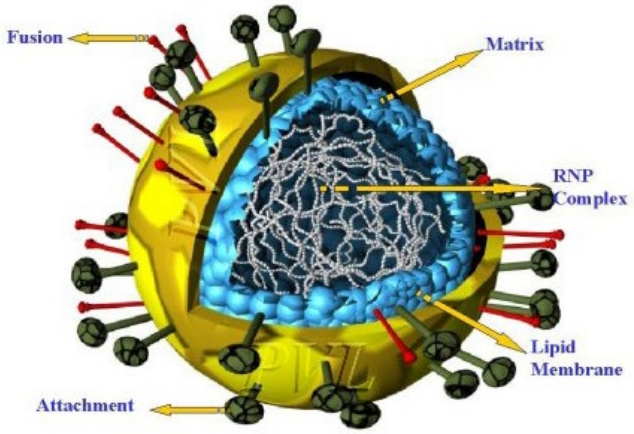
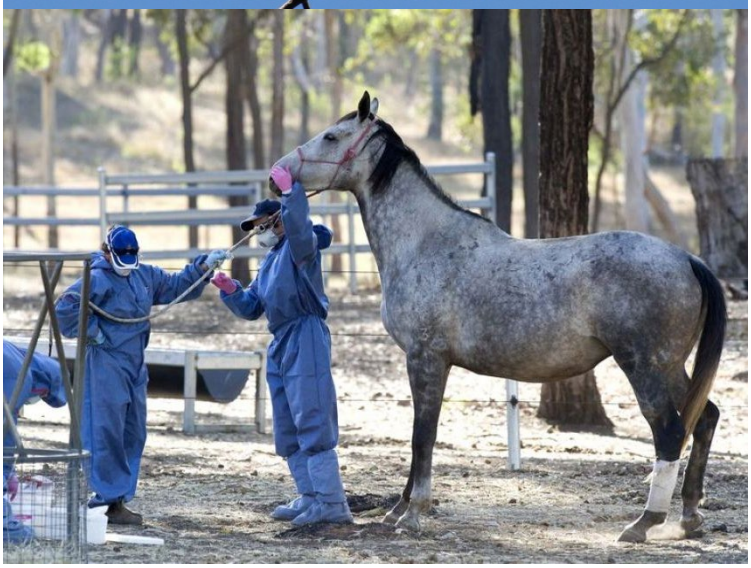
United States Department of Agriculture

Agricultural Research Service

November 2018

Henipavirus Gap Analysis
Workshop Report





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To cite this report:
Henipavirus Gap Analysis Workshop Report. 2018. U.S. Department of Agriculture, Agricultural Research Service, Washington, DC. http://go.usa.gov/xnHgR.

Contents

EXECUTIVE SUMMARY

Henipavirus is the taxonomic genus for a group of viruses in the family Paramyxoviridae that includes *Hendra virus* (HeV) and *Nipah virus* (NiV). These viruses are zoonotic agents that are highly pathogenic in humans with case fatality rates of 40% to 70%. As such, these viruses are classified as Biosafety Level 4 (BSL-4) agents, requiring the highest level of laboratory biocontainment. Importantly, they have many of the physical attributes to serve as potential agents of bioterrorism, and are also considered emerging zoonotic pathogens with increasing geographical distribution in Australia, New Caledonia, Southeast Asia, and Madagascar.

Hendra virus first emerged in 1994 in Australia spilling over from bats to horses to humans, causing several disease outbreaks since with significant fatality rates. Nipah virus emerged in Malaysia in 1999, resulting in nearly 300 human cases with over 100 deaths.

The Nipah virus outbreak in Malaysia was especially concerning, causing widespread panic and fear because of the high mortality rate in people and the inability to control the disease initially. There were also considerable social disruptions and tremendous economic loss to an important pig-rearing industry. This highly virulent virus, believed to be introduced into pig farms by fruit bats, spread easily and silently among pigs and was transmitted to humans who came into close contact with infected animals. A NiV outbreak in Bangladesh in 2001 resulted from direct bat to human transmission via contaminated date palm juice with further spread within the human population. From 2001 to 2012, the World Health Organization (WHO) reported a total of 209 cases, with 161 deaths due to of NiV infections. In 2014, the WHO reported a NiV outbreak in fourteen districts of Bangladesh, resulting in 24 cases and 21 deaths. In 2015, three fruit bats tested positive for NiV in New Caledonia at the Noumea National Park, including three bats at the Noumea Zoo.

This gap analysis report focuses primarily on NiV and its potential impact on agricultural swine production. However, information is also provided on the threat henipaviruses pose to public health, both as emerging zoonotic agents and as potential agents of bioterrorism. Included in this report is scientific information on *Henipavirus* virology, epidemiology, pathogenesis, immunology, and an assessment of the available veterinary medical countermeasures to detect, prevent, and control disease outbreaks. Importantly, gaps are provided to inform research needs and priorities. Some of the major gaps and obstacles for disease control can be summarized as follows:

Diagnostics

The availability of safe laboratory diagnostic tests are limited. Virus isolation and serum neutralization assays require live NiV; thus, BSL-4 containment laboratories are required. Nucleic acid-based assays, such as RT-PCR are available, but genetic variation amongst henipaviruses are reported to impact sensitivity and real time RT-PCR may not be able to detect all divergent and novel henipavirus strains. Serological assays are limited in their ability to differentiate between known and unknown henipaviruses, as cross-reactivity to one or more known viruses is possible. Commercial diagnostic test kits are not available. International standards for NiV assay validation are needed. Gaps include a lack of positive experimental and field samples for test validation (or even evaluation) and there are restrictions on material transfer (e.g., obtaining animal samples that could be used to validate tests) due to biosecurity concerns. Low biosafety level reference sera

against various isolates are not yet available. There is a need for high throughput antibody assays for disease outbreaks, recovery and surveillance purposes. There is also a need to develop operator-safe diagnostics tests and reagents that can be produced in low biocontainment facilities.

Vaccines

There is currently a commercial vaccine available for horses, but there are no vaccines for swine or humans. There are several experimental vaccine candidates that may be safe and effective in swine and other domestic animals. However, all these vaccine candidates will require further research to establish their efficacy, and they will need to be fully developed to be licensed and stockpiled for rapid use in an emergency disease outbreak in swine.

Surveillance

Surveillance is the first line of defense against a disease outbreak. Rapid and accurate detection affects the time when control measures can be implemented and affects the extent of the disease outbreak. Because of limitations with laboratory diagnosis, surveillance programs are dependent on the reporting of clinical signs in populations at risk. Diagnosis of NiV infections based on clinical presentation has a low positive predictive value as there are numerous etiologies for encephalitis in humans, and clinical signs in pigs are difficult to differentiate from many common endemic infectious diseases.

Depopulation

Depopulation is the primary countermeasure to reduce virus shedding and stop the spread of NiV in livestock. Disease outbreaks have shown that the control of NiV in pig populations through stamping out is complex due to the zoonotic nature of the agent. In addition, depopulation may be logistically difficult and may be impossible in a rapidly spreading outbreak in countries where there are pig dense regions with millions of pigs, such as the states of Iowa, North Carolina, and Minnesota in the United States, or South East China.

GROUP PICTURES

Henipavirus Gap Analysis Working Group, Winnipeg, Canada
November 14-17, 2017

The Nipah Virus Countermeasures Working Group, Geelong, Australia
March 17-19, 2009

GLOSSARY

APHIS: Animal and Plant Health Inspection Service, USDA, United States of America

ARS: Agricultural Research Service

AAHL: Australian Animal Health Laboratory

BSL-4: Biosafety Level 4

CDC: U.S. Centers for Disease Control and Prevention, HHS, United States of America

CFIA: Canadian Food Inspection Agency

DIVA: Differentiating between infected and vaccinated animals

ELISA: Enzyme-linked immunosorbent assay

FADDL: U.S Foreign Animal Disease Laboratory, Plum Island Animal Disease Center

FLI: Friedrich Loeffler Institute

GMP: good manufacturing practice

HeV: Hendra virus

HHS: Department of Human Health Services, United States of America

HSPD-9: Homeland Security Presidential Directive Nine

ICAR: Indian Council of Agricultural Research

Ig: Immunoglobulin

IEDCR: Institute of Epidemiology, Disease Control and Research in Bangladesh

MLV: Modified live virus vaccine

NAHLN: National Animal Health Laboratory Network, USA

NIHSAD: National Institute of High Security Animal Diseases, ICAR, India

NCFAD: National Center for Foreign Animal Disease, CFIA, Canada

NiV: Nipah virus

NiV-B: Nipah virus Bangladesh

NiV-M: Nipah virus Malaysia

NiV N: Nipah virus nucleoprotein

NVCWG: Nipah Virus Countermeasures Working Group

NVS: National Veterinary Stockpile

OIE: World Organisation for Animal Health

PCR: Polymerase Chain Reaction.

PPE: Personal Protective Equipment

RT-PCR: Reverse transcription-polymerase chain reaction

rRT-PCR: Real-time reverse transcription-polymerase chain reaction

sHeV G: recombinant soluble Hendra virus G protein

sNiV G: recombinant soluble Nipah virus G protein

USDA: United States Department of Agriculture, United States of America

INTRODUCTION

Nipah virus (NiV) is an emerging zoonotic virus. First isolated in pigs and people from an outbreak in Malaysia in 1998 (Ang *et al.* 2018), this emerging virus causes severe disease in humans. The source of transmission was determined to be from bats to pigs to humans, through close contact with infected animals. The virus is named after the location where it was first detected in Sungai Nipah, a village in the Malaysian Peninsula where exposed pig farmers became severely ill with encephalitis.

Nipah virus is closely related to another zoonotic virus called Hendra virus (HeV), formerly called Equine *Morbillivirus*, and named after the town where it first appeared in Australia. Hendra virus infection was first recognized in 1994, when it caused an outbreak of acute, fatal respiratory disease that killed 14 horses. Three human cases, leading to two deaths were recorded during the outbreak. The precise mode of virus transmission to the three Australian patients is not fully understood. All three individuals appear to have acquired their infection as a result of close contact with horses, which were ill and later died.

Although members of this group of viruses have only caused a few focal outbreaks, their ability to infect a wide range of animal hosts and to produce a high mortality rate in humans has made this emerging zoonotic viral disease a significant public health threat.

Certain species of bats of the genus *Pteropus* (fruit bats, also called flying foxes) are the principal natural reservoir hosts for NiV and HeV – see Table I. Bats are susceptible to infection with these viruses but do not develop disease. Fruit bats are distributed across an area encompassing Australia, Southeast Asia; including Indonesia, Malaysia, the Philippines and some of the Pacific Islands, the Indian subcontinent, and Madagascar (See Fig. 1). There is also growing evidence that viruses related to NiV and HeV circulate in non-pteropid fruit bats across the globe (Clayton, 2017).

The exact mode of transmission of henipaviruses is uncertain, but appears to require close contact with contaminated tissue or body fluids from infected animals. The role of domestic species other than pigs in transmitting NiV infection to other animals has not yet been determined. In 2014, an outbreak was reported in the Philippines involving the consumption of meat from NiV-infected horses, further expanding the potential routes of transmission for henipaviruses.

Despite frequent contact between fruit bats and humans there is no serological evidence of human infection among persons that are in contact with bats. Pigs were the apparent source of infection among most human cases in the Malaysian outbreak of NiV in 1998-1999. Nipah virus has continued to spillover over from animals with at least six outbreaks resulting in human fatalities in Bangladesh in 2013, one in India in 2014, and two in Bangladesh in 2015. The World Health Organization (WHO) had not reported any NIV cases 2016-2017, but in 2018 twenty three new cases and 21 deaths were reported in Kerala, India - See Table II.

The spread of henipaviruses to new geographical areas is a concern. In 2014, the Philippines reported an outbreak with a zoonotic paramyxovirus in horses and people. There is further evidence for broader distribution of NiV in pteropid fruit bats species. There is also growing evidence that viruses related to NiV and HeV also circulate in non-pteropid fruit bats worldwide.

BACKGROUND

Organization of the Gap Analysis Working Groups on Nipah Virus (2009 and 2017)

The United States Department of Agriculture (USDA) organized the first Nipah virus gap analysis workshop in Australia in 2009 with the support of the Australian Animal Health Laboratory (AAHL). The working group was charged by the USDA National Veterinary Stockpile Steering Committee with making recommendations on specific materials, commercially available and in the pipeline, which will ensure the United States has an arsenal of highly efficacious countermeasures to control and mitigate the impact of an outbreak of Nipah virus. Nipah virus experts representing laboratories in South East Asia, Australia, Canada, and the United States were invited to participate and contributed to this report. The second workshop was organized in 2017 by the Special Pathogens Unit, National Centre for Foreign Animal Disease, Canadian Food Inspection Agency (CFIA), in collaboration with BSL4ZNet and DISCONTOOLS (http://www.discontools.eu/). The participants were charged with assessing available veterinary medical countermeasures to control and respond to a Nipah virus disease outbreak. In addition, the workshop participants agreed to update the gap analysis conducted at the AAHL in Geelong, Australia, in 2009.

Report Updates

This report will be updated periodically with new scientific information, research breakthroughs, and/or the successful development of veterinary medical countermeasures. This report was last updated with the support of Henipavirus experts November 2018.

Reference Material

The following reports and websites are recommended:

OIE – World Organisation for Animal Health - Nipah in Animals
http://www.oie.int/en/animal-health-in-the-world/animal-diseases/Nipah-Virus/
Accessed July 22, 2018

FAO – Food and Agriculture Organization
Manual on the diagnosis of Nipah virus infection in animals
www.fao.org/DOCREP/005/AC449E/AC449E00.htm
Accessed July 22, 2018

CDC – Center for Disease Control and Prevention - Special Pathogens Branch
https://www.cdc.gov/vhf/nipah/index.html
Accessed July 22, 2018

WHO - World Health Organization
http://www.who.int/news-room/fact-sheets/detail/nipah-virus
Accessed July 22, 2018

Guidelines for Veterinarians Handling potential Hendra Virus infection in Horses (QDPI)
https://www.daf.qld.gov.au/__data/assets/pdf_file/0005/126770/2913_-Guidelines-for-veterinarians-handling-potential-Hendra-virus-infection-in-horses-V5.1.pdf

Accessed July 22, 2018

CFSPH – Center for Food Security and Public Health
Nipah Virus Infection
http://www.cfsph.iastate.edu/Factsheets/pdfs/nipah.pdf
Accessed July22, 2018

DEFINITION OF THE THREAT

The threat for a natural introduction of henipaviruses in the United States is low, but there is significant concern that henipaviruses could be used for nefarious purposes to harm agriculture and people. Both Hendra virus and Nipah virus are on the HHS and USDA list of overlap Select Agents and Toxins. Henipaviruses are listed as APHIS Tier 3 high-consequence foreign animal diseases and pests. Henipaviruses are promiscuous in their ability to cause severe morbidity in several animal species, including people, and human infections result in a very high mortality rate. The mortality rate in pigs is actually reported as about 2.5% in adult pigs – high morbidity, but low mortality. Mortality rates in humans range from 40% (Malaysia) to 75% (up to 100%) in Bangladesh. The animal reservoir includes several species of bats, and henipaviruses may thus be readily available in these wildlife reservoirs.

INFECTION IN PEOPLE

Between September 1998 and June 1999, a NiV outbreak in Malaysia resulted in severe viral encephalitis in 105 patients (Goh *et al.,* 2000; Epstein *et al.,* 2006). Ninety-three percent had had direct contact with pigs, usually within two weeks prior to the onset of illness, suggesting that there was direct viral transmission from pigs to humans and a short incubation period. The main presenting features were fever, headache, dizziness, and vomiting. Fifty-two patients (55%) had a reduced level of consciousness and prominent brain-stem dysfunction. Distinctive clinical signs included segmental myoclonus, areflexia and hypotonia, hypertension, and tachycardia. The initial cerebrospinal fluid findings were abnormal in 75% of patients. Antibodies against Hendra virus were detected in serum or cerebrospinal fluid in 76 percent of 83 patients tested. Thirty patients (32%) died after rapid deterioration in their condition. An abnormal doll's-eye reflex and tachycardia were factors associated with a poor prognosis. Death was probably due to severe brain-stem involvement. Neurologic relapse occurred after initially mild disease in three patients. Fifty patients (53%) recovered fully, and 14 (15%) had persistent neurologic deficits.

Unlike Malaysia, the NiV outbreaks in Bangladesh were strictly confined to human populations with significantly higher mortality rate (Hossain *et al.,* 2008). NiV outbreaks in Bangladesh have continued annually since 2008 resulting in a total of 207 reported cases, 152 of which were fatal resulting in a 70% mortality rate (Clayton,. 2017). In 2018, NiV infection was confirmed in Kerala, India, where 23 confirmed cases were reported and case fatality rates were 90% (Arunkumar *et al.,* 2018).

INFECTION IN PIGS

The NiV outbreak in Maylasia in 1999 was facilitated by the rapid spread of the virus in pig populations. Although some pigs demonstrated a febrile respiratory illness with epistaxis, dyspnoea, and cough, few animals exhibit neurological signs, and the majority of pigs had subclinical infections. There are no clinical signs in pigs that are specific for NiV infection. Both, apparently healthy pigs and pigs showing clinical signs shed significant amount of virus.

ECONOMIC IMPACT

The NiV outbreak in Malaysia in 1999 destroyed the main market for Malaysian hogs in Singapore. The Malaysia outbreak resulted in an 80% drop in pork consumption in the domestic market. Over half the standing pig population in the country was culled to halt the outbreak. Half the pig farms

went out of business. The cumulative economic losses based on government figures was estimated to be approximately $217 million USD.

BIOTERRORISM

NiV has many of the physical attributes needed for a biological weapon, including easy access to virus resulting from its wide distribution in nature and laboratories, easy to produce, easy to disseminate, and the potential for high morbidity and mortality in people.

GAP ANALYSIS

The following section summarizes what we know about henipaviruses, gaps in our knowledge, and the threat of bioterrorism.

VIROLOGY

The following summarizes our current knowledge of viral strains, taxonomy, reservoir, genome, morphology, determinants of virulence, host range, and tissue tropism.

Virus species

Nipah virus (NiV) was first isolated in 1999 from samples collected during an outbreak of encephalitis and respiratory illness among pig farmers. The name Nipah originated from Sungai Nipah, a village in the Malaysian Peninsula where pig farmers became sick. There are currently two genotypes identified: NiV-Malaysia and NiV-Bangladesh. Different strains/genotypes of NiV have emerged: Malaysia, Bangladesh, and Cambodia. NiV Malaysia resulted in the culling of a million pigs and 250 human cases (106 fatal). NiV Bangladesh is associated with outbreaks in people (Clayton, 2017).

Hendra virus (HeV) was first isolated in 1994 from specimens obtained during an outbreak of respiratory and neurologic disease in horses and humans in Hendra, a suburb of Brisbane, Australia.

Cedar virus (CedV) is a novel *Henipavirus* isolated from Australian bats, which appears to be non-pathogenic in lab animal experiments (Marsh et al. 2012).

Ghanaian bat henipavirus (GhV) is a species of henipaviruses assembled from sequences collected from *Eidolon helvum*, a bat species in the family Pteropodidae (Drexler *et al.* 2009; Drexler *et al*. 2012). No isolates have been reported, and both pathogenicity and the cross-species transmission remain unknown. Partial sequences of 19 phylogenetically novel African henipaviruses have also been discovered, suggestive of a further diversity of African henipaviruses.

Mòjiāng henipavirus (MojV) was discovered during retrospective surveillance for the etiologic agent responsible for cases of fatal respiratory illness in cave-miners, China. A full-genome was assembled from sequences detected from a cave-dwelling rodent species (Wu Z. *et al.* 2014). MojV is circumstantially associated with the fatal respiratory illness, however, pathogenicity studies have not been completed.

