NIPAH Baseline Situation Analysis

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Contents

List of abbreviations

AAV	Adeno-associated virus
AGM	African green monkey
CNS	central nervous system
ELISA	enzyme-linked immunosorbent serologic assay
HeV	Hendra virus
IFN	interferon
NK	Natural Killer
NiV	Nipah virus
NP	Nucleoprotein
NHP	Non-human primates
Poly-IC	Polyinosinic:polycytidylic acid
R&D	research and development
RT-PCR	real-time polymerase chain reaction
VEE	Equine Venezuelan Encephalitis
VLP	Virus-like particle
VSV	Vesicular Stomatitis Virus

Introduction

Epidemiology overview

Nipah virus (NiV) is a paramyxovirus (genus Henipavirus) that was first identified in 1999 in Malaysia, where it caused an outbreak of respiratory and neurological disease in pigs and encephalitis in people. Three years later, a genetically distinct NiV independently emerged in India as well as in Bangladesh, where human NiV outbreak events have been reported nearly every year since. A putative NiV also caused an outbreak of disease in horses and people in the Philippines in 2014. To date, there is no reported evidence of NiV outbreaks in humans emerging in any other country than Malaysia, Singapore, Bangladesh, India and Philippines. More than 600 cases of NiV human infections have been reported globally. However, given the delay in identification of the Indian outbreak and large distribution of bats that can carry NiV, it is possible that more human cases have occurred where NiV has not been detected. A total of 276 cases were reported with 106 fatalities (38%) in Malaysia, but case fatalities in later outbreaks in India and Bangladesh were associated with significantly higher case fatality rates of 43 to 100%. NiV infection in humans has a range of clinical presentations, from asymptomatic infection to acute respiratory syndrome and fatal encephalitis. The natural reservoir of the virus consists of the widely distributed fruit bats from the Pteropodidae family. Virus transmission from bats to humans occurs through inhalation, contact or consumption of NiV contaminated foods. NiV is transmitted by zoonotic (from bats to humans, or from bats to pigs and then to humans) as well as human-to-human routes. Human-to-human transmission is particularly notable in the outbreaks in India and Bangladesh, where it has been reported to account for 75% and 51% of cases, respectively. At present no vaccines or antiviral drugs are available for NiV disease and the treatment is just supportive. Current prevention strategies focus on raising disease awareness in affected areas.

The virus

NiV was first isolated by Chua et al. in 1999 after a severe outbreak of viral encephalitis among pig-farmers in Malaysia. The virus, cultured from the cerebrospinal fluid of two patients, was causing syncytial formation of Vero cells after 5 days, and it was found to be a previously undescribed paramyxovirus related to the Hendra virus (HeV). The Henipavirus genus in the subfamily Paramyxovirinae (family Paramyxoviridae) was then created for these two pathogenic viruses, HeV and NiV (Wang 2001). Subsequently, other viruses were added to this genus.

NIV is an enveloped, negative-sense, single-stranded RNA virus. The genome is unusually large, comprising more than 18 000 nucleotides. Its 6 genes code for the nucleocapsid (N), phosphoprotein (P), matrix protein (M), fusion glycoprotein (F), attachment glycoprotein (G) and the large polymerase. The viral G protein attaches to the host cell ephrin B2 and/or B3 receptor, and activates the F protein to initiate viral envelope and host membrane fusion and viral entry (reviewed by Ong 2015). Of note, membrane fusion is not only essential for viral entry but also for cell-cell fusion and the process of syncytia formation, which makes it an attractive target for therapeutic development. Numerous studies have been conducted to decipher the mechanisms of NiV and other paramyxoviruses replication.

The nucleotide sequence of a fair number of NiV isolates has been determined. A relative heterogeneity has been observed among nucleotide sequences obtained from Bangladesh/India as compared with sequences from samples obtained during the initial Malaysian outbreak. This observation led to the classification of two distinct lineages of NiV. Currently available sequences obtained from Malaysia and Cambodia were designated genotype M, while sequences obtained from Bangladesh and India were designated genotype B (Lo 2012, reviewed by Angeletti 2016). A 729 nucleotide region of the N protein gene of NiV has been proposed that can be used for such genotyping. Predicted amino acid identities between NiV-M and NiV-B range from 92% to 100% (reviewed by Rockx 2012).

Relatively few isolates of NiV have been obtained from human cases across different infection outbreaks

The variation of nucleotide and amino acid sequences within the Malaysia genotype (NiV-M) has been reported to range, respectively, between 0.19 and 2.21% and 0.18 and 3.67%, and within the Bangladesh genotype (NiV-B) between 0.28 and 1.06% and 0.28 and 0.56%. In Malaysia, very limited variation was observed between human NiV isolates and isolates obtained from bats years later. Interestingly, molecular evidence suggests that at least two major strains of NiV were circulating in pigs during the 1998 outbreak in Malaysia. No data are available to indicate whether these 2 strains represent 2 independent introductions of NiV into the pig population, or if the latter strain evolved from the initial NiV strain. Interestingly, the NiV diversity observed among isolates from bats in Malaysia and Thailand was associated with co-circulation of multiple strains within populations rather than co-evolutionary patterns (Angeletti 2016).

In Bangladesh, genetic heterogeneity in human isolates suggests multiple introductions of NiV in the human population from different colonies of fruit bats.

Sequence homology at the level of G and F, the glycoproteins involved in virus binding and entry, is sufficient to allow for antigenic cross-reactivity between HeV and NiV, but NiV-B and NiV-M G and F share a much higher homology

The NiV attachment glycoprotein G and fusion protein F are essential for virus binding and entry and as such are the primary targets for protective antibody responses. The 83% and 88% amino acid identity between HeV and NiV G and F, respectively, results in antigenic cross-reactivity between these viruses (Rockx 2012). The NiV-M and NiV-B strains share 95% amino acid homology in the G protein and 98% homology in the F protein.

Other henipaviruses

HeV was first isolated in 1994 in Australia from fatal cases of severe respiratory disease in horses. Since its first identification in 1994, it has caused 7 confirmed cases in humans and 4 deaths in Australia, all acquired from contact with infected horses (Broder 2013). Infection of horses results from spill-over events from the natural host reservoir, which has been identified as pteropid bats (Black flying-fox, Pteropus Alecto, and Spectacled flying-fox, Pteropus conspicillatus). In humans, HeV begins as an influenza-like illness, but the involvement of the lung and brain, as with Nipah, can also manifest as an acute severe respiratory syndrome, encephalitis or a combination of both.

Other henipaviruses are not known to cause human disease, except for Mojiang paramyxovirus (MojPV), a henipavirus-like virus, which has been implicated in the death of three miners in China in 2012, following potential zoonotic transmission from rats (Wu 2014).

Immune Responses to NiV Infection

Innate immunity

(adapted from Prescott 2012)

Innate immunity generally refers to the ability of cells to detect the invasion of a microbe by the engagement of pathogen-associated molecular patterns (PAMPs) with host encoded pattern recognition receptors (PRRs). This interaction leads to the expression of several antiviral proteins, as well as type I and type III interferons (IFNs), which activate additional antiviral responses via the Jak/STAT pathway. The NiV genomic RNA is recognized by cellular cytoplasmic RNA helicases. In vitro, endothelial cells (an important cell type targeted in vivo) infected with NiV produce IFNβ as well as innate chemokines and cytokines, including IP-10 and IL-6. IP-10 is a chemokine that attracts activated T lymphocytes, whereas IL-6 is a cytokine that stimulates acute-phase proteins and acts as an inflammatory molecule. These chemokines have the ability to functionally recruit T cells. This mechanism likely contributes to the extensive vasculitis reported in virtually all histopathology studies. In vivo, little is known about the activation of the innate immune system. The expression of innate immune genes has been documented as a response to NiV infection in animal models of disease, including the upregulation of IP-10 and IL-6.

Nevertheless, this innate response is balanced by the ability of NiV (as other RNA viruses causing disease in humans) to stimulate antagonising mechanisms. NiV has been extensively characterized in vitro for this ability to subvert innate immunity and several mechanisms have been identified, as presented below.

Mechanisms of inhibition of innate immunity

The P gene of NiV is transcriptionally edited to produce not only the phosphoprotein but also two alternate V and W proteins, and an alternate ORF expresses a C protein. These proteins possess multiple capabilities to inhibit IFN production, as well as downstream signalling. In addition, the expression of these antagonistic genes is temporally regulated during infection. Both STAT1 and STAT2, which are required for IFNβ signalling, are antagonized by P gene products. The antagonistic function of the P, V, W and C proteins has been assessed in vivo. Hamsters inoculated with recombinant virus lacking the ability to produce either the C or the V protein displayed no pathology, and the viral genome was almost undetectable in these animals, suggesting that the antagonistic properties for these proteins are responsible for pathogenesis. The results were subsequently confirmed by Satterfield (2015). A V-deficient virus was found strongly attenuated, behaving as a replicating non-lethal virus in ferrets.

Bharaj (2016) also reported that the matrix protein (M), which is important for virus assembly and budding, can also inhibit IFN-I responses by targeting TRIM6, IKKε and unanchored polyubiquitin chains.

Finally, the nucleoprotein (N) was shown to impact host innate immune responses by preventing the nucleocytoplasmic trafficking of STAT1 and STAT2, resulting in an antagonistic activity against the JAK/STAT signalling pathway (Sugai 2017).

Adaptive immunity

Adaptive immune responses to NiV infection in humans have been very poorly characterized

Little is known about the adaptive immune response to NiV infection in humans. Studies are lacking to identify protective responses. And the immunological mechanisms that may explain the different patterns of disease severity have not been better elucidated.

Sera from infected patients contain measurable NiV-specific IgM antibodies as early as four days after exposure, persisting for at least 3 months (reviewed by Ong 2015, Mathieu 2015, Prescott 2012). Of note, no difference has been observed in clinical features or mortality between seropositive or seronegative patients. Specific IgG can also be detected by day 25 following infection and were shown to persist for several years. The IgG3 and IgG1 subclass distribution observed in NiV infection is similar to the IgG subclass responses seen in measles (Wong 2001).

The presence of antibodies suggests that both B-cell and CD4+ T-cell responses are elicited in response to virus infection, but the cellular arm of the immune response has not been studied in convalescent subjects, and the role it may play in protection following natural infection or vaccination remains unknown.

The NiV N protein is considered as a highly immunogenic protein in human infections and therefore has been proposed as a target for serological diagnosis (Yu 2006). Of note, using immunoblot assays, the serum from one patient with a relapsing NiV encephalitis complication has recently been shown to not react as robustly with the NiV proteins as compared to the other positive patients'sera, and to bind mainly to the NiV G protein (Tiong 2017).

