pseudo-type assay.

Marketability of NiV vaccines for pigs may be challenging due to the sporadic nature of NiV outbreaks. An alternative strategy could be to include NiV antigen in a vaccine that is already widely used, creating a bivalent vaccine. Live attenuated porcine reproductive and respiratory syndrome virus (PRRSV) vaccines are extensively used in Southeast Asia, due to the impact of PRRSV on porcine reproduction. Reverse genetic engineering allows for expression of NiV G in PRRSV modified live virus (MLV), which could be an attractive vaccine for the veterinary market. The Pirbright Institute now hopes to test this bivalent vaccine in pigs.

Session 8 Q&A

Q: With a HeV antigen, as used in the HeV-sG-V Nipah vaccine, what are your thoughts for a HeV vaccine?

A: Regulatory work is in progress for use of the HeV-sG-V vaccine against HeV in Australia. The vaccine has been shown, pre-clinically, to be effective against both HeV and NiV.

Q: What is the intended vaccination schedule of the ChAdOx1 vaccine?

A: A single vaccination is sufficient in animals, and although both one and two doses will be tried in the phase I trial, the hope is that one dose will be sufficient to achieve protection.

Q: Can you explain the striking differences in vaccine efficacy seen with different MV strains?

A: This could potentially result from the different levels of attenuation of the different strains. For a lethal disease like Nipah, limiting side effects of vaccination through attenuation is not an objective, and one should focus on using the most efficacious strain. For less potent diseases, a broader range of the strains would perhaps provide sufficient protection, but for Nipah only a couple of the strains were potent enough.

Q: Is there a chance that VSV may persist or be shed from recipients?

A: In the clinical trials, saliva, urine, and blood will be tested for viremia. From the Ebola trials, we are aware of some shedding from adults and children. There is no precedent of VSV persisting.

Q: Can rVSV-Nipah cause neurovirulence?

A: Given the nature of NiV, neurovirulence studies will be conducted, and discussed further with the regulatory authorities.

Q: Has the molecular clamp approach been used for any other pathogen?

A: We aim to show that this approach works for a well characterised pathogen, with well-known correlates of protection, such as the flu virus.

Q: Is two-dose vaccination the standard approach when immunising pigs?

A: Maybe. We will see in a few months time if a one-dose regimen is effective.

Chair: There are a number of NiV vaccine candidates with very strong preclinical data, in a number of different animal models. This is encouraging in terms of succeeding in developing a Nipah vaccine, and it is encouraging to see that some of the vaccine candidates are about to commence clinical testing.

SESSION 9 – PROGRESS AND CHALLENGES IN THERAPEUTICS DEVELOPMENT

Chair: Professor Benhur Lee, Mount Sinai

Putative Nipah therapeutics: pathophysiologic, target profile, and trial design considerations

Dr. Libby Higgs, NIAID/NIH

NIAID's involvement in the development of therapeutics for Nipah was originally inspired by the 2018 Kerala outbreak, with WHO requesting our support for the development of a concept and protocol for the use of Nipah therapeutics in the context of the outbreak, along with the WHO R&D Blueprint Therapeutics Trial Design working group, ICMR, and Kerala Officials. The protocol was prepared quickly, leveraging previous work, but was only approved as the outbreak ended. Monoclonal antibody shipped from Australia to be tested therapeutically in the outbreak was left unused. The work continued, however, and the ambition is to establish a feasible multinational, multi outbreak Nipah treatment protocol for establishing the safety and efficacy of candidate Nipah therapeutics (and, ideally post-exposure prophylaxis [PEP], but this work has not yet started), with the support of WHO, CDC, ICMR, and NIAID. Currently, an IRB-approved treatment protocol exists that is suitable for use internationally, and linked to more than 50 SOPs developed by ICMR. India and Bangladesh have accepted the protocol and are working to implement it, and other interested countries are encouraged to join. The short-lived nature of Nipah outbreaks requires the immediate implementation of the protocol from the start

of an outbreak, and ongoing planning for such an event. Rapid implementation is facilitated by a stand-by multinational research capacity, as well as prepared sites in areas previously affected by an outbreak. In addition, mobile teams with specially adapted field equipment are preparing to respond to new outbreak areas.

