Chapter 4
Epidemiology of Henipaviruses

Stephen Luby and Emily Gurley

Core Message The henipaviruses are RNA viruses whose natural reservoir is large fruit bats. People occasionally become infected with these viruses by being exposed to body fluids of bats or other infected animals.

Henipaviruses are a recently discovered genus of paramyxovirus. At the time of drafting this chapter three henipaviruses had been isolated: Hendra virus [1], Nipah virus [2] and Cedar virus [3]. The reservoir for all three isolated henipaviruses is fruit bats of the genus *Pteropus* in the family *Pteropodidae* [3, 4]. Segments of RNA closely related to known henipaviruses, but likely representing different species of henipavirus have been identified from urine and saliva of *Pteropus giganteus* [5], and from feces and tissue samples from *Eidolon helvum*, a native African fruit bat in the family *Pteropodidae* [6, 7].

Neither Nipah nor Hendra virus causes any apparent disease in infected bats [4, 8, 9] and likely coevolved with these bats. The ephrin-B2 and ephrin-B3 molecules which henipaviruses exploit to enter epithelial cells [3, 10] are widely conserved across mammals, and many mammals are therefore susceptible to henipavirus infection [11].

Human infection and severe disease has been recognized occasionally with Hendra virus, repeatedly with Nipah virus, but has not yet been described with Cedar virus. In contrast to Nipah virus and Hendra virus which causes severe illness in laboratory animals that are experimentally infected, ferrets and guinea pigs that were infected with Cedar virus remained clinically well [3]. This chapter updates a previous chapter on the epidemiology of human henipavirus infection by these authors [12].

S. Luby, M.D. (✉)
Department of Medicine, Stanford University School of Medicine,
The Jerry Yang and Akiko Yamazaki Environment and Energy Building, 473 Via Ortega,
MC 4020, Stanford, CA 94305, USA
e-mail: sluby@stanford.edu

E. Gurley, Ph.D., M.P.H.
Centre for Communicable Diseases, ICDDR,B,
GPO Box 128 Mohakhali, Dhaka 1212, Bangladesh

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1 Hendra Outbreaks

Hendra virus, previously referred to as equine morbillivirus, was first identified in an outbreak in September 1994 in Hendra, a suburb of Brisbane, Queensland, Australia [13, 14]. The first recognized infection occurred in a pregnant mare that was staying in an open paddock when noted to be ill. The mare was moved into a stable in Hendra and died within 2 days. Between 8 and 11 days after the mare's death 18 other horses residing in or near the stable became ill. Affected horses had depression, loss of appetite, fever, ataxia, tachycardia, tachypnea, dyspnea, and a copious frothy nasal discharge. Among 18 horses with clinical illness, 14 died, 12 horses from the Hendra stable, 1 horse staying in the paddock adjoining the stable and 1 horse living on a neighboring property that had very close contact with horses from the Hendra stable. Autopsy findings from the horses were notable on gross pathology for heavy edematous lungs with hemorrhage and froth in the airway. The histopathological findings suggested interstitial pneumonia, with focal necrotizing alveolitis, and syncytial giant cells within the vascular endothelium [1].

Two employees at the stable, a 40-year-old male stable hand and a 49-year-old male horse trainer had particularly close contact with the index mare during the final stages of her fatal illness. The horse trainer, whose hands and arms had abrasions, attempted to force feed the mare by placing his bare hands with food into the sick mare's mouth. Both the stable hand and the horse trainer became ill 5–6 days after the death of the mare with fever, myalgia, headaches, lethargy, and vertigo. The stable hand remained lethargic for several weeks but eventually recovered. The horse trainer developed progressive respiratory failure and died. His autopsy findings were consistent with interstitial pneumonia, with focal alveolitis and syncytial formation [14]. An identical virus which was ultimately named Hendra virus was grown from samples from both the affected horses and the affected people [1].

Since its identification and the first two recognized human infections, five additional human infections with Hendra virus have been recognized, all in Queensland, Australia, though Hendra virus infection of horses has also been identified in New South Wales, Australia [15]. The third person with recognized Hendra infection was a 35-year-old male who lived on a horse stud farm [16]. He had cared for two sick horses, one with acute respiratory distress and the other with a rapid onset of neurological symptoms. Both horses died. He assisted a veterinary surgeon during the necropsy of the two horses. Throughout caring for the horses and the necropsy the assistant never wore gloves, mask or protective eyewear. A few days after assisting with the autopsies he became ill, and sought medical attention. Subsequent PCR evaluation of serum samples from that illness amplified a 500 nucleotide sequence of the matrix gene of Hendra virus.