NiV-neutralizing antibodies have been detected in patients' sera. Animal studies suggest that they are highly protective when elicited/administered before or shortly after infection.

Complement has been reported to significantly enhance neutralization by antibodies specific for the NiV F and G glycoproteins (Johnson 2011).

Pathophysiology and clinical disease, case definition

Clinical disease

(adapted from the reviews by Ong 2015, Kulkarni 2013, Wong 2012 and Rockx 2012)

In the majority of cases, the incubation period of Nipah has been reported to be 5 days to 2 weeks; however, a maximum delay of 2 months between exposure and the onset of illness has also been observed during the outbreak in Malaysia (Goh 2000). The majority of patients initially develop influenza-like signs and symptoms, including fever, headache, myalgia and vomiting. In general, the more severe clinical features manifest as either an acute encephalitic syndrome or less frequently a pulmonary syndrome. It is also very likely that a considerable number of infections remain asymptomatic but the exact prevalence is unknown.

Goh (2000) described the clinical features of the largest cohort of 94 patients with NiV encephalitis who were admitted to a single hospital during the outbreak in Malaysia. Clinical signs such as areflexia/hyporeflexia with hypotonia, pinpoint pupils with variable reactivity, tachycardia, hypertension and abnormal doll's eye reflex were more frequently seen in patients with reduced

level of consciousness. Segmental myoclonus characterized by focal, rhythmic jerking of the diaphragm and muscles in the limbs, neck and face was present in 32% of patients, and may be unique to acute NiV encephalitis. Other clinical features (e.g. meningism, generalized tonic-clonic convulsions, nystagmus and cerebellar signs) were also observed. A pulmonary syndrome has been described in some patients who presented with cough, atypical pneumonia, acute respiratory distress syndrome and abnormal chest X-ray findings.

Interestingly, a higher prevalence of respiratory disease was observed during the Bangladesh outbreaks with cases experiencing atypical pneumonia and developing an acute respiratory distress syndrome (Hossain et al., 2008). In Bangladesh, altered mental status and/or unconsciousness was reported in more than 70% of patients, severe weakness in 67% and areflexia/hyporeflexia in 65% of cases, consistent with a high prevalence of acute encephalitis.

In Bangladesh, based on a review of 196 cases, the median duration from onset of illness to death was 6 days (ranging from 1 to 47 days) (Rahman 2012). Overall, mortality was high, ranging from about 40% to 100%. In a large cohort of patients who survived, the majority had no or few sequelae. However approximately 20% of patients were reported to have neurological deficits, neuropsychiatric sequelae and gait/movement disorders. The most intriguing complication of Nipah is probably relapsing encephalitis which may occur weeks to years after symptomatic infection and even after asymptomatic NiV infection. So far, more than 20 cases of relapsing NiV encephalitis have been reported, one of which occurred 11 years after an asymptomatic infection. Clinical and radiological findings suggest that relapsing NiV encephalitis is distinct from acute NiV encephalitis. The brain MRI in relapsing encephalitis shows more extensive and confluent hyperintense cortical lesions. Virus could not be cultured from samples collected in a series of NiV relapsing encephalitis. There are currently no known risk factors for relapsed encephalitis, and the mechanisms for persistent CNS infection and disease recrudescence have not been elucidated.

Risk factors and mechanisms for persistent CNS infection and disease recrudescence are unknown

Pathological features are characterized by disseminated, multi-organ vasculopathy comprising endothelial infection/ulceration, vasculitis, vasculitis-induced thrombosis/occlusion, parenchymal ischemia/microinfarction, and parenchymal cell infection in the central nervous system (CNS), lung, kidney and other major organs. This unique dual pathogenic mechanism of vasculitis-induced microinfarction and neuronal infection causes severe tissue damage in the CNS.

Case definition

To our best knowledge, there is no standard case definition of Nipah disease. Epidemiological investigations have used various definitions. At least a temporal association with a disease outbreak and the need for laboratory confirmation were shared across definitions. For instance, the definition used during the 2004 outbreak in Bangladesh is presented below (WHO 2004)

Can a standard case definition of Nipah disease be developed and used widely?

*A **suspect case** was any person with fever AND one or more of the following isolated neurological signs: altered mental state, confusion, convulsions, unconsciousness or neck stiffness, focal weakness/paralysis after 15 December 2003 OR an individual with fever and vomiting or respiratory symptoms or headache AND having had physical contact or shared daily activities with or lived near to a probable or laboratory-confirmed case after 15 December2003.*
*A **probable case** was any patient, dead or alive, who developed fever AND one or more of the following isolated neurological signs: altered mental state, confusion, convulsions, unconsciousness or neck stiffness, focal weakness/paralysis after 15 December 2003 AND who was living in the same district as a laboratory-confirmed case after 15 December 2003.*
*A **laboratory-confirmed case** was any case positive for Nipah IgM antibody after 15 December 2003.*

Pathophysiology of Nipah

Viral tropism

Infections caused by henipaviruses are characterized by their ability to infect multiple organ systems, with a predominant neurological or respiratory tropism. In human cases of NiV infection, NiV can be detected in bronchiolar epithelial cells and is shed mainly by nasopharyngeal and tracheal secretions in the early phase of the illness (reviewed by Escaffre 2013). During the late stages of disease, virus replication spreads from the respiratory epithelium to the endothelium in the lungs. The infection can sometimes trigger a prominent vasculitis in small vessels and capillaries as characterized by endothelial syncytium and mural necrosis. Without being infected themselves, circulating leukocytes could transfer the virus to endothelial vascular cells (Mathieu 2015). NiV was shown indeed to not infect but to efficiently bind to human lymphocytes, and co-culturing with permissive endothelial cells resulted in virus amplification and infection of these target cells. Moreover, mononuclear leukocytes isolated from infected hamsters transferred lethal infection to naive animals. Leukocytes would thus be used as a vehicle to spread the virus to other organs by the haematogenous route. In addition to the lungs, important target organs are the brain, spleen and kidneys, and viremia following respiratory infection can lead to multi-organ failure.

Entry into the brain may actually occur through two distinct pathways: i.e. via the hematogenous route through the choroid plexus and cerebral blood vessels, or anterogradely via the olfactory nerve. In animal models of infection, early virus replication was indeed observed in the olfactory epithelium (Baseler 2016). In addition, Borisevich (2017) showed that human olfactory neurons are highly susceptible to infection with henipaviruses. Nevertheless, it is currently unknown whether the route of virus transmission seen in animal models is relevant in human infections, since the olfactory epithelial surface is relatively large in these species compared to man (Escaffre 2013).

Determinants of pathogenicity

The determinants of NiV pathogenicity are poorly understood. Clinical observations as well as data generated in non-human primates (NHPs) indicate pathogenic differences between NiV-M and NiV-B isolates. For instance, in African green monkeys (AGM), while NiV-B was uniformly lethal, only 50% of NiV-M-infected animals succumbed to infection. Histopathology of lungs and spleens from NiV-B-infected AGMs was significantly more severe than NiV-M-infected animals. These data showed that NiV-B is more pathogenic than NiV-M in AGMs, as observed in human subjects (Mire 2016). However, the mechanisms underlying these differences remain unknown.

Similarly experimental infections in ferrets suggest that NiV-B is shed at higher levels than NiV-M, which may be sufficient to enable the former virus to transmit more efficiently between humans (Hayman 2014).

As presented above, several gene products of NiV have the capacity to inhibit the early proinflammatory response at sites of infection, thereby preventing control of the infection by the immune system. Immunopathology is another possible mechanism. For instance, it was shown that NiV infection resulted in overexpression of CXCL10 (IP-10) in endothelial cells and perivascular infiltrating cells in the brain of patients, as well as Syrian hamsters. CXCL10 is an important chemoattractant involved in the generation of inflammatory immune response and neurotoxicity. Its expression likely plays a role in the accumulation of inflammatory cells in infected areas of the brain (reviewed by de Wit 2015).

Case detection

The epidemic of Nipah virus in Malaysia in 1998/1999 required more than six months before being effectively controlled, as a consequence of the misdiagnosis of the etiologic agent and the resulting implementation of incorrect control measures (Chua 2013). National systems for surveillance of infectious disease in countries such as Malaysia subsequently recognised Nipah as a zoonotic disease of interest, and included it in their list of notifiable diseases. Hospital-based surveillance was developed, as it is considered necessary to quickly detect NiV outbreaks and initiate appropriate control measures (Rahman 2011). However, surveillance in rural areas of the countries where NiV is endemic in bats can be expected to be of moderate quality, so that many cases likely go undetected (Satterfield 2016b). Veterinary surveillance of NiV encephalitis in pigs is organised as well, as the disease appears among the "OIE-Listed diseases, infections and infestations" in force in 2017.

Case management

Guidelines for the clinical management of Nipah patients have been made available in various countries, including affected countries such as Bangladesh (Rahman 2011) and Malaysia. Such management is based on general, supportive treatment (ensuring fluid and electrolyte balance, oxygen inhalation if required…etc.), symptomatic treatment (fever, convulsions, shock…etc), and procedures to prevent further spread of the disease (isolation, barrier nursing, safe handling of deceased bodies…etc.).

Special populations

To our knowledge, there has been no evidence of increased incidence or increased severity of Nipah in special populations such pregnant women, infants or immunocompromised subjects. However, in the case series reported by Goh (2000) older patients, especially those having diabetes mellitus and those with severe brain-stem involvement, carried a poorer prognosis than other Nipah patients.

Animal models

Small animal models

Mice

Standard laboratory mouse strains such as Balb/c and C57BL/6 do not develop disease upon intraperitoneal or intranasal inoculation with NiV (reviewed by de Wit 2015). However, intracerebral

inoculation of these animals results in a lethal infection. In aged mice, intranasal NiV inoculation did not result in clinical disease, but most animals seroconverted and vRNA and infectious virus could be detected between 2 and 15 days after inoculation in the lungs of most animals. In IFNAR-KO mice lacking the type I IFN receptor, intraperitoneal inoculation resulted in neurological disease. Histologically, these animals developed vasculitis, perivascular cuffing and meningeal inflammation; the lungs exhibited bronchointerstitial pneumonia.

Of note, a human lung xenograft model has also been developed to study the pathogenesis of NiV infection in human lung in vivo (Valbuena 2014). The model uses the severely immunodeficient NOD/SCID/γcnull (NSG) mice as hosts to support the successful engraftment of human lung xenografts.