Early identification of infected individuals is key to success with therapeutics. With delayed seroconversion (IgM at day 4), there is a need for rapid POC diagnostics (such as the mobile NAT developed by ICMR) and active surveillance of both pulmonary and CNS disease. We already have a fairly good understanding of the Nipah pathogenesis, supported by prospective natural history studies in NHP and humans, which will help inform the TPP for therapeutics. To facilitate access of therapeutics to future outbreaks, ICMR has taken the lead in ensuring free production and supply, as well as putting in place shipping agreements with manufacturers.

In designing a treatment protocol, a number of factors need to be considered: 1) What is the objective of the treatment (reducing morbidity/mortality, preventing infection); 2) What clinical phenotypes can be expected, based on viral tropisms;

3) How to account for NiV strain differences. There are currently a limited number of therapeutics with efficacy data in NHP and at least phase I study in humans, of which m102.4 and remdesivir are the most prominent candidates. A protocol for acute Nipah disease has now been established, but protocols for PEP in high-risk contacts and HCW are still missing. In the latter, the m102.4 is likely to be a good candidate.

Acute clinical Nipah disease phenotypes include vasculitis, encephalitis, pulmonary disease, and myocarditis, and represent a broader clinical spectrum than is accounted for in the current clinical case definitions. Thus, we may need to take on a broader set of case definitions in Nipah surveillance. The AGM model is not perfect, but it does emulate the clinical disease and is helpful for screening therapeutics. The late onset of viremia seen in animal models, due to the tropism of NiV for the smaller endothelial cells found in microvasculature, suggests that therapeutics should be able to penetrate organs and tissues. Also, it means that rapid diagnostics cannot depend on testing of peripheral blood.

The differences between NiV-B and NiV-M are challenging to account for in a multinational, multi outbreak protocol for

clinical testing of therapeutics: Which strain should the studies be powered for, given the differences in mortality rates (75-100 % for NiV-B, 47 % for NiV-M)? With a DSMB with close oversight one can do both, stratified by outbreak and with different rules for the different pathogens: The DSMB can stop the study in the event of higher mortality rates, which allows for a smaller n with a successful therapeutic. Alternatively, one can do a pre-planned sample size readjustment. Both of these approaches have been tested in clinical trials against Ebola.

Delivering therapeutics to the brain for treatment of NiV encephalitis (and in general) has proven very difficult, due to the BBB. Tailored transporters are being developed, but are not available at this time.

Ideal characteristics for a Nipah therapeutic are: 1) Quick onset of antiviral activity; 2) Effectiveness against both NiV-B and NiV-M; 3) Penetrates affected tissues and cells; 4) Long half-life for easier and safer clinical management; 5) Acceptable safety profile in humans. Monoclonal antibodies do not cross the BBB, and use in combination with a therapeutic that does, such as remdesivir, may be necessary. One could also consider using a cocktail of mAbs.

The idea is for the study protocol to run continuously, across outbreaks/strains and in different countries, to continuously optimise Nipah therapy as new treatments become available. The participants will be randomised to standard of care (SOC) plus placebo or SOC plus treatment at a 1:1 ratio. Once an endpoint has been reached, the

study will be notified by the DSMB, and the study can continue with testing the new standard of care (if changed as a result of the study, for example by including m102.4 in the standard of care) with the next therapeutics candidate. For the study to be able to compare different study sites and settings, it is important that clinicians come together to establish a common standard of care. The issue with strain diversity is resolved through stratification on outbreak. In addition, one will stratify for neurologic involvement, based on a simple yes/no assessment. Due to the possibility of relapse of NiV infection (as has been the case for Ebola), a new treatment paradigm is considered where acute treatment is followed by maintenance therapy to prevent relapse from residual virus.

