

Nipah Virus

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Abstract

Nipah virus is an emerging zoonosis with the potential to cause significant morbidity and mortality in humans and major economic and public health impacts. According to World Organisation for Animal Health (Office International des Épizooties: OIE), Nipah virus is a notifiable disease of importance to international trade.

Keywords: *Henipavirus*; Nipah virus; *Paramyxoviridae*

Introduction

More than 60% of the newly identified infectious agents that have affected people over the past few decades have been caused by pathogens originating from animals or animal products. Of these zoonotic infections, 70% originate from wildlife. Bats have been recognized to be important reservoir of zoonotic viruses, including Ebola, Marburg, SARS and Melaka viruses [1-4]. Furthermore, bats may be the source of the new Middle East Respiratory Syndrome (MERS) coronavirus recently reported responsible of lethal cases in humans in Middle-East and Europe [5]. In this context, Nipah Virus (NiV) represents another new emerging zoonosis, one of the most important bat-borne pathogens discovered in recent history. In 1998 a dangerous new virus emerged in Malaysia [6]. Initially thought to be a form of Japanese Encephalitis, it was later identified as a new zoonotic disease and named Nipah after the village of “Sungai Nipah” where it was first identified [7]. Similarly, at the beginning in pigs it was confused with Classical swine fever [7]. In infected people, Nipah virus causes severe and commonly lethal illness. It can also cause severe disease in animals such as pigs, and may require the application of stamping out policy, thus resulting in significant economic losses for farmers. The first outbreak in Malaysia resulted in the eventual culling of about 1.1 million pigs [8]. Categorized as zoonotic biosafety level 4 (BSL4) agent [9], depending upon the geographic locations of outbreaks, it is responsible of case mortality between 40% to 100% in both humans and animals [10,11], thus one of the most deadly virus known to infect humans.

Aetiology

The Nipah virus is closely related to Hendra virus (HeV) and Cedar virus [12,13]. They are the three recognized species members of the genus *Henipavirus*, a new class of virus in the *Paramyxoviridae* family.

Among Paramyxoviruses, henipaviruses are characterized by a wider host range and a larger genome [12], when compared to the other members of the family, such as measles virus and canine distemper virus, showing generally a narrow host range and genetically stable with an almost uniform genome size shared by all members of *Paramyxovirinae* [9].

Nipah is an envelope, negative-sense, single-stranded RNA virus, with a genome sequence size of about 18,000 nucleotides. NiV genome organization comprises six major genes present in all *Paramyxovirus*: RNA polymerase and nucleocapsid genes (N, P and L); envelope membrane protein genes (F and G); and matrix protein (M). The attachment (G) glycoprotein which binds the viral receptor, and the fusion (F) glycoprotein which drives virus-host cell membrane fusion,

are the two membrane-anchored envelope glycoproteins responsible for host cell infection by NiV [9]. Virions are pleomorphic, ranging in size from 40 to 600 nm in diameter [14].

As other animal *Paramyxovirus*, the virus is inactivated by 60°C for 60 minutes. It is stable between pH 4.0 and 10.0. It survives for long periods in favourable conditions, for days in fruit bat urine and contaminated fruit juice. It is susceptible to common soaps and disinfectants. Lipid solvents, such as alcohol and ether, and sodium hypochlorite solutions were used effectively in outbreaks for disinfection [15].

Species Susceptible to NiV

Humans, pigs, bats, dogs, cats, goats and horses are sensible to NiV infection [16,17]. NiV infection has been reported also in sheep [18], but the observation could not be further confirmed and remains controversial [19,20]. Clinical disease can be observed in experimental conditions in ferret (*Mustela putorius furo*) [21], guinea pig (*Cavia porcellus*) [22], squirrel monkey (*Saimiri sciureus*) [23], African green monkey (*Chlorocebus aethiops*) [24,25], hamster (*Cricetinae*) [26], and in suckling mouse (*Mus musculus*), or deleted for the type I interferon receptor (IFNAR) [27,28].

Natural Host

Fruit bats (*Macrochiroptera*) of the family *Pteropodidae*—particularly species belonging to the *Pteropus* genus—are the natural hosts for Nipah virus. There is no apparent disease in fruit bats. Bats belonging to the genus *Pteropus* are widely distributed. They live in the tropics and subtropics of Asia, including the Indian subcontinent, Australia, Indonesia, Madagascar, and a number of remote oceanic islands in both the Indian and Pacific Oceans.