Guinea pigs

NiV infection in guinea pigs has been reported to be often mild and variable, ranging from an inapparent infection following challenge to sudden death (reviewed by Geisbert 2012). One study demonstrated that intraperitoneal inoculation with high doses of NiV resulted only a transient fever with minor weight loss after 5–7 days. Guinea pigs are thus not considered a suitable animal model of NiV infection.

Hamster

The Golden Syrian Hamster is a commonly used model to study henipaviruses pathogenesis as the clinical signs and pathological lesions observed following inoculation highly resemble those observed in humans, even though the disease course seems to be affected by the route of inoculation and the inoculum dose. The virus used for infection also influences the disease course, as NiV-M-infected Syrian hamsters show accelerated virus replication, pathology and death when compared to NiV-B-infected animals (DeBuysscher 2013).

Like human patients, the main histopathological finding in Syrian hamsters infected with NiV is systemic vasculitis. Moreover, animals develop encephalitis, with infection of neurons in the CNS. In hamsters that develop severe respiratory disease, usually upon intranasal inoculation with a high inoculum dose, rhinitis can be observed, with virus replicating in the respiratory as well as olfactory epithelium; bronchointerstitial pneumonia develops in the lungs of these animals (reviewed by Dhondt 2013 and de Wit 2015).

Ferrets

Ferrets inoculated oronasally with NiV develop respiratory as well as neurological signs of disease. Systemic vasculitis is present in infected ferrets. Histologically, the upper respiratory tract, with rhinitis, tonsillitis and nasopharyngitis, as well as the lower respiratory tract, with necrotizing bronchointerstitial pneumonia, are affected; focal necrosis has been detected in the spleen. Encephalitis has not been detected, but non-suppurative meningitis occurred in a subset of animals and viral antigen was detected in brain endothelial cells and meninges, and occasionally in neurons and glial cells close to infected endothelium (de Wit 2015).

Non-Human Primates

Preclinical tests of vaccines and therapeutic products are ideally conducted in an animal species that not only develops similar symptoms of the disease as observed in humans, but also shares similar pathophysiological and immunological mechanisms. Non-human primates typically best meet these

requirements. The first NHP model described for NiV infection was the Squirrel monkey. However, more recent studies favoured AGMs.

African Green Monkey

A consistent lethal infection and disease is observed in AGMs with low dose NiV challenge. Infection by intratracheal inoculation results in a rapid spread of the virus within 3–4 days and the establishment of infection in multiple organ systems (reviewed by Dhondt 2013, Geisbert 2012, Geisbert 2010). Severe respiratory pathology, neurological disease, endothelial syncytia, and generalized vasculitis all occur in AGMs infected with NiV, providing an accurate reflection of what is observed in infected humans. This conclusion was further confirmed by a comprehensive study reported in 2015 (Johnston), that captured physiological data together with clinical pathology information and virological testing in 4 AGMs inoculated by intra-tracheal route. Increases in heart rate and respiratory rate seen in most animals during acute phase suggested responses to fever, to a developing hypoxic state, and/or to a state of compensatory shock. Animals that succumbed appeared to enter a state of irreversible decompensated shock with hypothermia, azotemia, hyperglycemia, and signs of liver disease. Similar to NiV disease in humans, pneumonitis, systemic vasculitis, and coagulopathy were prominent findings in these animals, and significant histopathologic lesions were present in most tissues assessed, including lymphoid tissues and the lungs. Virus antigen was often associated with areas of necrosis and/or inflammation. Interestingly, in all animals that survived beyond the acute disease phase, relapse encephalitis was identified.

Other animals

Cats

Cats inoculated with NiV subcutaneously or intranasally and orally develop signs of respiratory disease. Histologically, cats develop bronchointerstitial pneumonia and, in some animals, meningitis (reviewed by de Wit 2015). The virus can be found in the lungs, but also in kidneys, spleen and lymph nodes. The model may be used for evaluation of vaccines or therapies, but its interest in limited by the poor availability of reagents and limited characterization of the pathogenic mechanisms in this species.

Pigs

Efforts have been undertaken in view of the development of a NiV veterinary vaccine for pigs, which explains the large number of studies conducted in this animal species. Oronasal and ocular routes of NiV infection mimic quite well natural infection and most infected animals remain asymptomatic even if viral shedding is observed following challenge (reviewed by Dhondt 2013). Sick animals present fever, depression, cough, shivering, and rarely, neurological signs including abnormal posture and seizures. A subcutaneous route of inoculation was also tested, causing symptomatic infections in pigs. The disease shares a number of features with human infection. However, a number of species-specific differences of NiV replication in porcine and human cells have been identified. Sauerhering (2016) found that NiV growth substantially differs in primary airway epithelial cells between pigs and humans, with a more rapid spread of infection in human airway epithelia. Increased replication in human cells correlated with higher endogenous expression levels of the main NiV entry receptor ephrin-B2. On the other hand, porcine monocytes, natural killer cells, and CD6+/CD8+ T cells have been found to support NiV replication, which may facilitate dissemination of the virus (Prescott 2012).

Comparison of models

The advantages and disadvantages of the various animal models described in the literature are presented in Table 1 below.

Table 1. Summary of advantages / disavantages of the different animal models of NiV infection

Model	Advantages	Disadvantages
Mice	Low cost	Intracranial challenge route not relevant to human infection
IFNAR-/- mice	Low cost Some resemblance with pathology in humans (vasculitis, bronchointerstitial pneumonia, meningeal inflammation but no encephalitis)	Not suited to vaccine studies Intraperitoneal route of infection
Syrian hamsters	Low cost Good resemblance with infection in humans (extensive lesions, respiratory and neurological disease; systemic vasculitis)	Limited availability of reagents
Ferrets	Some resemblance with infection in humans (respiratory disease, systemic vasculitis, but encephalitis not detected)	Limited availability of reagents
AGM	Good resemblance with disease in humans (infection in multiple organ systems, neurological disease and severe respiratory pathology along with generalized vasculitis) Resemblance with immune response in humans	High cost Small sample size
Cats	Intranasal or oral route of challenge Very limited resemblance with disease in humans (respiratory disease, but no neurological signs)	Limited availability of reagents, limited characterization of the model
Pigs	Some resemblance with disease in humans (respiratory disease, encephalitis, as well as congestion and haemorrhagic lesions)	Various species-specific differences of NiV replication identified

More detailed tables describing the disease and histopathological lesions in each animal model can be found in the reviews by Dhondt (2013) and Geisbert (2012). While there is today no ideal animal model to study novel vaccines or therapies against NiV infections, various options are available for preclinical testing of such candidates.

Preclinical models are available for evaluation of NiV vaccine and therapy candidates

Epidemiology, Prevention and Control

Transmission and maintenance

The widely distributed fruit bats from Pteropodidae family are considered to form the natural reservoir of NiV. Serological studies demonstrated evidences of NiV infection in multiple bat species, including frugivorous and insectivorous bats. As summarized by Wacharapluesadee (2016), NiV-B has been found in Pteropus giganteus (India), and Pteropus lylei (Thailand). NiV-M has been found Pteropus hypomelanus (Malaysia and Thailand), Pteropus vampyrus (Malaysia and Indonesia), Pteropus lylei (Cambodia and Thailand), Hipposideros larvatus (Thailand), Taphozous species (Thailand) and Rousettus amplexicaudatus (East Timor). However, viral isolation and molecular characterization was usually only successful in Pteropus species (Simons 2014). Henipavirus infection in bats seems to be asymptomatic. Experimental infection by subcutaneous route has been followed by seroconversion in the majority of animals in the absence of visible clinical disease, while a range of histopathological changes was observed within the tissues. Nipah virus was excreted in urine. As suggested by Middleton (2007), this intermittent, low-level excretion may be sufficient to sustain the net reproductive value of the virus in a species where there is regular urine contamination of the fur, mutual grooming, and where urine droplets are a feature of the environment.

Introduction of NiV infection into the human population occurs by two mechanisms of spill-over from bats: transmission via an intermediate animal host, which precipitated the outbreak in Malaysia; and bat-to-human transmission, which has occurred in Bangladesh and India (Clayton 2017).

In Malaysia, the highest risk of human NiV infection was associated with activities involving close contact with and handling of pigs, and transmission to people is thought to have occurred through direct contact with infectious secretions or excretions of pigs. Importation of infected pigs into Singapore from Malaysia precipitated a cluster of infections in abattoir workers in Singapore, whose exposure risks were also associated with close contact with pigs or their excretions. In Malaysia, NiV was highly infectious for pigs, with all classes of pigs susceptible. The pattern of on-farm infection was consistent with respiratory transmission; between-farm spread was generally associated with the movement of pigs. An extensive post-outbreak surveillance program in Malaysia showed that farms that did not receive pigs at the time of the outbreak generally remained uninfected, even when neighbouring farms were infected (Breed 2006). Human infections were predominantly attributed to contact with live pigs. A small number of infected people had no history of contact with or proximity to pigs, and human-to-human transmission is suspected in a few cases. No human case was attributed to contact with bats.

In Bangladesh, a most common risk factor for human NiV infection is drinking contaminated date palm sap or its fermented product. Date palm sap is harvested from December through to March by cutting into the tree trunk and allowing the sap to flow overnight into an open clay pot. Infrared camera studies have demonstrated that Pteropus giganteus bats frequently visit date palm sap trees and lick the sap during collection, potentially contaminating it with NiV from saliva and/or urine (Simons 2014). The other common risk factor for human infection is contact with a patient with Nipah encephalitis. Overall, these two risk factors reflect the two main mechanisms of human infection observed in Bangladesh as well as in India: bat-to-human and human-to-human transmission. In a small number of instances, direct contact with infectious material left by foraging

Possible NiV outbreak in the Philippines in 2014

(adapted from Ching 2015)

In April 2014, 2 human deaths were reported in the municipality of Senator Ninoy Aquino, island of Mindanao in the Philippines. An outbreak investigation identified additional human deaths and non-fatal infections as well as concurrent neurologic disease and sudden deaths in several horses. Subsequently, a human case was defined as illness in any person with an epidemiologic link to the municipality of Senator Ninoy Aquino and who had experienced acute encephalitis syndrome, severe influenza-like illness, or meningitis during the period from March 3 to May 24, 2014. This case definition was met by 17 persons (11 acute encephalitis syndrome, 5 influenza-like illness, 1 meningitis).

Of the 17 case-patients, a total of 7 (41%) had participated in horse slaughtering and horse meat consumption, and 3 (18%) had only consumed horse. Five (29%) case-patients had been exposed to other human case-patients, but not to any horses.