Monoclonal antibody countermeasures for pathogenic henipaviruses

Professor Christopher Broder, Uniformed Services University, Bethesda

NiV tropism is conferred by the Ephrin-B2 and Ephrin-B3 receptors expressed on the surface of endothelial cells, which bind NiV G. The sG was developed to study these receptors, and also with the intent of testing its use in vaccines. As it turned out, immunisation with sG elicits strong immune responses resulting in good titres of cross-reacting, neutralising antibodies in animal models. The m102.4 monoclonal antibody was identified through recombinant phage-display human antibody isolation, using HeV sG to fish out monoclonal Fab fragments from human antibody libraries. Among the identified Fab fragments, the m102.4 Fab was selected for its ability to detect both HeV and NiV with high affinity. Subsequently, it was light-chain shuffled, affinity matured, put into an IgG1 format, and m102.4 IgG1 was produced in CHO cells for use in passive

immunisation experiments.

Passive immunisation with NiV antibodies was first piloted in hamsters, using hamster serum and mouse mAbs. Prof. Broder and his team started testing with human antibodies in parallel, in the newly developed ferret model (the first study was published in 2009). They showed that intravenous transfer of m102.4, 10 hours after infection, provided complete protection against NiV in ferrets. Later, it was shown that m102.4 blocked the binding of HeV and NiV to the Ephrin B2/B3 receptors (and, thus, viral cell entry) through binding to the Ephrin B2/B3 binding sites of HeV and NiV G. Passive transfer of m102.4 also protected against NiV-M challenge in AGM, even when administered after the onset of disease symptoms (the animals survived). Thus, it is believed

that the administration of a NiV neutralising antibody provides a critical window of relief in the about 10 days it takes the host's own adaptive immune system to mount an effective response against the virus. The recovered animals, even when receiving the antibody after the onset of disease symptoms, showed no signs of viral antigen in autopsied tissues. In contrast, when challenged with NiV-B, protection was only afforded when the antibody was administered prior to the onset of disease symptoms.

The demonstrated efficacy of m102.4 in animals has spurred compassionate use on a few occasions where people have been known to be exposed to HeV or NiV, with the first case being a mother and daughter having had close contact with a horse dying from HeV infection. None of the

recipients got infected, and none of them had any adverse effects. The m102.4 CHO-K1 cell line has since been donated to Queensland Health for GMP manufacture of m102.4 and controlled clinical testing. Results from a dose escalation study in humans is now accepted for publication, and the antibody seems to have been well tolerated.

A cross-reactive neutralising mAb for NiV F has also been identified, the humanised version being known as h5B3.1. This, too, neutralises NiV-B and NiV-M, as well as HeV. Human 5B3.1 has been found to bind the pre-fusion conformation of F, blocking transition to the extended intermediate conformation, and thereby preventing viral entry. The antibody has been found to bind a highly conserved epitope of F, which explains its cross-reactivity. In the extended form of F, the epitope is broken.

Animal data and results from clinical testing suggest a role for mAbs as therapeutics agents against henipavirus infection.

The ability to protect from disease even when administered post infection is key to the use of these antibodies as therapeutics or PEP. It is conceivable that new, optimised antibodies and the use of mAb cocktails can further enhance the therapeutic efficacy of such treatment.

Therapeutic remdesivir treatment protects African green monkeys from lethal Nipah virus, Bangladesh challenge

Dr. Emmie de Wit, Laboratory of Virology, NIAID, NIH

Notes from this presentation are not available.

Inhibition of Nipah virus infection by targeting viral cell attachment and entry

Dr. Branka Horvat — INSERM

The Ephrin B2/B3 receptors are highly conserved among different mammalian species, which is why the henipaviruses have such broad species tropisms. Using recombinant NiV expressing enhanced green fluorescent protein (EGFP), it was shown that among the leukocytes, NiV only infects dendritic cells (DCs), at low levels. It remains possible, however, that leukocytes could capture NiV particles on their surface and trans-infect other susceptible cells. The question is if this could be used to limit NiV infection therapeutically.

