



Breaking the Chain In Bangladesh

Nipah virus has struck again in western Bangladesh, as it has done almost every winter since 2001. Scientists believe they know why the killer stalks this region—and are testing a simple intervention

RAJBARI, BANGLADESH—When Anwara Begum came down with a high fever on 28 December, she and her husband thought it was a cold or the flu. As the hours passed, she grew too weak to eat or get out of bed, and she couldn't move her fingers. On the 30th, Begum's husband, Ramjan Ali, took her to a clinic, where doctors administered fluids for her diarrhea and discharged her. By the time she got home, she was unable to speak. "Both of us were very scared," says Ali.

Early in the morning on 1 January, Begum died. Three days later, her 2-year-old daughter, Dilruba, started running a fever. The toddler grew more and more lethargic, then could no longer move her limbs. Before doctors at Faridpur Medical College Hospital could move her to an isolation ward, Dilruba was dead. The hospital sent blood samples to the Institute of Epidemiology, Disease Control and Research (IEDCR) in Dhaka, the capital. An antibody test confirmed the worst: Dilruba had succumbed to Nipah virus, a rare pathogen discovered in 1999. IEDCR dispatched a team to probe how she and her mother contracted the disease and to scour

Rajbari here in western Bangladesh for more cases. The evidence uncovered heightens the mystery about why this region has become ground zero for Nipah.

Nipah claims few lives, but in South Asia it is a dreaded malady. The virus kills almost three-quarters of those it infects in Bangladesh, where nearly all known cases in the past decade have occurred, and leaves many survivors with crippling neurological disorders. "This virus is a bad actor. It causes a striking degree of anxiety and panic," says epidemiologist Stephen Luby of the U.S. Centers for Disease Control and Prevention (CDC), who has been on assignment with the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) in Dhaka since late 2004. Nipah's hallmark symptom is brain inflammation, or encephalitis. "Previously healthy young people die and die in groups," says Luby. "This really is a community crisis."

Nipah first emerged in Malaysia in 1998, but only in Bangladesh has it become a peren-



Risky business. *Agachhi* taps a date palm in western Bangladesh. Bamboo skirts (above) appear to prevent bats from contaminating collected sap.

nial scourge. Almost every winter since 2001, the virus has flared up in the world's most densely populated nation. It's more brush-fire than inferno: All told, the virus has killed 111 people in Bangladesh during the past decade. Fifty people died in 2004, the worst year. This winter is shaping up to be bad, with the death toll at 27 as *Science* went to press—and 2 months to go in the Nipah season.

Nipah is also a scientific puzzle. Disease hunters believe they have pinned down the virus's natural reservoir—fruit bats—and they have nailed a transmission route in Bangladesh: consumption of contaminated date palm sap. But some things don't add up. Fruit bats test positive for Nipah antibodies across southern Asia, and date palm sap is a delicacy throughout Bangladesh. Yet the virus mostly haunts only what investigators

call "the Nipah belt," a clutch of districts near the Ganges River in western Bangladesh. "There is something about this area, at this time of year, that puts people at particular risk," says Luby.

A gnawing fear is that Nipah virus will break out of its geographic box. IEDCR Director Mahmudur Rahman is optimistic that won't happen. "I believe that we will be able to keep the disease under control," he says. "But if we fail, it will be a real disaster for the country."

Out of hiding

Nipah first garnered attention in September 1998, when pigs on farms in Malaysia started

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Caught in the act. *Pteropus* fruit bats (top) are the natural reservoir for Nipah virus. Infrared cameras have observed *Pteropus* and other bats licking sap from tapped date palms.

getting sick in droves. Public health authorities assumed it was swine fever: The pigs had classic symptoms, such as high fever, muscle spasms, and abnormal gait. But it differed in one way: Many pigs developed loud barking coughs. Piglets readily succumbed to the disease, but most grown pigs recovered.

Then farmers started getting ill—and dying. Their symptoms included high fever, muscle pain, and severe encephalitis. Malaysian health authorities assumed the killer was Japanese encephalitis (JE), a mosquito-borne virus—pigs are a reservoir—and ordered widespread pesticide fogging. Pig handlers were vaccinated and told it was safe to go back to work. But some of them came down with encephalitis and died, too. Still convinced that they were dealing with JE, health officials assumed that batches of the inactivated vaccine were ineffective and turned to a live-attenuated JE vaccine. Another red herring was that some victims tested positive for JE antibodies. These may have been false positives, says Kaw Bing “Paul” Chua, a virologist at Temasek Life Sciences Laboratory in Singapore, or else pig farmers had been exposed to JE in the past without becoming ill.