McCormack and colleagues evaluated people who had contact with Hendra infected humans and horses during these first two recognized outbreaks of Hendra. They collected serum samples from 159 people who had contact with Hendra infected human patients, 16 who participated in necropsies on Hendra virus infected

horses, 6 who had other close contact with Hendra infected horses and 113 who had other variable contact with horses [17]. None of the tested study subjects had neutralizing antibody to Hendra virus.

The fourth recognized human infection with Hendra occurred in a recent veterinary graduate who conducted a limited autopsy on a 10-year-old horse that died of a rapidly progressive respiratory illness with large amounts of blood stained frothy nasal secretions [18]. Although she initially wore gloves, she soon removed them because they were not appropriately designed and had become contaminated inside. She did not use any other personal protective equipment. She reached deep into the carcass to examine internal organs and became heavily contaminated with the horse's body fluids. After completing the autopsy, the veterinarian returned home and showered. Seven days later she developed a dry cough, sore throat, fever, body aches, and fatigue. She recovered after 8 days. Serial serological samples demonstrated seroconversion of IgM and IgG antibodies against Hendra virus. The two autopsy assistants and an adult family member who held the dying animal's head and were exposed to frothy bloody nasal secretions did not develop clinical illness and were seronegative for Hendra virus infection [18].

The fifth and sixth recognized human infections with Hendra virus were a 33-year-old male veterinarian and a 21-year-old female veterinary nurse who worked at a veterinary practice in Thornlands, Queensland during an outbreak of Hendra virus that affected five horses in the practice [19]. Both the veterinarian and the nurse performed nasal cavity lavage to a horse during the 3 days before the horse developed symptoms of ataxia, depression, and disorientation and was later confirmed to be infected with Hendra virus [20]. The veterinarian developed fever, myalgia, and headache which progressed to confusion, ataxia, respiratory failure, and death. The nurse developed fever, confusion, and ataxia. She survived with substantial neurological deficits. Both the veterinarian and nurse had Hendra virus RNA detected by reverse transcription PCR from both serum and nasopharyngeal aspirate specimens. The outbreak investigation identified 83 other people who had contact with the sick horses. Sixteen reported mild symptoms, but none developed a clinical illness. None had Hendra virus RNA or Hendra antibodies. Among the 28 persons who reported contact with potentially infected equine body fluids only the two cases developed Hendra virus infection. One veterinary worker who had a percutaneous blood exposure from an infected horse also had no evidence of infection.

The seventh recognized human infection with Hendra virus was a veterinarian who examined a horse that died the next day. A pony and a horse on the same property died of confirmed Hendra infection in the subsequent 11 days [21].

These seven people infected with Hendra virus were infected through contact with only five Hendra virus infected horses. Most infected horses do not transmit Hendra virus to people. Indeed, of the 84 recognized equine Hendra virus infections through July 2013, only 5 have resulted in human infection [15, 22]. In the originally identified outbreak in the Hendra stable, all of the infected horses developed illness within one incubation period (8–11 days after the death of the index mare). This suggests that the mare was a superspreader [23], though we do not know if this

exceptionally efficient transmission was due to unusual viral shedding in this mare, care practices by its animal handlers or both. The absence of a successive wave of infection among horses, and the low attack rate of Hendra virus among people who had contact with Hendra virus infected horses suggest that such superspreaders are exceptional. All seven recognized human cases of Hendra virus had intimate contact with Hendra virus infected horses, usually with heavy exposure to respiratory secretions and without wearing personal protective equipment. Other people with close contact with these same horses did not develop Hendra virus infection. These observations suggest that Hendra virus is not easily transmitted from horse to human. It apparently requires a horse that is an unusually efficient transmitter and a person with a high exposure to infectious secretions.

All humans confirmed with Hendra virus infections had contact with Hendra virus infected horses. The absence of human cases among healthcare workers and among family members suggests that Hendra virus is not easily transmissible from person to person. Selvey and colleagues identified 128 people who cared for Pteropid bats, the wildlife reservoir of Hendra virus [24]. The bat carers included volunteers who cared for injured or orphaned bats and professionals who cared for captive bats. Bat carers had a median 48 months of bat contact. Seventy-four percent reported daily contact with flying foxes. Seventy-four percent reported having been bitten, 88 % scratched, and 60 % reported exposure to flying fox blood. None of the bat carers tested positive for antibodies to Hendra virus. While, direct transmission of Hendra virus from flying foxes to humans could not be excluded, the study suggested that it was extremely rare.