With an in vitro transinfection assay – which involved 1) co-culture of NiV and lymphocytes; 2) washing and treating the lymphocytes with different enzymes; and 3) co-culture of the lymphocytes with Vero cells, which are susceptible to NiV – it was determined that the NiV attachment receptor (assuming a receptor was involved) was susceptible to pronase and heparinase treatment. Proteoglycans (also known as glycosaminoglycans [GAG]), of which heparan sulphate (HS; similar to heparin, except membrane-bound instead of secreted) is one example, were deemed the most likely receptor candidate. It was found that CHO cells, which do not express the Ephrin B2/B3 receptors, were fully capable of transinfection, though that this ability was cancelled with treatment of heparinase. Furthermore, cells deficient of HS had a much smaller ability to trans-infect with NiV. Heparin was also demonstrated to bind NiV, and treatment of cells with heparin pre- or post infection resulted reduced cellular transinfection. Thus, transinfection was dependent on HS and could be inhibited by heparin in a competitive manner. In vivo, treatment with heparin devoid of anticoagulant activity (achieved by periodate oxidation treatment of the heparin) improved survival of hamsters infected with NiV. Thus, heparin is suggested as a potential low-cost co-treatment against henipavirus infection.

The function of the henipavirus F (fusion) protein involves a conformational change resulting in the insertion of a so-called "fusion peptide" domain of the protein into the cell membrane of the target cell. The domain next to the fusion peptide, the N-terminal Heptad repeat (HRN) domain, then interacts with the C-terminal Heptad repeat (HRC) domain to initiate the fusing of viral and

target cell membranes. However, lipid-anchored peptides have been designed that prevent the viral fusion, consisting of a 35 amino-acid peptide, a polyethyleneglycol (PEG) sequence, and a lipid moiety (cholesterol or tocopherol). A β-galactosidase complementation reporter assay was conducted to measure fusion of viral particles to HEK-293 cells, and the best peptide candidates were tested in animal studies. Here, it was shown that intranasally administered lipid-tagged peptides resulted in 50 % survival of NiV-infected hamsters (compared to 0 % in controls). Similarly, in AGM, the lipid-tagged peptides resulted in 33 % survival of monkeys subjected to an excessively lethal NiV inoculum. Administration of the peptide by aerosolisation instead of parenteral injection reduced the observed development of neutralising Abs against the peptide. Now, the ambition is to develop a fusion-blocking peptide in the form of a nasal spray that can be used prophylactically against henipavirus infection.

Characterization of novel inhibitors of Nipah virus

Dr. Michael Lo, Viral Special Pathogens Branch, US CDC

Notes from this presentation are not available.

Session 9 Q&A

Q: Developing standard clinical treatment guidelines is important. Will there be any stage in the multinational, multi-outbreak study where treatment is only given to some patients, based on randomisation?

A: Any patient would be given treatment. It is likely that late-stage patients do not respond to treatment, but they will still be included, so that clinicians will not question whether or not to include them.

C: With the experiences made in other viruses, such as HIV, it seems prudent to also use henipavirus mAbs in combination, rather than alone.

A: Actually, the fact that evidence of antigen is never seen after recovery of treated individuals suggests the opposite.

Q: Do the ferrets ever manifest neurologic disease, and have you been able to treat them at that stage?

A: The ferrets do exhibit neurologic disease. An experiment where ferrets with such symptoms have been subjected to therapy has not yet been conducted. The closest we have come, so far, is successfully treating NHP with fever.

Q: Have you measured mAb levels in the brain?

A: No, we have never looked for the presence of mAb in the brain. Based on the damage observed in the microvasculature, however, it is likely that the mAb has leaked into the CNS. We have given up on the work of tailoring mAbs for increased passage across the BBB.