Taxonomy

NiV and HeV are members of the family Paramyxoviridae, order *Mononegavirales*. Comparison of nucleic acid and deduced amino acid sequences with other members of the family confirms that NiV and HeV are members of the family Paramyxoviridae, but with limited homology with members of the *Morbillivirus*, *Rubulavirus* and *Respirovirus* genera (See Fig. 2). The name *henipavirus* was recommended for the genus of both HeV and NiV (Wang *et al.,* 2000). HeV appear to be less diverse that NiV but molecular epidemiology studies are needed to identify new isolates that may bridge the gap between HeV and NiV.

Reservoir

The natural reservoir, or primary animal host, of the henipaviruses are fruit bats mainly from the genus *Pteropus* (flying foxes). Nucleic acid and antibody signatures of exposure to NiV or NiV-like viruses has been documented in a diversity of bat species across the globe (Table 1).

Genome

The complete genomes of both HeV and NiV have been sequenced (Wang *et al.,* 2001). Henipaviruses have a large non-segmented genome comprised of single-stranded negative-sense RNA. Their genomes are 18.2 kb in size and contain six genes corresponding to six structural proteins. All genes are of similar size to homologues in the respirovirus and morbillivirus genera, with the exception of P which is 100-200 amino acids longer (See Fig. 3). Most of the increase in genome length is due to longer untranslated regions between genes, mainly at the 3' end of each gene. The role of these long untranslated regions are not understood. Henipaviruses employ an unusual process called RNA editing to generate multiple proteins from a single gene. The process involves the insertion of extra guanosine residues into the P gene mRNA prior to translation. The number of residues added determines whether the P, V or W proteins are synthesized. The C protein is made via an alternative translational initiation mechanism. The functions of the V, W, and C proteins are unknown, but they may be involved in disrupting host antiviral mechanisms (see Immunology below). The function of the G protein is to attach the virus to the surface of a host cell via the major receptors ephrin-B2 and ephrin-B3 ligands, highly conserved proteins present in many mammals. G glycoprotein is the major neutralizing antigen and the target protein for vaccine development. X-ray crystal structure for NiV G complex with ephrin-B3 has been determined. This interaction is highly conserved between NiV and HeV. This interaction is a prime candidate for developing henipavirus specific therapeutics. The F protein fuses the viral membrane with the host cell membrane, releasing the virion contents into the cell. It also causes infected cells to fuse with neighboring cells to form large multinucleated syncytia.

The genome size and organization of CedPV is very similar to that of HeV and NiV. The nucleocapsid protein displays antigenic cross-reactivity with henipaviruses and CedPV uses the same receptor molecule (ephrin-B2) for entry during infection. Clinical studies with CedPV in *Henipavirus* susceptible laboratory animals confirmed virus replication and production of neutralizing antibodies although clinical disease was not observed. In this context, it is interesting to note that the major genetic difference between CedPV and HeV or NiV lies within the coding strategy of the P gene, which is known to play an important role in evading the host innate immune system. Unlike NiV and HeV, and almost all known paramyxoviruses, the CedPV P gene lacks both RNA editing and also the coding capacity for the highly conserved, interferon pathway antagonists, V or W proteins (Marsh *et al.* 2012).

Although, GhV and MojV have not yet been isolated from hosts, sequence constructed genomes are similar in size, organization, and coding capacity to HeV, NiV, and CedV (Wu Z *et al.* 2014, Drexler *et al*. 2012). Like HeV and NiV, both GhV and MojV are predicted to possess the RNA editing site in the P gene and presumably coding capacity for V and W proteins. Receptor-usage studies with recombinant GhV G glycoprotein demonstrated that like CedV, GhV G was capable of binding to

ephrin-B2, but not ephirn-B3 (Lee B *et al.* 2015). A receptor remains undiscovered for MojV; however, ephrin-B2, -B3 appear to be unlikely candidates (Rissanen I *et al.* 2017).

Morphology

Henipaviruses are pleomorphic ranging in size from 40 to 600 nm in diameter. They possess a lipid membrane overlying a shell of viral matrix protein. At the core is a single helical strand of genomic RNA tightly bound to the nucleocapsid (N) protein and associated with the large (L) and phosphoprotein (P) proteins, which provide RNA polymerase activity during replication. Embedded within the lipid membrane are spikes of fusion (F) protein trimers and attachment (G) protein tetramers.

Determinants of virulence, host range, and tissue tropism

Molecular determinants of virulence, host range and cell tropism have been extensively studied and are well understood for many paramyxoviruses. Infectivity is determined by the cell-attachment and fusion glycoproteins and the presence of appropriate P gene products modulate virulence by antagonizing the cellular interferon response.

Henipaviruses have a large host range, unlike other members of the Paramyxoviridae, which generally have a very narrow host range. The cell attachment protein, unlike many other members for the paramyxovirus subfamily, does not have haemagglutinating activity and as a consequence does not bind sialic acid on the surface of cells.

The receptor for henipavirus is present on many different cultured cell types from many different species. The receptors for HeV and NiV are the same and have been identified as ephrin-B2 and ephrin-B3. Ephrin-B2 or -B3 are highly conserved across vertebrate species and are members of a family of receptor tyrosine kinase ligands. Ephrin-B2 is highly expressed on neurons, smooth muscle, arterial endothelial cells and capillaries, which closely parallels the known tissue tropism of HeV and NiV *in vivo*. Ephrin-B3 is also widely expressed but particularly in specific regions of the central nervous system and may facilitate pathogenesis in certain neural subsets.

Virology Research Priorities

- Molecular epidemiology and determinants of strain variation
- Need sequencing of henipaviruses from bats, especially Bangladesh
- Determine molecular basis for virulence

PATHOGENESIS

The following summarizes our current knowledge of viral pathogenesis, including routes of infection, tissue tropism, pathogenesis, clinical signs, and clinical pathology reported in naturally acquired infections. It should be noted that experimental infection in other animal models have been developed. NiV and HeV (henipaviruses) are distinguished from all other paramyxoviruses particularly by their broad species tropism and ability to cause fatal disease in multiple vertebrate hosts including humans, monkeys, pigs, horses, cats, dogs, ferrets, hamsters and guinea pigs, spanning 6 mammalian Orders (Broder CC *et al.,* 2012; Geisbert TW *et al.,* 2012).

NiV infections in humans and pigs are linked to contact with bats. Clinical signs in human cases indicate primarily involvement of the central nervous system with 40% of the patients in the Malaysian outbreak having also respiratory syndromes, while in pigs the respiratory system is considered the primary virus target, with only rare involvement of the central nervous system.

Humans

The main histopathological findings include a systemic vasculitis with extensive thrombosis and parenchymal necrosis, particularly in the central nervous system (Wong *et al.,* 2002). Endothelial cell damage, necrosis, and syncytial giant cell formation are seen in affected vessels. Characteristic viral inclusions are seen by light and electron microscopy. Immunohistochemistry (IHC) analysis shows the widespread presence of NiV antigens in endothelial and smooth muscle cells of blood vessels (Hooper *et al*., 2001). Abundant viral antigens are also seen in various parenchymal cells, particularly in neurons. The brain appears to be invaded via the hematogenous route and virus has been isolated from the cerebrospinal fluid of patients with NiV encephalitis (Wong *et al*., 2002). Infection of endothelial cells and neurons as well as vasculitis and thrombosis seem to be critical to the pathogenesis of this new human disease.

NiV infection can rarely cause a late-onset encephalitis up to a couple of years following a non-encephalitic or asymptomatic infection, or a relapsed encephalitis in patients who had previously recovered from acute encephalitis (Wong *et al*., 2001; Goh *et al*., 2000; Tan *et al*., 2002).

The most recent NiV outbreak, and first reported in South India, resulted in 23 human cases with a case-fatality rate of 91% (Arunkumar *et al*., 2018). The clinical manifestations and high fatality rate among people were similar to those of earlier NiV outbreaks in India and Bangladesh, and the NiV isolate from this outbreak showed a 97% genetic similarity to the NiV-B lineage. All human cases, following the index case, were due to nosocomial transmission in three different hospitals. Although it was not possible to establish the exact NiV transmission event to the identified index case, the most likely zoonotic route was from *P.* giganteus (Indian flying fox). It was noted that in Kerala, date palms are not used for obtaining sap, and the narrow-mouthed vessels used to collect sap from coconut and Asian Palmyra palm do not allow access by bats. The human-to-human transmission rate was very high in this recent outbreak, and the index case transmitted NiV to 19 contacts (primary cases), while three cases were reported as secondary (Arunkumar *et al*., 2018). These nosocomial transmissions to the primary cases were concomitant with the index case presenting with a persistent cough and near the terminal stage of NiV illness. Of the 23 cases, 20 (87%) had respiratory symptoms presumably increasing the possibility of human-to-human transmission by droplet, and it was reported that only those with direct exposure to the patient's coughing appeared to have acquired NiV infection.

Pigs

Experimental challenge studies in piglets conducted at the National Centre for Foreign Animal Diseases, Winnipeg, Canada, demonstrated neurological signs in several inoculated pigs (Weingartl *et al.,* 2005; Berhane et al., 2008; Weingartl, H.M., personal communication of unpublished data). The rest of the pigs remained clinically healthy. NiV was detected in the respiratory system (turbinates, nasopharynx, trachea, bronchus, and lung), the lymphoreticular system (endothelial cells of blood and lymphatic vessels), submandibular and bronchiolar lymph nodes, tonsil, and spleen, with observed necrosis or lymphocyte depletion in lymphoid tissues, most importantly in lymph nodes (Hooper et al.,

2001, Weingartl et al., 2006; Berhane et al., 2008). NiV presence was confirmed in the nervous system of both sick and apparently healthy animals (cranial nerves, trigeminal ganglion, brain, and cerebrospinal fluid). No virus was detected urine, although NiV antigen was found in kidneys of field swine cases (Tanimura et al., 2004). This study suggests NiV invaded the porcine host central nervous system via cranial nerves after initial virus replication in the upper respiratory tract, and later in the infection also by crossing the blood-brain barrier as a result of viremia. Additional information on NiV and HeV pathogenesis in pigs are summarized in Middleton and Weingartl, 2012.

Cats

Cats were recognized as a naturally susceptible host for NiV during the 1998-99 Malaysian outbreak (Hooper *et al.*, 2001). Experimental infections of cats revealed they are highly susceptible to productive infection by both HeV and NiV and disease is severe. HeV infected cats develop fever and elevated respiratory rates, and there is rapid progression to severe illness and death within 24 hours of the onset of clinical signs (Westbury *et al.,* 1996). HeV disease in cats is similar to that observed in horses, with wide-spread vasculitis and parenchymal lesions in a wide range of organ systems particularly the lungs (Hooper *et al.,* 2001; Hooper *et al.,* 1997). Experimental NiV infection in the cat is essentially identical in outcome as compared to HeV infection and closely resembles most of the pathogenic processes seen in cases of henipavirus infection of people (Broder *et al.,* 2012).

Dogs

Middleton *et al*., 2017, conducted experimental infections with HeV in dogs and determined that the virus is not highly pathogenic in dogs but their oral secretions pose a potential transmission risk to people. The time window for potential oral transmission corresponded to the period of acute infection.

Horses

The pathology caused by HeV or NiV in horses (natural or experimental infection with HeV or natural infection with NiV) is more severe than that caused by either virus in pigs. Naturally acquired HeV infection in horses is often associated with severe disease, and experimental infections are essentially uniformly fatal. Animals initially become anorexic and depressed with general uneasiness and ataxia, with a developing fever with sweating. Respiration becomes rapid, shallow and labored with pulmonary edema and congestion with nasal discharge being a common terminal feature 1 to 3 days following the onset of clinical signs. Neurologic disease is also present but less frequent and noted in both terminally ill horses and in those that recovered from respiratory infection (Rogers *et al.,* 1996; Williamson *et al.,* 1998). Infection is wide-spread with an endothelial cell tropism with syncytia (Hooper *et al.,* 2001; Hooper *et al.,* 1997; Marsh *et al.,* 2011; Murray *et al.,* 1995; Williamson *et al.,* 1998). Experimental infection of horses with NiV has not been carried out, but the brain and spinal cord of one naturally infected horse was confirmed and revealed non-suppurative meningitis (Hooper *et al.,* 2001).

Bats

Fruit bats in the *Pteropus* genus have been identified as the reservoir hosts for HeV, NiV, and CedV. Henipaviruses have been isolated to date in *Pteropus* spp. from Australia (HeV, CedV) and Malaysia/Bangladesh/Cambodia/Thailand (NiV). Serological evidence of NiV or NiV-like exposure was detected in bats sampled in Madagascar and Ghana (Iehle C., *et al.,* 2007, Hayman *et al.,* 2008). Subsequently, 19 novel henipavirus sequences and one full-length genome of an African henipavirus,

GhV, were identified from related pteropodid bats, *Eidolon helvum*, sampled in Ghana (Drexler *et al.*, 2009; Drexler *et al.*, 2012). Nucleic acid and antibody signatures of henipaviruses have been detected serologically and by PCR in non-*Pteropus*, but related pteropodid bats in Central and West Africa, China, and Southeast Asia (Table 1); however the role that these non-*Pteropus* spp. play in the maintenance and transmission of henipaviruses remains unclear. The genome of MojV was constructed from sequences collected from a rodent, *Rattus flavipectus*, but comprehensive surveys have not been performed to rule out whether bats also host MojV.

There is no significant pathology in bats, and the frequency of viral shedding from wild bats is rare, with prevalence ranging from (1-3%) with temporal variation of infection and viral shedding observed among different bat populations (Gurley *et al.,* 2017 and Wacharaplusadee *et al.* 2010, 2016). Henipavirus isolation from bat excreta is challenging, potentially due to low viral load.

Pathogenesis Research Priorities

- Identify determinants of virulence in pigs
- Develop experimental infection models in bats to study shedding
- Comparative genomic studies of contemporaneous NiV strains collected from bats and humans during outbreaks.
- Expand knowledge of spectrum of henipaviruses in bat hosts in NiV hotspots (e.g. western Bangladesh & West Bengal India)
- Determine whether the innate immune system in bats is responsible for limiting viral replication
- Determine how the net reproductive value of henipaviruses are sustained in bats
- Determine how transmission effected within bats, and between bats and other species

IMMUNOLOGY

The following summarizes our current knowledge of NIV immunology, including innate and adaptive immune responses to wild-type virus, immune evasion mechanisms, and protective immunity.

Innate and adaptive immune responses to wild-type NiV

Viral RNA can be detected by both cytoplasmic and endosomal pattern recognition receptors (PRRs), resulting in innate immune Type I IFN induction/ and signaling pathways:

- Retinoic Acid-inducible Gene I (RIG- I)- recognizes 5' triphosphorylated RNA
- Melanoma Differentiation Antigen 5 (Mda-5)-recognizes cytosolic dsRNA
- RNA-dependent Protein Kinase (PKR)- recognizes cytosolic dsRNA
- Toll-like Receptor (TLR) 3- recognizes endosomal dsRNA
- TLR 7-8- recognizes endosomal ssRNA

Immune evasion mechanisms

The NiV uses unusual processes called RNA editing and internal translational initiation to generate multiple proteins from the phosphoprotein (P) gene, resulting in 4 proteins (P, C, V, and W) that function in inhibiting Type I interferon pathways:

- NiV P, V, and W have individually been shown to bind STAT1 and STAT2, effectively preventing STAT1 phosphorylation in type I IFN-stimulated cells.
- The V protein localizes to the cytoplasm, while the W protein localizes to the nucleus.

- The C protein can partially rescue replication of an IFN-sensitive virus, but the mechanism is unknown.
- Nuclear localization of W enables it to inhibit both dsRNA and TLR 3 (IRF-3 dependent) IFN-β induction pathways.
- A single point mutation in the V protein abrogates its ability to inhibit of IFN signaling.
- The V proteins of paramyxoviruses interact with the intracellular helicase Mda-5, and inhibits its IFN-β induction, but not with RIG-I.
- NiV V, W, and P bind polo-like kinase (PLK) via the STAT1 binding domain (Ludlow *et al.,* 2008).
- The P, V, and W proteins of NiV Malaysia and NiV Bangladesh inhibit IFN-stimulated response element (ISRE), which have a role in inducing transcription of IFN-stimulated genes (ISGs). Some of these ISGs include IRF-7, 2'5' Oligoadenylate Synthetase (OAS), RnaseL, p56, and double-stranded RNA-induced protein kinase (PKR). These ISGs all contribute to the generation of an 'antiviral state' in the cell.

Protective immunity

The G and F protein induce neutralizing antibodies that protect against challenge. Recent evidence from vaccination challenge studies indicates that both serum neutralizing antibody and T cell-mediated immunity are needed for protection from NiV infection in pigs (Pickering *et al.,* 2016).

Research needs

- Innate immunity and immunosuppression
  - Need studies in NiV infected cells and animal models
  - Need to study infection in various cell types, including cells of the immune system and bat cells
  - Use infectious clone to study virulence determinants
  - Identify targets for antiviral agents
  - Cytokine response to infection in human and bat cell lines
  - Need to study the potential for type 1 interferon or other cytokines to provide early protection from Nipah virus infection, transmission and/or clinical signs.

- Protective Immunity
  - Need to better define correlates of protection
  - Study T lymphocyte subset responses and cellular targets (e.g., N)

EPIDEMIOLOGY

Certain species of fruit bats of the genus *Pteropus* are the principal natural reservoir hosts for NiV and HeV. Bats are susceptible to infection with these viruses but do not develop disease. Other zoonotic viruses like Ebola, Marburg, and SARS virus, have also been identified in various bats (Leroy *et al*. 2005; Towner *et al*. 2009; Li W *et al*. 2005). Fruit bats are distributed across an area encompassing Australia, Southeast Asia; including Indonesia, Malaysia, the Philippines and some of the Pacific Islands, the Indian subcontinent, and Madagascar (See Fig. 1). There is further evidence for broader

distribution of NiV in pteropid fruit bats species across their range (Wacharapluesadee S. and Hemachudha T., 2007). There is also growing evidence that viruses related to NiV and HeV also circulate in non-pteropid fruit bats worldwide.

Hendra Virus

Hendra virus infection was first recognized in 1994 in Australia, when it caused an outbreak of acute, fatal respiratory disease that killed 14 horses. Three human cases, leading to two deaths were recorded during the outbreak. In 1995, a horse was infected with associated human cases. The precise mode of virus transmission to the three Australian patients is not fully understood. All three individuals appear to have acquired their infection as a result of close contact with horses, which were ill and later died.

There have been several recognized outbreaks in Australia since 1994. Hendra virus reemerged in 1999, 2004, and 2006-2010. All known HeV cases have occurred in Queensland or northern New South Wales. From 1994 to 2010, HeV was confirmed on 11 premises in Queensland and one premise in northern New South Wales. In Australia, GlobalincidentMap.com reported: 21 cases in 2011; 12 cases in 2012; 10 cases in 2013; four cases in 2014; three cases in 2015; one case in 2016; and four cases in 2017. Al cases have involved horses as an intermediate host along with some additional human infections, resulting in several fatalities. The Australian Veterinary Association's national president, Dr. Ben Gardiner, was quoted as stating "no state or territory was immune from the virus."

The natural reservoirs for HeV are flying foxes found in Australia. Bats are susceptible to infection with these viruses but do not develop disease.

Hendra virus infection has also been detected in two dogs that were in close contact with infected horses. Both dogs remained clinically normal with no history of related illness.

Updated statistics on HeV outbreaks, including locations, dates and confirmed human and animal cases may be found on the Australian Veterinary Association website (Assessed July 22, 2018).