2 Nipah Virus Outbreaks

2.1 Malaysia/Singapore

Human Nipah virus (NiV) infection was first recognized in a large outbreak in peninsular Malaysia from September 1998 through May 1999 [25–27]. The initial human cases were identified among pig farmers who lived near the city of Ipoh within the state of Perak in northwestern peninsula Malaysia in late September 1998. Patients presented with fever and headache. Over half developed a reduced level of consciousness; 42 % had seizures [28]. Among 28 early cases, 4 had IgM antibodies against Japanese encephalitis. The government declared the outbreak was due to Japanese encephalitis and initiated widespread mosquito control measures. By December 1998 larger clusters of similar cases were reported within the Port Dickson District of Negri Sembilan, 300 km south of Ipoh [29]. In March 1999 a novel paramyxovirus was isolated from the cerebrospinal fluid of a patient from Sungai Nipah village [2] that was confirmed to be the cause of the outbreak [25]. Ultimately the Malaysian Ministry of Health reported 283 cases with 109 (39 %) fatalities [27].

Parashar and colleagues conducted a case–control study to explore the risk factors for human illness with NiV during the outbreak [30]. They enrolled 110 NiV antibody confirmed cases from Port Dickson and two sets of controls, 147 community farm controls from among persons who either lived or worked on pig farms with no reported human encephalitis cases, and 107 case-farm controls who were selected from among NiV antibody negative persons who lived on farms where there was a known case of human NiV infection. Case patients were more likely than community farm controls to report increased numbers of sick or dying pigs, dogs and chickens on their farms. Case patients were more likely than case farm controls to have direct contact with pigs that appeared sick and to have close contact with pigs through feeding pigs, processing baby pigs, assisting in breeding of pigs, assisting in birth of pigs, injecting or medicating pigs, and handling dead pigs.

In contrast to the severe illness manifested by Hendra virus infected horses, most pigs infected with NiV had mild illness. Forty-one percent of human NiV infected cases who worked on pig farms reported no increase in sick or dying pigs on their farm [30]. Case fatality among adult infected pigs was low, ranging from <1 to 5 % [31]. Among three pigs infected with NiV through experimental oral inoculation or sharing a cage with an inoculated pig, all developed asymptomatic infections [32]. A subset of NiV infected pigs were severely affected and developed fever, agitation, trembling, and twitching accompanied by rapid labored respirations, increased drooling and a non-productive loud barking cough [31]. Pathological examination of severely affected pigs demonstrated extensive involvement of the lungs with a giant cell pneumonia with multinucleated syncytial cells containing NiV antigen in the lungs and epithelial cells lining the upper airways [25]. NiV was recovered from respiratory secretions of infected pigs, and NiV antigen was detected in renal tubular epithelial cells [25, 32].

Between March 10 and 19, 1999 eleven workers in one of Singapore's abattoirs developed NiV associated with encephalitis or pneumonia [26]. One worker died. Compared to controls who were also abattoir workers, cases were more likely to be exposed to pig urine or feces from pigs that had been imported from Malaysia during the Malaysian NiV outbreak. NiV RNA recovered from autopsy specimens from the one worker who died, had a nucleotide sequence that was identical to the sequences of NiV isolates from humans and from pigs in Malaysia [26].

The isolation of NiV from pigs' lungs and respiratory secretions combined with the observation that human cases of NiV infection had closer contact with pigs and so more contact with pigs' secretions and excretions than controls suggests that NiV was transmitted from infected pigs to humans through contaminated saliva and possibly urine. The human outbreak of NiV infection ceased after widespread deployment of personal protective equipment to people contacting sick pigs, restriction on livestock movements, and culling over 900,000 pigs [33]. Since the outbreak ended through December 2014 no human or porcine NiV infections have been reported from Malaysia.

Mathematical modeling suggests that multiple spillovers into the pig population were necessary to create a dynamic population with sufficient newly susceptible pigs to sustain NiV transmission within pigs for months [34]. All human NiV

infections in the outbreak in Malaysia/Singapore in 1998–1999, may have been linked to a single NiV transmission from an infected bat to an immunologically primed pig population, leading to a sustained porcine epidemic which in turn led to a human epidemic. NiV isolates from pigs and people were nearly identical [25, 35].

Not all people infected with NiV in Malaysia had contact with pigs. In the Port Dickson case control study, two human NiV infected cases reported no contact with pigs [30]. KS Tan provided details on two additional NiV patients who had no direct contact with pigs [36]. One NiV patient who did not enter or go near a pig farms prior to his illness, worked repairing pig cages. His illness suggests that pig secretions/excretions remain infectious at least for hours and perhaps for days. The Port Dickson case control study noted an increased risk of dying dogs on farms where NiV cases were confirmed [30]. Serological studies in dogs in Malaysia demonstrated that they were commonly infected [37, 38]. One NiV patient who had no pig exposure worked as a cabinet maker and lived near a pig farm. His two pet dogs became seriously ill and died before the patient became ill with NiV infection [36].