Neutralizing antibodies against NiV and correspondingly lower neutralizing antibody titers against HeV were found for 3 patients. A serum sample from a patient was positive by real-time PCR for NiV, and a 71 nucleotides long sequence of the P gene of NiV was detected from another patient. This short segment had 99% nucleotide identity with NiV-M and 94%-96% identity with NiV-B. Further attempts to amplify additional genome and isolate the virus were unsuccessful.

This outbreak led to the conclusion that ongoing surveillance is needed to help with prompt response to future potential additional outbreaks of Nipah, including not only diagnosis, but also investigation of risk factors associated with spill-over and virus transmission, implementation of control measures, and further characterization of the virus involved. Another important lesson from this outbreak is the fact that a NiV isolate not identical to the one currently circulating in Bangladesh has the potential to emerge and cause disease in human.

bats in trees or on fruit may have also played a role in bat-to-human transmission, and domesticated animals such as cows, goats and pigs may also pose infection risks to people.

Person-to-person transmission has been identified as a pathway for infection of people in India and Bangladesh. In Siliguri, India, 75% of the patients had a history of hospital exposure, i.e. they were members of the hospital staff or had attended to or visited patients in the hospital (Chadha 2006). Similarly, a study of human NiV infections between 2001 and 2007 in Bangladesh attributed 51% of all cases to onward transmission from an infected person, although only 7% of people were identified as having transmitted their infection. In these instances, close contact between infected and naïve individuals appears to drive transmission, and specific risk factors for exposure are associated with direct contact with a clinically unwell patient or their secretions, in particular patients with severe illness and who later died as a result of their infection. Several studies evaluated the risk factors associated with transmission and suggested that the main mode of human-to-human transmission is via direct contact with the infectious secretions of a patient with clinical disease; this is further supported by isolation of NiV from respiratory secretions and urine of patients (Clayton 2017). So far, the basic reproductive number (R_0) of the strains of NiV that have spilled over in Bangladesh has remained very low, averaging 0.48 (Luby 2013).

What is the potential for NiV and other hendraviruses to cause a pandemic ?
(adapted from Luby 2013)

Human-to-human transmission of NiV remains very limited in current outbreaks. However, humans are susceptible to the virus; and as an RNA virus, NiV is characterized by a high rate of mutation, which has the potential to lead to the emergence of a strain better adapted to spread from human to human. Moreover, if such adapted virus were to infect communities in South Asia, high population densities and global interconnectedness could be expected to rapidly spread the infection.

At risk populations

As demonstrated by the Ebola epidemic in 2013-2016, there is potential for highly pathogenic emerging infections that normally cause only small, isolated and containable outbreaks to occasionally result in large epidemics that inflict a significant mortality and morbidity burden. In this regard, the number of people at risk of acquiring NiV in Bangladesh and the neighboring West Bengal state of India (regions that experience more frequent outbreaks) exceeds 250 million. The total number of people at risk, including all countries that experience NiV outbreaks and in which the Pteropus bats occur naturally, exceeds two billion (Satterfield 2015).

Due to the different mode of virus transmission during the outbreaks in Malaysia and Singapore compared to more recent outbreaks, the definition of at risk populations has evolved. Following the NiV outbreak in March 1999 in Singapore, a serological survey screened individuals potentially exposed to NiV, and found that all of the antibody positive individuals had direct contact with pigs (Chan 2002). Subsequent observations in Bangladesh and India led to very different results.

The risk of NiV transmission is underpinned by social and environmental factors, which should be taken into account for control measures

A review of 196 cases in Bangladesh indicated that cases were distributed in all age groups, with a median age of 25 years (range: 6 months to 75 years) and 124 (63%) cases were observed in males (Rahman 2012).

Human cases of Nipah were found to generally occur in areas near inland water, predominantly the Ganges, which provides a suitable habitat for date palm trees (Simons 2014). The disease was also characterized by seasonality in Bangladesh, with cases occurring only between December and April, roughly coinciding with the time that palm sap is collected (Simons 2014, Stone 2011). The central role for palm sap consumption as a risk factor was identified by a number of studies. For instance, Hedge (2016) analysed a large dataset of 157 cases and 632 controls over the period 2004–2012 in Bangladesh, and found that Nipah cases were 4.9 times more likely to consume raw date palm sap.

Human-to-human NiV transmission is the source of additional risk factors, as illustrated by the study by Hedge (2016), who observed that cases in Bangladesh were 7.3 times more likely to have contact with a Nipah case than controls. Specific cultural practices may underlie the fact that transmission commonly occurs between infected patients and their caregivers. In Bangladesh, societal norms dictate that such care is provided by patients' family members even in hospital settings, where trained health-care professionals adopt a "hands off" approach to patient management. A number of cases of human infection in Bangladesh were attributed to corpse-to-human transmission, through

embracing the body of a loved one soon after their death, or through ritual preparation of a corpse for religious burial (Clayton 2017).

Distribution

Table 2 below presents the human cases of NiV that have been confirmed since first identification of the virus.

Table 2. Morbidity and mortality due to Nipah or Nipah-like virus encephalitis

(adapted from http://www.searo.who.int/entity/emerging_diseases/links/nipah_virus_outbreaks_sear/en/)

Year(s)	Country	Reported number of human cases	Reported number (%) of deaths among cases
1998-1999	Malaysia Singapore	276	106 (38%)
2001	India	66	45 (68%)
2001	Bangladesh	13	9 (69%)
2003	Bangladesh	12	8 (67%)
2004	Bangladesh	67	50 (75%)
2005	Bangladesh	12	11 (92%)
2007	Bangladesh	18	9 (50%)
2007	India	5	5 (100%)
2008	Bangladesh	11	9 (82%))
2009	Bangladesh	4	1 (25%)
2010	Bangladesh	16	14 (88%)
2011	Bangladesh	44	40 (91%)
2012	Bangladesh	12	10 (83%)
2013	Bangladesh	24	21 (88%)
2014	*Philippines*	*17**	*9 (53%)*
2014	Bangladesh	18	9 (50%)
2015	Bangladesh	9	6 (67%)

* possible NiV outbreak

Since 2001, outbreaks of Nipah have occurred almost annually in Bangladesh, affecting from 4 to more than 60 subjects (reviewed by Clayton 2017).

NiV has been shown to circulate in Pteropus bats in the countries where outbreaks of human disease have occurred. A 3-year longitudinal study of Pteropus hypomelanus bats (Rahman 2013) in Malaysia indicated an overall NiV seroprevalence of 9.8%, with nonseasonal temporal variation. Viral circulation was demonstrated within the study period.

Evidence of hendravirus or hendravirus-like infection was demonstrated by serological and/or molecular testing in bat samples collected from numerous countries (reviewed by Breed 2013), such as Bangladesh, India, Thailand, Cambodia, Vietnam, Indonesia or Madagascar where samples were found positive for NiV; China with a NiV-like virus; or Australia, Papua New Guinea and Indonesia with HeV. Bat infection was even detected in West African countries, with samples from Ghana and Equatorial Guinea found henipavirus-positive. These bat viruses may have the potential to spill-over into other mammalian hosts, including human. Actually, Pernet (2014) showed evidence of henipavirus infection in human samples from Cameroon.

Current prevention and control strategies

Current strategies for the management of henipaviruses are generally directed at minimising direct or indirect contact with the natural host (e.g. with palm sap), monitoring intermediate hosts,

improving biosecurity on farms, and better disease recognition and diagnosis (Breed 2006). Prevention efforts also focus on interventions to interrupt transmission of NiV through person-to-person contact.

In Malaysia, the central strategy has been the implementation of sound farm management practices, such as monitoring herd health and early recognition of disease syndromes (Breed 2006). Several simple on-farm measures can also be taken to reduce the likelihood of spill-over events occurring. They include removal of fruit tree plantations from the immediate vicinity of pig sheds, wire screening of open-sided pig sheds, or ensuring roof run-off does not enter pig pens to avoid contact with bat urine or faeces or partially eaten fruits.

In Bangladesh, interrupting bat-to-human transmission is a main objective. People's knowledge of NiV was found low in a study conducted in randomly selected villages in Bangladesh (Nahar 2015), indicating a need for implementing strategies to increase awareness about the risks associated with this virus. Research was also conducted on methods to protect palm sap from bats. Studies compared methods to protect sap (Khan 2012). Bamboo, dhoincha, jute stick and polythene skirts covering the sap producing areas of a tree effectively prevented bat-sap contact. Pasteurizing date palm sap is another option. Moreover, the Government of Bangladesh issued a recommendation to abstain from drinking raw sap. However, such prevention methods rely on behaviour modifications. While these approaches appear to be effective strategies of prevention, their implementation is often problematic due to cultural factors (Satterfield 2015). Research was then conducted on behaviour change communication interventions. Nahar (2017) reported for instance that a 'do not drink raw sap' message is less effective than an encouragement to only drinking sap if it has been protected from bat contamination, 'safe palm only'. In addition, Parveen (2016) found that during outbreaks, one-way behaviour change communication without meaningful causal explanations is unlikely to be effective. Based on the cultural context, interactive communication strategies in lay language with supporting evidence may help make prevention messages credible in affected communities.

Similar communication is also necessary during outbreaks to interrupt person-to-person transmission. The National Guideline for Management, Prevention and Control of NiV Infection developed in Bangladesh identified a variety of prevention messages and levels of communication, to address risks associated with patient to care giver/contact, patient to health care worker, touching objects used by patients or handling deceased bodies (Rahman 2011). Specific measures are also taken in the hospital setting to avoid further spread of the disease, including isolation of patients, using personal protective equipment and applying standard precautions such as environmental decontamination and safe waste disposal. Another precaution is the safe handling of corpses of suspected Nipah patients.

Summary of epidemiology, prevention and control

Because of their wide distribution and flying range that can cover huge areas of human habitat, pteropid bats are highly effective in NiV dissemination. Moreover, the changing climate can be expected to affect the distribution of this virus reservoir in the future. Hence, the likelihood of additional henipavirus outbreaks in new areas/countries can be considered very high (Ong 2015). In view of the very high case fatality associated to the disease and the lack of safe and effective vaccine and therapies, this is a serious source of concern.

To date, outbreaks have resulted from two main transmission modes, consumption of contaminated palm sap (Bangladesh, India) or direct contact with infected pigs (Malaysia, Singapore). Since 2001, outbreaks of Nipah have been observed almost annually in Bangladesh. Current prevention of disease essentially relies on behaviour modifications to prevent spill-over from bats, even though the implementation of such measures is often challenging.