Nipah Virus

Nipah virus is a recently-recognized, zoonotic paramyxovirus that causes severe disease and high fatality rates in people. Outbreaks have occurred in Malaysia, Singapore, India and Bangladesh, and a putative Nipah virus was also recently associated with human disease in the Philippines (Clayton, 2017). The following summarizes our current knowledge of NiV epidemiology taking into account disease outbreaks in Malaysia and Bangladesh.

Malaysia

Nipah virus was first described in 1999 in Malaysia. The outbreak in Malaysia resulted in over a million pigs culled, 800 pig farms demolished, 36,000 jobs lost, $120+ million exports lost, and over 300 human cases (106 fatal, ~35% mortality) in pig farmers (Chinese) and Singapore abattoir workers (Field *et al.,* 2001). The NiV outbreak in pigs was described as highly infectious, frequently asymptomatic, low mortality rate (~5%), with respiratory and neurological syndromes. The pig farm pattern of disease included 30% morbidity and 5% mortality with sows first affected, followed by weaners, growers and finishers. The duration of clinical disease on a farm lasted ~ 2 weeks with a sero-prevalence approaching 100% in some cases. The outbreak in Malaysian pigs was associated

with an increased incidence of human viral encephalitis cases, strongly associated with pig farm workers, with temporal and spatial links to disease in pigs.

During the outbreak, evidence of NiV infection was found in domestic animals such as goats and cats, but especially dogs (Field *et al.,* 2001). After pig populations were destroyed, but before residents were allowed to return to their homes, studies were undertaken in the epidemic area to determine whether domestic animal populations maintained active infection in the absence of infected pigs (Mills *et al.,* 2009). Dogs were especially suspected because they live commensally with both pigs and humans. However, serologic screening showed that in the absence of infected pigs, dogs were not a secondary reservoir for NiV.

Although human-to-human transmission of NiV during the 1998-1999 outbreak in Malaysia was not reported, a small number of infected people had no history of contact with pigs, suggesting human-to-human transmission occurred in these cases (Clayton, 2017).

The reservoir and natural host of NiV was determined to be fruit bats. Fruit bats have a wide geographic distribution, high antibody prevalence (17-30%), but no apparent clinical disease. A NiV neutralizing antibody study (Yob *et al.,* 2001) from 237 wild-caught bats surveyed on Peninsular Malaysia April 1–May 7, 1999, found four different species of fruit bats, and one species of insectivorous bats, tested positive for NiV (see Table I).

The routes of NiV excretion in bats include urine, saliva, and foetal tissues and fluids but the exact modes of transmission have yet to be determined.

The drivers of the emergence of NiV in Malaysia were determined to be large piggery (30,000+) adjacent to primary forest/fruit bat habitat and a network of other large farms close by. The stages of emergence associated with the outbreak included a spillover from flying foxes to domestic pigs near Ipoh (see Fig. 4), where farming practices and high pig densities facilitated the dissemination of the infection. Transportation of pigs for commerce led to the southern spread of the outbreak with the amplifying pig host facilitating the transmission of the virus to humans.

The epidemic enhancement of the outbreak included the initial introduction of infection in a naive pig population resulting in a rapid epidemic peak, followed by burn-out and localized human infections. Subsequent introduction(s) into partially immune pig populations resulted in a lower epidemic peak but prolonged duration and increased total number of infectious pigs, increasing the chances of spread to surrounding farms.

Bangladesh

Bangladesh experienced its first reported NiV outbreak in Siliguri and Naogaon in 2001 (Fig. 5). Unlike Malaysia, outbreaks in Bangladesh appeared to be strictly confined to human populations and significantly higher mortality rate. From 2001 to 2018, the WHO reported a total of 261 cases, with 198 deaths (76% mortality) due to NiV infection (see Table II).

The transmission of NiV to humans in Bangladesh was determined to be associated with drinking date palm juice, considered a delicacy in this region of the world. In the Tangail outbreak of 2005, it was

estimated that persons drinking raw date palm sap had a 7.0 odds ratio of developing a NiV infection when compared to controls (95% confidence level, 1.6).

NiV cases in Bangladesh have been seasonal, with human cases reported between the months of January and April. This coincides with the season for collecting date palm sap, late November through April. However, there is significant heterogeneity in the number of spillovers detected by district and year that remains unexplained. Cortes et al., in 2018 analyzed data from all 57 spillovers occurring during 2007–2013 and found that temperature differences explained 36% of the year-to-year variation in the total number of spillovers each winter, and that distance to surveillance hospitals explained 45% of spatial heterogeneity. January, when 40% of the spillover events occurred, was the month with the lowest mean temperature during every year of the study.

Bats are recognized as a nuisance and frequently drink the juice, defecate into juice, and occasionally drown in the palm sap collecting pot. Measures have been put in place to prevent bats access to the sap collecting pot, which has been very effective in reducing the spread of NiV from bats to humans in Bangladesh.

India

In 2001, an outbreak occurred within a hospital in Siliguri, West Bengal. Nosocomial transmission likely occurred, though it is unknown how primary cases were infected. Another outbreak in 2007 was reported in Nadia, West Bengal. Consumption of date palm sap was identified as the likely route of infection of primary cases there. In May of 2018, another outbreak was reported in Kerala. A total of 85 cases were reported in these three outbreaks in 2001, 2007, and 2018, with 62 deaths (73% mortality) due to NiV infection (see Table II).

In 2012, Yadav et al. surveyed the Indian states of Maharashtra and West Bengal to evaluate the presence of viral RNA and IgG against NiV in different bat populations belonging to the species Pteropus giganteus, Cynopterus sphinx and Megaderma lyra. The authors found NiV RNA in Pteropus bat thus suggesting it may be a reservoir for NiV in India.

In 2018, an outbreak of 23 cases of NiV disease was reported in Kerala, India. This was the first spillover in NiV in South India. 18 cases were lab-confirmed and the case fatality rate during this outbreak was 91% (Arunkumar G *et al*. 2018).

Philippines

In 2014, the Philippines reported an outbreak with a zoonotic paramyxovirus in horses and people that is very closely related to NiV based on sequence analysis. Virus isolation was unsuccessful so it was impossible to confirm that there was transmission from presumably bats to horses, from horses to people, and also human to human (Ching P.K., *et al.*, 2015; Clayton, 2017).

New Caledonia

In 2015, three fruit bats tested positive for NiV in New Caledonia at the Noumea National Park, including three bats at the Noumea Zoo.

Research needs

- Improved understanding of infection dynamics in flying foxes: modes of transmission, immune response, evidence of disease, and the implications of co-infection with NiV and other henipaviruses
- Better understanding of co-circulation of different strains / species of henipaviruses within Pteropus populations and the effect of waning herd immunity on outbreaks.
- Other animals such as infected dogs and cats need to be further studied to determine their potential role in the transmission of NiV.
- Improved understanding of infection dynamics in humans: modes of transmission, implications of genetic diversity of the virus for infection, transmission & pathogenicity
- Research into bat populations: additional research regarding bat distributions & ecological impacts
- Research aimed at improving the capacity to diagnose henipavirus infections and improve human health outcomes
- Research into infection and clinical signs in pigs in Bangladesh and potential for pig to human and human to pig transmission.

BIOTERRORISM

The following summarizes the rationale for considering NiV as a potential agent of bioterrorism.

NiV is classified by CDC as a Category C pathogen – emerging pathogens that could be engineered for mass dissemination in the future. Category C include pathogens are readily available, easy to produce, easy to disseminate, and have the potential for high morbidity and mortality with major health impact.

NiV has many of the physical attributes to serve as a potential agent of bioterrorism. The outbreak in Malaysia caused widespread panic and fear because of its high mortality and the inability to control the disease initially. There were considerable social disruptions and tremendous economic loss to an important pig-rearing industry. This highly virulent virus, believed to be introduced into pig farms by fruit bats, spread easily among pigs and was transmitted to humans who came into close contact with infected animals. From pigs, the virus was also transmitted to other animals such as dogs, cats, and horses.

Nipah Virus Bioterrorism Threat Assessment

Virology

- Reverse genetic methods are available for negative strand RNA viruses, including Nipah, and all genomic sequence data is available.
- Many laboratories are actively engaged in research programs on the cell biological properties of the henipaviruses.

- Virus can be amplified to reasonably high unconcentrated titers (>10^7). Several cell culture lines can be used, Vero cell use most often reported, and wild-type virus can be grown and harvested from cell cultures.
- A major constraint in handling Nipah is the requirement for BSL4 facilities; , however, potential terrorists may not respect this need.
- Inactivation of virus can be achieved with a variety of agents typically used for envelope viruses; but extensive environmental stability testing not reported.
- Vaccines and passively-delivered countermeasures are under development both for human and veterinary use. A commercial Hendra virus vaccine is available for horses, and the soluble G protein based vaccine has shown experimental efficacy against Nipah virus in nonhuman primates.
- Bats are sold (often live) in markets throughout their range, providing a potential source of virus; and serological tests are available for identifying henipaviruses

Economic Impact
- Destroyed the main market for Malaysian hogs in Singapore
- ~80% drop in pork consumption in the domestic market.
- Over half the standing pig population in the country was culled to halt the outbreak.
- Half the pig farms went out of business.
- During the outbreak cumulative economic losses based on government figures >$217 million USD.
- Cumulative government costs in operations and lost revenues >$298 million USD.
- Other countries in South East Asia often have a higher pig density than Malaysia. China, with approximately half of the pigs in the world, is especially vulnerable to an economic and public health disaster if NiV were to emerge and be rapidly transmitted between pigs and from pigs to people.

Epidemiology and Clinical Disease
- In outbreaks to date henipaviruses do not appear to be highly infectious. Infection requires close contact with secretions of diseased animals. Many infections can be mild to asymptomatic.
- In the initial 1998-99 outbreak the virus was *initially misdiagnosed* as Japanese Encephalitis; amplification occurred from veterinary reuse of needles in immunization programs to control JE, increasing outbreak severity and extent.
- Time from exposure to signs of infection averages ~2 weeks for humans and seroconversion occurs within a month of onset (dose / route dependent).
- Transmission directly to the vascular system could occur through bites from infected animals or broken skin exposed to secretions of infected animals.
- It is quite likely that an outbreak in animals would result in transmissions to humans.
- An outbreak of Nipah pneumonia or ARDs-like disease with human-to-human transmission as demonstrated in the Bangladesh outbreak could cause significant mortality. Nipah could cause more severe or different disease presentations in older or sick populations.

Viral Transmission
- Deliberate release of virus in some manner is possible.

- Aerosol delivery might transmit the disease effectively to domestic animals, but the environmental requirements for maintaining virus stability are not well known.
- Transmission to humans through consumption of contaminated food has been documented.
- The veterinary reuse of needles in the Japanese Encephalitis immunization campaign and in artificial insemination may have been a factor in the near 100% infection level of Nipah in pigs observed on affected farms.
- Deliberate contamination of veterinary needles could initiate an outbreak in susceptible domestic animals.
- Human-to-human transmission through travel has not been documented, but is possible.
- Transport of infected pigs on trucks was a transmission route in the Malaysian outbreak. Generalizing-- transportation of infected humans on crowded airplanes, buses or trains could also transmit the disease. Human cases have been transported to highly populous cities (e.g. Dhaka) where risk of exposure and spread among the public is increased.

Summary

- Nipah (henipaviruses) can be isolated from animal hosts.
- Several species of fruit bats, including *Pteropus spp.* widely distributed throughout Southeast Asia. The live animals are sold in food markets.
- A Nipah outbreak in swine producing areas can cause an economic crisis, even if human cases do not occur.
- Nipah virus can be amplified in permissive cell cultures (e.g., Vero cells) providing adequate laboratory facilities are available (Biosafety Level 4), although a bioterrorist group would not be restricted from growing the virus because of the lack of BSL-4 facilities.
- Effective aerosol delivery is likely possible but unpredictable on the basis of publicly available information. General unknowns are-- titers necessary for infection, virion stability in vitro, and how infectious the virus would be with this delivery.
- Effective surveillance programs, particularly in pig farming areas, are the best defense for early detection and containment of infection, whatever the source.

SUMMARY OF OBSTACLES TO PREVENTION AND CONTROL

The 2017 gap analysis working group determined that the following countermeasures were important but several weaknesses were identified.

DIAGNOSIS

NiV and HeV are zoonotic paramyxoviruses capable of causing severe disease in humans and animals. These viruses require biosafety level 4 (BSL-4) containment. The availability of safe laboratory diagnostic tests is limited. Sequence variation effects molecular diagnostics; both Clifton Beach (2007) and Redlands (2008) reported that Hendra virus strains failed in AAHL Hendra virus specific real-time PCR. Most published diagnostic PCRs only detect HeV or NiV, but not both. There is a need for a more general PCR to detect divergent and novel strains. Pan-paramyxovirus PCR assays exist and are in use to detect henipaviruses, but limitations in sensitivity limit diagnostic value. The USAID PREDICT program previously used its pan-paramyxovirus PCR assay for surveillance in more than 20 countries in Africa and Asia. Virus isolation and serum neutralization assays require live NiV. There is a need for diagnostics that can be used safely in the laboratory. There is a need for rapid nucleic acid-based assays that can detect all henipaviruses. There is also a critical need for improved antibody-based assays for disease outbreaks and disease surveillance. Importantly, there is a need to develop operator-safe diagnostic tests for which reagents can be produced without requiring high containment facilities.

Currently there are no expectations that validated tests will become available for livestock (or other species) in the near future. Nothing has been done in terms of test harmonization since 2009; however, a number of technology transfers have occurred: from AAHL to laboratories in Asia (Malaysia mainly); limited transfer from NCFAD to India (Bhopal High Containment Animal Health Laboratory); limited transfer from AAHL to the FLI and bilateral transfers between NCFAD and FLI.

VACCINATION

There is currently a commercially available vaccine for horses but no vaccines for swine or human vaccines. The goal for a HeV vaccine for horses is to vaccinate horses in areas at risk for transmission from bats to horses in order to prevent bat to horse transmission and subsequent horse to human transmission. The goal for a NiV vaccine for swine is to have a large stockpile of vaccine available for rapid use in an outbreak situation to prevent swine to swine, swine to human, and perhaps human to swine transmission to control the outbreak. A large stockpile of NiV vaccine, or vaccine antigen concentrate, for rapid emergency use in swine to control a potential outbreak that spreads too quickly to be stamped out in swine dense areas is needed. The vaccine should be licensed in the U.S., E.U or Australia for stockpiling as well as in the countries where NiV is endemic in bats. The stockpile should be available for use internationally where ever it may be needed.

SURVEILLANCE

Passive surveillance is the primary and most economical method used. Passive surveillance in commercial swine herds based on clinical signs has many weaknesses due to the difficulty of differentiating NiV from many common endemic infectious diseases of pigs; e.g., classical swine

fever, porcine reproductive and respiratory syndrome, pseudorabies, swine enzootic pneumoniae, and porcine pleuropneumonia.

In the case of infections in swine where recognition of Nipah symptoms is less likely, surveillance activities must be based on diagnostic testing to supplement surveillance based on clinical signs.

Active surveillance programs are expensive and would have to rely on direct diagnostic tests such as viral isolation and nucleic acid-based assays but available tests have significant weaknesses and have not been validated.

Rapid confirmation of cases is essential. Knowledge on serological cross-reactions with other henipaviruses and/or morbilliviruses in bats is improving. There is an urgent need to establish diagnostic capacity for Nipah virus in countries that are most likely to experience spillovers from the bat reservoirs.

DEPOPULATION

Depopulation is the primary countermeasure to reduce virus shedding and stop the spread of Nipah virus in swine. Recent outbreaks have shown that the control of Nipah virus in pig populations through stamping out is complex due to the zoonotic nature of the agent and may be very expensive, particularly in areas with high pig densities. Because Nipah virus spreads rapidly and silently in pigs, a large number of animals would need to be pre-emptively culled if an outbreak occurred in the U.S, or in other swine dense countries in order to minimize the virus spread in the vicinity of infected herds. Thus, this method of control would have significant financial implications due to the culling of thousands or millions of animals.

COUNTERMEASURES ASSESSMENT

ASSUMPTIONS

The following captures assumptions made by the gap analysis working group to assess potential countermeasures to enhance our ability to contain and eradicate an outbreak of NiV.

Situation
Countermeasures assessed for worst case scenario: A coordinated intentional distribution of NiV-contaminated material in a high density highly populated pig region of the United States.

Target Population
Countermeasures assessed for target pig production segments in priority order:

1. Backyard pigs
2. Comprehensive commercial swine operations (farrowing, nursery, and finishing)
3. Commercial indoor farrowing operations
4. Large intensive indoor pig farms
5. Valuable commercial genetic swine stock

Scope of Outbreak
Countermeasures assessed for multiple outbreaks occurring simultaneously in backyard pigs, three farrowing commercial operations, a finishing pig commercial operation, a sow replacement operation, and evidence of infection in feral swine.

DECISION MODEL

The quantitative Kemper-Trego (KT) decision model was used to assess available vaccines and diagnostics. For the criteria and weights used to assess NiV vaccines and diagnostics (See Appendices II, III).

Criteria
The following critical criteria were selected to enable the comparison of countermeasures using a pertinent and valid analysis, as follows:

<u>Vaccines</u>

- Efficacy
- Safety
- One dose
- Manufacturing safety
- DIVA compatible
- Manufacturing yield
- Rapid production
- Reasonable cost
- Short withdrawal period
- Long shelf life

Diagnostics

- Sensitivity
- Specificity
- DIVA detection
- Multispecies
- Validation to purpose
- Speed of scale-up
- Throughput
- Pen-side test
- Rapid result
- No need for a confirmatory test
- Easy to perform
- Safe to operate
- Availability
- Storage/Distribution
- Low cost to implement
- Perform at BSL-2
- Does not require use of live virus to prepare reagents

Weight

Each criterion was weighted to allow a quantitative comparison of the impact of the selected interventions (See Appendices II and III).

Product profile

To ensure a consistent and meaningful assessment, the desired product profile (i.e., the benchmark) was identified for each countermeasure:

Desired Vaccine Profile

1. Highly efficacious: prevent transmission; efficacy in all age animal target hosts, including maternal antibody override; cross protection across all henipavirus strains; quick onset of immunity; multiple animal target hosts; one year duration of immunity
2. Safe in all age animal target hosts; no reversion to virulence for live vaccines
3. One dose
4. Safe vaccine strain for manufacturing
4. DIVA compatible
5. Manufacturing method yields high number of doses
6. Rapid speed of production and scale-up
7. Reasonable cost
8. Short withdrawal period for food consumption
9. Long shelf life

Desired Diagnostic Test Profile
1. Detect all henipavirus
2. Identify Nipah virus specific strains
3. Direct tests for control and eradication
4. Indirect tests for post-control monitoring
5. Rapid test
6. >95% specificity
7. >95% sensitivity
8. Pen-side test
9. DIVA Compatible
10. Field validated
11. Easy to perform/easily train NAHLN's personnel
12. Scalable
13. Reasonable cost
14. Operator safe
15. Reagents can be produced in low containment

Values
The values assigned for each of the interventions reflect the collective best judgment of members of the gap analysis working groups (See Appendices I and II)

VACCINES
The human infections in the 1999 outbreak in Malaysia were linked to transmission of NiV from pigs. Accordingly, a swine vaccine able to prevent virus transmission would be an important tool to safeguard commercial swine operations and people at risk. In addition, since henipaviruses have a very broad host range, a vaccine that is efficacious in multiple susceptible animal species would be desirable. Although the 2017 gap analysis working group determined that there are still no NiV commercial vaccines available, there are several vaccine candidates that may be safe and effective in swine and other domestic animals that were recently reviewed in: (Weingartl H.M., 2015; Broder, C.C., *et al, 2016*; and Satterfield, B.A., *et al.,* 2016). After these reviews were published, a manuscript was published demonstrating the efficacy of a virus-like-particle (VLP) Nipah virus vaccine in hamsters for inducing virus neutralizing antibodies and protection from challenge (Walpita P., *et al.,* 2017). Another manuscript was published that concluded that an adjuvanted Hendra soluble G vaccine in pigs induced neutralizing antibody titers considered to be protective against Nipah virus without detectable T cell-mediated immunity to Nipah, which did not protect from challenge with Nipah virus. However, pigs that had been previously challenged with a low dose of NiV developed neutralizing antibodies and cell-mediated immune memory and were protected from a high challenge dose of NiV. The conclusion of this manuscript was that both virus neutralizing antibodies and cell-mediated immunity were necessary for protection from NiV challenge (Pickering B.S., *et al.,* 2016). Subsequent unpublished research demonstrated that a different adjuvant used with the soluble Hendra G vaccine caused the induction of both high tittered virus neutralizing antibody and detectable T cell-mediated immunity in pigs to NiV. Challenge studies were not conducted (J.A. Roth, personal communication). All of these vaccine candidates would need further research and development to be licensed, and would need to be made available as a stockpile for rapid use in an emergency if an outbreak in swine were to occur that could not be effectively stamped out. A swine vaccine would

also be needed if the Nipah virus were to mutate to be efficiently transmitted between people and between people and pigs.