There was limited evidence of person-to-person transmission of NiV in Malaysia. Multiple cases in families may have resulted from shared exposures. A large cohort study enrolled healthcare workers from the three hospitals that admitted over 80 % of patients with suspected NiV encephalitis [39]. The study enrolled 363 health care workers who provided direct patient care to encephalitis patients. More than 60 % reported contact with encephalitis patients before the institution of infection control measures on March 19, 1999. Many reported episodes of high risk exposure including skin exposure to body fluids of NiV infected patients ($n=89$), splash of patient body fluids to mucosal membranes ($n=39$), or needle stick injuries ($n=12$). None reported any serious illness, encephalitis or hospital admission. None of the first serum samples were positive by EIA for NiV IgG or IgM antibody. In the second round of antibody testing conducted 30 days later 3 of 293 serum samples (1 %) from exposed health care workers were positive for NiV IgG antibody, though none had detectable IgM and all three were negative for anti-NiV neutralizing antibodies. All three were nurses who cared for outbreak related encephalitis patients for more than 30 days compared with a mean of 10 days in nurses with negative IgG antibodies [39]. One of the nurses with NiV IgG antibody reported a febrile illness before the first serum sample was obtained, and the second reported a febrile illness between the two serum samples. One of the nurses reported a mucosal splash exposure. In a separately reported investigation a nurse who cared for hospitalized NiV infected patients and had antibody against NiV but was asymptomatic, had MRI findings characteristic of NiV infection [40]. Eleven years after the Malaysian outbreak a 32-year-old women presented with characteristic MRI findings of late onset NiV encephalitis and NiV IgG antibody [41]. Her family had stopped pig farming and moved away from the outbreak area 10 years before the outbreak, but she visited her aunt and uncle during the NiV outbreak and cared for her aunt who became ill and died. The woman reported no contact with pigs or other domestic animals.

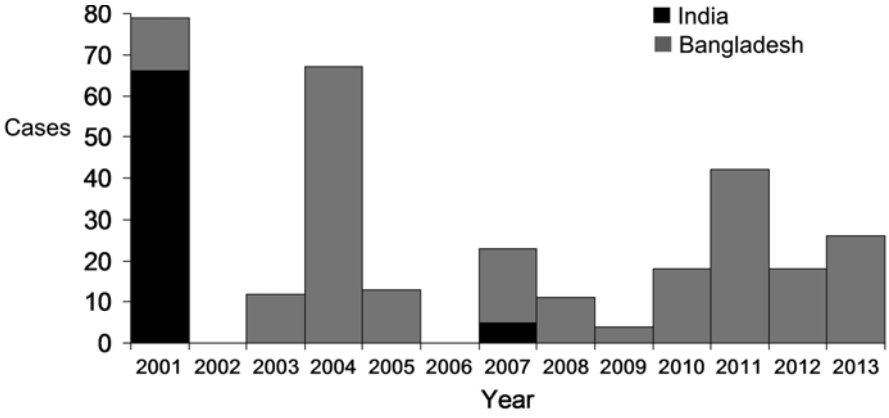

Fig. 4.1 Human infections with Nipah virus in South Asia 2001–2013

2.2 NiV Epidemiology India/Bangladesh

The epidemiology of NiV in Bangladesh/India has been quite different than in Malaysia. Since 2001 human infections with Nipah virus have been recognized in South Asia most years (Fig. 4.1). The cases in Bangladesh have largely clustered in western/northwestern Bangladesh (Fig. 4.2). The two recognized Indian outbreaks occurred in West Bengal, remarkably near where cases have been repeatedly identified in Bangladesh (Fig. 4.2).

2.2.1 NiV Transmission Through Date Palm Sap

Outbreak investigations in Bangladesh have identified drinking raw date palm sap as the most common pathway of NiV transmission from *Pteropus* bats to people. In the 2005 outbreak investigation in Tangail, Bangladesh NiV cases were 7.9 times more likely to report drinking raw date palm sap in the 10 days before they developed illness than neighborhood matched controls [42]. Similarly in the 2008 outbreak in Manikgonj and Rajbari districts in Bangladesh cases were 25 times more likely than controls to report drinking raw date palm sap [43]. In outbreaks in Faridpur, Bangladesh in 2010, and in Lalmonirhat in 2011 cases were again significantly more likely than controls to report drinking raw date palm sap in the 2 weeks prior to the onset of illness [44, 45]. The outbreaks of human NIV infection in Bangladesh and India coincide with the date palm sap harvesting season [46].