Among the genus *Pteropus*, the Indian Flying Fox (*Pteropus giganteus*) (wingspan 1.5 m and up to 1.2 kg) and the relatively smaller Greater short-nosed fruit bat or Short-nosed Indian fruit bat (*Cynopterus sphinx*) (wingspan 48 cm), widespread and very common

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species in South Asia, have been identified as the main natural reservoir [29]. Various other pteroid bats have been recognized NiV host carriers. The grey-headed flying fox (*Pteropus poliocephalus*) and the black flying-fox (*Pteropus alecto*), both *Pteropus* spp. occurring in Malaysia were found seropositive for NiV [30]. Neutralizing antibodies, and the virus has been isolated from the small flying fox or variable flying fox (*Pteropus hypomelanus*) and the large flying fox (*Pteropus vampyrus*) [30-32]. NiV has been isolated from urine of Lyle's flying fox (*Pteropus lylei*) in Cambodia [33].

Serological evidences indicate that circulation of henipaviruses in bats is not limited to species belonging to the genus *Pteropus*, but also extended to a wider range of both frugivorous and insectivorous bats [30,34,35]. An example is represented by the Lesser Asiatic yellow house bat (*Scotophilus kuhlii*) (wingspan up to 5.2 cm, weight up to 22 gr), insectivorous bat (*Microchiroptera*) of the genus *Scotophilus* (yellow bats), family Vespertilionidae, diffuse in Bangladesh, India, Indonesia, Malaysia, Pakistan, Philippines, Sri Lanka, and Taiwan, reported as Nipah virus carrier [30]. Furthermore, in China, the prevalence of anti-NiV or closely related virus antibodies was especially prominent among Daubenton's bat (*Myotis daubentoni*) and Rickett's big-footed bat (*MYOTIS ricketti*), two species of insectivorous bats of the genus *Myotis*, family Vespertilionidae [35]. Daubenton's bat (*MYOTIS daubentoni*) is widely distributed throughout Britain, Europe, and as far as Japan and Korea. The presence of the Rickett's big-footed bat (*MYOTIS ricketti*) is limited to in China and Laos. A relatively high prevalence of anti-henipavirus antibody was also found in China among Leschenault's Rousette fruit bat (*Rousettus leschenaultia*) of genus *Rousettus* [35], and in Ghana in the straw-coloured fruit bat (*Eidolon helvum*) of genus *Eidolon* [34], both of the family Pteropodidae.

In Bangladesh the disease has become endemic and also in this country bats represent a risk factor. The following species of bats are present in Bangladesh: *Pteropus giganteus*, *Cynopterus sphinx*, *Macroglossus sobrinus*, *Rousettus leschenaulti*, *Megaderma lyra*, *Pipistrellus* sp., *Scotophilus heathii*, *S. Kuhlii* and *Taphozous saccolaimus*. Among the reported species are included recognized natural hosts of the virus.

Epidemiology

Intensive agriculture has been implicated in the transmission of the deadly Nipah virus to humans. Between the 1970s and the 1990s, pig and mango production tripled in Malaysia. Mango trees were typically planted near pig enclosures, attracting fruit bats to the area. As bats fed and roosted in the trees, nearby livestock became infected with Nipah virus, which eventually spread to farm labourers. It is assumed that the geographic distribution of henipaviruses overlaps with that of *Pteropus* category. This hypothesis was reinforced with the evidence of henipavirus infection in *Pteropus* bats from Australia, Bangladesh, Cambodia, China, India, Indonesia, Madagascar, Malaysia, Papua New Guinea, Thailand and Timor-Leste [30,33,35,36]. Furthermore, the detection of antibodies against Nipah and Hendra viruses in straw-coloured fruit bat (*Eidolon helvum*) [34], indicates that these viruses might be present within the geographic distribution of *Pteropodidae* bats, not only in Asia, but extended to Africa, Arabian peninsula coast, Middle-East, Cyprus and Southern Turkey.

Outbreaks

Although Nipah virus has caused relatively few outbreaks, it infects a wide range of animals and causes severe disease and death in people, making it a public health concern. Nipah virus was first recognized in

1998 during an outbreak among pig farmers in Malaysia. Since then, there have been various outbreaks, all in South Asia. The chronology of outbreaks due to Nipah virus is summarized in (Table 1) [37, 38].