Summary

- Vaccination against NiV has been successfully demonstrated
- Experimental henipavirus vaccines can prevent clinical disease
- Experimental henipavirus vaccines elicit systemic and mucosal immunity
- Experimental henipavirus vaccines prevent viral replication in target tissues
- HeV commercial vaccine Equivac® HeV does not cross protect against NiV infection in swine
- Henipavirus vaccines appear to be effective in several mammalian animal species

Assessment of Commercial Vaccines

A commercial vaccine (Equivac® HeV) against Hendra virus approved for use in horses (Middleton D.J. *et al.,* 2014) was registered by Zoetis in Australia in 2015. A six month booster dose is required for full protection, followed by annual vaccination. The vaccine is also approved for pregnant mares. There is currently no NiV vaccine approved for swine. Likewise, there is no vaccine against HeV or NiV approved for human use.

Assessment of Experimental Vaccines

The working group felt that limited information was available to assess and contrast experimental vaccines that have been reported in the literature. Experimental animal vaccines under investigation are summarized in Table I. Experimental vaccines for humans are summarized in Table II. Several of the working group members have directly or indirectly been involved in the research associated with these vaccines so that an assessment could be made (See Appendix I). The following describes some of the most promising experimental vaccine technologies.

1) Canarypox-vectored NiV Vaccines

The ALVAC canarypox virus-based recombinant vaccine vector (Taylor *et al.,* 1994) was used to construct two experimental NiV vaccines (Weingartl *et al.,* 2006). These experimental vaccines were engineered by Merial.

The first construct carries the gene for NiV attachment glycoprotein G (ALVAC-G). The second construct carries the NiV fusion protein F (ALVAC-F).

The efficacy of both the ALVAC-G and ALVAC-F were tested in pigs either as monovalent vaccine or in combination (ALVAC-G/F). The vaccine dose used was 10(8) PFU. The vaccine regimen was two doses administered 14 days apart. Both non-vaccinated controls and vaccinated pigs were challenged with 2.5 x 10(5) PFU of NiV two weeks later.

The combined ALVAC-F/G vaccine induced the highest levels of neutralization antibodies. Despite the low neutralizing antibody levels induced by ALVAC-F all vaccinated animals were protected against challenge. Virus was not isolated from the tissues of any of the vaccinated pigs post-challenge, and a real-time reverse transcription (RT)-PCR assay detected only small amounts of viral

RNA in several samples. In challenge control pigs, virus was isolated from a number of tissues or detected by real-time RT-PCR. Vaccination of pigs with the ALVAC-F/G stimulated both type 1 and type 2 cytokine responses. No virus shedding was detected in vaccinated animals, in contrast to challenge control pigs, from which virus was isolated from the throat and nose.

Based on the data generated in this one study, both the ALVAC-G or the combined ALVAC-F/G vaccine appears to be a very promising vaccine candidate for swine.

2) Soluble G Henipavirus Vaccine

HeV and NiV infect cells by a pH-independent membrane fusion event mediated by their attachment (G) and fusion (F) glycoproteins. Scientists at the Uniformed Services University of the Health Sciences in Bethesda, Maryland, in collaboration with the Australian Animal Health Laboratory characterized HeV- and NiV-mediated fusion activities and detailed their host-cell tropism characteristics. These studies suggested that a common cell surface receptor was utilized by both viruses. To further characterize the G glycoprotein and its unknown receptor, soluble forms of HeV G (sG) were constructed by replacing its cytoplasmic tail and transmembrane domains with an immunoglobulin kappa leader sequence coupled with an S-peptide tag (sG) to facilitate purification and detection. Expression of sG was verified in cell lysates and culture supernatants by specific affinity precipitation. Analysis of sG by size exclusion chromatography and sucrose gradient centrifugation demonstrated tetrameric, dimeric, and monomeric species, with the majority of the sG released as a disulfide-linked dimer. Immunofluorescence staining revealed that sG specifically bound to HeV and NiV infection-permissive cells. The scientists further reported that administration of sG to rabbits can elicit a potent cross-reactive neutralizing antibody response against infectious HeV and NiV (Bossart *et al.* 2005). The HeV sG subunit vaccine has been the most extensively studied NiV/HeV vaccine platform because of its ability to elicit a potent cross-protective immune response to NiV and has been shown to induce potent cross-reactive neutralizing antibody responses in a variety of animals including mice, rabbits, cats, ferrets, monkeys and horses.

Experimental subunit vaccine formulations containing either HeV sG or NiV sG were first evaluated as potential NiV vaccines in the cat model. Two cats were immunized with HeV sG and two cats were immunized with NiV sG. Immunized animals and two additional naïve controls were then challenged subcutaneously with 500 TCID50 of NiV. Naive animals developed clinical disease 6 to 13 days post-infection, whereas none of the immunized animals showed any sign of disease (Mungall *et al.,* 2006).

In a subsequent experiment, an experimental subunit formulation containing HeV sG and CpG adjuvant was evaluated as a potential NiV vaccine in the cat model. Vaccinated animals demonstrated varying levels of NiV-specific Ig systemically and importantly, all vaccinated cats possessed antigen-specific IgA on the mucosa. Upon oronasal challenge with NiV (50,000 TCID50), all vaccinated animals were protected from disease although virus was detected on day 21 post-challenge in one animal. (McEachern *et al.,* 2008).

Additional studies with the HeV-sG vaccine in the ferret model formulated in CpG and Allhydrogel™ and could provide complete protection from a 5,000 TCID50 dose of HeV (100 times the minimal lethal dose) with no disease or evidence of virus or viral genome in any tissues or body fluids and only a low level of HeV genome detected in the nasal washes from 1 of 4 animals in a low-dose vaccine

group, and no infectious HeV could be recovered from any immunized ferrets (Pallister J, et al. A recombinant Hendra virus G glycoprotein-based subunit vaccine protects ferrets from lethal Hendra virus challenge. Vaccine. 2011;29:5623-30) In a similar study with NiV-B, vaccinated ferrets remained disease free, and virus or viral genome was undetectable in all tissues and fluids with no observed pathology in examined tissues. The study also revealed good durable immunity with other ferrets challenged 434 days post-vaccination, with 5 of 5 animals were disease free following challenge and viral genome was detected only from the nasal secretions of one ferret and the bronchial lymph nodes of another ferret that were given an intermediate vaccine dose (Pallister JA, et al. Vaccination of ferrets with a recombinant G glycoprotein subunit vaccine provides protection against Nipah virus disease for over 12 months. Virol J. 2013;10:237).

The HeV-sG subunit vaccine has also been evaluated in the African green monkey (AGM), which is the only nonhuman primate model that has uniformly recapitulated human disease for both NiV and HeV infection (Rockx B, et al. A novel model of lethal Hendra virus infection in African green monkeys and the effectiveness of ribavirin treatment. J Virol. 2010;84:9831-9; Geisbert TW, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS One. 2010;5:e10690). HeV-sG was initially tested by formulation in Allhydrogel^{TM} and CpG and animals were challenged by intratracheal administration with a 10-fold lethal dose of NiV (1×10^5 TCID50). Complete protection was observed in all vaccinated animals with no evidence of clinical disease, virus replication, or pathology in any vaccinated subject with some having pre-challenge NiV neutralizing titers as low as 1:28. A second study demonstrated HeV-sG vaccination and protection from a HeV in the AGM model and also showed that HeV-sG in Allhydrogel^{TM} alone was suffient to confer complete protection from infection and disease (Mire CE, et al. A recombinant Hendra virus G glycoprotein subunit vaccine protects nonhuman primates against Hendra virus challenge. J Virol. 2014;88:4624-31). The HeV-sG subunit vaccine is now being evaluated as a NiV/HeV vaccine for human use with support from the Coalition for Epidemic Preparedness Innovations (CEPI) (Hum Vaccin Immunother. 2017 Dec 2;13(12):2755-2762. doi: 10.1080/21645515.2017.1306615. Vaccines for epidemic infections and the role of CEPI. Plotkin SA)

A recent publication demonstrated that an adjuvanted HeV-sG vaccine in pigs induced SN antibody titers considered to be protective against NiV without detectable T cell-mediated immunity to NiV which did not protect from challenge with NiV. Pigs which had been previously challenged with a low dose of NiV developed SN antibodies and cell-mediated immune memory and were protected from a high challenge dose of NiV. The conclusion of this manuscript was that both SN antibodies and cell-mediated immunity were necessary for protection from NiV challenge (Protection against henipaviruses in swine requires both, cell-mediated and humoral immune response, B.S. Pickering, J.M. Hardham, G. Smith, E.T. Weingartl, P.J. Dominowski, D.L. Foss, D. Mwangi, C.C. Broder, J.A. Roth, H.M. Weingartl, Vaccine 34(40): 4777-4786, 2016). Subsequent unpublished research demonstrated that a different adjuvant used with the soluble HeV-sG vaccine caused the induction of both high tittered SN antibody and detectable T cell-mediated immunity in pigs to NiV. Challenge studies were not conducted (J.A. Roth, personal communication).

3) Vaccinia-vectored NiV Vaccine

The NYVAC vaccinia virus-based recombinant vaccine vector (Tartaglia *et al.,* 1992) was used to construct an experimental NiV vaccine where the vaccinia virus expresses both the NiV glycoproteins G and F (Guillaume *et al.,* 2004). This experimental vaccine was engineered by the Pasteur Institute.

Scientists at the Pasteur Institute in collaboration with University of Malaysia scientists showed that both of the NiV glycoproteins G and F when expressed as vaccinia virus recombinants induced an immune response in hamsters that protected against a lethal challenge with NiV. Furthermore, this team of scientists demonstrated passive transfer of antibody induced by either of the glycoproteins protected the animals.

DIAGNOSTICS

The gap analysis working group determined that the availability of validated diagnostic tests for surveillance, early detection, and recovery during a NiV outbreak were critical to minimize the spread of disease and reduce the economic and public impact.

Currently the diagnosis of NiV infection is by virus isolation, detection of viral RNA, or demonstration of viral antigen in tissue collected at necropsy. Specific antibody detection can also be useful, particularly in pigs where NiV infection may go unnoticed. Demonstration of specific antibody to NiV in either animals or humans is of diagnostic significance because of the rarity of infection and the serious zoonotic implication of NiV transmission.

Summary

- Antibody responses to NiV take at least 14 days and therefore early diagnosis based on serology will be less reliable than antigen or molecular tests
- Recombinant N-ELISA will likely not pick up all infected pigs
- The concept of a pen-side test is attractive, but the development and regulation of such a test will be extremely challenging

Assessment of Laboratory Diagnostic Tests (See Appendix II)

Details in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2015, Chapter 2.1.14 Hendra and Nipah Virus Diseases, provides recommendations for the following tests.

Identification of the agent

1. Virus isolation and characterization
    1.1. sampling and submission of specimens
    1.2. isolation in cultured cells
    1.3. Identification: immunostaining and Immuno EM
2. Viral identification: differentiation of HeV and NiV
    2.1 comparative immunostaining
    2.2. immunofluorescence
    2.3. microtiter neutralization

3. Molecular methods
    3.1. real-time RT-PCR
    3.2. Conventional RT-PCR and Sanger sequencing

4. Immunohistochemistry

Serological tests
1. Virus neutralization tests
2. Enzyme-linked immunosorbent assay
3. Bead-based assays

Histopathology
1. Veterinary diagnostic labs might use histopathology to make the first diagnosis
2. NiV does not produce pathognomonic lesions, but a generalized vasculitis with fibrinoid necrosis in several tissues (e.g. lung and kidneys) is characteristic; NiV might be considered in the initial differential diagnosis by experienced veterinary pathologists.

Assessment of Available Diagnostic Tests
Australia, Canada, and Germany have diagnostic capability for henipaviruses in livestock; India (e.g. NIHSAD) is building its veterinary diagnostic capability; U.S. veterinary diagnostic laboratories do not have diagnostic capability to detect NiV in livestock, although the Center for Disease Control (CDC) in Atlanta, Georgia, is an OIE collaborating center for NiV.

Currently, there are no expectations of validated tests for livestock (or other species). Nothing has been done in terms of test harmonization for serological, antigen, or nucleic acid detection assays; however, successful technology transfers have taken place, as follows: from AAHL to laboratories in Asia (Malaysia mainly); limited transfer from NCFAD to India (Bhopal High Containment Animal Health Laboratory); limited transfer from AAHL to FLI and bilateral transfers between NCFAD and FLI.

Serologic testing plays an important role in the diagnosis and detection of NiV infections. Serologic tests are the most straightforward and practical means to confirm acute cases of disease and serologic evidence of infection is used in screening programs for reservoir hosts and domestic animals. However, serological assays are limited in their ability to differentiate between known and unknown henipaviruses, as cross-reactivity to one or more known viruses is possible. Both serum neutralization and Luminex assays have shown positive reactivity to both NiV and HeV in bats where the presence of a yet-to-be characterized henipavirus could not be ruled out.

Several standard and new experimental technologies that are currently being used or considered for the detection of NiV in the laboratory or as pen-side tests for field use. Shedding of NiV in oral fluids starts early post-infection and rope sampling could prove convenient for collecting samples that could be used to test larger numbers (i.e., pen tests) of pigs. Suitability of oral fluid samples for various test platforms should be investigated. There is a need to develop a formalized worldwide structure for test validation and ring trials (i.e., inter-laboratory comparisons).

The following describes some of the most promising diagnostic platforms with potential application for NiV detection.

1) *Quantitative (q) real-time PCR*

Real-time PCR is a sensitive and useful approach to the detection of henipavirus genome in specimens. Due to its nature, rRT-PCR may not be able to detect all divergent and novel henapivirus strains, although adaptation of molecular tests to new virus variants could be rapid. Test methods and primers used depend on the technology platform and associated chemistry being used in individual laboratories. Test procedures have been described by different laboratories (Mungall *et al.,* 2006; Wacharapluesadee and Hemachudha, 2007; Guillaume *et al*., 2004; Chang *et al*., 2006; Feldman *et al*., 2009).

The AAHL has developed a quantitative real-time PCR to detect NiV or HeV RNA synthesis. The most commonly targeted amplification regions are directed against the N gene (Feldman et al., 2009).

RT-PCR targeting the N gene of NiV will detect both, NiV-M and NiV-B, with somewhat lower sensitivity for NiV-B. Confirmatory RT-PCR targeting the F gene specific only for NiV-B has therefore been developed (publication in preparation; H.M. Weingartl, personal communication).

2) *Conventional PCR*

Classical RT-PCR followed by sequencing may be more successful in detecting novel henipavirus strains. Combination of both approaches may need to be considered. Genomic RNA detection can be performed on blood or serum samples collected from live animals as well as tissues from dead animals. RNA is extracted using an RNA extraction kit [e.g., RNeasy Mini Kit (Qiagen)]. Extracted total cellular RNA is first subjected to first-stand cDNA synthesis using a reverse transcriptase kit [e.g., SensiScript (Qiagen)] and a reverse transcriptase primer. The resulting cDNA is amplified using a Master Mix PCR kit (Qiagen) and primers that are designed to target HeV and NiV positive-sense mRNA from the N, M and G genes and negative-sense genomic viral RNA (vRNA) at the N/P, M/F and F/G gene junctions.

3) *Field PCR*

Not available. Isothermal real-time RT-PCR is promising as a field deployable assay.

While this will be costly and not be practical to have in large numbers, it is worth considering having the capabilities to establish in several strategically located regions across the nation to response rapidly in an emergency situation. Technically it will not be difficult to achieve if there is the will and financial support.

4) *Virus isolation (VI)*

Virus isolation in permissive cell culture is considered the "gold standard" for isolating all strains of henipaviruses. Virus isolation greatly facilitates identification procedures and definitive diagnosis should be undertaken where operator safety can be guaranteed. Isolation is especially relevant in any new case or outbreak, particularly in countries or geographical areas where infection by NiV or HeV has not been previously documented. Implication of wildlife species as natural hosts of the viruses requires positive serology, PCR or virus isolation from wild-caught animals (Daniels *et al.,* 2007). The range of tissues yielding virus in natural and experimental cases include the brain, lung, kidney and spleen (Crameri G., *et al.* 2002).

Henipaviruses grow rapidly to high titers in a large number of cell lines. African green monkey kidney (Vero) and rabbit kidney (RK-13) cells have been found to be particularly susceptible. A CPE usually develops within 3 days, but two 5-day passages are recommended before judging the attempt unsuccessful. After low multiplicity of infection, the CPE is characterised by formation of syncytia that may, after 24–48 hours, contain over 60 or more nuclei. Syncytia formed by NiV in Vero cell monolayers are significantly larger than those created by HeV in the same time period. Although the distribution of nuclei in NiV-induced syncytia early in infection resembles that induced by HeV, with nuclei aggregated in the middle of the syncytia, nuclei in mature NiV-induced syncytia are distributed around the outside of the giant cell (Hyatt *et al.*, 2001).

Very low virus load in bats makes isolation very difficult. Linfa Wang and colleagues at the AAHL have increased sensitivity of cell lines by "rational engineering," consisting of a single point mutation in ephrinB2 resulting in enhanced affinity for NiV.

5) *Pen-side test*

Not yet developed.

While the concept is attractive, it is a huge challenge technically and in regulatory sense, especially considering how presumable false positive results would be handled.

6) *N and G ELISA*

Indirect recombinant N- ELISA and G-ELISA have been developed, and are now in the stage of diagnostic evaluation (Fisher K., *et al.*, 2018). The N-ELISA protocol was transferred to HSADDL (India) and validated and used for surveillance (Kulkarni *et al.,* 2016).

Problems with specificity (i.e., false positives) could arise. For example, swine sero-surveillance in West Bengal, India, appears to be negative; however, 8/328 samples tested positive (i.e., presumably false positive) using the anti-N antigen ELISA antibody detection test. Evaluation of the indirect IgG ELISA based on the recombinant NiV-N antigen using swine samples from Canada yielded similar results, including an indirect IgG ELISA based on the G glycoprotein. In Canadian context, the problem is the diagnostic specificity, with 5% false positives, resulting in the decision to complement with the G-ELISA. Only sera positive on both tests are considered

positive. Confirmatory testing may be required, if this was to be the first case reported in non-endemic area.