SESSION 10 – PANEL DISCUSSION: COLLABORATION & SYNERGY

Chair: Professor Linfa Wang & Dr. Raúl Gómez Román

1: Exchange of materials and SOPs, biobanking

Professor Benhur Lee, Mount Sinai

Throughout Nipah@20, we have heard people mention the difficulties they have experienced getting access to samples and standards. For example, Professor Steve Luby (WHO NiV R&D Roadmap) mentioned the limited availability of clinical samples as a major hurdle in the development of new diagnostics, and the WHO R&D Blueprint suggesting to create a virtual repository of clinical reference samples for use in R&D. Dr. Jillian Sacks (FIND) and Dr. Laura Mazzola (WHO) both pointed to the need for international reference standards and accessible sample repositories. Dr. John Klena (CDC) addressed the increased difficulty of sharing positive controls internationally. Thus, a centralisation of this function to an "approved" facility may be advantageous, for instance abiding to the Clinical Laboratory Improvement Amendments (CLIA) standards for laboratories.

Dr. Kim Halpin (AAHL), representing the only OIE henipavirus reference lab, advocated for an "informal" network of reference labs for henipaviruses. How about one that is formal, modelled after the WHO network of influenza laboratories? Dr. Yoshihiro Kaku (NIID) told of his generation of IgM positive controls from immunised macaques, but could this also be achieved through the sequencing and synthesis of mAbs? Their development of a pseudotyped VSV neutralisation assay allows for both screening and confirmatory test, in the absence of BSL-4. It is important that such techniques are shared internationally, like they offer to do, for more efficient development of diagnostics and therapeutics. Dr. Emily Gurley (Johns Hopkins) points to a weak point of current research on NiV; the inaccurate reporting of the strains used. Notably, in particular the NiV-B strain really constitutes a number of different strains, each with small genetic differences that may impact the pathogenicity and tropisms of the virus.

The best way forward in henipavirus R&D involves more proactive collaboration across fields and nations, and avoiding a practice of working in scientific silos. Biobanking and exchange of materials through a centralised entity will be beneficial, both in terms of access and the ease with which reagents may be shared. With a centralised function, legal and IP issues may be negotiated ahead of time, allowing for straight-forward sharing with vetted institutions across the world. The function could be assigned to an existing entity, such as BEI Resources or the National Collection of Pathogenic Viruses (NCPV), or to a new one. Finally, it is important to secure funding for all of the prioritised activities mentioned, such as standardised reagent generation, sequence curation, and so on.

2: Inter-nation collaboration Sino-French NiV taskforce

Dr. Zhengli Shi, Wuhan Institute of Virology

A Sino-French agreement to collaborate on emerging infectious diseases was established in 2004, following the SARS outbreak. The collaboration involves three Chinese and ten French institutions, and has resulted in the building of a BSL-4 laboratory in Wuhan – the first of its kind in China. Pathogens studied at this facility include Lassa, Nipah, Hendra, Ebola, and other EIDs, with work conducted on cells and in small animals. The Wuhan facility offers repositories of biological resources, conducts R&D and biosafety training, and aims to be accredited as a WHO reference laboratory for one or more BSL-4 pathogens.

Nipah represents a priority pathogen for the Wuhan facility, due to 1) its ability to infect animals and humans; 2) its high mortality in humans; and 3) the prevalence of henipaviruses and henipa-like viruses in countries of Southeast Asia, including China. Work on Nipah is divided into six work packages: 1) NiV pathogenesis; 2) Epidemiology; 3) Development of a DC-based prophylactic mucosal vaccine; 4) Therapeutics development; 5) Diagnostics development; 6) Biostatistics analyses.

The Wuhan BSL-4 facility has an annual call around April for external organisations wanting access to the facility to test their own samples or use samples from the in-house repositories. In addition, they offer an international BSL-4 biosafety training course in an annual call around September. The 9th International Symposium on Emerging Viral Diseases will be held in Wuhan, China, on October 18-20, 2020.