The Nipah virus infection has become endemic in Bangladesh, causing regularly outbreaks, in particular in districts where date palm

Year	Country	State or District	Cases	Deaths	Case fatality
1998-1999	Malaysia	Perak, Selangor, Negeri Sembilan states	265	105	40%
1999	Singapore	Singapore	11	1	9%
2001	India	Siliguri district, West Bengal	66	49	74%
2001	Bangladesh	Meherpur district	13	9	69%
2003	Bangladesh	Naogaon district	12	8	67%
2004	Bangladesh	Faridpur and Rajbari districts	67	50	75%
2005	Bangladesh	Tangail district	12	11	92%
2007	Bangladesh	Thakurgaon, Naoga and Kushtia districts	18	9	50%
2007	India	Nadia district, West Bengal	5	5	100%
2008	Bangladesh	Manikgonj, Rajbari and Faridpur district	11	9	82%
2009	Bangladesh	Rajbari, Gaibandha, Rangpur and Nilphamari districts	4	1	25%
2010	Bangladesh	Faridpur, Rajbari, Gopalganj and Madaripur districts	16	14	88%
2011	Bangladesh	Lalmonirhat, Dinajpur, Comilla, Nilphamari and Rangpur districts	44	40	91%
2012	Bangladesh	Joypurhat Rajshahi, Natore, Rajbari and Gopalganj districts	12	10	83%
2013	Bangladesh	Gaibandha, Jhainaidaha, Kurigram, Kushtia, Magura, Manikgonj, Mymensingh, Naogaon, Natore, Nilphamari, Pabna, Rajbari and Rajshahi districts	24	21	87%

Table1: Chronology of outbreaks due to Nipah virus (1998-2013) [37, 38].

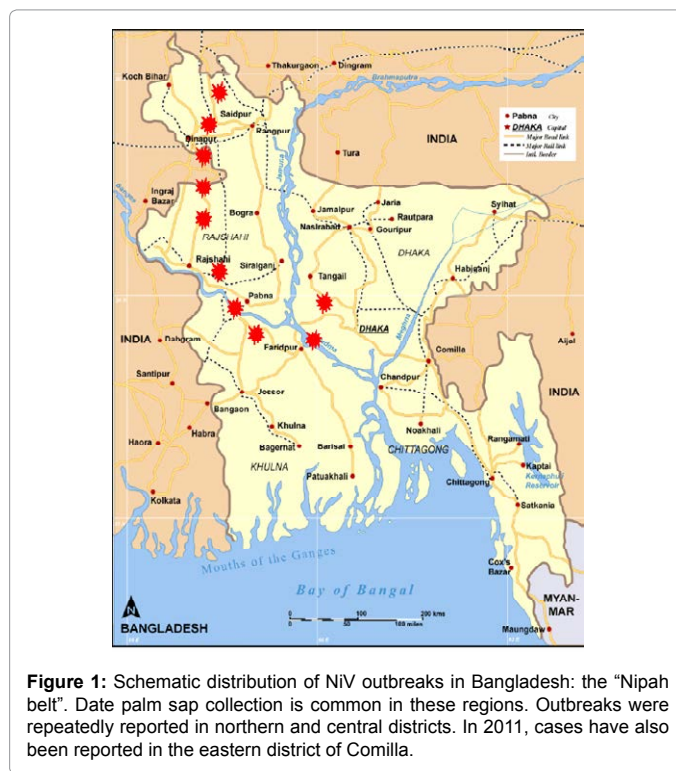


Figure 1: Schematic distribution of NiV outbreaks in Bangladesh: the "Nipah belt". Date palm sap collection is common in these regions. Outbreaks were repeatedly reported in northern and central districts. In 2011, cases were also been reported in the eastern district of Comilla.

sap is seasonally produced. Various reports referred to outbreaks since 2001 [37,38]. The NiV epidemiology in Bangladesh can be schematically referred to the so called “Nipah belt” (Figure 1), corresponding to northern-central districts of the country where date palm sap collection is also common [39]. Nipah virus outbreaks in Bangladesh are most common during the winter season [39]. In 2009, encephalitis and severe respiratory disease broke out in Rajbari and Manikganj districts. Twenty two fatal cases were reported from Faridpur district in January 2010 [40]. Nipah virus infections in central Bangladesh caused four dead in January 2011. In February 2011, Nipah has become a nightmare in border district Lalmonirhat of northern Bangladesh. The spread of the infection could not be arrested by experts, and death toll of the fatal virus resulted in 17 victims [39]. Between 2011 and 2012, an outbreak of the Nipah virus in northern Bangladesh has killed 30 people, prompting national health warnings against the fatal pathogen spread by fruit bats. Six people from the Northern Joypurhat district have died thus far in 2012 and 24 during the same period in 2011 [41]. Apparently, everyone who got infected died. However, it is actually difficult to have a real assessment because not everybody went to the hospital to be diagnosed as a confirmed case of NiV infection. Furthermore, it is probable that other patients remained undiagnosed because they died before admission and/or because necropsies were considered a high risk of exposure.