A diagnostic test for differentiating infected from vaccinated animals (DIVA) would have to most likely target the N antigen, or alternatively P gene coded products depending on the level of expression and antigenicity in animals, and the number of reactors in non-endemic areas.

The N ELISA assay could fulfill DIVA requirements if the canarypox vectored NiV-G-NiV-F vaccine is used because antibodies to N would only occur after NiV infection.

7) *IgM ELISA*

The U.S Center for Disease Control and Prevention (CDC) developed an IgM ELISA for human serology. Detection of IgM was used to confirm recent infection with NiV in both Malaysia and Bangladesh. NiV-infected cells that have been inactivated by gamma irradiation are used as antigens.

In theory the same can be done for different animal species as long as we have the right anti-species antibodies. For bats, that is still a challenge.

8) *Virus neutralization test (VNT)*

VNT serves as the traditional gold standard of serological investigations. The VNT requires live virus and thus BSL-4 containment facilities are required (Crameri *et al.,* 2002). It has proven to be a very valuable specific and sensitive tool in the diagnosis of NiV.

VNT rely on quantification methods. Three different procedures are available to titer HeV and NiV. In the traditional plaque and microtiter assay procedures, the titer is calculated as plaque forming units (PFU) or the tissue culture infectious dose capable of causing CPE in 50% of replicate wells ($TCID_{50}$), respectively.

In an alternative procedure, the viruses are titrated on Vero cell monolayers in 96-well plates and after 18–24 hours, foci of infection are detected immunologically in acetone-fixed cells using anti-viral antiserum (Crameri G., *et al.* 2002). The virus titre is expressed as focus-forming units (FFU)/ml.

Neutralisation assays using these three methods are described in the OIE Manuel of Diagnostic Tests and Vaccines for Terrestrial Animals.

Virus quantification procedures should be conducted at BSL4. A new version of the differential neutralisation test has been recently described, which avoids the use of infectious virus by the use of ephrin-B2-bound biospheres (Bossart *et al.,* 2007). Although the test has yet to be formally validated, it appears to have the potential to be a screening tool for use in countries without BSL4 facilities.

9) Pseudotype virus plaque reduction neutralization test (PRNT)

The standard plaque reduction neutralization assay (PRNT) used to detect NiV and HeV must be performed in BSL-4 containment and takes several days to complete. The CDC and the AAHL have modified the PRNT by using recombinant Vesicular Stomatitis Virus (VSV) derived from the cDNA of VSV Indiana to construct pseudotype particles expressing the F and G proteins of NiV (pVSV-NiV-F/G) as target antigens (Chang *et al.,* 2006; Tamin *et al.,* 2009; Kaku *et al*., 2009). This rapid assay can be performed at BSL-2. The PRNT was evaluated using serum samples from outbreak investigations and more than 300 serum samples from an experimental NiV vaccination study in swine. The results of the neutralization assays with pVSV-NiV-F/G as antigen showed a good correlation with those of standard PRNT. The PRNT titers give an indication of protective immunity. Therefore, this new method has the potential to be a rapid and cost-effective diagnostic method, especially in locations that lack high containment facilities, and will provide a valuable tool for basic research and vaccine development. A similar assay has been developed by the Japanese-Australian group (Kaku et al., 2009), which proved to be as specific as the VNT and much more sensitive than VNT.

10) Serological Binding Assay

Currently, a Luminex®-based (e.g. Bio-Rad Bio-Plex) multiplex microsphere immunoassay for the detection of antibodies specific to HeV and NiV G glycoproteins is used for bat surveillance at the AAHL, and by other research investigators. This multiplex microsphere immunoassay detects antibodies to recombinant soluble G (sG) proteins from NiV and HeV in a multiplexed assay. In contrast to traditional ELISAs, these Luminex-based platforms are more sensitive and require less sample sera to generate results with multiple analytes. The sG proteins retain their ability to bind the cellular receptor molecule, indicating their native conformation is maintained, which is important for the detection of neutralizing antibodies. Since the G specific antibody response to both NiV and HeV can be measured simultaneously, this assay can differentiate between the serologic responses to NiV and HeV. A variety of statistical models have been developed to determine thresholds to determine the cutoff value between negative and positives median fluorescence intensities (MFI). Instances when negative control sera is available, a MFI value three standard deviations above the z score can be used to interpret the cutoff for positive valuves.

11) Luminex® multiplexed nucleic acid detection assay

Foord et al, 2012, reported microsphere suspension array systems enable the simultaneous fluorescent identification of multiple separate nucleotide targets in a single reaction using commercially available oligo-tagged microspheres (Luminex® MagPlex-TAG) to construct and evaluate multiplexed assays for the detection and differentiation of HeV and NiV. Assays were developed to target multiple sites within the nucleoprotein (N) and phosphoprotein (P) encoding genes. The relative specificities and sensitivities of the assays were determined using reference isolates of each virus type, samples from experimentally infected horses, and archival veterinary diagnostic submissions. Results were assessed in direct comparison with an established qPCR. Foord reported the microsphere array assays achieved unequivocal differentiation of HeV and NiV

and the sensitivity of HeV detection was comparable to qPCR, indicating high analytical and diagnostic specificity and sensitivity.

12) Blocking Luminex® Assay

This is an extension of the Binding Luminex Assay, developed as a surrogate VNT in the sense that it measures antibodies that block the binding of the soluble henipavirus G protein to the ephrin-B2 receptor molecule. It is highly specific, but needs further validation with field samples.

DEPOPULATION

Preemptive culling of herds in the neighborhood of an infected herd is an effective and even indispensable measure in the control of a NiV epidemic in areas with high pig densities. The purpose of this measure is to prevent infection of new herds, which would generate massive infectious virus production, and thus to reduce the virus infection load in an area. This reduced infection load subsequently results in a reduction of the between-herd virus transmission. However, recent outbreaks have shown that the control of Nipah virus in pig populations through stamping out is complex due to the zoonotic nature of the agent. In addition, depopulation may be logistically difficult and very expensive in swine dense area, and would not be effective if the Nipah virus mutates to become easily transmitted between people and from people to pigs. Depopulation will not be possible in situation like those that occurred in Bangladesh in which NiV was transmitted from bats to humans without an amplifying host. Depopulation of swine may be impossible in a rapidly spreading outbreak in a pig dense region with hundreds of millions of swine, such as in southeast China (Vergne T. *et. al*. 2017).

SURVEILLANCE

The initial expression of NiV in U.S swine would be variable and unpredictable due to the myriad of host factors and the broad diversity of virulence among strains of henipaviruses. Different surveillance strategies will be required to detect the different clinical manifestations.

For acute infection, surveillance activities can be based on clinical signs, but signs are unlikely to be noticed by producers and practitioners. It would be prudent to develop surveillance activities based on diagnostic testing to supplement surveillance based on clinical signs.

The following surveillance programs are in place to meet the objective of rapid detection of henipaviruses in Malaysia and Australia:

1. Population-based passive reporting of suspicious NiV cases. Efforts to enhance reporting will be focused on high risk areas.
2. Laboratory-based surveillance of serum and tissue submitted from sick pigs.

There is no diagnostic capability for henipaviruses in United States veterinary diagnostic laboratories due to the lack of BSL-4 laboratory space. The only diagnostic capability for henipaviruses in the U.S is the Center for Disease Control and Prevention (CDC). There are no active or passive surveillance

programs. Henipavirus suspect samples would be sent to the CDC, the OIE reference laboratory at the Australian Animal Health Laboratory, or the National Canadian Foreign Animal Disease Center, in Winnipeg, Canada.

DRUGS

There are no licensed anti-viral drugs available to treat people or animals against Henipaviruses.

DISINFECTANTS

People: Soaps and detergents.

Fomite disinfection: Sodium hypochlorite to supply 10,000 ppm chlorine or Virkon.

PERSONAL PROTECTIVE EQUIPMENT (PPE)

PPE should be suitable to prevent farm-to-farm virus spread by diagnostic or vaccination teams.

RECOMMENDATIONS

RESEARCH

The 2017 gap analysis working group recommended the implementation of the following research priorities.

Viral Pathogenesis

- Determine early events of NiV infection, immune evasion and identify determinants for virulence and host susceptibility

Immunology

- Characterize the antibody and cell-mediated immune response to NiV infection and vaccination
- Develop the basic knowledge of the mechanisms NiV uses to evade the innate immune response
- Characterize the ability of interferons to inhibit virus replication and shedding early in infection.

Vaccine Discovery and Development Research.

- Implement comprehensive vaccine research program to deliver next generation NiV vaccines (e.g., DIVA [differentiate infected from vaccinated animals] capable), and specifically design strategies for control in priority susceptible hosts
- Investment in Nipah vaccine development needs to include conducting studies to demonstrate safety and efficacy necessary for licensure by authorities in countries that may have an emergency need for vaccine in swine.

Diagnostics

- Develop a panel of reference standards for both molecular and serologic tests that can be made available to all of the laboratories performing diagnostic tests for henipaviruses. This panel should also include monoclonal antibodies and recombinant antigens that would be readily available as low biosecurity BSL-2 reagents.
- Develop a formalized structured worldwide network for reference panel development and assay validation and harmonization.
- Develop and validate broadly reactive PCR assays targeting highly conserved genetic targets within the henipaviruses. Evaluate the relative sensitivity and specificity of the currently used PCR assays.
- Develop and validate field tests (both protein- and nucleic acid-based) to detect henipaviruses.
- Explore new antigen detection assays, including antigen capture, Loop Mediated Isothermal Amplification Protocol (LAMP) suitable for resource limited situations, and nanotechnology.
- Develop species specific reagents to improve the quality of serologic assays.
- Evaluate the relative sensitivity and specificity of molecular and serologic tests, especially new serologic tests that could replace serum neutralization titers (SNT) and meet DIVA requirements.
- Explore the use of serological assays based on recombinant antigens that could be produced at BSL-2. Classical serological tests using low biosecurity (recombinant) reagents produced at BSL-2 facilities could be developed reasonably quickly and at a reasonable cost.
- Develop species independent serologic assays using recombinant antigens.

Epidemiology

- The epidemiology of NiV in disease outbreaks needs to be assessed and modeled on the level of the individual pig, the herd, and the demographics of the region.
- Epidemiological investigations should be performed on the implementation of emergency vaccination and the use of 'DIVA' and other diagnostic tests to detect infected pigs in vaccinated populations
- Risk assessments need to be performed with regard to control or spread of henipaviruses
- The epidemiological evaluation of wildlife needs to be carried out in order to improve the risk estimates of outbreaks in domestic animal and human populations

PREPAREDNESS

Many of the countermeasures discussed in this report will require preparation and integration in a coordinated disease control program and funding for a stockpile for use in an emergency response plan for an outbreak of NiV infection. The Henipavirus gap analysis working group recommends investing in the implementation of the following preparedness plan to ensure the effective use of the countermeasures in the NVS:

- See the Ausvetplan: https://www.animalhealthaustralia.com.au/our-publications/ausvetplan-manuals-and-documents/ Assessed July 22, 2018
- See Guidelines for Veterinarians Handling potential Hendra Virus infection in Horses (QDPI): https://www.daf.qld.gov.au/__data/assets/pdf_file/0005/126770/2913_-Guidelines-for-veterinarians-handling-potential-Hendra-virus-infection-in-horses-V5.1.pdf Accessed July 22, 2018

Surveillance

Routine surveillance for NiV is now limited to serologic screening of pigs in several Southeast Asian countries.

- Develop a regional surveillance strategy, including laboratory, to detect spillovers of NiV into domestic and agricultural animals.
- Determine the optimal surveillance strategy to detect circulation of NiV in the bats reservoirs and other wild life.
- Improve surveillance capacity to detect henipaviruses in high risk countries.
- Establish a formal laboratory network for henipavirus surveillance that includes standardized specimen collection, laboratory testing scheme, quality control, specimen referral and accreditation.

Biosecurity

Design NiV-specific on-farm biosecurity programs to implement in a disease outbreak situation.

Personal Protective Equipment and Decontamination

- See Australian procedures https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0003/494202/Hendra-virus-ppe-procedures.pdf Assessed July 22, 2018

- FAO (Food and Agriculture Organization) – Manual on the Diagnosis of Nipah Virus in Animals: Chapter 2: Working safely with Nipah Virus
  http://www.fao.org/docrep/005/AC449E/ac449e05.htm#bm05
  Assessed July 22, 2018

Depopulation and Disposal

Develop plans for handling disposal of animals infected with a zoonotic agent, including an emergency plan to dispose of infected swine and decontaminate facilities and equipment determined to be infected.

- FAO (Food and Agriculture Organization) – Manual on the Diagnosis of Nipah Virus in Animals: Chapter 5: Control and eradication
  http://www.fao.org/docrep/005/AC449E/ac449e08.htm#bm08

CONCLUSION

The threat of an outbreak with a henipavirus in the United States due to a natural transmission from a reservoir host is very low since the reservoirs are known to be bats in South East Asia, South Asia, and Asia. However, an outbreak that is not controlled in swine or in people in Asia could result in infection being introduced accidentally into North America or Europe. There is considerable concern that henipaviruses could be used as a weapon of mass destruction (WMD) because they have many of the characteristics of the ideal biological weapon, including causing one of the highest mortality rate in people known for an infectious disease. The possibility of an intentional criminal spread at least in short clusters of terrorist attacks is a distinct possibility, for example by aerosolization in confined public spaces, or through infection of pigs. Surveillance brings challenges and weaknesses of diagnostic methods may impede the early detection of an outbreak in the United States. There are no commercially available diagnostic tests and although laboratory tests are available they have not been field validated. Depopulation is the primary method to eradicate NiV but present very high risks since henipaviruses are BSL-4 zoonotic agents. There are commercially available vaccines for horses, but none for swine and people. Accordingly, the gap analysis working group recommends investing in the research and development of countermeasures and ensure their use and integration in planning for preparedness and future control campaigns. Priority should be given to funding research to improve surveillance, diagnostics, and vaccines. Specific goals include 1) improving diagnostic tests to rapidly identify new disease outbreaks; 2) epidemiological research to better understand virus transmission in wildlife and maintain a passive surveillance program in high risk commercial livestock operations; and 3) develop safe and effective vaccines specifically designed for control and eradication. The United States should stockpile NiV vaccines when they become available for use in contact herds to create a buffer zone as an additional control measure to prevent the spread of henipaviruses should an outbreak ever occur.

FIGURES

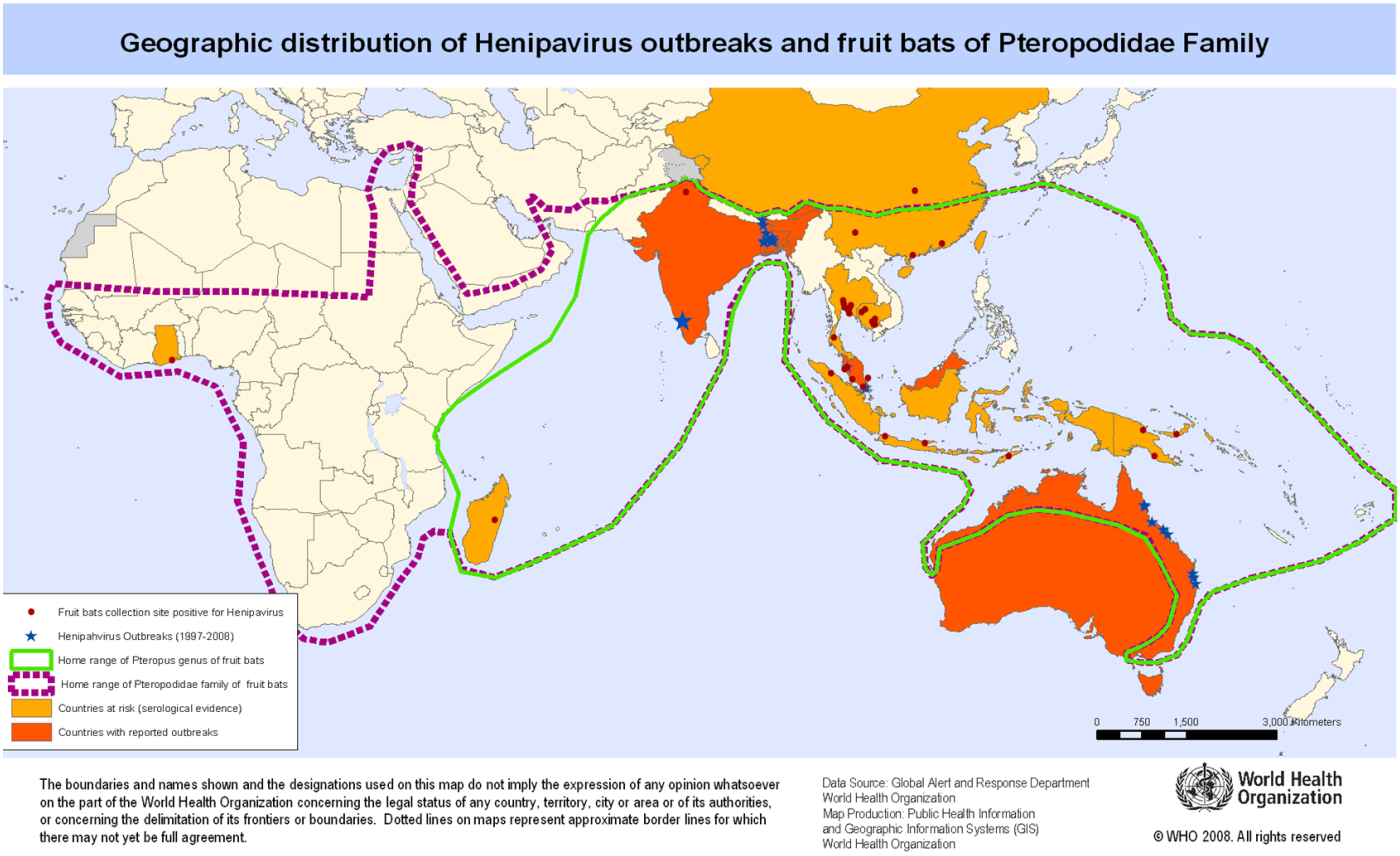

Figure 1: Geographic distribution of fruit bats of the Pteropodidae family. WHO: Nipah virus infections: http://www.who.int/csr/disease/nipah/en/ (Assessed and modified November 26, 2018).