Human-to-human transmission has contributed significantly to the spread of NiV in Bangladesh and India, as well as during the first recognised (putative) outbreak in the Philippines. Human-to-human transmission is driven by close contact with patients who are clinically unwell, and factors such as patient care and basic infection control measures will be critical considerations for the mitigation of onward transmission of NiV infection in future outbreaks in people (Clayton 2017).

Diagnostics

Reliable detection of markers of NiV infection remains a key to establishing diagnosis both in animals and humans because the diagnosis may not be suspected initially, and disease manifestations can be rather nonspecific (Ong 2015).

Diagnostic Technologies

Various assays have been developed for laboratory confirmation of NiV infections. ELISA and RT-PCR are preferred methods. Virus isolation in cell culture is complicated by the requirement for high containment facilities.

Serological methods

ELISA

Several ELISA systems have been developed for serological testing after the initial investigations of NiV outbreaks in Malaysia and Singapore. Subsequently in Bangladesh, the first NiV outbreaks were only suspected after these tests were found to be positive in patients. These tests have also been used widely for investigative field studies in bats and other animals. ELISAs have been used both for detection of the viral antigen and evaluation of antibody responses (see Table 3 below). Chiang (2010), for instance, reported the development of monoclonal antibody-based antigen capture ELISAs for virus detection and for differentiation between NiV and HeV. Such viral antigen capture ELISAs provide a high throughput format at relatively low cost for screening of samples, and thereby could serve as an alternative to PCR for rapid diagnostic and virus detection (Kulkarni 2013). ELISAs developed for serology testing (IgM or IgG) may use infected cell lysate antigens for coating the plates. However, their use is limited to BSL4 laboratories. To overcome this problem, NiV recombinant proteins, and the N protein in particular, have been produced as an alternative antigen for serological detection of infection.

The specificity and sensitivity of available ELISAs has not been often reported. One such validation study concluded in 92% assay sensitivity and specificity of a NiV-N protein-based IgM capture ELISA, but used as a reference method the ELISA developed at CDC (Yu 2006), which performance documentation had not been disclosed.

Luminex

Two different Luminex assay formats were developed for detection of NiV-specific antibodies, a binding assay and a blocking or inhibition assay (reviewed by Wang 2012). The inhibition assay measures antibodies that block ephrin-B2 receptor binding. Such assay can be considered as a surrogate neutralization test. Similar Luminex assays have been used successfully for HeV diagnosis in human as well as veterinary settings.

Rapid test

From an outbreak investigation/control point of view, it is most useful in some situations to have access to a rapid (point-of-care) test for both human and animal applications. Rapid tests, usually consisting of lateral flow immunochromatographic assays, have been developed for detection of antigen and/or antibody corresponding to a variety of infectious diseases. However, as of today, no such test has been made available for Nipah serology.

Virus neutralization

Serum neutralization tests are considered as the reference for serology testing, and NiV neutralization tests were developed soon after the first outbreak in Malaysia. In a conventional NiV neutralization test, sera are incubated with virus in the wells of 96-cell microtiter plates prior to the addition of Vero cells. Those sera that completely block development of a cytopathic effect are designated as positive. A more rapid plaque assay neutralization procedure has also been developed to detect NiV-neutralizing antibodies, in which a specific number of plaque-forming units of the virus are incubated with dilutions of test serum prior to adsorption to the cell monolayers, and foci of infection detected immunologically after fixation of the cells with methanol (reviewed by Wang 2012, Daniels 2001).

Neutralization testing may not be performed in many laboratories because these tests involve handling live viruses, which requires high containment facilities. Several pseudotyped viruses bearing the NiV F and G proteins have been developed to obviate this requirement, and shown to be more sensitive than conventional serum neutralization assays (Wang 2012). However, further validation of the pseudotype assays, at large scale, would be required to corroborate their high sensitivity and specificity before considering them as a reliable alternative to other serological assays.

Molecular methods

Specific and reliable PCR-based methods and sequencing to identify henipaviruses are now more widely available for outbreak investigations and diagnosis, and have begun to replace serological testing (Ong 2015). A real time RT-PCR TaqMan assay for NiV was first developed in 2004, based on the N gene sequence. The sensitivity of the test was close to 1 plaque forming unit, and it was demonstrated that the assay was able to detect NiV RNA in blood specimens from infected hamsters. The assay was also shown NiV-specific since it failed to detect HeV RNA. A commercial kit based on this technology has obtained CE marking and been made available, for the detection of NiV in serum, plasma, infected animal tissue or secretion by using real time PCR systems.

Subsequently, in another study, several consensus quantitative PCRs were developed for detection of both HeV and NiV. In this study, the N gene SYBR Green assay was the most useful assay for investigation of potentially unknown henipaviruses and the P TaqMan assay was preferred when high sensitivity was required to detect infection by know strains of NiV (reviewed by Wang 2012).

Two versions of a Taqman array card have also been recently developed for simultaneous detection of more than 20 pathogens (including NiV) in cerebrospinal fluid or blood samples (Onyango 2017, Liu 2016). The assays showed good performance when compared to standard PCR, with better specificity (96-99% overall) than sensitivity (< 90% overall). The TaqMan array card could be used in field settings with different objectives: as a rapid screen in case of suspected neurological disease, for outbreak investigations or for the surveillance of specific pathogens including NiV.

Tissue culture method

Virus culture can be used for diagnosing NiV infections. Particularly in any new case or outbreak, isolation of the virus is highly desirable. Brain, lung, kidney and spleen samples should be tested. A cytopathic effect usually develops within 3 days of culture in Vero cells. Initially after low multiplicity infection of cell monolayers, the cytopathic effect is manifested by the formation of syncytia that may contain up to 20 or more nuclei. Subsequently syncytia lift from the substrate, leaving punctate holes in the cell monolayer. Identification methodologies for virus isolates include immunostaining of fixed infected cells, neutralization with specific antisera, PCR of culture supernatants, and electron microscopy (Daniels 2001).

Immunohistochemistry

Where infected animal or human tissues are available, immunohistochemistry using specific anti-henipavirus antibodies is sometimes useful to confirm the diagnosis (Ong 2015). In NiV infections there is a wide range of tissues in which viral antigen can be detected (Daniels 2001), since the primary pathology occurs in the vascular endothelium. However, this method has been more frequently reported for detection of HeV than NiV infections.

Summary and challenges

Table 3 provides an overview of the advantages and disadvantages of the different technologies used for diagnosis of NiV, and table 4 provides a summary of the techniques available in various laboratories for diagnosis of NiV infections. Despite the very narrow geographical distribution of the disease, a variety of diagnostic tools have been made available by various laboratories.

Table 3. Summary of advantages / disavantages of the main NiV assays (for diagnosing human infections)

Technique	Advantages	Disadvantages
ELISA (IgM)	May provide Nipah diagnosis if performed between 4 days and 3 months after exposure	
ELISA (IgG)	May provide Nipah diagnosis	Requires a second serum sample (convalescent serum)
Nucleic acid-based assays	Becoming the clinical diagnostic standard Performed rapidly	
Viral culture	Virus isolation important when a new case or outbreak occurs	Requires too much time to be of use in clinical setting Requires BSL4 precautions

Table 4. Overview of diagnostic tests developed in various laboratories

(adapted from Kulkarni 2013)

Technique	Assay	Laboratory
ELISA (detection of viral antigen)	Antigen-capture ELISA using antibodies produced by DNA immunization	CDC, USA
	Antigen-capture ELISA using antibodies produced by DNA immunization	Nat. Inst. Inf. Dis., Japan
	Monoclonal antibody-based antigen-capture ELISA	CDC, USA
ELISA (detection of IgM and IgG)	Solid-phase blocking ELISA	DVS, Malaysia
	Indirect ELISA based on E coli-expressed N	Chinese Nat. Diagn. Center for Exotic Animal Dis.
	Indirect IgG ELISA based on recombinant N	Institute Trop. Med., Japan
	IgM capture ELISA based on recomb. N	Institute Trop. Med., Japan
Luminex	Binding assay	CSIRO, Australia
	Receptor inhibition (blocking) assay	CSIRO, Australia
Neutralization	Plaque assay	CSIRO, Australia
Pseudotype neutralization	VSV pseudotypes expressing NiV F /G	CDC, USA
	VSV pseudotypes expressing NiV F /G	Nat. Inst. Inf. Dis., Japan
	VSV expressing secreted alkaline phosphatase pseudotyped with NiV F/G	Nat. Inst. Inf. Dis., Japan
Immunohistochemistry	Monoclonal antibody-based immunohistochemistry	Nat. Inst. of Animal Health, Japan
PCR	Real-time RT-PCR (Taqman) using primers in N gene	Institut Pasteur, France
	Real-time RT-PCR (Taqman) using primers in P gene	Inst. of Zoology, UK
	SYBR Green assay using primers in N gene	Inst. of Zoology, UK
	Duplex nested RT-PCR (bat urine specimen)	Chulalongkorn Uni. Hosp., Thailand
	Taqman array card (multiplex)	CDC, USA
Virus culture	Plaque assay	CSIRO, Australia

Many countries in the South-East Asia region did not have adequate facilities for diagnosing the virus when the first outbreaks occurred. However, Bangladesh, India and Thailand developed laboratory capacity and addressed this issue. For instance, a Nipah laboratory was set up in Bangladesh (IEDCR) for safe specimen handling and testing by ELISA to identify IgM and IgG antibodies against NiV (Rahman 2012). In India, a BSL4 laboratory at National Institute of Virology (ICMR), Pune, has prepared to diagnose NiV in the country. The OIE Reference Laboratory for Henipaviruses in Asia–Pacific region is located at Australian Animal Health Laboratory, Geelong, and the High Security Animal Disease Laboratory, in Bhopal, with BSL3 + facility caters the need for exotic animal disease diagnosis (Kulkarni 2013).

Of note, NiV diagnostic tests have to be deployed under a range of circumstances: they are involved in studies of the natural history of infections, diagnosis of the disease in pigs; in special circumstances they may facilitate international trade in known susceptible species and they are a key to the diagnosis of human infection and disease. An additional constraint is that diagnostic technologies should be appropriate to resource-limited environments (Wang 2012).

NiV diagnostic validation studies are still lacking to fully characterize the performance of available assays. In particular, assay cross-reactivity appears as a largely unaddressed topic. The antibody cross-reactivity between HeV and NiV can be expected to decrease NiV assay specificity. However, in some instances, assays providing wide coverage, across all henipaviruses, may be desirable. Surveillance studies indicated the presence of a diverse group of henipavirus or henipa-like viruses in bats at different geographic locations around the world, from Australia, Southeast Asia to China, Madagascar, and West Africa. It is highly possible that any of these bat viruses have the potential to spill-over into other mammalian hosts, including human, and cause diseases. These viruses may induce cross-reactive, but not cross-neutralizing, antibody responses against known henipaviruses (Wang 2012).