In 2013, 24 cases of Nipah virus infection have been reported in Bangladesh since the beginning of the year, of which 21 cases have died. The age distribution of cases is from 8 months to 60 years. Sixteen cases were male and eight were females. These cases were from 13 different districts [38].

Transmission

During the initial outbreaks in Malaysia and Singapore, most human infections resulted from direct contact with sick pigs or their contaminated tissues. Transmission is thought to have occurred via respiratory droplets, contact with throat or nasal secretions from the pigs, or contact with the tissue of sick animals [42]. In swine, vertical transmission across the placenta, by iatrogenic means and in semen has been suggested but not confirmed [15].

While the outbreak in Malaysia had progressed from the natural host (fruit bats), to amplification host (livestock) and finally to humans, in Bangladesh no amplification host was needed. People were somehow being directly infected by fruit bats. In the Bangladesh and India outbreaks, consumption of fruits or fruit products (e.g. raw date palm sap) contaminated with urine or saliva from infected fruit bats



Figure 2: Date palm sap harvesters, known as gachis in Bangladesh.

was the most likely source of infection [17]. Other people seem to have been infected while working in the trees [43].

In Bangladesh, date palm sap has been identified as the most relevant risk factor related with the epidemiology of Nipah virus [40]. In this country, it is very popular, used to make products like molasses, used as a sweetener in traditional cakes and desserts, and often consumed raw. Date palm sap is collected during the coolest months of the year, typically mid-December through Mid-February when humidity and temperatures permit efficient sap collection. Harvesters, known as *gachis* in Bangladesh, collect sap by cutting a v-shaped gouge into a date palm tree and hanging a container overnight (Figure 2).

During the later outbreaks in Bangladesh and India, Nipah virus spread directly from human-to-human through close contact with people's secretions and excretions. In Siliguri, India, transmission of the virus was also reported within a health-care setting, where 75% of cases occurred among hospital staff or visitors [42]. From 2001 to date, around half of reported cases in Bangladesh were due to human-to-human transmission by close contact. Most of these infections occurred due to a small number of human transmitters, including one (“Patient F”) linked to 22 other human cases. Such persons are reminiscent of “super spreaders” in other diseases, most recently SARS [44,45].

Sources of Virus

Nipah virus has been found in urine and uterine fluids of wild *pteropid* bats, experimentally isolated from urine, kidney and uterus of infected bats [15]. Virus may be found in fruit or juice (e.g. unpasteurised date palm sap) contaminated with bat saliva or urine. Other sources for infection are contaminated drinking water and aborted bat fetuses or other fluids/tissues of parturition. Infected pigs shed Nipah virus in respiratory secretions, saliva and urine. Role of other animals as a source of virus in outbreaks is less clear though virus has been isolated from feline respiratory secretions, urine, placenta and embryonic fluids [15].

Signs and Symptoms

Humans

The incubation period generally varies from four days to 2 weeks [7], but may be extended up to 45 - 60 days [7,42]. The clinical course is characterized by high fever followed by seizure and death due to encephalitis or respiratory disease. Human infections range from asymptomatic infection to fatal encephalitis. Infected people initially develop influenza-like symptoms of high fever, headache, myalgia, sore throat and weakness. This can be followed by impairment in spatial perception and stability, feeling abnormally sleepy, altered consciousness, and neurological signs, sometimes accompanied by nausea and vomiting, that indicate acute encephalitis [42]. Some patients infected with NiV Bangladesh strain can also experience atypical pneumonia and severe respiratory problems, including acute respiratory distress [46]. Seriously affected patients can develop septicaemia, gastrointestinal bleeding, and renal impairment [15]. Encephalitis and seizures occur in severe cases, progressing to coma within 24 to 48 hours [42]. The case fatality rate estimates remain ~40-100% during sporadic outbreaks (Table 1). Most people who survive acute encephalitis make a full recovery, but around 20% are left with residual neurological consequences such as persistent convulsions and personality changes [42,47]. A limited number of recovered patients

may experience encephalitic relapse up to years later and subclinically infected individuals may show central nervous signs up to 4 years later. [15,48].