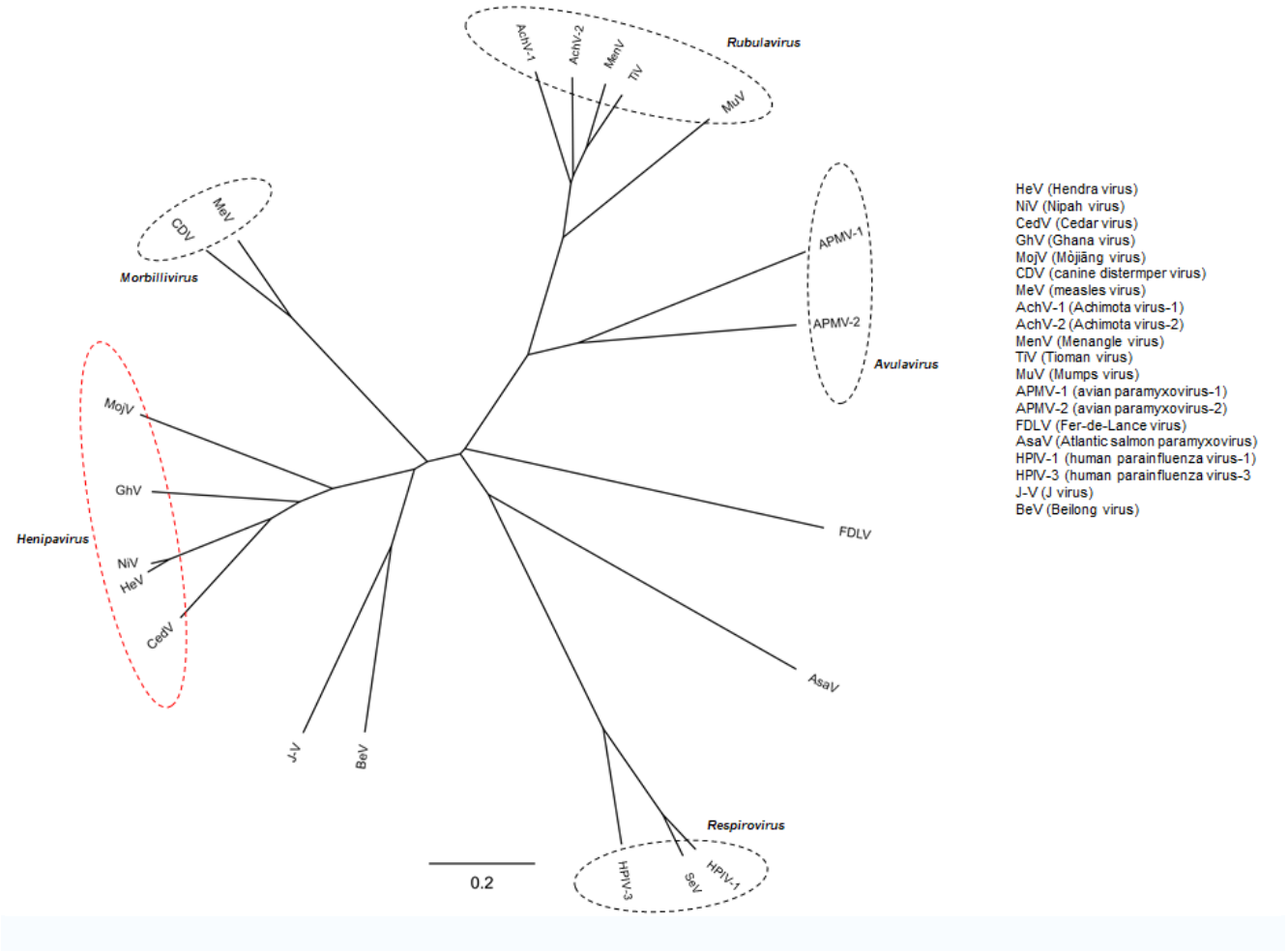

Figure 2: Phylogenetic tree based on alignment of amino acid sequence of the N-gene of selected *Paramyxovirinae* subfamily members.

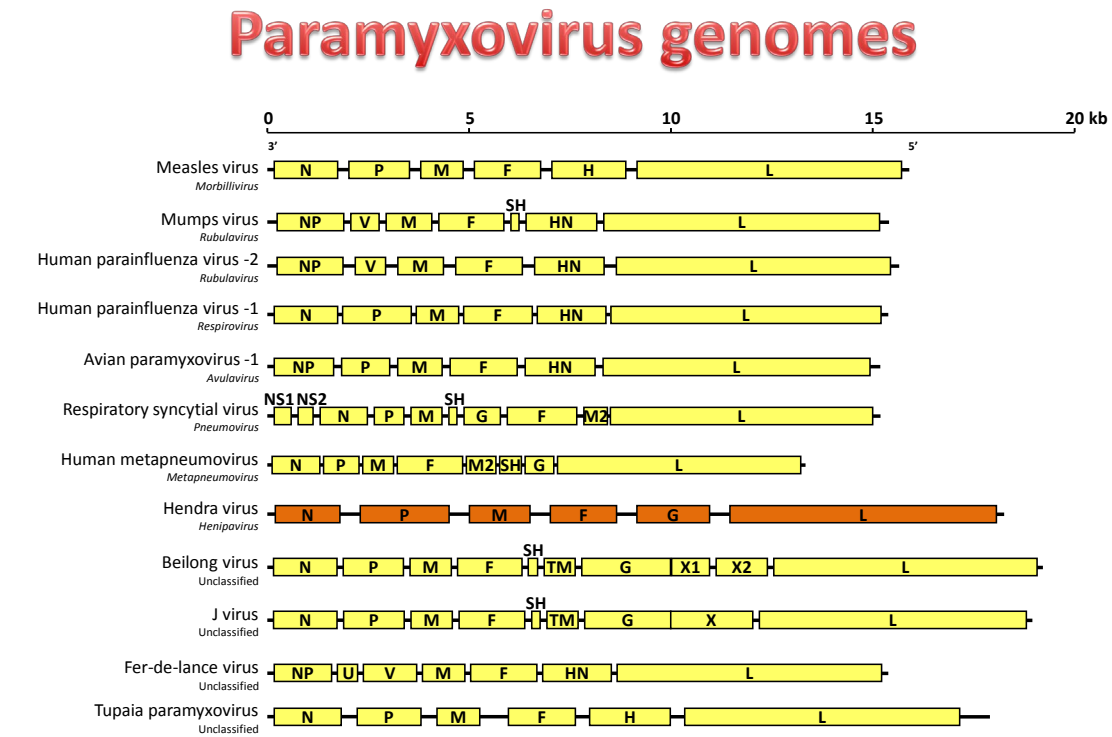

Figure 3: Comparison of *Paramyxoviridae* viruses genomes (Provided by Glen Marsh, AAHL)

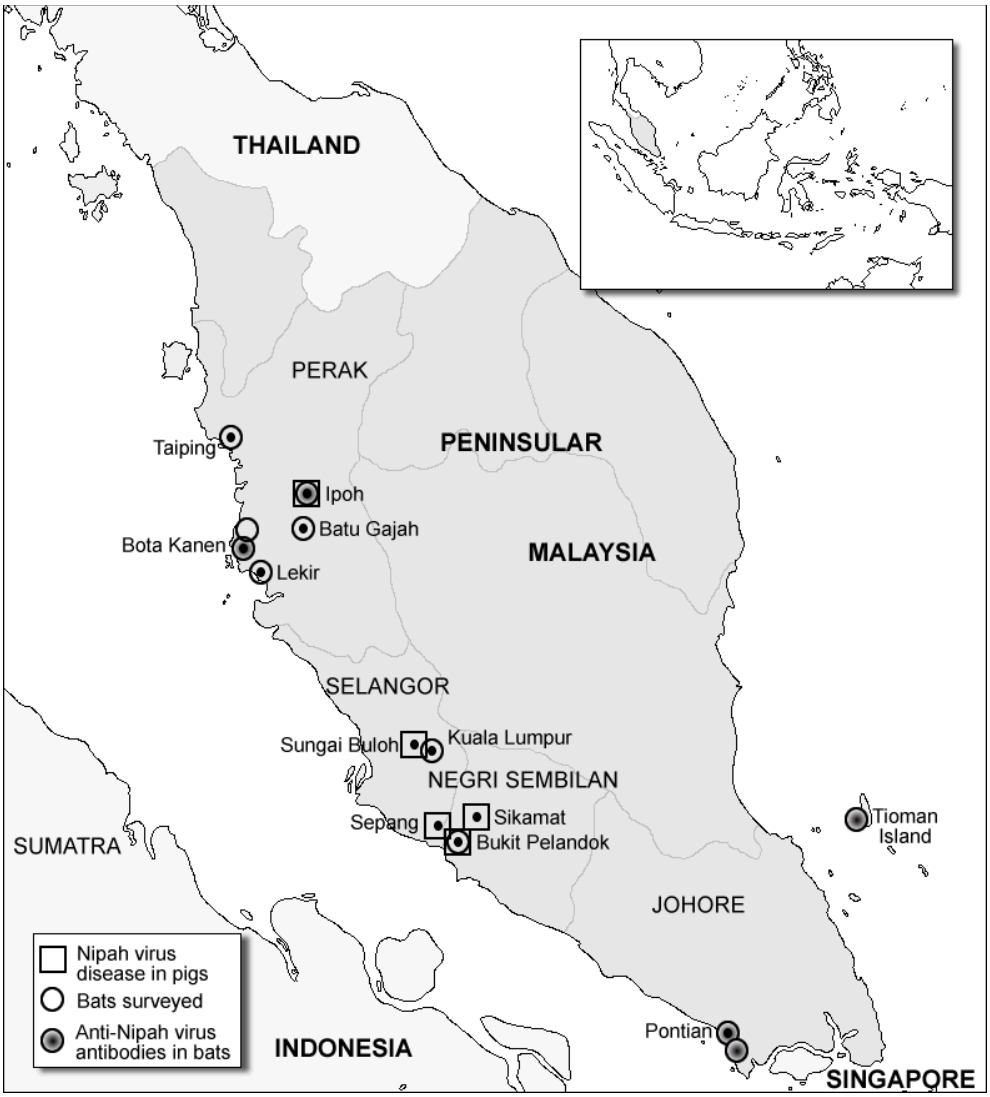

Figure 4: Descriptive map of NiV in Malaysia (Yob *et al.,* 2001)

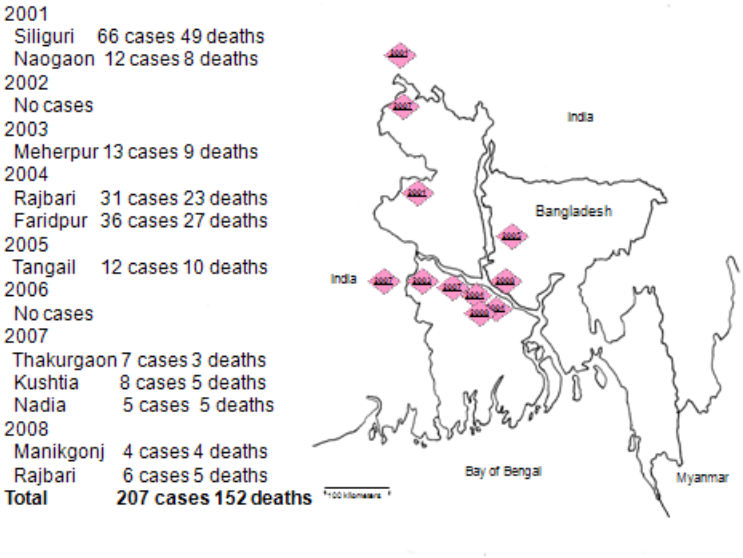

Figure 5: Epidemiology of Nipah Virus Infections in Bangladesh (Source: Steve Luby, icddr,b)

TABLE I: NIPAH VIRUS INFECTION IN BATS

Suborder	Species	No. of bats	No. Positive
Pteropodiformes	*Balionycterus macul*	4[*]	0
	Cynopterus brachyotis	56[*], 1[†]	2[*], 0[†]
	C. horsfieldi	24[*]	0
	C. sphinx	2[ǂ], 34[§], 68[†]	0[ǂ], 0[§], 0[†]
	Eidolon dupreanum	73[#]	14
	E. helvum	59[¶], 215[Δ]	23[¶], 3[Δ]
	Eonycteris spelaea	38[*], 64[§]	2[*], 0[§]
	Epomophorus gambianus	89[¶]	1
	Epomops buettikoferi	7[¶]	0
	E. franqueti	29[¶]	0
	Hipposideros armiger	63[*], 88[§], 1[†]	2[*], 0[§], 0[†]
	H. bicolor	1[*]	0
	H. larvatus	21[ǂ], 95[§], 81[†]	0[ǂ], 2[§], 0[†]
	H. pomona	60[*], 2[†]	1[*], 0[†]
	Hypsignathus monstrosus	18[¶]	1
	Macroglossus sobrinus	4[*], 1[†]	0, 0[†]
	Megaderma lyra	1[ǂ]	0
	Megaderma spasma	13[§]	0
	Megaerops ecaudatus	1[*]	0
	Nanonycteris veldkampii	4[¶]	0
	Rhinolophus acuminatus	2[†]	0
	R. affinis	6[*], 94[ǂ]	0[*], 1[ǂ]
	R. ferrumequinum	3[ǂ]	0
	R. luctus	11[ǂ], 1[†]	0[ǂ], 0[†]
	R. macrotis	3[ǂ]	0
	R. pearsoni	35[ǂ]	0
	R. pusillus	35[ǂ]	0
	R. refulgens	1[*]	0
	R. rex	1[ǂ]	0
	R. sinicus	51[*]	1
	Rousettus leschenaulti	52[*], 11[§], 15[†]	5[*], 0[§], 0[†]
	R. madagascariensis	5[#]	0
	Pteropus hypomelanus	35[*], 36[§]	11[*], 4[§]
	P. lylei	857[§], 408[†]	83[§], 50[†]
	P. medius	2790[«]	100
	P. rufus	349[#]	6
	P. vampyrus	29[*] 39[§]	5[*], 1[§]
Vespertilioniformes	*Chaerephon plicatus*	153[†]	0
	Emballonura monticola	14[§]	0
	Ia io	7[ǂ]	0
	Miniopterus spp.	32[ǂ]	5
	Myotis altarium	2[ǂ]	0
	M. daubentoni	89[ǂ]	9
	M. ricketti	84[ǂ]	8
	Murina cyclotis	1[†]	0
	Nyctalus velutinus	1[ǂ]	0
	Scotophilus heathi	3[§]	0
	Scotophilus kuhlii	33[*], 20[ǂ], 98[†]	1[*], 0[ǂ], 0[†]
	Tadarida plicata	50[§]	0
	Taphozous melanopogon	4[*], 69[†]	0[*], 0[†]
	T. saccolaimus	1[*]	0
	T. theobaldi	121[†]	0

[*]Yob JM, et al. 2001; , [ǂ]Yan L, et al. 2008; [§]Wacharapluesadee S, et al. 2005; [†]Reynes JM, et al. 2005; [¶]Hayman DTS, et al. 2008; [Δ]Drexler JF, et al. 2009; [#]Ihele C, et al. 2007; [«]Epstein JH, et al. 2016

TABLE II – NIPAH VIRUS CASES 2001-2018

Morbidity and mortality due to Nipah or Nipah-like virus encephalitis in WHO South-East Asia Region, 2001-2018

Country: Bangladesh

Month/Year	Location	No. cases	No. deaths	Case Fatality Rate
April, May 2001	Meherpur	13	9	69%
January 2003	Naogaon	12	8	67%
Jan 2004 Apr 2004	Rajbari Faridpur	31 36	23 27	74% 75%
Jan- Mar 2005	Tangail	12	11	92%
Jan-Feb 2007 Mar 2007 Apr 2007	Thakurgaon Kushtia Pabna, Natore and Naogaon	7 8 3	3 5 1	43% 63% 33%
Feb 2008 Apr 2008	Manikgonj Rajbari	4 7	4 5	100% 71%
Jan 2009	Gaibandha, Rangpur and Nilphamari Rajbari	3 1	0 1	0% 100%
Feb-Mar 2010	Faridpur Faridpur, Rajbari, Gopalgonj, Kurigram,	8 8 1	7 7 1	87.50% 87.50% 100%
Jan-Feb 2011	Lalmohirhat, Dinajpur, Comilla Nilphamari, Faridpur, Rajbari	44	40	91%
Jan 2012	Joypurhat	12	10	83%
Jan- Apr 2013	Pabna, Natore, Naogaon, Gaibandha, Manikganj	24	21	88%
Jan-Feb 2014	13 districts	18	9	50%
Jan-Feb 2015	Nilphamari, Ponchoghor, Faridpur, Magura, Naugaon, Rajbari	9	6	67%

Country: India

Month/Year	Location	No. cases	No. deaths	Case Fatality Rate
Feb 2001	Siliguri	66	45	68%
Apr 2007	Nadia	5	5	100%
May 2018	Kerala	23	21	91%

WHO (World Health Organization). Morbidity and mortality due to Nipah or Nipah-like virus encephalitis in WHO South-East Asia Region, 2001-2018. Available at: http://www.who.int/csr/disease/nipah/en/.

TABLE III – VACCINE PLATFORMS

C.C. Broder et al. / Vaccine 34 (2016) 3525–353

Table 1
Advanced active vaccination and passive immunization platforms tested in Hendra virus and/or Nipah virus animal challenge models.

Platform	Viral antigen target or immunogen	Animal challenge model
Active vaccination		
Recombinant vaccinia virus	Nipah F and/or G glycoprotein	Hamster[a] (NiV)
Recombinant canarypox virus	Nipah F and/or G glycoprotein	Pig[b] (NiV)
Recombinant VSV	Nipah F and/or G glycoprotein	Ferret[c] (NiV), Hamster[d] (NiV), nonhuman primate[e] (NiV)
Recombinant AAV	Nipah G glycoprotein	Hamster[f] (NiV, HeV)
Recombinant measles virus	Nipah G glycoprotein	Hamster and nonhuman primate[g] (NiV)
Recombinant subunit	Hendra soluble G glycoprotein	Cat[h] (NiV), Ferret[i] (HeV, NiV), nonhuman primate[j] (HeV, NiV), horse[k] (HeV)
Passive immunization		
Human monoclonal antibody m102.4	Hendra/Nipah G glycoprotein	Ferret[l] (NiV) Nonhuman primate[m] (HeV, NiV)

[a] Hamsters immunized with NiV F and/or G glycoprotein encoding recombinant vaccinia viruses were protected against disease following intraperitoneal challenge with 10^3 PFU of NiV [137].
[b] Pigs immunized with NiV F and/or G glycoprotein encoding recombinant canarypox viruses were protected against intranasal challenge with 2.5×10^5 PFU of NiV [138].
[c] Ferrets immunized with NiV F and/or G glycoprotein encoding recombinant vesicular stomatitis virus (VSV) vectors were protected against lethal intranasal challenge with 5×10^3 PFU of NiV [141]
[d] Hamsters immunized with NiV F and/or G glycoprotein encoding recombinant vesicular stomatitis virus (VSV) vectors were protected against lethal intraperitoneal challenge with 10^5 $TCID_{50}$ of NiV [143]; or 6.8×10^4 $TCID_{50}$ of NiV [142].
[e] African green monkeys immunized with a NiV G encoding recombinant VSV vector were protected against lethal intratracheal challenge with 10^5 $TCID_{50}$ of NiV [156].
[f] Hamsters immunized with a NiV G encoding recombinant adeno-associated virus (AAV) vector were protected against lethal intraperitoneal with 10^4 PFU of NiV [139].
[g] Hamsters and African green monkeys immunized with a NiV G encoding recombinant measles virus vector were protected against lethal intraperitoneal challenge with 10^3 $TCID_{50}$ of NiV (hamsters) or 10^5 $TCID_{50}$ of NiV (monkeys) [140].
[h] Hendra virus soluble G glycoprotein (HeV-sG) used to immunize cats protects against lethal subcutaneous (500 $TCID_{50}$) [120] or oronasal (5×10^4 $TCID_{50}$) NiV challenge [145].
[i] HeV-sG used to immunize ferrets protects against lethal oronasal challenge with 5×10^3 $TCID_{50}$ of HeV [124] or 5×10^3 $TCID_{50}$ of NiV challenge [146].
[j] HeV-sG used to immunize African green monkeys protects against lethal intratracheal challenge with 10^5 $TCID_{50}$ of NiV [157] or 5×10^5 PFU of HeV [147].
[k] HeV-sG used to immunize horses protects against lethal oronasal challenge with 2×10^6 $TCID_{50}$ of HeV [15].
[l] A NiV and HeV cross-reactive G glycoprotein specific neutralizing human mAb (m102.4) protects ferrets against lethal oronasal challenge with 5×10^3 $TCID_{50}$ of NiV [125] or 5×10^3 $TCID_{50}$ of HeV (J. Pallister and C. Broder, unpublished) by post-exposure infusion.
[m] Human mAb m102.4 protects African green monkeys by post-exposure infusion following lethal intratracheal challenge with 4×10^5 $TCID_{50}$ of HeV [153] or lethal intratracheal challenge with 5×10^5 PFU of NiV [154].