In February 1999, Chua, then at the University of Malaya in Kuala Lumpur, joined the investigation when a patient with acute encephalitis was admitted to the university’s medical center. His serum also tested positive for JE antibodies. But anti-JE interventions appeared to be having little or no

effect, so Chua suspected an unusual or novel pathogen—and went fishing. He inoculated cell lines with cerebrospinal fluid and serum from their patient and five others. The pathogen ravaged Vero cells from African green monkey kidney tissue, making them fuse and form syncytial cells. That’s the calling card of paramyxoviruses, a diverse family that includes measles virus, mumps virus, and respiratory syncytial virus. Chua bombarded the Vero cells with monoclonal antibodies against several paramyxoviruses. Nothing stuck, which suggested that the virus might be new to science.

The University of Malaya’s aging electron microscope produced only blurred images of the viral particles. On 12 March, Chua packed samples in dry ice and flew to the United States. At CDC’s laboratory in Fort Collins, Colorado, microbiologist C. Bruce Cropp put the samples under their electron microscope, and when the image appeared, Chua immediately recognized the ringlike structures of paramyxovirus nucleocapsids. “Great fear overwhelmed me,” he says. Paramyxoviruses, he knew, are spread by close contact, via saliva or sputum. No wonder the JE control measures had failed. A few weeks later, Chua and CDC collaborators identified a novel paramyxovirus.

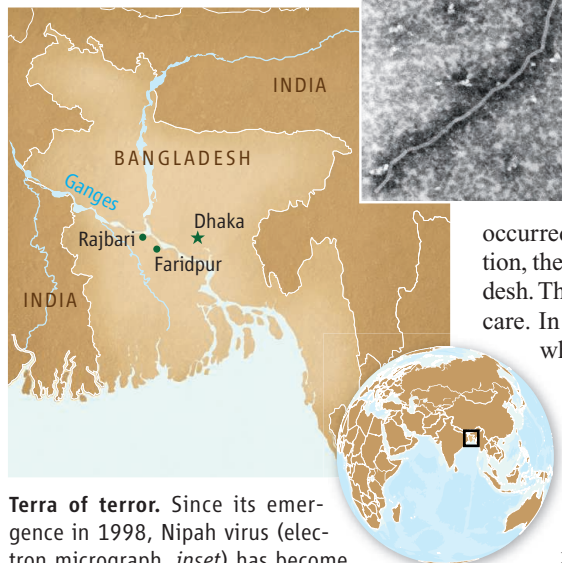
Malaysian authorities quickly changed tack. They ordered a mass cull of some 1 million pigs and sought to limit human contact with pigs to break the transmission chain. The measures worked. Through June 1999 the virus—named Nipah, after the village where it was first isolated—had infected 265 people in Malaysia and 11 pig slaughterhouse workers in

Singapore, killing 106. The tally does not include several encephalitis victims in 1997 whose stored sera were later found to have anti-Nipah antibodies, says Chua.

The virus was no longer infecting pigs or humans—but it had to be hiding somewhere. During the outbreak, Nipah virus had also been isolated from other livestock and domestic animals like cats and dogs. But its natural reservoir was unknown.

Scientists had a good lead though. Nipah’s closest kin is Hendra virus, which emerged in the mid-1990s in Australia. That virus had infected several horses and their handlers, causing an illness marked by severe encephalitis that killed two people. Scientists in Australia found live Hendra virus in *Pteropus* fruit bats, commonly called flying foxes, and concluded that this species is the virus’s natural reservoir. In Malaysia, researchers uncovered Nipah antibodies in scores of fruit bats roosting in trees near the pig farms where the outbreak had occurred. The bats would eat durian flowers and mangoes and other fruits in groves near pig farms; presumably their Nipah-tainted urine or saliva infected the pigs, says Chua. After months of searching, in early 2000 they found live virus in one bat from a large colony on Tioman Island, off peninsular Malaysia’s east coast. The team declared that it had uncovered Nipah’s reservoir; some experts were dubious. “There was skepticism about the single isolation from Tioman,” says Luby.

For 2 years, Nipah virus laid low before reemerging far from the original epicenter—and with a whole new *modus operandi*. Western Bangladesh, 2500 kilometers northwest of Malaysia, experienced its first outbreak in early 2001. This time, pigs had nothing to do with disease transmission. Victims were contracting Nipah from an unknown source, and it was spreading from person to person—something that rarely occurred in the Malaysia outbreak. In addition, the mortality rate was higher in Bangladesh. The likely explanation is inferior health care. In Malaysia, many acutely ill patients who were put on ventilators pulled through. Bangladesh has only a handful of ventilators, and to date, only one patient has been put on one, Luby says. “This is a terrible virus,” he says, “but I don’t think there’s something qualitatively more virulent about the strains we’re seeing here compared to the strain that was seen in Malaysia.”



Terra of terror. Since its emergence in 1998, Nipah virus (electron micrograph, inset) has become a recurring nightmare only in western districts of Bangladesh.