Nipah virus in domestic animals

Nipah outbreaks in pigs and other domestic animals (horses, goats, sheep, cats and dogs) were first reported during the initial Malaysian outbreak in 1999 [18,42]. Many pigs had no symptoms, but others developed acute febrile illness, laboured breathing, and neurological symptoms such as trembling, twitching and muscle spasms [49].

Swine

Nipah virus is highly contagious in pigs. Pigs are infectious during the incubation period, which lasts from 4 to 14 days [7]. Generally, mortality was low except in young piglets [15]. Available observations of clinical signs in swine would suggest a respiratory and neurologic involvement. Clinical manifestations are associated with age groups [7,15]. Suckling pigs and piglets (<1 month old): laboured breathing and muscle tremors with limb weakness. Mortality in piglets can be high (40%). Young swine (1 to 6 months old): begins as an acute fever with respiratory signs, laboured breathing, nasal discharge and loud nonproductive cough (“barking pig syndrome” and “one-mile cough”). Accompanying neurologic signs: muscular fasciculation, myoclonus, limb weakness, and spastic paresis, and in some cases, lateral recumbency with paddling and tetanic spasms. Disease presentation can be mild to fulminant with high morbidity and low mortality (<5%). Older animals (>6 months old): acute febrile course with marked neurologic signs. Central nervous system involvement: nystagmus, bruxism, head pressing, aggressive behaviour, tetanic spasms and seizures. Respiratory signs may include open-mouthed breathing, nasal discharge and sialorrhoea (possibly due to pharyngeal paralysis). Sudden death in this age group with few signs has been reported. Abortions during the first trimester have also been reported [15]. Morbidity in confined animals approaches 100% [49].

Other species

Limited clinical information exists for other species. In dogs, distemper-like syndrome was described with pyrexia, depression, dyspnoea and conjunctivitis with purulent ocular-nasal discharge [6]. Severe disease with mortality was also reported. NiV infection was confirmed by immunohistochemical examination of 1 dead and 1 dying dog from the epidemic area in Malaysia. Both showed histologic evidence of severe disease [50]. Morbidity in dogs during outbreaks in Malaysia was interestingly high, with a seroprevalence from 15% up to 46% [8]. Nipah affected cats were observed on farms during outbreaks in Malaysia and some of these resulted in death [49]. Experimental intranasal and oral inoculation of cats produced clinical disease characterized by acute febrile course with respiratory complications [51]. Fruit bats show no serious signs of infection.

Lesions

In humans: different pathological features have been observed, primarily at the level of central nervous system. Confirmed NiV patients showed marked vasculitis with endothelial damage, up to cellular lyses, in the arterioles, venules, and capillaries of various organs. The brain was the most severely affected organ [6]. In one study, evaluation at autopsy of microscopic features in the CNS showed necrotic lesions, perivascular cuffing, thrombosis, and vasculitis in 80% to 90% of the 30 cases examined; endothelial syncytia were present in 27% and meningitis in 57% of the patients [52]. The severity of the CNS

pathology was demonstrated also by Magnetic Resonance Imaging (MRI) analysis of encephalitis patients in the Malaysian outbreak [53,54]. Investigations by MRI revealed a pattern similar to ischaemic infarction caused by obstruction of small cerebral blood vessels. Patients had multiple small (less than 1 cm in maximum diameter) bilateral abnormalities within the subcortical and deep white matter; in some patients, the cortex, brainstem, and *corpus callosum* were also involved. However, relapse and late-onset cases in Malaysia, and other outbreaks of Nipah virus in Bangladesh, showed a different pattern of predominantly confluent cortical lesions [53].