Gap analysis: Diagnostics

Due to the high case fatality rate associated with NIV infections, and the risk of human-to-human transmission, accurate tests, with very high levels of both sensitivity and specificity are needed. The consequences of false negative or false positive assay results are indeed difficult to manage. Available data remain insufficient to determine whether available tests have adequate performance for optimal diagnosis of human NiV infections. Detection of IgM by ELISA has been most widely applied in the context of the latest outbreaks in Bangladesh. However, very limited information has been disclosed about the performance of these assays. Laboratories seem to be more concerned by a possible lack of assay specificity than sensitivity, as IgM ELISA positive results are usually reported to be confirmed by another method (Sazzad 2015, Homaira 2010, Gurley 2007).

What are the sensitivity and specificity of current ELISA IgM assays?

Laboratories offering henipavirus testing pay detailed attention to quality assurance issues, but NiV disease may occur in remote locations without ready access to sophisticated laboratory facilities, and therefore reproducible diagnostic test accuracy can sometimes be challenging to deliver at the point of care (Wang 2012). Continued efforts are therefore needed to ensure better access to suitable diagnostics in all areas where NiV outbreaks are likely to occur.

Future directions for research in NiV diagnostics should include assay validation vs. international reference standards (RNA, antibody), point-of-care diagnostic development, and expansion of multiplex assays to distinguish Nipah from other diseases with similar clinical presentations.

Therapeutic interventions

Antiviral drugs

Ribavirin and chloroquine

Ribavirin is a guanosine analogue and broad spectrum nucleoside antimetabolite antiviral drug which features on the WHO Essential medicines list. An inhalation solution of ribavirin is also indicated for the treatment, in young children, of severe lower respiratory tract infections due to respiratory syncytial virus, another paramyxovirus. During the NiV outbreak in Malaysia in 1998/99, ribavirin was

given empirically to treat 140 patients. The trial was not randomized. Patients who were managed prior to the availability of ribavirin or refused ribavirin were taken as controls. There were 45 deaths in the treated group (32%) and 29 in the controls (54%), corresponding to 36% reduction in mortality (Chong 2001). On Cox regression analysis, younger age and use of ribavirin were independently associated with better survival (p = 0.011 and 0.013, respectively). To our knowledge, this is the only available report on the efficacy of ribavirin in patients. In subsequent animal studies, ribavirin was found to only delay NiV disease and death (reviewed by Ong 2015, Broder 2013, Steffen 2012).

Ribavirin was also tested in combination with the antimalarial drug chloroquine. Chloroquine was indeed shown early on to block the critical proteolytic processing needed for the maturation and function of the HeV F glycoprotein (Pager 2004), and later shown to inhibit NiV infection in cell culture (Porotto 2009). However, in vivo data proved disappointing. Chloroquine did not protect hamsters when administered either individually or in combination with ribavirin (Freiberg 2010).

Other antiviral drugs

In view of the questionable efficacy of ribavirin and/or chloroquine and the severity of NiV infections in people, considerable effort has been spent in developing and exploring new therapeutic options against henipaviruses. Such research has primarily focused on targeting the fusion and entry steps of the virus infection process, as done by F glycoprotein-targeted peptide fusion inhibitors. A 36 amino acid **HR2-based fusion inhibitor** (NiV-Fc2), analogous to the approved HIV-specific therapeutic peptide enfuvirtide, has been proposed as a specific therapy against henipaviruses (Steffen 2012).

Subsequent studies focused on peptide optimization, leading to the identification of the cholesterol tagged hPIV3-based HR2-derived peptide as a promising candidate (Porotto 2010). Such cholesterol-tagged peptides could penetrate the CNS and exhibited some effective therapeutic activity against NiV infection in the hamster model. However, relatively large amounts of HR2-cholesterol peptides (2 mg/kg) were needed to achieve 60% or less survival of hamsters infected with NiV, when administered simultaneously or prior to NiV inoculation. Additional studies were conducted more recently in view of better understanding the properties underlying the peptides' potency (Mathieu 2017). The observation of direct correlation between the length of the linker, the antiviral effect of the molecule and its protease sensitivity (leading to decreased stability in vivo) illustrates the major challenges remaining to be addressed by this type of research.

Nearly two decades of research on the molecular mechanisms of membrane fusion have not resulted in the identification of a lead candidate with successful proof of concept data in an animal model of NiV infection

Small-molecule nucleoside inhibitors have also been evaluated against NiV. Very recently, Hotard (2017) evaluated 4'-Azidocytidine (R1479), an antiviral initially developed for hepatitis C therapy, against a range of paramyxoviruses and reported similar levels of in vitro activity against henipaviruses as against flaviruses.

Other candidate drugs evaluated in vitro for their activity against henipavirus infections include cationic compounds and calcium influx inhibitors (Aguilar 2011).

Biologicals

Convalescent plasma

The recent pathogen outbreaks, such as Ebola viral disease or Middle East respiratory syndrome coronavirus, have renewed attention to convalescent plasma and immunoglobulins. In case of severe disease, when no treatment with a proven record of safety and efficacy is available, they may appear as the only available therapeutic option. However, convalescent plasma has not been investigated clinically during outbreaks of NiV infections, and research efforts are rather focused on monoclonal antibodies.

Monoclonal antibodies

Poly-IC12U has not reached 100% protection in the hamster model of NiV infection. Can it be considered a promising drug candidate?

Data from the phase 1 trial of m102.4 will help estimate its therapeutic window, and the future commercial cost of a treatment

Monoclonal antibodies targeting the surface glycoproteins of HeV have shown efficacy against both HeV and NiV as pre- and post-exposure prophylaxis in animal models, but since these antibodies must be administered before the onset of clinical signs, they are unlikely to be useful for treating symptomatic patients, while probably beneficial for post-exposure prophylaxis in potentially exposed individuals (Satterfield 2015). Presently, the only reported and most promising post-exposure therapy against NIV infection is a human monoclonal antibody known as m102.4, which was isolated from a recombinant human phage-displayed Fab library (reviewed by Broder 2013).
The m102.4 mAb has exceptionally potent neutralizing and cross-neutralizing activity against both NiV and HeV viruses and its epitope maps to the ephrin receptor binding site. Testing of m102.4 has confirmed its neutralization activity against several isolates including NiV-M and NiV-B. Effective post-exposure efficacy with m102.4 has been demonstrated in both ferrets and NHPs (AGMs) infected with either HeV or NiV. Of note, a study conducted in AGMs confirmed the higher pathogenicity and lethality of NiV-B compared to NIV-M, and showed that the therapeutic window for m102.4 was much shorter with NiV-B than NiV-M, with all animals succumbing to NiV-B infection if treated later than on days 3 and 5 post inoculation (Mire 2016).

Over the past years, eleven human subjects have been reported to receive high-dose m102.4 therapy on an emergency use basis because of high-risk exposure to HeV in Australia (10 people) or NiV in the United States (one person), and all have remained well with no associated adverse events (Broder 2013). A phase 1 clinical trial has been initiated for this product in April 2015 in Australia in a total of 40 subjects (5 groups of 8 subjects receiving 1 or 2 intravenous infusion(s) of different doses of m102.4) (https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=368110&isReview=true). Assessments include safety/tolerability, pharmacokinetics and immunogenicity parameters. To our knowledge, the outcome of this trial has not been disclosed yet, but the results will be essential for determining the feasibility of using such monoclonal antibody for pre- or post-exposure prophylaxis.

Host directed interventions

Immunomodulators

The innate immune response to NiV infection is thought to alter the pathogenic process that is induced, offering the option of a therapeutic approach based on immunomodulation. A derivative of

synthetic polyinosinic:polycytidylic acid (poly-IC12U or Rintatolimod), an analogue of double-stranded RNA which strongly activates IFN production, has been shown effective in limiting disease and increasing survival of NiV-infected hamsters. When administered at 3 mg/kg of body weight daily from the day of infection to 10 days post-infection, prevented mortality in 5 of 6 infected animals (Georges-Courbot 2006). This drug candidate is not new. It was evaluated clinically as early as in the 1980s, and at that time associated to significant toxicity (with adverse events such as fever, arthritis, liver toxicity, thrombocytopenia, or neurotoxicity). Nevertheless, poly-IC12U underwent further clinical development for chronic fatigue syndrome, with more than 90 000 doses administered, and these trials concluded in an acceptable safety profile (Mitchell 2016). Poly-IC12U may thus hold promise for Nipah therapy.

Another strategy, targeting the activation of IRF3-dependent signalling mechanisms with small molecule agonists, has been proposed more recently by Pattabhi (2016), but so far it has not advanced beyond a very early stage of development.

Adjunctive therapies

As for other severe diseases of viral origin, aggressive supportive care may help improve patient survival. NiV infections, especially as seen in Bangladesh, are associated to respiratory disease and respiratory failure. Oxygen supplementation and eventually transfer to ICU are part of the management guidelines of this infection. Ensuring patient access to the best medical practices in this area should remain a priority.

Gap analysis: Therapeutics

Without any other currently available therapeutic options, ribavirin is still considered today as an option for treatment of NiV infections in emergency settings, but its impact on disease progression is highly questionable. Research on alternative treatments appears therefore as critical. Numerous studies have uncovered determinants important for various steps in the NiV replication cycle. While each step of the replication process represents a potential target for the development of antiviral drugs, most efforts have been focused on the mechanisms leading to productive membrane fusion. Until now, such research has not resulted in the identification of many promising candidates, and none of them has been reported successful in an animal model of NiV infection.

The most advanced alternative treatment under development is a monoclonal antibody, m102.4, which was identified by panning a large nonimmune antibody library against the HeV glycoprotein G. This monoclonal antibody was then affinity maturated, converted to IgG1 and proved highly neutralizing against NiV (Zhu 2008). It has now reached clinical phase 1 evaluation. Following high-risk NiV exposure or cases of infection in India or Bangladesh in recent years, there has been an interest in compassionate use of the m102.4 antibody, which was found difficult to orchestrate as the product was still at a preclinical stage of development. A possible strategy for access to this therapy would be to further develop, manufacture and stockpile the antibody so as to deploy it rapidly when and where another outbreak occurs (Broder 2013). However, more data from clinical studies will be required to determine what could be the indication and usage of this monoclonal antibody.