In Bangladesh date palm sap harvesters collect sap beginning in December with the first cold night and continue collecting most regularly through January and early February, though some harvesters continue to collect in at least a few trees through

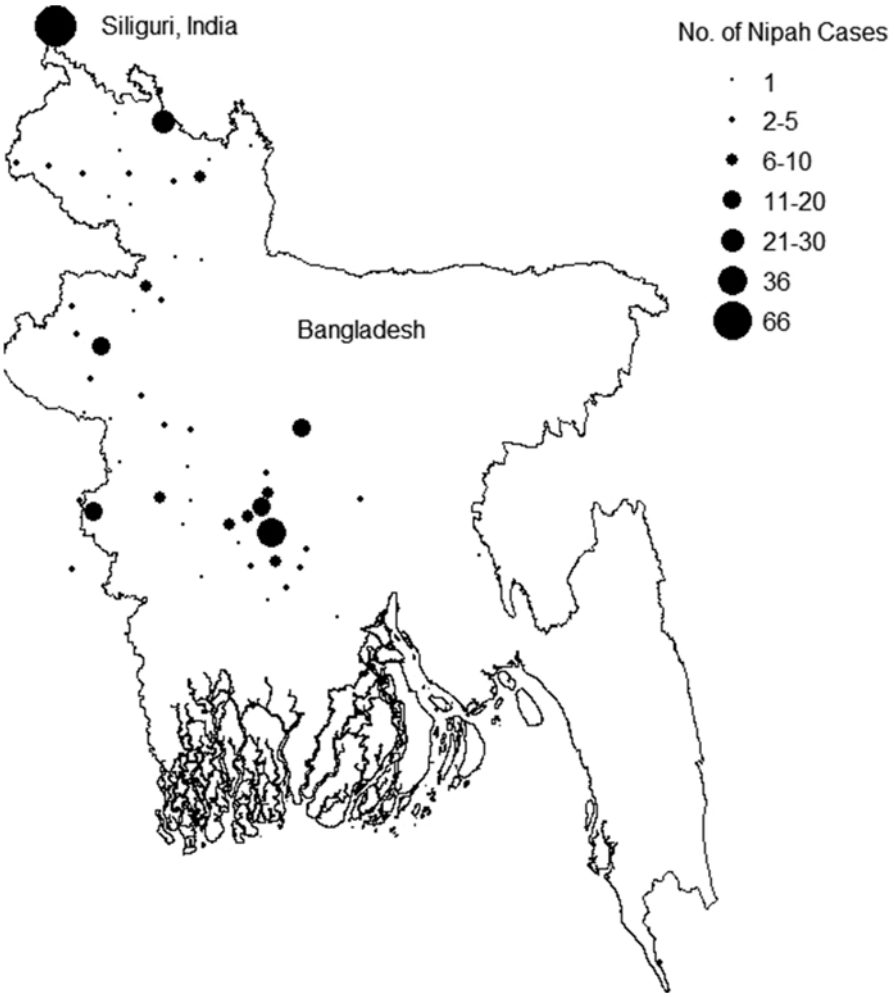

Fig. 4.2 Location of Nipah cases, Bangladesh/India 2001–2013

March and early April. At the beginning of the season, the bark is shaved off of one side of the tree (*Phoenix sylvestris*) near the top in a V shape and a small hollow bamboo tap is placed at the base of the V [47]. In the late afternoon, the date palm sap harvester climbs the tree, scrapes the area where the bark is denuded so the sap can flow freely, and ties a 2–4 l clay pot underneath the tap. During the night as the sap rises to the top the tree, some sap oozes out from where the bark is denuded, flows through the tap and drips into the clay pot. Palm sap collectors climb the trees at daybreak to gather the clay pots.

Most date palm sap in Bangladesh is cooked and made into molasses that is a popular sweetener for cakes and other desserts [47, 48]. A smaller amount of date palm sap is sold fresh for immediate consumption. Indeed, after a few hours, likely

because of fermentation, the date palm sap is less sweet and sap sellers have to lower the price. Collectors will often share fresh sap as a treat with family members and walk house to house near where the sap was collected and offer to sell it to neighbors.