Other affected organs were the kidney, lung, and heart [6,52]. The respiratory disease was reported in up to 63% of confirmed case during the outbreaks in Bangladesh [40]. In the lung, vasculitis was seen in 62% of cases and fibrinoid necrosis was found in 59% of cases. Fibrinoid necrosis often involved several adjacent alveoli and was frequently associated with small vessel vasculitis. Multinucleated giant cells with intranuclear inclusions were occasionally noted in alveolar spaces adjacent to necrotic areas. Alveolar hemorrhage, pulmonary edema, and aspiration pneumonia were often encountered. Histopathological changes of bronchiolar epithelium were uncommon [52]. In the kidney, focal glomerular fibrinoid necrosis was seen in 34% of cases. In some cases, the glomeruli were totally destroyed by inflammation. Vasculitis, thrombosis, and interstitial inflammation were occasionally seen. Syncytial formation involving the periphery of the glomerulus and tubular epithelium was rarely seen [52]. In the heart, vasculitis was noted in 31% of cases. A large myocardial infarction associated with vasculitis was found in a patient comatose for >2 weeks. In another patient who survived more than a month, focal myocardial fibrosis associated with vasculitis was noted [52].

In animals: principal gross and microscopic lesions associated with Nipah in swine are found in lungs and/or central nervous system [7,49]. Lung lesions may vary from mild to severe pulmonary consolidation with petechial or ecchymotic haemorrhages and distended interlobular septa. Trachea and bronchi may be filled with frothy exudate which varies in appearance from clear to blood-tinged. Meningeal oedema with congestion of the cerebral blood vessels has been observed in the brain. Some cortical renal congestion may be evident [7,49].

Histologically, epithelia of all the major respiratory pathways are affected with presence of syncytial multinucleated cells in vascular endothelium. A mononuclear vasculitis with fibrinoid necrosis is often observed associated with thrombosis. Principal histologic changes in the brain, if present, are perivascular cuffs and gliosis. Generalised vasculitis in cats and non-suppurative meningitis in horses have been also reported [15].

Reported lesions from experimentally infected animals resemble the lethal disease observed in humans, increasing the information on pathogenesis and representing suitable models to develop new immunotherapeutic approaches using antiviral drug testing and vaccine development against acute NiV infection [55]. For example, golden hamsters develop systemic vasculitis, pulmonary disease, and encephalitis. Ferrets develop severe respiratory and neurological disease [56]. NiV is similar to HeV infection in cats except there is more involvement of the upper and lower respiratory tract [51]. Cats may be a suitable model for the respiratory aspects of NiV, but they are not useful for studying the encephalitic form. NiV is highly pathogenic to chicken embryos, a useful animal model for studying NiV and the effects on the vascular endothelium or neurons [57]. Whereas allantoic inoculation of NiV results in considerable variation and only partial mortality, yolk sac inoculation results in generalized fatal disease

of chicken embryos, with gross lesions of petechial to ecchymotic hemorrhages and congestion in the kidneys. Mice are not a suitable model of NiV disease. Swiss mice inoculated either by the intranasal or the intraperitoneal routes do not develop clinical signs, but NiV antibodies can be produced after repeated infection [55]. However, NiV can be lethal if administered intracranially into suckling mice [58].

Diagnosis

Nipah virus infection can be diagnosed by a number of different tests. Since Nipah is classified as a biosafety level 4 (BSL4) agent, special precautions must be undertaken in the collection, submission and processing of samples. Biosafety considerations require that this work be carried out only in a physical containment level 4 (PC4) facilities. Various strategies have been developed to reduce the risk of laboratory sera, including gamma-irradiation or sera dilution and heat-inactivation. Henipavirus antigens derived from tissue culture for use in ELISA can be irradiated with 6 kilo Greys prior to use, with negligible effect on antigen titre [59].

Identification of the agent

virus isolation by cell culture can be performed from brain, lung, kidney and spleen samples transported at 4°C in 48 hours or frozen if over 48 hours, using African green monkey kidney (Vero) and rabbit kidney (RK-13) cells [59]. Cytopathic effect (CPE) usually develops within 3 days. Monolayers are examined for the presence of syncytia after incubation for 24–48 hours at 37°C. Henipavirus-induced syncytia are characterised by presence of large multinucleated cells containing viral antigen. In absence of CPE, two 5-day additional passages are recommended to confirm negative results. Immunostaining or virus neutralization tests (plaque reduction, microtitre neutralization, immune plaque assay) are applied to characterize the virus isolate and differentiate cross reactivity within henipaviruses [59].

Polymerase Chain Reaction (PCR) assay and real-time PCR can be applied with the advantage of not propagating live infectious virus. Immunohistochemistry can be applied on formalin-fixed tissues or formalin-fixed cells of vascular endothelium from brain, lung, mediastinal lymph nodes, spleen, kidney, uterus, placenta and foetus, using antisera to NiV, rabbit antisera to plaque-purified NiV or biotin-streptavidin peroxidase-linked detection system [59].