Another therapeutic approach may be based on immunomodulation, as suggested by the efficacy of poly-IC12U in the hamster model of NiV infection. However, for a better understanding of the

probability of success of this approach, poly-IC12U should be evaluated in the same AGM model as used for the m102.4 antibody.

Overall, the development of therapies against NiV infections remains at a very early stage. Research efforts in this area should be encouraged, especially when considering the high case fatality rate associated to NiV infections and the risk of a large outbreak occurring in the future. One may also consider that antiviral drugs usually have the potential for broad virus coverage, so that progress made on NiV research can be expected to bring additional benefits for the management of other henipa- or paramyxovirus infections.

Vaccines

All R&D activities for NiV vaccines are in the pre-clinical stage. Many vaccine candidates including different live recombinant vaccine and subunit vaccines have been produced and tested in various animal models including hamsters, pigs, cats, ferrets and AGMs. These vaccines are based on the F and/or G glycoproteins, and essentially target the induction of NiV neutralizing antibodies, as done with other human paramyxovirus vaccines such as mumps or measles vaccines.

Despite the high level of identity between NiV-M and NiV-B, shouldn't a vaccine be based on Niv-B sequences rather than NiV-M?

Live-attenuated vaccine candidates (reassortant/recombinant)

Due to the extreme lethal nature of NiV, producing a safe, live attenuated vaccine with no potential of reversion is generally considered a difficult approach, although recombinant-derived NiV mutants have been produced that are attenuated in hamster and ferret models. A live-vectored vaccine approach has been preferred.

Vaccinia virus-derived vectors

The first vaccination and challenge experiments were carried out with NiV in the hamster model using recombinant vaccinia viruses (Guillaume 2004). NiV F and G encoding recombinant vaccinia viruses (highly attenuated NYVAC strain) were examined individually and in combination. All animals were completely protected following intraperitoneal NiV challenge, regardless of whether they were immunized with the G or F or both vaccinia virus recombinants. Of note a humoral anamnestic response was observed following virus challenge suggesting that vaccine-induced immune responses did not prevent virus replication, i.e. sterile immunity was not induced. As passive transfer of antisera from vaccinated animals also demonstrated protection, a major role of specific neutralizing antibody in protection was inferred. Subsequently, a recombinant canarypox (Alvac)-based candidate, another highly attenuated vaccinia virus vector suited to veterinary applications, was also tested successfully in a pig model of NiV infection (Weingartl 2006).

Vesicular Stomatitis Virus Vectors

The most studied vectored vaccine candidate is the VSV vector, which uses a similar strategy to that being employed in the Ebola virus vaccine candidate in current clinical trials. However, the VSV vector used has not been identical among studies (reviewed by Satterfield 2016). Three types of vaccines can be distinguished : replication-incompetent VSV pseudotypes expressing NiV G or F proteins (Lo 2013), VSV virions with F and G that can undergo a single round of replication (produced by co-infection of two VSV pseudotypes, one expressing F and one expressing G, which generates a

chimeric virus pair that expresses both VSV F and G on the virus surface) (Mire 2013, Chattopadhyay 2011), and replication competent VSV recombinants, where the VSV-G is replaced in the vaccine vector by the Ebola GP so as to overcome the lack of a functional surface protein for virus entry (DeBuysscher 2014).

A study tested the protective efficacy of the replication competent VSVs expressing either N, F or G of the Malaysian strain of NiV. Following a single dose administration, the vaccine vectors expressing G and F fully protected Syrian hamsters from lethal NiV challenge, whereas the N expressing vector conferred only partial protection (DeBuysscher 2014). Another study in AGMs confirmed the protective capacity of the same recombinant virus expressing the G protein of NiV (rVSV-EBOV-GP-NiV-G) (Prescott 2015). Interestingly, in the hamster model, this candidate, when used for single-dose vaccination 1 day before challenge, reduced viral load, limited pathology and fully protected hamsters from NiV infection. The vaccine was even partially protective when administered at early time points following challenge with NiV, suggesting the potential of the vaccine in an outbreak setting or for post-exposure prophylaxis. Moreover, induction of cross-protection between NiV-B and NiV-M has been demonstrated for another VSV-vectored vaccine (single round replication) in AGMs (Mire 2013).

Hence, VSV recombinant vaccines may appear as the most promising vaccine candidates for Nipah. However, a recent toxicity study conducted in mice (Van Den Pol 2017) pointed to a significant safety concern of neurotropism for such recombinant vaccine. A combination of VSV recombinant viruses expressing both Nipah F and G (as described by Mire 2013 and Chattopadhyay 2011) was indeed found even more neurotropic than wild-type VSV after intracranial injection, evoking a rapid lethal response in the adult brain. The observation is in contrast with an earlier study that showed that both the VSV-Ebola and VSV-Lassa viruses were devoid of neurotropic actions in mice and rats, and suggests that a vaccine expressing a single NiV glycoprotein should be preferred over a combination of F and G.

Measles Virus vector

Live-attenuated measles vaccines have been used since the 1960's worldwide, and have appeared as attractive candidates for development of vectored vaccines. In particular, measles virus induces strong cellular immunity and durable responses. A concern related to the use of a measles vector is due to the possible negative effect of pre-existing immunity in human populations on the immunogenicity of such vector vaccine. Nevertheless, the vaccine has been considered to offer the potential to elicit long-term immunity against both MV and NiV, and has been proposed as a way to reach general childhood vaccination against NiV. The pathway of clinical development for such combination vaccine remains largely unclear, and most likely would require too significant investments to appear as a feasible strategy. In any case, protection data in AGMs for this candidate have appeared suboptimal, as after 2 injections of the vaccine, animals were not protected against the brain lesions induced by NiV challenge (Yoneda 2013).

The neurotropic nature of a VSV vaccine expressing both F and G is a critical safety concern

Venezuelan Equine Encephalitis virus vector

A replicon vaccine vector system was developed from an attenuated strain of Venezuelan equine encephalitis virus (VEE). Alphavirus replicon vectors offer several potential advantages as vaccine delivery systems. They typically express heterologous genes to high levels and induce self-limiting infection. Replicons expressing HIV or cancer target antigens have been evaluated in human subjects, and some promising data generated. A VEE-vectored NiV vaccine candidate has been evaluated in mice and found to induce NiV neutralizing antibodies following a 3-dose vaccination schedule (Defang 2010). To date, the protective potential of this vaccine candidate remains unknown.

Adeno-Associated Virus

Adeno-associated viruses are probably more popular as gene-therapy vectors than vaccine platforms. Nevertheless, a number of AAV-based vaccine candidates have been shown to induce promising data in animal models. Ploquin (2013) demonstrated that a single injection of the AAV8-NiV.G vector is sufficient to protect the hamsters against lethal challenge with NiV.

Subunit vaccines

A subunit vaccine incorporating recombinant glycoprotein G has been approved as a veterinary vaccine for HeV in Australia in 2015. This vaccine is formulated using glycoprotein G with a proprietary immunostimulatory complex adjuvant. Currently, the vaccine is administered to horses by the intramuscular route as two immunizations, three to six weeks apart followed by boosting six months later. The effectiveness of this vaccine in horses suggests this approach may also be of interest in humans (Satterfield 2015). Various formulations of an experimental subunit G vaccine against Nipah have been evaluated successfully in animal models, including AGMs, confirming the potential of this type of vaccine. Some of these studies, with challenge performed up to more than a year after vaccination, demonstrated the durability of protection induced by these vaccine.

HeV is a zoonotic infection affecting horses and humans in Australia. A vaccine has been made available for horses in 2012 (Broder 2013). Horse owners in Australia are encouraged to vaccinate their horses to both reduce the risk of HeV infection, and to prevent potential transmission to humans. Vaccine uptake has been limited though, with an estimated 11-17% of horses in Australia vaccinated (Manyweathers 2017). Spill-over events of HeV in horses continue to occur.

NiV virus-like particles (VLPs) have also been obtained by co-expression of the G, F and M proteins under optimized conditions. They appeared as an alternative vaccine candidate, especially in view of their ability to induce a neutralizing antibody response in mice (Walpita 2011).

A vaccine candidate requiring multiple injections does not seem very compatible with the targeted indication for at-risk persons in the area of an ongoing outbreak

Vaccine summary

Table 5 below presents the various vaccine candidates evaluated in preclinical models.

Table 5. Nipah vaccine candidates with preclinical data (adapted from Satterfield 2015)

Candidate name/ identifier & institution	Efficacy or immunogenicity data in animal model	Developer	Reference
VV-NiV.F and/or G	100% protection in hamster model, following 2 vaccinations (1 month apart) starting 4 months before challenge	INSERM	Guillaume 2004
ALVAC-F/G*	100% protection in pig model, no evidence of clinical illness, following 2 vaccinations (14 days apart) starting 28 days before challenge	CFIA-NCFAD	Weingartl 2006
VSV-NiVM F and G	High neutralizing antibody titers induced in mice following single intranasal vaccination	Yale University	Chattopadhyay 2011
VSV-NiVB F and/or G	100% protection in ferret model, no evidence of clinical illness, following single vaccination 28 days before challenge	UTMB	Mire 2013
VSV-NiVM F or G	100% protection in hamster model, no evidence of clinical illness, following single vaccination 32 days before challenge	CDC	Lo 2014
rVSV-EBOV-GP-NiV-G	100% protection in hamster model following single-dose vaccination 1 day before challenge	RML	DeBuysscher 2014
	100% protection in AGM model following single-dose vaccination, 29 day before challenge		Prescott 2015
rMV-Ed-G	100% protection in hamster model, no evidence of clinical illness, following 2 vaccinations (21 days apart) starting 28 days before challenge 100% protection in AGM model, but no protection against infection (pathological changes observed in brain), following 2 vaccinations (28 days apart) starting 35 days before challenge	UoT	Yoneda 2013
V-NiVG (VEE)	High neutralizing antibody titers induced in mice following 3 vaccinations at weeks 0, 5 and 18	USU	Defang 2010
AAV-NiVM G	100% protection in hamster model, no evidence of clinical illness, following single vaccination 32 days before challenge	INSERM	Ploquin 2013
rLa-NiVG and/or rLa-NiVF**	High neutralizing antibody titers induced in pigs following 2 vaccinations (4 weeks apart)	CAAS-SKLVB	Kong 2012
HeV sG (subunit) + CpG	100% protection in ferret model, no evidence of clinical illness, following 2 vaccinations (20 days apart) 20 or 434 days before challenge	Zoetis, Inc. /USU	Pallister 2013
HeV sG (subunit) + Alum + CpG	100% protection in AGM model, no evidence of clinical illness, following 2 vaccinations (21 days apart) starting 42 days before challenge		Bossart 2012
	100% protection in cat model, no evidence of clinical illness, following 2 vaccinations (21 days apart) starting 42 days before challenge		McEachern 2008
HeV sG (subunit) + Quil A + DEAE-dextran + Montanide	100% protection in cat model, no evidence of clinical illness, following 3 vaccinations (14 days apart) starting 104 days before challenge		Mungall 2006
VLPs (F/G/M)	High neutralizing antibody titers induced in mice following 3 vaccinations at weeks 0, 2 and 4	UTMB	Walpita 2011

** Canarypox vector, suited to veterinary applications*

*** Newcastle Disease Virus vector, suited to veterinary applications*

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).