Update from SEARO meeting and the International Research Collaboration Platform in India/Bangladesh (requirements for therapeutics trials)

Dr. Nivedita Gupta, Scientist F, Division of Epidemiology & Communicable Diseases, ICMR

Conducting a multi-country, multi-outbreak, multi-intervention clinical trial on NiV therapeutics requires regional collaboration. Thus, on three occasions so far in 2018 and 2019, scientists from across Southeast Asia have convened to discuss issues related to Nipah R&D. Preparations for a clinical Nipah trial, with support from NIH, NIAID, and the WHO Southeast Asia Regional Office (SEARO), were on the agenda. The ICMR has committed to conducting a trial, through 1) dedicated funding; 2) establishment of a multi-disciplinary trial team; 3) adaptation of trial protocol to the Indian setting; 4) developing necessary SOPs; 5) developing and validating indigenous POC PCR diagnostics; 6) signing an MOU with the Henry Jackson Foundation (U.S.) and the Serum Institute of India for production of m102.4; 7) adapting the trial protocol to the Bangladesh setting (where chances of recruiting a sufficient number of participants are the best).

The Regional Enabler for the Southeast Asia Research Collaboration for Health (RESEARCH) Platform was established on August 28, 2019, with ICMR as secretariat. The goal of the platform is to facilitate clinical research across the region, leveraging the unique capabilities of each country and sharing expertise. With the platform in place, it will be easier to mount rapid responses to new outbreaks, and to conduct clinical trials – not only on NiV, but also other EIDs. The platform has decided on dengue fever as the first priority disease, with more to come. Of the 11 member states in the WHO Southeast Asia Region (SEAR), 10 participated in the second meeting of the RESEARCH Platform, on December 3-4, 2019. Here, concept proposals on six tracks concerning dengue were developed: 1) Clinical management of dengue; 2) Entomology and vector control; 3) Epidemiology and vaccines; 4) Community engagement; 5) PoC diagnostics; 6) Governance.

Comments from representatives from WHO Regional Offices

SEARO & WIPRO

WHO were not available for comment at this time.

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3: Challenges in vaccine safety monitoring
Need to maximise safety monitoring across programmes

Dr. Robert Chen, Brighton Collaboration

The Brighton Collaboration was formed in 2000 with the goal to build trust in the safety of vaccines via rigorous science. Unlike efficacy, safety generally cannot be measured directly, but is inferred from the relative absence of adverse events following immunisation (AEFI). Rare AEFI are easily missed in the absence of a standard case definition. Thus, the mission of the Brighton Collaboration is to develop internationally accepted and harmonised standards for monitoring vaccine safety throughout the vaccine life cycle. They do this with the help of > 750 volunteers. In its 20 years of operation, the Brighton Collaboration has delivered > 60 AEFI case definitions, tiered by 3 levels of evidence, and including guidance for the collection and reporting of vaccine safety data. These case definitions have been endorsed by key regulatory stakeholders such as FDA, EMA, and WHO.

The Brighton Collaboration's Safety Platform for Emergency vACcines (SPEAC), which is being tailored to CEPI's needs, has the following goals: 1) To provide coordinated safety data review capacity, with a fully functional pool of potential DSMB members; a liaison observer for each CEPI vaccine trial; and a meta-DSMB to integrate safety knowledge across different products, pathogens and platforms; 2) To harmonise vaccine safety monitoring during CEPI preclinical and clinical trials, with landscape analyses for potential vaccine issues; an online vaccine safety resource of standards, templates, and tools; identifying relevant vaccine safety issues for CEPI vaccine candidates; developing new adverse event case definitions for CEPI clinical trials; and completing a standard template for communicating risk/benefit data; 3) To provide a continuous improvement framework.

4: General discussion

There was no time for a general discussion.

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