Serological tests

Serum Neutralisation (SN) test is designated as the reference standard for anti-henipavirus antibody detection [59]. Cultures are read at 3 days, and those sera that completely block development of CPE are designated as positive. Immune plaque assay is an option in case of cytotoxicity. Indirect or capture enzyme-linked immunosorbent assay (ELISA) can be applied on for detection of IgG and IgM, respectively. Due to false-positives related to specificity of ELISA, positive reactions have to be confirmed by SN [59].

Treatment

There are currently no antiviral drugs or vaccines available to treat Nipah virus infection for either people or animals. Intensive supportive care with treatment of symptoms is the main approach to managing the infection in people. Experimentally, the therapeutic use of a neutralizing human monoclonal antibody, the m102.4, which recognizes the receptor binding domain of the NiV G glycoproteins, appeared promising in a ferret animal model [21]. Furthermore, the m102.4 was also successfully tested in Non Human Primate (NHP)

models against challenge with related Hendra virus [60].

Prevention

There is no vaccine against Nipah virus. A number of researches have been successfully conducted on the development of vaccines [61,62]. Experiments have been conducted also in African green monkeys [63]. However, results are limited to experimental condition and further progress is required to obtain protection against NiV in humans and animals. Only recently, a vaccine for the prevention of Hendra virus in horses has been licensed in Australia by Pfizer Animal Health under the name Equivac® HeV [64].

To date, prevention of Nipah virus infection relies on veterinary measures in domestic animals and public health education.

Control of Nipah Virus in Domestic Animals

Taking into account the human health implications, all field investigations should take necessary precautions to prevent infection. This includes prompt and accurate veterinary investigations on suspected clinical cases especially in pigs. Any respiratory or neurological conditions of swine in an area known to have pteropid bats, should consider Nipah as a rule out. Nipah should be suspected if pigs also have an unusual barking cough or if human cases of encephalitis are present. Symptoms in pigs are not dramatically different from other respiratory and neurological illnesses of pigs. Differential diagnosis should be applied in case of deaths of suckling pigs and piglets, sudden death in boars and sows, abortions and other reproductive dysfunction, respiratory diseases with harsh, non-productive coughing, and in cases with encephalitic manifestations of trembling, muscular incoordination and myoclonus leading to lateral recumbency.

In pig farms contact with fruit bats and their secretions should be avoided using screens at open-air access. Control of any access to swine by other wild or domestic animals should be also ensured. Routine cleaning and disinfection of animal farms (with sodium hypochlorite or other detergents) is expected to be effective in preventing infection. If an outbreak is suspected, the animal premises should be quarantined immediately. Culling of infected animals, with close supervision of burial or incineration of carcasses, may be necessary to reduce the risk of transmission to people. All materials and equipment from affected farms should be cleaned and disinfected. Restricting or banning the movement of animals from infected farms to other areas has to be applied to reduce the spread of the disease.

Public health education

In countries like Bangladesh where Nipah virus is endemic, authorities stress the importance of public awareness. An explicit warning has been made by the Health Minister A.F.M. Ruhul Haque: “Only by stopping the consumption of the raw sap, can this disease be stopped. Despite our many attempts at raising awareness, people are ignoring the warnings and as a result, are getting infected” [41], underlining the importance of providing information and the difficulties encountered to obtain behavior changes in target populations.

In the absence of a vaccine, the only way to reduce the risk of infection in people is by raising awareness of the risk factors and educating people about the measures they can take to reduce exposure to the virus.

Public health educational messages should focus on: i) Reducing the risk of bat-to-human transmission: Efforts to prevent transmission

should first focus on decreasing bat access to date palm sap. Freshly collected date palm juice should also be boiled and fruits should be thoroughly washed and peeled before consumption. ii) Reducing the risk of human-to-human transmission: Close physical contact with Nipah virus-infected people should be avoided. Masks, gloves and protective equipment should be worn when taking care of ill people. Regular hand washing should be carried out after caring for or visiting sick people. iii) Reducing the risk of animal-to-human transmission: Masks, gloves and other protective clothing should be worn while handling sick animals or their tissues, and during slaughtering and culling procedures [42].