TABLE IV – CURRENT VACCINE CANDIDATES

B.A. Satterfield et al. / Vaccine 34 (2016) 2971–2975

Table 1
Development status of current vaccine candidates.

Candidate name/ identifier: institution	Preclinical	Developers	Ref
Subunit vaccine			
HeV sG	X	Zoetis, Inc./USU	[16,18,34,39]
Vectored vaccines			
VSV-NiV_B F and/or G	X	UTMB	[17]
VSV-NiV_M G	X	CDC	[15]
VSV-NiV_M G	X	RML	[14,19]
VSV-NiV_M F and/or G	X	Yale University	[40]
VSV-HeV G:	X	TJU/RML	[41]
RABV-HeV G:	X	TJU/RML	[41]
ALVAC-F/G	X	CFIA-NCFAD	[20,42]
AAV-NiV_M G	X	INSERM	[43]
rMV-Ed-G	X	UoT	[44]
V-NiVG	X	USU	[45]
rLa-NiVG and/or rLa-NiVF	X	CAAS-SKLVB	[21]
Passive antibody transfer			
Polyclonal serum NiV F or G	X	INSERM	[46]
Mouse mAbs NiV F or G	X	INSERM	[47]
Human mAb m102.4 Henipah G	X	USU	[35,48]

Abbreviations: USU (Uniformed Services University of the Health Sciences); UTMB (University of Texas Medical Branch); CDC (Centers for Disease Control and Prevention); RML (Rocky Mountain Laboratories); TJU (Thomas Jefferson University); CFIA-NCFAD (Canadian Food Inspection Agency – Centre for Foreign Animal Diseases); Institut national de la santé et de la recherché médicale (INSERM); UoT (University of Tokyo); CAAS-SKLVB (Chinese Academy of Agricultural Sciences (CAAS) – State Key Laboratory of Veterinary Biotechnology (SKLVB)).

APPENDIX I: COUNTERMEASURES WORKING GROUP INSTRUCTIONS

Decision Model

We will use a decision model to assess potential countermeasures to stockpile. These countermeasures must significantly improve our ability to control and eradicate an outbreak of Nipah virus in a disease-free country. The decision model is a simple tool that will allow us to focus on critical criteria for the National Veterinary Stockpile, and rank the available interventions relative to each other. The decision model is available as a Microsoft Excel spread sheet, which has been prepared to quantitatively assess the rankings we assign to a set of selected criteria that will lead to the selection of the highest cumulative option. We can use as many criteria as we want but the objective is to get down to the ones that will make or break success. The criteria for each intervention will be selected by the Countermeasures Working Group, but a preliminary set has been identified to expedite the process. You are encouraged to review the criteria prior to coming to the meeting and be prepared to modify the criteria as needed with the working group. The following provides an example of criteria and assumptions for assessing vaccines.

Criteria

If a vaccine is going to be used as an emergency outbreak control tool for Nipah virus, then we need to know: 1) is it efficacious (does it effectively eliminate shedding or just reduce shed by a known log scale); 2) does it work rapidly with one dose (probably do not have time for a second dose); 3) whether it is available today from the perspective of having a reliable & rapid manufacturing process (need to know it can be up & running rapidly and will yield a predictable amount of vaccine; 4) can we get the product to the outbreak site rapidly & safely; 5) once at the site, can we get it into the target population rapidly; 6) type of administration- mass or injected, people and equipment to do the job become important); and 7) are diagnostics available to monitor success and or DIVA compliant. While cost is important, the cost of the vaccine in an outbreak will be small in comparison to the other costs. In addition, how fast the product can be made is important because that will have a big impact on how big a stockpile will be needed. Accordingly, you will see from the Excel sheets that have been prepared for vaccines that the following critical criteria and assignment of weights for each criterion are proposed.

Weight	Critical Criteria
10	Efficacy
6	Safety
8	One dose
6	Speed of Scale up
2	Shelf life
2	Distribution/storage
10	Quick Onset of Immunity
8	DIVA Compatible
2	Withdrawal
2	Cost to Implement

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APPENDIX II – VACCINES ASSESSMENT

Experimental Veterinary Vaccines For Nipah Virus - USDA/ARS, 03-19-09								
	Rank each Intervention (2,4,6,8, or 10) as to its importance to making a decision, only one "10" rankings allowed							
Weight	**Critical Criteria**	CPV-G	CPV-F	VV-G	Soluble G			
10	Efficacy	6	4	2	6			
6	Safety	10	10	2	10			
8	One dose	4	4	4	2			
8	Manufacturing safety	8	8	6	8			
10	DIVA Compatible	8	8	8	8			
8	Manufacturing yield	8	8	8	6			
6	Rapid production	8	8	4	4			
4	Reasonable cost	6	6	4	2			
2	Short withdrawal	8	8	2	4			
8	Long shelflife	8	8	8	4			
Rank each Criteria 2,4,6,8 or10 on each criterion -- no more than two "10" rankings allowed								
	Critical Criteria	CPV-G	CPV-F	VV-G	Soluble G	0	0	0
	Efficacy	60	40	20	60	0	0	0
	Safety	60	60	12	60	0	0	0
	One dose	32	32	32	16	0	0	0
	Manufacturing safety	64	64	48	64	0	0	0
	DIVA Compatible	80	80	80	80	0	0	0
	Manufacturing yield	64	64	64	48	0	0	0
	Rapid production	48	48	24	24	0	0	0
	Reasonable cost	24	24	16	8	0	0	0
	Short withdrawal	16	16	4	8	0	0	0
	Long shelflife	64	64	64	32	0	0	0
	0	0	0	0	0	0	0	0
	Value	512	492	364	400	0	0	0

APPENDIX III – DIAGNOSTICS ASSESSMENT

Experimental Diagnostics For Nipah Virus - USDA/ARS, 03-19-09

Rank each Intervention (2,4,6,8, or 10) as to its importance to you in making a decision, no more than one "10" rankings allowed

Weight	Critical Criteria	qPCR	conv PCR	field PCR	VI	penside	v ELISA	N ELISA	IgM ELISA	VNT	ps VNT	bind lum	block lum
10	Sensitivity	10	10	8	8	4	10	4	8	8	8	8	8
8	Specificity	8	6	8	10	6	6	6	8	10	8	8	8
2	DIVA	8	8	8	8	8	2	10	6	2	2	8	2
6	multispecies	8	8	8	8	8	6	6	2	8	8	6	8
8	Validation to purpose	8	8	8	8	4	8	4	10	8	10	8	10
4	Speed of Scaleup	8	4	4	2	6	8	8	8	2	4	4	4
4	Throughput	8	2	2	2	4	8	8	8	2	4	6	6
4	Flock Side Test	2	2	10	2	10	2	2	2	2	2	2	2
10	Rapid Result	6	4	8	2	8	6	6	6	4	4	10	8
4	No need to Confirm	6	4	4	8	2	6	4	6	8	8	8	8
8	Easy to perform	8	6	6	4	8	8	8	6	6	6	8	8
8	safe to operate	8	8	6	2	6	8	8	8	2	8	8	8
8	Availability	8	8	2	2	2	6	8	4	2	6	4	4
6	Storage/Distribution	4	6	6	2	6	6	6	6	2	4	4	4
4	Low Cost to Implement	2	4	2	2	4	6	8	6	2	4	4	2

Rank each Criteria 2,4,6,8 or10 on each criterion -- no more than two "10" rankings allowed

Critical Criteria	qPCR	conv PCR	field PCR	VI	penside	v ELISA	N ELISA	IgM ELISA	VNT	ps VNT	bind lum	block lum
Sensitivity	100	100	80	80	40	100	40	80	80	80	80	80
Specificity	64	48	64	80	48	48	48	64	80	64	64	64
DIVA	16	16	16	16	16	4	20	12	4	4	16	4
multispecies	48	48	48	48	48	36	36	12	48	48	36	48
Validation to purpose	64	64	64	64	32	64	32	80	64	80	64	80
Speed of Scaleup	32	16	16	8	24	32	32	32	8	16	16	16
Throughput	32	8	8	8	16	32	32	32	8	16	24	24
Flock Side Test	8	8	40	8	40	8	8	8	8	8	8	8
Rapid Result	60	40	80	20	80	60	60	60	40	40	100	80
No need to Confirm	24	16	16	32	8	24	16	24	32	32	32	32
Easy to perform	64	48	48	32	64	64	64	48	48	48	64	64
safe to operate	64	64	48	16	48	64	64	64	16	64	64	64
Availability	64	64	16	16	16	48	64	32	16	48	32	32
Storage/Distribution	24	36	36	12	36	36	36	36	12	24	24	24
Low Cost to Implement	8	16	8	8	16	24	32	24	8	16	16	8
Value	672	592	588	448	532	644	584	608	472	588	640	628

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REFERENCES

Ang, B.S.P., Lim, T.C.C., Wang L. 2018. Nipah virus infection. J Clin Microbiol 56:e01875-17. https://doi.org/10.1128/JCM.01875-17.

Angeletti S, Presti AL, Cella E, et al. Molecular epidemiology and phylogeny of Nipah virus infection: a 654 mini review. Asian Pac J Trop Dis 2016;9(7):630-4. doi: 10.1016/j.apjtm.2016.05.012

Anonymous (2008) Nipah virus, fatal - Bangladesh (03). *Pro-MED* (International Society for Infectious Diseases) (March 11, 2008, archive no. 20080311.0979. Available at www.promedmail.org.

Anonymous (2008) Hendra Virus, Human, Equine - Australia (07): (Queensland). *Pro-MED* (International Society for Infectious Diseases) (August 21, 2008, archive no. 20080821.2606). Available at www.promedmail.org.

Anonymous (2009) Hendra Virus, Human, Equine – Australia (04): (Queensland) Fatal. *Pro-MED* (International Society for Infectious Diseases) (September 3, 2009, archive no. 20090903.3098. Available at www.promedmail.org.

Arunkumar G, Chandni R, Mourya DT, Singh SK, Sadanandan R, Sudan P, Bhargava B., NIPAH: Nipah Investigators People And Health. (2018). Outbreak investigation of Nipah Virus Disease in Kerala, India, 2018.J Infect Dis. doi: 10.1093/infdis/jiy612. [Epub ahead of print]

Berthane Y., Weingartl H.M., Lopez J., Neufeld J., Czub S., Embury-Hyatt C., Goolia M., Copps J., Czub M. 2008. Bacterial Infections in Pigs Experimentally Infected with Nipah Virus. Transboundary and Emerging Diseases. 55 (2008) 165–174.

Bossart K.N., McEachern J.A., Hickey A.C., Choudhry V., Dimitrov D.S., Eaton B.T. & Wang L.F. (2007). Neutralization assays for differential henipavirus serology using Bio-Plex protein array systems. *J. Virol. Methods*, 142, 29–40.

Bossart K. N., Crameri G., Dimitrov A. S., Mungall B. A., Feng Y-R, Patch J. R., Choudhary A., Wang L-F., Eaton B. T., and Broder C. C. (2005). Receptor Binding, Fusion Inhibition, and Induction of Cross-Reactive Neutralizing Antibodies by a Soluble G Glycoprotein of *Hendra Virus*. J Virol.; 79(11): 6690–6702

Bossart KN, Bingham J, Middleton D. 2007. Targeted strategies for henipavirus therapeutics. *Open Virol J.* 1, 14-25.

Bossart KN, Tachedjian M, McEachern JA, Crameri G, Zhu Z, Dimitrov DS, Broder CC, Wang LF. 2008. Functional studies of host-specific ephrin-B ligands as Henipavirus receptors. Virology. 372(2): 357-71.

Bossart KN, Rockx B, Feldmann F, Brining D, Scott D, LaCasse R, Geisbert JB, Feng YR, Chan YP, Hickey AC, Broder CC, Feldmann H, Geisbert TW. 2012. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med. 8;4(146).

Broder CC, Geisbert TW, Xu K, Nikolov DB, Wang LF, Middleton D, Pallister J, Bossart KN. (2012). Immunization strategies against henipaviruses. Curr Top Microbiol Immunol.; 359:197-223. doi: 10.1007/82_2012_213

Broder, C.C., Weir, D.L., Reid, P.A. 2016. Hendra Virus and Nipah Virus Animal Vaccines, Vaccine 34:3525-3534,

Chang, L.Y., Ali, A.R., Hassan, S.S. & AbuBakar, S. Quantitative estimation of Nipah virus replication kinetics in vitro. *Virol J* **3**, 47 (2006).

Ching P.K., de los Reyes V.C., Sucaldito M.N., Tayag E., Columna-Vingno A.B., Malbas F.F., Bolo G.C., Sejvar J.J., Eagles D., Playford G., Dueger, E., Kaku, Y., Morikawa, S., Kuroda, M., Marsh, G.A., McCullough, S., Foxwell A.R. 2015. Outbreak of henipavirus infection, Philippines, 2014. Emerg Infect Dis. 21:328-331.

Chua K.B., Bellini W.J., Rota P.A., Harcourt B.H., Tamin A., et al. (2000) Nipah virus: A recently emergent deadly paramyxovirus. Science 288: 1432–1435

Clayton, B.A. 2017. Nipah virus: transmission of a zoonotic paramyxovirus. Current Opinion in Virology: 22: 97-104.

Cortes M.C., Cauchemez S., Lefrancq N., Luby S.P., Hossain M.J., Sazzad H.M.S., Rahman M., Daszak P., Salje H., Gurley E.S. 2018. Characterization of the spatial and temporal distribution of Nipah virus spillover events in Bangladesh 2007 - 2013. J Infect Dis. 2018 Jan 17. doi: 10.1093/infdis/jiy015.

Crameri G., Wang L-F, Morrissy C., White J., and B. T. Eaton. 2002. A rapid immune plaque assay for the detection of Hendra and Nipah viruses and anti-virus antibodies. J Virol Methods; 99(1-2): 41-51.

Daniels P., Halpin K., Hyatt A. & Middleton D. (2007). Infection and disease in reservoir and spillover hosts: determinants of pathogen emergence. *Curr. Top. Immunol. Microbiol.*, 315, 113–131.

DeBuysscher et al. 2014. Recombinant VSV, with Nipah G or F in hamsters. Vaccine. 32(22):2637-44.

Drexler JF, Corman VM, Gloza-Rausch F, Seebens A, Annan A, Ipsen A, Kruppa T, Müller MA, Kalko EK, Adu-Sarkodie Y, Oppong S, Drosten C. (2009). Henipavirus RNA in African bats. PLoS One.; 4(7):e6367. doi: 10.1371/journal.pone.0006367.

Drexler JF, Corman VM, Müller MA, Maganga GD, Vallo P, Binger T, Gloza-Rausch F, Cottontail VM, Rasche A, Yordanov S, Seebens A, Knörnschild M, Oppong S, Adu Sarkodie Y, Pongombo C, Lukashev AN, Schmidt-Chanasit J, Stöcker A, Carneiro AJ, Erbar S, Maisner A, Fronhoffs F, Buettner R, Kalko EK, Kruppa T, Franke CR, Kallies R, Yandoko ER, Herrler G, Reusken C, Hassanin A, Krüger DH, Matthee S, Ulrich RG, Leroy EM, Drosten C. (2012). Bats host major mammalian paramyxoviruses Nat Commun.; 3:796. doi: 10.1038/ncomms1796.

Eaton, B.T., Broder, C.C., and L-F Wang. 2005. Hendra and Nipah viruses: pathogenesis and therapeutics. Curr Mol Med.; 5(8): 805-16

Eaton, B.T., Broder, C.C., Middleton, D., and L-F Wang. 2006. Hendra and Nipah viruses: different and dangerous. Nat Rev Microbiol.; 4(1): 23-35.

Eaton, B.T., Mackenzie, J.S. and Wang, L-F. 2007. Henipaviruses. In: Fields Virology 5th Ed (eds, D.M. Knipe, D.E. Griffin, R.A. Lamb, S.E. Straus, P.M. Howley, M.A. Martin and B. Roizman), pp. 1587-1600, Lippincott Williams & Wilkins, Philadelphia, USA.

Epstein J.H., Field H.E., Luby S., Pulliam J.R., Daszack P. 2006. Nipah virus: impact, origins, and cuases of emergence. Curr. Infect. Dis. Rep.; 8(1): 59-65

Epstein JH., Anthonyb SJ., Islamc A., Kilpatrick AM., Khan SA., Ross N., Smith L., Barr J., Zambrana-Torreliog C., Taoh Y., Islam A., Quan PL., Olival K., Gurley E., Hossain M.J., Field HE., Fielder M., Briese T., Rahman M., Crameri G., Wang L-F., Luby S., Lipkin WI., Daszak P. (2016). Nipah virus ecology and infection dynamics in its bat reservoir, *Pteropus medius*, in Bangladesh. International Journal of Infectious Diseases. Volume 53, Supplement, Pages 20-21. http://dx.doi.org/10.1016/j.ijid.2016.11.055

Feldman, K.S., Foord, A., Heine, H.G., Smith, I.L., Boyd, V., Marsh, G.A., Wood J.L., Cunningham, A.A. and Wang, L.-F. 2009. Design and evaluation of consensus PCR assays for henipaviruses. *J. Virol. Meth.* 161, 52-57.

Field, H., Young, P., Yob, J.M., Mills, J., Hall, L., and J. Mackenzie. 2001. The natural history of Hendra and Nipah viruses. Microbes Infect; 3(4): 307-14.
Fischer K, Diederich S, Smith G, Reiche S, Pinho dos Reis V, Stroh E, Groschup MH, Weingartl HM, and Balkema-Buschmann A. 2018. Development of Indirect ELISAs based on Hendra and Nipah virus proteins for the detection of henipavirus specific antibodies in pigs. PLoS ONE 13(4): e0194385. https://doi. org/10.1371/journal.pone.0194385

Foord, A., White J., Colling A., Heine, H. 2013. Microsphere Suspension Array Assays for Detection and Differentiation of Hendra and Nipah Viruses. Biomed Res. Int. 2013, 2013:289295. doi: 10.1155/2013/289295. Epub 2013 Feb 6

Geisbert TW, Feldmann H, Broder CC. (2012). Animal challenge models of henipavirus infection and pathogenesis. Curr Top Microbiol Immunol.; 359:153-77. doi: 10.1007/82_2012_208

Goh, K.J., C.T. Tan, N.K. Chew, P.S. Tan, A. Kamarulzaman, S. A. Sarji, K. T. Wong, B. J. Abdullah, K. B. Chua, and S. K. Lam. 2000. Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. N. Engl. J. Med. 342:1229-1235

Guillaume et al. 2004. Recombinant vaccinia virus (NYVAC), with NiV F or G, in hamsters. J Virol. 78:834-40.

Guillaume, V. et al. 2004. Specific detection of Nipah virus using real-time RT-PCR (TaqMan). *J Virol Methods* **120**, 229-37.

Guillaume V., Contamin H., Loth P., Georges-Courbot M.-C., Lefeuvre A., Marianneau P., Chua K. B., Lam S. K., Buckland R., Deubel V., and Wild T. F. 2004. Nipah Virus: Vaccination and Passive Protection Studies in a Hamster Model. J. Virol.; 78(2): 834–840.

Gurley E.S., Hegde S,T,, Hossain K,, Sazzad H,M,S,, Hossain M,J,, Rahman M,, Sharker M,A,Y,, Salje H,, Islam M,S,, Epstein J,H,, Khan S,U,, Kilpatrick A,M,, Daszak P,, Luby S,P. 2017. Convergence of humans, bats, trees, and culture in Nipah virus transmission, Bangladesh. Emerg Infect Dis.;23(9):1446-1453. doi: 10.3201/eid2309.161922.