Sap harvesters and villagers report that bats and other animals sometimes visit the trees during sap collection. Sap harvesters commonly find bat excrement outside of the clay pot or floating in the sap and occasionally find drowned dead bats floating in the pots [42, 47]. Infrared wildlife photography confirms that *Pteropus* bats, the presumed reservoir of NiV in Bangladesh, commonly visit date palm trees during collection and lick the sap stream [49]. Infrared cameras placed in the seven trees that were the source of fresh date palm sap drunk by the human NiV cases in the Manikgonj/Rajbari outbreak in 2008, identified an average of four *Pteropus* bat visits per tree where the bat licked the sap stream, per night of observation [43].

Date palm sap is a plausible vehicle for transmission of NiV from *Pteropus* bats to people. *Pteropus* bats occasionally shed NiV in their saliva [8, 50, 51]. The infrared camera studies confirm that *Pteropus* bats directly lick raw date palm sap and occasionally urinate in the sap collection pot [49]. NiV inoculated in mango flesh, mango juice, pawpaw juice, and lychee juice for up to 3 days was recoverable at high concentrations [52]. NiV that was inoculated into a solution of 14 % sucrose and 0.21 % bovine serum albumin to mimic date palm sap, survived for 8 days at 22 °C with no reduction in titer [53]. To date, in outbreak investigations NiV has not been isolated directly from date palm sap [43]. This is not surprising, because *Pteropus* shedding of NiV is intermittent [54], and with the median 10 day incubation period from exposure to date palm sap to illness [43], and the time required to recognize an outbreak of NiV, outbreak investigation teams have only been able to collect sap samples from trees weeks after the likely transmission event.

Some date palm sap in Bangladesh is fermented into palm wine (*tari*). One NiV case in India [55] and an outbreak in Bangladesh [56] have been tied to drinking this fermented date palm sap. Apparently, at least in some cases, the alcohol content of the fermented sent sap is insufficient to inactivate the virus.

Other direct pathways of NiV transmission from *Pteropus* to people have not been confirmed. In the 2004 outbreak in Rajbari District, Bangladesh, cases were more likely to climb trees than controls (83 % versus 51 %, $p=0.025$) [57]. It is possible that children climbing trees had direct contact with NiV contaminated bat urine or bat saliva that subsequently infected their respiratory or gastrointestinal tract and led to infection; however, this pathway of transmission has been assessed but not implicated in any of the subsequent outbreak investigations through 2014. Moreover, 91 % of cases in the Rajbari outbreak reported drinking raw date palm sap [57]. The father of two of the cases was a date palm sap harvester and the outbreak was centered on his friends and family (Emily Gurley personal communication). Although there was insufficient statistical power to implicate date palm sap in the Rajbari outbreak investigation (91 % versus 72 %, $p=0.328$), the subsequent repeated implication of date palm sap as the vehicle of transmission in other outbreaks, and the high level of exposure among cases (91 %) in Rajbari suggests that fresh date palm sap was the primary vehicle of NIV infection in this outbreak.

2.2.2 NiV Transmission from Domestic Animals

A second route of transmission for NiV from bats to people in Bangladesh is via domestic animals. Fruit bats commonly drop partially eaten saliva-laden fruit. Domestic animals in Bangladesh forage for such food. Date palm sap that is contaminated with bat feces and so is unfit for human consumption is also occasionally fed to domestic animals [47]. Animal husbandry practices in Bangladesh are quite different than in Malaysia. In Malaysia, thousands of pigs were raised together on large factory farms. By contrast, in Bangladesh many rural families keep just a few domestic animals. If a domestic animal in Bangladesh contracts NiV, there are few susceptible mammals physically close enough to become infected, so rather than sustained transmission as was observed in the Malaysian outbreak, in Bangladesh the chain of transmission would be expected to be short.

Nevertheless, there have been human NiV cases linked to apparent domestic animal infections in Bangladesh. The index case in the Meherpur District 2001 outbreak developed illness on April 20, the latest post winter onset of any confirmed NIV outbreak in Bangladesh, past the end of the date palm sap season in most communities. NiV cases in Meherpur were eight times more likely to report contact with a sick cow than controls [58]. In the Naogaon outbreak in 2003, NiV cases were six times more likely than controls to report contact with a pig herd that visited the community 2 weeks before the human outbreak [59]. In 2004 a child developed NiV infection 2 weeks after playing with two goats that developed an illness that began with fever, and progressed to difficulty walking, frothing at the mouth and death [60].

2.2.3 NiV Person-to-Person Transmission

In contrast to limited evidence of person-to-person transmission of NiV in Malaysia, person-to-person transmission of NiV has been repeatedly identified in Bangladesh/India. The first NiV outbreak recognized in the Indian subcontinent was a large outbreak affecting 66 people in Siliguri, India in 2001. The outbreak apparently originated from an unidentified patient admitted to Siliguri District Hospital who transmitted infection to 11 additional patients, all of whom were transferred to other facilities. In two of the facilities, subsequent transmission infected 25 staff and 8 visitors [61].