International norms and approaches in non endemic countries

Due to the significant morbidity and mortality, and rapid spread potential in domestic animals, and evidence of zoonotic properties, recently, Nipah virus has been included in the list of diseases with relevance for international trade of the World Organisation for Animal Health (Office International des Épizooties: OIE) [65]. Therefore, NiV outbreaks of has to be immediately notified to OIE by the veterinary authority of the member states.

In non endemic countries scientific attention is high on henipaviruses, but practical field implications are less obvious. Despite the recognized importance of Niv, inclusion in national monitoring plans remains questionable. For example, when compared to other zoonotic pathogens circulating in Europe, such as *Campylobacter* or *C. burnetii* (Q fever), NiV appears to be the most dangerous agent (Table 2). In contrast, taking into account the very high incidence in human population with about 200,000 confirmed cases per year [66], *Campylobacter* results the most important among these considered pathogens, justifying the inclusion in a monitoring plan (Table 3).

Patogen	Nipah Virus	<i>C. burnetii</i>	<i>Campylobacter</i>
OIE notifiable disease	Pig diseases Nipah virus encephalitis	Multiple species diseases Q Fever	Bovine diseases genital campylobacteriosis
Zoonosis	YES	YES	YES
Pathogenicity in man	+++	+	+
Therapeutic or prophylactic means	NO	YES	YES
Risk Category	4	2	2
Domestic animals	YES	YES	YES
Wild animals	YES	YES	YES

Table 2: Comparison between NiV and other zoonotic pathogens. NiV appears to be the most dangerous agent.

Patogen	Nipah Virus	<i>C. burnetii</i>	<i>Campylobacter</i>
Presence in Europe?	NO	YES	YES
Incidence in human population	very low	low	High

Table 3: Example of applicable criteria for the inclusion of pathogens in monitoring plans. *Campylobacter* results the most important among the considered pathogens, justifying the inclusion in a monitoring plan.

Possible diffusion of Nipah Virus in free countries?
Fruit bats absent-Insectivorous bats?
Transmission from pig to man-Wild boar?
Ferrets sensible to NiV-Other mustelids?
Person-to-person direct transmission

Table 4: Elements suggesting potential for NiV epidemiological changes with increasing impact on public health and animal health in currently free countries, thus justifying monitoring of epidemiological evolution.

However, the introduction of Nipah virus in non endemic areas and in particular in Europe remains a plausible reality, primarily taking into account the presence of potentially NiV susceptible animal species. Intensive swine farming is widespread and transmission from pigs to humans was a key epidemiological feature of outbreaks in Malaysia and Singapore. Theoretically, wild boars might also play a role of amplification host. With concern to epidemiological perspectives, the presence in Europe of potentially NiV carrier bats of the species Daubenton's bat (*MYOTIS daubentoni*) represents another important aspect. Bat species in the genus *Myotis* naturally reside in trees, buildings, and caves that can be in close proximity to human residential areas, which increases the potential of transmission of zoonotic pathogens from bats to humans. Furthermore, susceptibility of ferrets to Nipah virus raises the possibility that the epidemiology could change further, evolving in natural condition and extending to other mustelids, and if a readily respiratory-transmissible Nipah virus could be created by serial passage in these wild animal species, as suggested by experiments in ferrets with H5N1 avian influenza [67]. In summary, it cannot be excluded that the virus might be introduced and diffused through insectivorous bats, domestic pigs or other wild animals such as wild boars or mustelids, and finally might circulate in the human population on the base of person-to-person transmission capacity. Therefore, these elements suggest the importance to monitor the NiV epidemiological evolution, in terms of variation of geographical distribution and acquisition of new transmission ways (Table 4).

In conclusion, knowledge and awareness on the disease should be improved and disseminated to health services, veterinarians, farmers and consumers. Nipah virus, as other zoonotic agents, might be included in monitoring plans, in particular for wild animals. Prioritization may drive the attention to other pathogens showing for example higher incidence in the population. However, field investigations may demonstrate radical and unexpected epidemiological changes. For example, the discovery of a novel ebolavirus-like filovirus in Spanish microbats demonstrated that the potential for such spill over events is not limited to Africa or Asia [68]. It is therefore important to enhance our preparedness to counter potential future introduction of exotic pathogens as henipaviruses in non endemic areas by conducting active pre-emergence research. Of utmost importance, monitoring the evolving epidemiology of a dangerous pathogen like the Nipah virus is an essential element to be able to promptly adapt control plans in the case that it might become a new public health priority.

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