Hayman D.T.S., Suu-Ire R., Breed A.C., McEachern J.A., Wang L.F., et al. 2008. Evidence of Henipavirus Infection in West African Fruit Bats. PLoS ONE 3(7): e2739. doi:10.1371/journal.pone.0002739

Hooper, P. T., Ketterer, P. J., Hyatt, A. D., and Russell, G. M. (1997). Lesions of experimental equine morbillivirus pneumonia in horses. Vet Pathol 34(4): 312-22

Hooper, P.T., Zaki, S., Daniels, P., and D. Middleton. 2001. Comparative pathology of the diseases caused by Hendra and Nipah viruses. Microbes Infect.; 3(4): 315-22.

Hossain, M.J., Gurley, E.S., Montgomery, J.M., Bell, M., Carroll, D.S., Hsu, V.P., Formenty, P., Croisier, A., Bertherat, E., Faiz, M.A., Azad, A.K., Islam, R., Molla, M.A., Ksiazek, T.G., Rota, P.A., Comer, J.A., Rollin, P.E., Luby, S.P., and R.F. Breiman. 2008. Clinical presentation of nipah virus infection in Bangladesh. Clin Infect Dis.; 46(7): 977-84.

Hyatt A.D., Zaki S.R., Goldsmith C.S., Wise T.G. & Hengstberger S.G. (2001). Ultrastructure of Hendra virus and Nipah virus within cultured cells and host animals. *Microbes Infect. ,* 3, 297–306.

Iehlé C, Razafitrimo G, Razainirina J, Andriaholinirina N, Goodman SM, Faure C, Georges-Courbot MC, Rousset D, Reynes JM. (2007). Henipavirus and Tioman virus antibodies in pteropodid bats, Madagascar. Emerg Infect Dis; 13(1):159-61.

Kaku, Y., Noguchi, A., Marsh, G.A., McEachern, J.A., Okutani, A., Hotta, K., Bazartseren, B., Fukushi, S., Broder, C.C., Yamada, A., Inoue, S. and Wang, L.-F. 2009. A neutralization test for specific detection of Nipah virus antibodies using pseudotyped vesicular stomatitis virus expressing green fluorescent protein. *J. Virol. Meth.* 160, 7-13.

Kong, 2012. Recombinant Newcastle disease virus with Nipah G or F, in mice and pigs. Virology 432(2):327-35.

Kulkarni S., Volchkova V., Basler C.F., Palese P., Volchkov V.E., Shaw M.L. 2009. Nipah virus edits its P gene at high frequency to express the V and W proteins. J Virol.;83(8):3982-7.

Kulkarni D.D., Venkatesh G., Tosh C., Patel P., Mashoria A., Gupta V., Gupta S. 2016. Development and Evaluation of Recombinant Nucleocapsid Protein Based Diagnostic ELISA for Detection of Nipah Virus Infection in Pigs. J. of Immunoassay Immunochem. 2016;37(2):154-66. doi: 10.1080/15321819.2015.1074922.

Kurup et al. 2015. Recombinant rhabdoviruses (VSV and Rabies) with Hendra G, in mice.., J Virol. Jan;89(1):144-54.

Lam SK. Nipah virus - a potential agent of bioterrorism? Antiviral Res. 2003 Jan; 57(1-2):113-9.

Lee B., Pernet O., Ahmed AA., Zeltina A., Beaty SM., Bowden TA. (2015). Molecular recognition of human ephrinB2 cell surface receptor by an emergent African henipavirus. Proc Natl Acad Sci U S A.; 112(17):E2156-65. doi: 10.1073/pnas.1501690112. Epub 2015 Mar 30.

Leroy EM, Kumulungui B, Pourrut X, Rouquet P, Hassanin A, Yaba P, Délicat A, Paweska JT, Gonzalez JP, Swanepoel R. (2005). Fruit bats as reservoirs of Ebola virus. Nature; 438(7068):575-6.

Li W, Shi Z, Yu M, Ren W, Smith C, Epstein JH, Wang H, Crameri G, Hu Z, Zhang H, Zhang J, McEachern J, Field H, Daszak P, Eaton BT, Zhang S, Wang LF. (2005). Bats are natural reservoirs of SARS-like coronaviruses. Science; 310(5748):676-9. Epub 2005 Sep 29.

Lo M.K., Harcourt B.H., Mungall B.A., Tamin A., Peeples M.E., Bellini W.J., Rota P.A. 2009. Determination of the henipavirus phosphoprotein gene mRNA editing frequencies and detection of the C, V and W proteins of Nipah virus in virus-infected cells. J Gen Virol.; 90(Pt 2):398-404.

Ludlow, L.E., Lo, M.K., Rodriguez, J.J., Rota, P.A., and C.M. Horvath, 2008. Henipavirus V protein association with Polo-like kinase reveals functional overlap with STAT1 binding and interferon evasion. J Virol.; 82(13): 6259-71.

Marsh GA, Haining J, Hancock TJ, Robinson R, Foord AJ, Barr JA, Riddell S, Heine HG, White JR, Crameri G, Field HE, Wang LF, Middleton D. (2011). Experimental infection of horses with Hendra virus/Australia/horse/2008/Redlands. Emerg Infect Dis.; 17(12):2232-8. doi: 10.3201/eid1712.111162.

Marsh G.A., de Jong C., Barr J.A., Tachedjian M., Smith C., Middleton D., Yu M., Todd S., Foord A.J., Haring V., Payne J., Robinson R., Broz I., Crameri G., Field H.E., Wang L.F. 2012. Cedar virus: a novel Henipavirus isolated from Australian bats PLoS Pathog. 8(8):e1002836. doi: 10.1371/journal.ppat.1002836.

McEachern, J.A., Bingham, J., Crameri, G., Green, D.J., Hancock, T.J., Middleton, D., Feng, Y.R., Broder, C.C., Wang, L.F., and K.N. Bossart. 2008. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. Vaccine; 26(31): 3842-52.

von Messling V. and Cattaneo R. 2012, A Henipavirus Vaccine in Sight. *Science;* Vol. 337, Issue 6095, pp. 651-652.

Middleton D.J., Westbury H.A., Morrissy C.J., van der Heide B.M., Russell G.M., Braun M.A., et al. 2002. Experimental Nipah virus infection in pigs and cats. J Comp Pathol;126(2-3):124-36.

Middleton D.J. and Weingartl H.M. 2012. Henipaviruses in their natural hosts. Curr Top Microbiol Immunol. 359:105-21. doi: 10.1007/82_2012_210.

Middleton D.J, Pallister J., Klein R., Feng Y.R., Haining J., Arkinstall R., et al. 2014. Hendra virus vaccine, a One Health approach to protecting horse, human, and environmental health. Emerg Infect Dis.; 20(3): 372–379. http://dx.doi.org/10.3201/eid2003.131159

Middleton D.J., Riddell S., Klein R., Arkinstall R., Haining J., Frazer L., Mottley C., Evans R., Johnson D., Pallister J.. 2017. Experimental Hendra virus infection of dogs: virus replication, shedding, and potential for transmission. Aust Vet J. 2017 Jan;95(1-2):10-18. doi: 10.1111/avj.12552.

Mills J.N., Alim A.N.M., Bunning M.L., Lee O.B., Wagoner K.D., Amman B.R., Stockton P.C., Ksiazek T.G. 2009. Nipah virus infection in dogs, Malaysia, 1999. Emerg Infect Dis [serial on the Internet]. Available from http://www.cdc.gov/EID/content/15/6/950.htm

Mire et al. 2013. Recombinant VSV, with NiV F or G in ferrets. Virol J. 10:353.

Mungall B. A., Middleton D., Crameri, G., Bingham J., Halpin K., Russell, G., Green D., McEachern J., Pritchard L. I., Eaton B. T., Wang L-F., Bossart K. N., and Broder C. C. (2006). Feline Model of Acute Nipah Virus Infection and Protection with a Soluble Glycoprotein-Based Subunit Vaccine. J Virol.; 80(24): 12293–12302.

Murray, K., Selleck, P., Hooper, P., Hyatt, A., Gould, A., Gleeson, L., Westbury, H., Hiley, L., Selvey, L., Rodwell, B., and et al. (1995). A morbillivirus that caused fatal disease in horses and humans. Science 268(5207): 94-7

Negrete O.A., Wolf M.C., Aguilar H.C., Enterlein S., Wang W., Mühlberger E., Su S.V., Bertolotti-Ciarlet A., Flick R., Lee B. 2006 Two Key Residues in EphrinB3 Are Critical for its Use as an Alternative Receptor for Nipah Virus. PLoS Pathog 2(2): e7. doi:10.1371/journal.ppat.0020007

Negrete, O.A., Chu, D., Aguilar, H.C, and B. Lee. 2007. Single Amino Acid Changes in the Nipah and Hendra Virus Attachment Glycoproteins Distinguish EphrinB2 from EphrinB3 Usage. Journal of Virology, 81 (19): 10804-10814

Pickering, B.S., et al. 2006. Recombinant soluble Hendra G, in swine, HeV or NiV challenge. Vaccine. 34(40): 4777-86.

Pickering, B.S., Hardham, J.M., Smith, G., Weingartl, E.T., Dominowski, P.J., Foss, D.L., Mwangi, D., Broder, C.C., Roth, J.A., Weingartl, H.M. (2016). Protection against henipaviruses in swine requires both, cell-mediated and humoral immune response. Vaccine 34(40): 4777-4786.

Ploquin et al. 2013. Adeno-associated virus (AAV), with NiV G in hamsters, but only low level cross-protection (50%) against a HeV challenge. J Infect Dis. 207(3):469-78.

Reynes J-M., Counor D., Ong S., Faure C., Seng V., Molia S., Walston J., Georges-Courbot M-C., Deubel V., Sarthou J.L. (2005). Nipah Virus in Lyle's Flying Foxes, Cambodia. Emerg Infect Dis. 2005 Jul; 11(7): 1042–1047. doi: [10.3201/eid1107.041350]

Rissanen I., Ahmed AA., Azarm K., Beaty S., Hong P[3], Nambulli S., Duprex WP., Lee B., Bowden TA. (2017). Idiosyncratic Mòjiāng virus attachment glycoprotein directs a host-cell entry pathway distinct from genetically related henipaviruses. Nat Commun.; 8:16060. doi: 10.1038/ncomms16060.

Rogers, R. J., Douglas, I. C., Baldock, F. C., Glanville, R. J., Seppanen, K. T., Gleeson, L. J., Selleck, P. N., and Dunn, K. J. (1996). Investigation of a second focus of equine morbillivirus infection in coastal Queensland. Aust Vet J 74(3): 243-4

Satterfield, B.A., Dawes, B.E., Milligan, G.N. (2016). Status of Vaccine Research and Development of Vaccines for Nipah Virus, Vaccine 34:2971–2975.

Tamin A., Harcourta B.H., Loa M.K., Roth J.A., Wolf M.C., Lee B., Weingartl H., Audonnet J-C., Bellini W.J., Rota P.A. 2009. Development of a neutralization assay for Nipah virus using pseudotype particles. Journal of Virological Methods; 160: 1–6.

Tan, C.T., Goh, K.J., Wong, K.T., Sarji, S.A., Chua, K.B., Chew, N.K., Murugasu, P., Loh, Y.L., Chong, H.T., Tan, K.S., Thayaparan, T., Kumar, S., and M.R. Jusoh. 2002. Relapsed and late-onset Nipah encephalitis. Ann Neurol.; 51(6):703-8.

Tanimura N., Imada T., Kashiwazaki Y., Shahirudin S., Sharifah S.H., Aziz A.J. 2004. Monoclonal antibody-based immunohistochemical diagnosis of Malaysian Nipah virus infection in pigs. J Comp Path, 131: 199-206.

Tartaglia J., Perkus M. E., Taylor J., Norton E. K., Audonnet J-C., Cox W. I., Davis S. W., van der Hoeven J., Meignier B., Riviere M., et al. (1992). NYVAC: a highly attenuated strain of vaccinia virus. Virology; 188(1):217-32

Taylor J, Tartaglia J, Rivière M, Duret C, Languet B, Chappuis G, Paoletti E. (1994). Applications of canarypox (ALVAC) vectors in human and veterinary vaccination. Dev Biol Stand. 1994;82:131-5.

Tong S., Chern S., Li Y., Pallansch M., Amderson L., Sensitive and Broadly Reactive Reverse Transcriiptase-PCR Assays To Detect Novel Paramyxoviruses. 2008. Journal of Clinical Microbiology. Vol. 46, No. 8: 2652-2658.

Towner JS, Amman BR, Sealy TK, Carroll SA, Comer JA, Kemp A, Swanepoel R, Paddock CD, Balinandi S, Khristova ML, Formenty PB, Albarino CG, Miller DM, Reed ZD, Kayiwa JT, Mills JN,

Cannon DL, Greer PW, Byaruhanga E, Farnon EC, Atimnedi P, Okware S, Katongole-Mbidde E, Downing R, Tappero JW, Zaki SR, Ksiazek TG, Nichol ST, Rollin PE. (2009). Isolation of genetically diverse Marburg viruses from Egyptian fruit bats. PLoS Pathog; 5(7):e1000536. doi: 10.1371/journal.ppat.1000536. Epub 2009 Jul 31.

Vergne, T. et. al. (2017). Pig Empire Under Infectious Threat: Risk of African Swine Fever Introduction into the People's Republic of China. Veterinary Record, 181:117.

Wacharapluesadee S., Lumlertdacha B., Boongird K., Wanghongsa S., Chanhome L., Rollin P., Stockton P., Rupprecht CE., Ksiazek TG., Hemachudha T. (2005). Bat Nipah Virus, Thailand. Emerg Infect Dis; 11(12): 1949–1951. doi: [10.3201/eid1112.050613]

Wacharapluesadee S. and Hemachudha T. (2007). Duplex nested RT-PCR for detection of Nipah virus RNA from urine specimens of bats. *J. Virol. Methods*, 141, 97–101.

Wacharapluesadee S., Boongird K., Wanghongsa S., Ratanasetyuth N., Supavonwong P., Saengsen D., Gongal G.N., Hemachudha T. 2010. A longitudinal study of the prevalence of Nipah virus in Pteropus lylei bats in Thailand: evidence for seasonal preference in disease transmission. Vector Borne Zoonotic Dis. 2010 Mar;10(2):183-90. doi: 10.1089/vbz.2008.0105.

Wacharapluesadee S., Samseeneam P., Phermpool M., Kaewpom T., Rodpan A., Maneeorn P., Srongmongkol P., Kanchanasaka B., Hemachudha T. 2016. Molecular characterization of Nipah virus from Pteropus hypomelanus in Southern Thailand. Virol J. 2016 Mar 25;13:53. doi: 10.1186/s12985-016-0510-x.

Walpita, P, 2011. Nipah virus-like particles (M, F and G) in mice. PLoS One. 6(4).

Walpita, P., Cong, Y., Jahrling, P.B., Rojas, O., Postnikova, E., Yu, S., Johns, L., Holbrook, M.R. (2017). A VLP-based vaccine provides complete protection against Nipah virus challenge following multiple-dose or single-dose vaccination schedules in a hamster model, Vaccines 2:21.

Wang, L-F, Yu, M., Hansson, E., Pritchard, L. I., Shiell, B., Michalski, W. P., and B. T. Eaton. 2000. The Exceptionally Large Genome of Hendra Virus: Support for Creation of a New Genus within the Family *Paramyxoviridae.* J Virol; 74(21): 9972–9979.

Wang L.F., Harcourt B.H., Yu M., Tamin A., Rota P.A., Bellini W.J. & Eaton B.T. 2001. Molecular biology of Hendra and Nipah viruses. *Microbes Infect.,* 3, 279–287.

Wang, 2006, DNA immunization Nipah F or G, in mice. Ann N Y Acad Sci. 1081:243-5.

Weingartl, H., Czub, S., Copps, J., Berhane, Y., Middleton, D., Marszal, P., Gren, J., Smith, G., Ganske, S., Manning, L., and M. Czub. 2005. Invasion of the central nervous system in a porcine host by nipah virus. J Virol.; 79(12): 7528-34.

Weingartl H. M., Berhane Y., Caswell J. L., Loosmore S., Audonnet J-C., Roth J. A., and Czub M. 2006. Recombinant canarypox virus (ALVAC), with NiV F or G, in pigs. J Virol.; 80 (16): 7929–7938.

Weingartl H. M. 2015. Hendra and Nipah viruses: pathogenesis, animal models and recent breakthroughs in vaccination. Vaccine: Development and Therapy; 2015:5, 59-74.

Westbury, H. A., Hooper, P. T., Brouwer, S. L., and Selleck, P. W. (1996). Susceptibility of cats to equine morbillivirus. Aust Vet J 74(2): 132-4

WHO (World Health Organization)**.** Morbidity and mortality due to Nipah or Nipah-like virus encephalitis in WHO South-East Asia Region, 2001-2018**.** Available at: http://www.who.int/csr/disease/nipah/en/. (Accessed on July 22, 2018).

Williamson, M. M., Hooper, P. T., Selleck, P. W., Gleeson, L. J., Daniels, P. W., Westbury, H. A., and Murray, P. K. (1998). Transmission studies of Hendra virus (equine morbillivirus) in fruit bats, horses and cats. Aust Vet J 76(12): 813-8

Wong S.C., Ooi M.H., Wong M.N., Tio P.H., Solomon T., Cardosa M.J. 2001. Late presentation of Nipah virus encephalitis and kinetics of the humoral immune response. J Neurol Neurosurg Psychiatry; 71(4):552-4.

Wong KT, Shieh WJ, Kumar S, Norain K, Abdullah W, Guarner J, Goldsmith CS, Chua KB, Lam SK, Tan CT, Goh KJ, Chong HT, Jusoh R, Rollin PE, Ksiazek TG, Zaki SR; Nipah Virus Pathology Working Group. Nipah virus infection: pathology and pathogenesis of an emerging paramyxoviral zoonosis. Am J Pathol. 2002 Dec; 161(6):2153-67.

Wu Z, Yang L, Yang F, Ren X, Jiang J, Dong J, Sun L, Zhu Y, Zhou H, Jin Q. (2014). Novel Henipa-like virus, Mojiang Paramyxovirus, in rats, China, 2012. Emerg Infect Dis. 2014 Jun; 20(6):1064-6. doi: 10.3201/eid2006.131022.

Yadav P.D., Raut C.G., Shete A.M., Mishra A.C., Towner J.S., Nichol S.T., *et al.* 2012. Detection of Nipah virus RNA in fruit bat (*Pteropus giganteus*) from India. Am J Trop Med Hyg, 87: 576-578

Yan Li, Jianmin Wang, Andrew C. Hickey, Yunzhi Zhang, Yuchun Li, Yi Wu, Huajun Zhang, Junfa Yuan, Zhenggang Han, Jennifer McEachern, Christopher C. Broder, Lin-Fa Wang, and Zhengli Shi. (2008). Antibodies to Nipah or Nipah-like Viruses in Bats, China. Emerg Infect Dis.; 14(12): 1974–1976. doi: [10.3201/eid1412.080359]

Yob J.M., Field H., Rashdi A.M., Morrissy C., van der Heide B., Rota P., bin Adzhar A., White J., Daniels P., Jamaluddin A., Ksiazek T. 2001. Nipah virus infection in bats (order Chiroptera) in peninsular Malaysia. Emerg Infect Dis. 2001 May-Jun; 7(3):439-41.

Yoneda et al. 2013. Recombinant measles virus, with NiV G in African green monkeys with NiV challenge. PLoS One. 8(3).