The longest sustained chain of person-to-person transmission of NiV so far identified in Bangladesh occurred in an outbreak in Faridpur District in 2004. Friends and family members who provided direct care to NiV infected patients, or helped to carry them or transport them to health facilities when they were near death, sustained a chain of transmission through five generations [62] (Fig. 4.3). One NiV patient was a popular religious leader who was visited by many of his family members and followers when he became ill. Twenty-two of these visitors developed NiV infection.

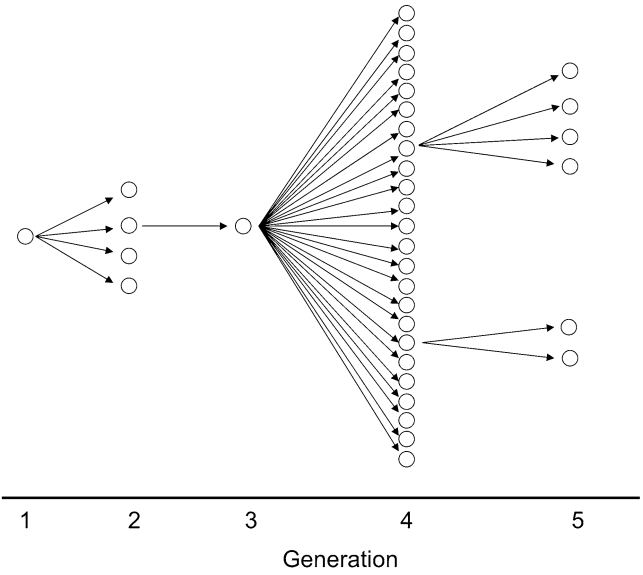

Fig. 4.3 Chain of person-to-person transmission in NiV outbreak in Faridpur, Bangladesh, 2004. (From [60])

While the outbreaks in Siliguri in 2001 and Faridpur in 2004 were the largest examples, person-to-person transmission has been identified in other outbreaks in Bangladesh [45, 63]. In a review of the 122 NiV cases identified in Bangladesh from 2001 through 2007, 62 (51 %) developed illness 5–15 days after close contact with another NiV patient [46].

Outbreak investigations in Bangladesh suggest that respiratory secretions are the primary vehicle of person-to-person transmission of NiV. Patients in Bangladesh were more likely to have respiratory symptoms then were patients in Malaysia. In a review of cases in the first four recognized outbreaks in Bangladesh, 62 % of patients had cough and 69 % had respiratory difficulties [64]. By contrast, in Malaysia only 14 % of patients presented with cough [28]. In the 2004 Faridpur outbreak, cases were more likely than controls to report touching an NiV infected patient who later died (OR 5.5, 95 % CI 2.1, 16) [62]. Similarly, in Thakurgaon in 2007 six family members and friends who cared for an NiV infected patient developed NiV infection. Cases were more likely than controls to have been in the same room when the index case was coughing (100 % versus 0 %, $p=0.04$) [63]. Across all recognized outbreaks in Bangladesh from 2001 through 2007, NiV patients who had difficulty breathing during their illness were more likely to transmit NiV than NiV patients who did not have difficulty breathing (12 % versus 0 %, $p=0.03$) [46].

NiV RNA has been frequently identified in the saliva of NiV patients [65, 66]. In Bangladesh family members and friends without health care or infection control training provide nearly all the hands on care to ill patients both at home and in the hospital [67]. During the Faridpur 2004 outbreak care providers shared eating

utensils, ate leftovers of food offered to NiV patients, commonly slept in the same bed with a sick, coughing NiV patient, and often fed and hugged the dying patient [68]. During an outbreak in Faridpur in 2010 a person whose only contact with an NIV infected patient was cleaning the corpse in preparation for burial became infected with NIV [45].

2.2.4 Other Plausible Pathways of NiV Transmission

There are a number of plausible pathways of NiV transmission from *Pteropus* bats to people that have been explored in outbreak investigations in Bangladesh, but have not been implicated as pathways of transmission. One of these pathways is living underneath a bat roost. Pteropus bats intermittently shed NiV in their urine [54]. Although some homes are located quite close to bat roosts, living near a bat roost has not been identified frequently in outbreak investigations, and has not been found more commonly among cases than controls [60].