Another summary of the present status of knowledge associated to the different vaccine candidates under evaluation for Nipah considers the advantages and disadvantages of these vaccines platforms, as shown in Table 6 below.

Table 6. Advantages and disadvantages of the different vaccine platforms under evaluation for Nipah (human vaccination)

Platform	Advantages	Disadvantages
Vaccinia-based vectors	Promising data in animal models of protection MVA strain well known in humans	2-dose schedule Concerns linked to anti-vector immunity
VSV-vectored vaccines	Promising data in animal models of protection Efficacy in animals vaccinated 1 day prior to challenge Platform used successfully in the context of an Ebola outbreak Single dose schedule	Neurotropism issue identified with vector expressing a combination of F and G
Measles-vectored vaccine	Promising data in animal models of protection Vector consisting of the measles vaccine strain	2-dose schedule Suboptimal protection observed in AGM model Pre-existing immunity against the vector in human populations
VEE replicon	Self-limiting infection Phase 1 clinical data available for VEE combined with HIV or prostate antigen	No protection data in NiV challenge model available 3-dose schedule
Subunit vaccine	Promising data in animal models of protection Expected safety	Need for an adjuvant, impacting perception of safety and potentially complicating clinical development Need for several injections less compatible with use in emergency setting

Gap analysis: Vaccines

As of today, no NiV vaccine candidate has advanced to a clinical phase of development. Consequently, it remains difficult today to assess the likelihood that any of these candidates will demonstrate characteristics compatible with the Target Product Profile developed by WHO in June 2017 for a Nipah vaccine (http://www.who.int/blueprint/priority-diseases/key-action/nipah/en/). Nevertheless, the probability of success of a vaccine against Nipah is expected to be very high. Other related paramyxoviruses, such as measles and mumps viruses, yield long-term immunity after natural infection, and live attenuated vaccines have been developed that have been used safely and effectively against these pathogens for decades. It has also been suggested that the likelihood of success of viral vaccines targeting viruses with average incubation periods of at least 5–7 days, as seen with NiV, is higher than that of vaccines for viruses with shorter incubation periods. In addition, various candidate vaccine platforms have demonstrated the feasibility of using one or both of the NiV glycoprotein (G) and fusion (F) protein as the antigen(s) to stimulate a protective immune response in various preclinical challenge models including hamsters, cats, ferrets, AGMs and pigs. Little or no clinical signs of disease were observed in vaccinated animals after NiV challenge, and protection against mortality often reached 100% (Satterfield 2016).

One of the most promising vaccine candidates is a VSV-G recombinant. In the hamster model, this vaccine was found protective following single-dose vaccination 1 day before challenge, suggesting the potential of this product for use in an emergency setting. However, despite encouraging efficacy data in animals, the proof of concept of such vaccine is still far from being established, as evidenced by the recent observation of a neurotropic effect with a VSV vector system expressing both NiV F and G. Another candidate of interest is a subunit vaccine containing adjuvanted recombinant G glycoprotein. The success of such vaccine candidate in experimental animal models of NiV infection and the use of Equivac HeV® as an effective subunit vaccine in horses suggest this approach may also be efficacious in humans (Satterfield 2016). However, the requirement for a multiple-dose schedule of immunization with this type of vaccine does not appear very compatible with the targeted indication for immunization of at-risk persons in the area of an ongoing NiV outbreak.

In any case, the epidemiology and sporadic nature of NiV outbreaks will make large scale clinical efficacy trials very difficult to organise, so that the pathway towards licensure for a Nipah vaccine will most likely require creative approaches. Specific opportunities, such as the procedure for Emergency Use Assessment and Listing (WHO EUAL), to facilitate access to critically needed vaccines in case of emergency are likely to be of help. In the USA, the Food And Drug Administration's Animal Rule pathway is intended to enable approval of drugs against highly lethal infections in situations where definitive human efficacy studies cannot be conducted (Satterfield 2016). In this instance, efficacy is to be demonstrated via protection in one or two experimental animal models that replicate key characteristics of the human disease and are predictive of the mechanism of protection in humans. The AGM, ferret, and hamster models are well established and accurately model human disease. However, no vaccines have been approved through this mechanism to date. And even with the Animal Rule, human clinical trials are still required for demonstrating vaccine safety.

The key issues with vaccines are delivery and costs (Hayman 2014). The question of how to finance such product development has to be raised, as a NiV vaccine would likely find use primarily in the low and middle income countries where the virus is currently endemic. In view of the current low disease incidence, a reasonable objective may be to advance the development of a vaccine candidate to early phases of clinical development, which would aim at better preparing for a potential epidemic of larger size. In parallel, it would be important to clarify expectations for a NiV vaccine. Answering questions such as "what role could a NiV vaccine play in controlling the disease? with what cost/effectiveness?" would help define the best way forward in terms of vaccine research and development.

Vector Control

The NiV outbreaks in Malaysia in 1998-1999 have been associated with infection of pigs with subsequent spread to human populations, while the outbreaks in Bangladesh resulted from direct contamination from infected fruit bats and human-to-human transmission. Theoretically, vector control efforts may target these 2 species. In any case, a transdisciplinary approach will be required to develop appropriate host management strategies that both maximise the conservation of bat populations as well as minimise the risk of disease outbreaks in domestic animals and humans (Breed 2006).

Pigs

NiV was first described following the 1998-1999 outbreaks of disease in pigs and humans in peninsular Malaysia. Over one million pigs were culled to contain the outbreak (Breed 2006), which had an enormous economic and social impact. To date, no other disease outbreak has been observed in this animal species, but following an outbreak of NiV observed in horses in 2014, the potential for new spill-over into swine can be considered as threatening. Vaccinating pigs has been proposed as a means to prevent spread to human populations, but it is also a veterinary option, aimed at protecting swine herds in the event of a new outbreak. As of today, a swine vaccine against NiV is not available, but promising results have been obtained with the canarypox vectored vaccine candidate and R&D efforts targeting the development of a veterinary vaccine against Nipah are still ongoing.

Bats

(adapted from Breed 2006)

Management of a bat-borne zoonotic disease has been previously attempted for rabies virus. Historically, population reduction was used as a control strategy for vampire bats in Latin America. The methods used at that time are now generally considered inhumane, unethical and ineffective and some of the targeted species have become endangered in their natural environment. Trap, vaccination and release programs using parenteral vaccination have been employed for rabies control of a few terrestrial wildlife species, but this approach is considered prohibitively expensive for large populations and logistically impossible for non-terrestrial species. An oral vaccine has been shown capable of inducing a protective immune response to rabies in vampire bats following oral vaccine delivery, and hence an oral vaccination approach may be plausible for other bat species. However, such program would be very ambitious. A specific vaccine would have to be developed, including a biomarker for discriminating between vaccinated and naturally infected individuals (Mackenzie 2003). Moreover, various aspects of bat ecology and behaviour would require further study before an oral vaccine, suitable bait and vaccination strategy can be deployed. The feasibility of using a NiV vaccine in wild bats for controlling outbreaks in human populations is still far from being established.

General conclusion and recommendations

This document gathers available data pertaining to NiV epidemiology, diagnostics, as well as vaccines, therapeutics and other methods for prevention and control. It identifies research and knowledge gaps which are meant to be used as a basis for discussion on the development of a NiV R&D roadmap.

Nipah is one of eleven diseases considered as in urgent need of R&D attention in the revised priority list of pathogens issued by WHO in January 2017 (http://www.who.int/blueprint/priority-diseases/en/). Since 2001, it has caused almost annual outbreaks in Bangladesh, and the infection has been associated to a case fatality rate of up to 100%. Nipah is a zoonotic disease. As its reservoir, the Pteropus bat, has a flying range that can cover huge areas, the risk of further spread of the disease is perceived as very high.

Since the first identification of the virus in 1999, significant efforts have already been devoted to development of diagnostic methods, outbreak preparedness and response as well as epidemiological

surveillance. The outcome of research has been impressive as well, in terms of characterizing the virus, elucidating its mechanisms of replication, identifying strategies for development of novel therapies and conducting preclinical studies for an array of vaccine candidates.

However, more progress is still needed to offer the most adequate tools for controlling Nipah. In terms of diagnostics, validation of methods and standardization across laboratories would improve currently available tests. Moreover, it would be useful to further invest in strategies that can facilitate access of patients to suitable diagnostics in all areas where NiV outbreaks are likely to occur.

In terms of therapies, animal models have suggested that the m102.4 human monoclonal antibody can prevent NiV infection and/or disease. The antibody is being developed for human use as both a NiV and HeV.countermeasure. Clinical data are still required to define how m120.4 would be used in patients (pre- or post-exposure), and what would be the cost of a treatment. It has been suggested that pre-positioning of the product in NiV endemic areas would facilitate access to this therapy in the event of another outbreak. However, the feasibility of such stockpiling would largely depend on international cooperation and financial support (Broder 2013).

Research on alternative therapeutic candidates should also be encouraged. An advantage of antivirals and immunomodulators compared to biologicals is their expected breadth of coverage. While NiV infections are the focus of this report, therapies that would also prove efficient against other henipaviruses appear as highly desirable.

Among the different vaccine candidates tested in preclinical models, VSV recombinant viruses appear as the most promising. However, the neurotropic potential of such vaccine should be investigated in detail before the product can be advanced to a clinical phase of development. Alternative vaccine approaches may also built on the experience gained with the subunit vaccine againt HeV.

Finally, an essential strategy for controlling Nipah should focus on preventing virus transmission from bats to humans. Controlling the virus in its wild reservoir does not seem a feasible approach. However, establishing or reinforcing surveillance systems is of utmost importance to ensure that NiV outbreaks can be detected quickly and appropriate control measures promptly initiated. Also essential are the efforts on behaviour change communication interventions, so as to increase awareness on the risks associated with this virus.

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