Another plausible pathway of transmission is consumption of bat-bitten fruit. Both birds and *Pteropus* bats often drop fruit after taking a single bite. In Bangladesh, where child malnutrition is widespread [69], ripe tasty dropped fruit is commonly picked up and consumed by rural Bangladeshi residents. In each of the outbreak investigations in Bangladesh consumption of dropped fruit has been evaluated as a potential exposure, but in none of the outbreaks have cases been reported to have eaten dropped fruit significantly more commonly than controls [70].

3 Open Questions in Henipavirus Epidemiology

Both Hendra and Nipah virus are widely distributed among *Pteropus* bats, but spillover occurs in a much more restricted region. Apparently the frequency of a specific human behavior that is uncommon across the human population but more common in these areas provides an opportunity for henipavirus transmission. In Queensland, the popularity of horse racing leads to many horses sharing the natural environment with *Pteropus* bats, and people come in close contact with symptomatic ill horses. In Bangladesh, *Pteropus* bats are present across the entire country, and presumably shed virus throughout the year [54]. We hypothesize that people living in the outbreak infected regions in Bangladesh are more likely to drink fresh date palm sap or have other activities that put them in more contact with bat secretions compared with people living in other regions with *Pteropus* bats, but without recognized human NiV cases.

Among the most important open question in Henipavirus epidemiology is estimating the magnitude of risk that a strain of Nipah virus would develop sufficient capacity for person-to-person transmission to cause a high mortality global outbreak [71]. NIV is a stage III zoonotic disease that is an agent that normally lives in its animal reservoir, but occasionally spills over into people and is capable of

non-sustained person-to-person transmission [72]. Because its basic reproductive number (R_0), i.e., the average number of people who a new case infects is <1, spillovers result in stuttering chains of person-to-person transmission. While stage III zoonotic agents are infecting humans, the virus comes under selection pressure favoring characteristics that facilitate person-to-person transmission [73]. Humanity has a deadly historical example of a different zoonotic paramyxovirus, rinderpest, whose ancestor virus spilled over into humans as measles virus between the eleventh and twelfth century [74] and was subsequently a major cause of human mortality for centuries [75]. The Henipaviruses are widely distributed across species of bats and there is no evidence that they cause illness in bats [4, 8]. Thus, these viruses likely coevolved with the bats. Date palm sap has been collected in the area that is now Bangladesh for centuries [76] and so while NiV disease is newly recognized, there have likely been occasional human infections for a long time, none of which have resulted in pandemic transformation of the virus. Nevertheless, population density in South Asia has reached unprecedented levels, and so there is increased opportunity for sustained person-to-person transmission. Better understanding the frequency of spillover of Henipavirus from bats to other mammals in the environment, and the rate of change in adaptation of those viruses can provide a more precise estimate of the risk of a NIV pandemic, which, in turn, could prioritize and inform policy to reduce risks.

A related question to pandemic risk is how much strain differences in Henipavirus are responsible for observed epidemiological differences. There is substantial heterogeneity among Nipah strains in Bangladesh compared with much less strain heterogeneity associated with the single large Nipah outbreak in Malaysia [77]. Nipah patients in Bangladesh were much more likely to have severe respiratory symptoms and much more likely to transmit Nipah person-to-person compared with Nipah patients in Malaysia [64]. In animal experiments inoculating Syrian hamsters and African green monkeys, animals exposed to a lower dose of Nipah virus were more likely to develop encephalitis; animals exposed to a higher dose of Nipah virus were more likely to develop severe respiratory disease [78, 79]. In human infections it is unclear if dose of exposure increases the proclivity for respiratory infection and subsequent person-to-person transmission. Alternatively, specific risk behaviors, especially the frequency of intimate personal contact with people who are dying in Bangladesh [68] may be the primary determinant of person to person transmission. It is also possible that some strains of Nipah that have characteristics which favor pulmonary tropism or other characteristics that facilitate person-to-person transmission. We do not yet have enough strains of henipavirus, paired with careful epidemiological data to resolve these questions, but continued careful outbreak investigation and collection of additional isolates could provide additional insight. If there are structural differences in viral proteins that facilitate person-to-person transmission, then better understanding the variability of these structures and capacities and their rate of change in different contexts can help to estimate pandemic risk and provide targets for intervention.

4 Conclusion

Careful investigation over the last 20 years have clarified the basic transmission pathways of Hendra and Nipah virus infection, and found evidence of other henipaviruses. These organisms are not easily transmitted to people. When humans do become infected, only occasional superspreaders infected with NiV transmit illness. To date transmission has not been sufficiently efficient to maintain person-to-person transmission. However, these agents are newly recognized. Human infection with either Hendra virus or Nipah virus is commonly fatal and their pandemic potential is poorly defined. Henipaviruses warrant ongoing public health and scientific attention.

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