A Startling Villain

DHAKA—When villagers in northeastern Bangladesh began dying 3 years ago, disease sleuths were stumped. “Usually we have a good idea of what we’re dealing with,” says Emily Gurley, an epidemiologist with the International Centre for Diarrhoeal Disease Research, Bangladesh, here. “This was not one of those outbreaks.” The surprising culprit they uncovered sends a chilling warning to regions facing food shortages.

Alarm bells rang on 4 November 2007, when a woman and her 3-year-old child were taken unconscious to a hospital in the Sylhet District, on the Indian border. Early that morning, relatives explained, an older daughter had died; all three had vomited and were restless. Within hours, the mother and younger girl were dead. More cases cropped up; because people were succumbing quickly and without a fever, says Gurley, “we suspected something toxic.”

After a few days, a common thread began to emerge. Victims had eaten a plant that locals call *gaghra shak*. University of Dhaka botanist Mohammad Zashim Uddin determined that villagers had consumed seedlings of common cocklebur (*Xanthium strumarium*). Scouring the literature, the scientists learned that livestock have died after grazing on these seeds and seedlings; there was one report of three children in Turkey who died after eating seeds.

Villagers in Sylhet said they commonly used leaves or stalks of mature cocklebur to flavor curries or for medicine. That’s fine, as the toxin, carboxyatryloside, is present in large amounts only in seeds and seedlings. But that summer, monsoon flooding had reduced rice yields. Impoverished villagers resorted to eating cocklebur seedlings. “As the flood waters receded, these were the only plants that were sprouting,” says Gurley, lead author of a report on the outbreak in the March 2010 issue of *PLoS ONE*.

Scientists briefed villagers on the danger in town hall meetings; newspapers picked up the story, too. All told, 76 people took ill; of 19 deaths, 13 were children under age 10. The unforeseen consequence of food insecurity was a sobering lesson for Gurley. “I tried to imagine what it would be like to prepare a meal of weeds for my two young children because we had no food,” she says. “I was unable to imagine it.”

—R.S.

Taking their cue from Malaysia, researchers zeroed in on *Pteropus* fruit bats, which are common across Bangladesh. Hendra virus and Nipah virus are now classified as Henipaviruses.

Antibodies to Henipaviruses have been found in *Pteropus* bats across Southeast Asia and from as far away as Madagascar and the African continent. A few years ago, live Nipah virus was isolated from a fruit bat in Thailand, which has never reported a human case. In Bangladesh, scientists haven’t been so lucky. Working with colleagues from the nonprofit EcoHealth Alliance, Luby says, “We have identified an awful lot of bats that are antibody-positive. It’s just a matter of time until we find live virus.”

Bangladesh had a Nipah-free year in 2002. The virus returned in 2003 and struck with a vengeance in 2004. The outbreak that year in Faridpur “confirmed the substantial risk of person-to-person transmission,” Luby says. Meanwhile, Malaysia hasn’t reported a case since 1999. The only other country where Nipah is known to have jumped into humans is India. It has reported several sporadic cases and one outbreak, when the virus killed four dozen people in Siliguri, a city just across the border from Bangladesh, in early 2001.

That presented a riddle. The virus appeared to be endemic to a broad swath of Asia. “Fruit bats are everywhere,” Luby says, and have similar levels of Nipah antibodies wherever researchers have looked. It’s possible that Nipah flies under the radar elsewhere. In more than half of encephalitis deaths in Asia, says Luby, the cause is unidentified. “It’s plausible that sporadic cases are going unrecognized,” he says. Yet only western Bangladesh is hit with recurring outbreaks.

Russian roulette

When Badsha Shaikh was a child, he says he had a talent for climbing trees. Now he’s nearing 50 and still spry. A curved dagger tied to his waist, Shaikh scampers up a 20-meter-tall date palm almost like he is dashing straight up the trunk. “It’s like he’s running up stairs,” anthropologist Rebeca Sultana of ICDDR,B says with a laugh. In several seconds, he’s near the top and slings in place a leather harness. Shaikh is a *gachhi*, or date palm sap collector. During the winter months here in

Ramchandrapur village, when the sap is running sweet, Shaikh tends a couple of dozen palms; the rest of the year, he makes a living as a rickshaw driver.

Fruit bats once were common here, says Shaikh, but some locals enjoy eating them, so there are fewer now. In the afternoon, Shaikh shimmies up his date palms, slashing v-shaped cut marks in patches of trunk stripped of bark just below the fronds. The rest of the day and overnight, trees ooze sap through the wounds, which funnel the liquid into a clay jug slung below the bare patch. Just before dawn, Shaikh clammers back up to retrieve the jugs. Most sap will be boiled down to molasses in big kettles.

The best stuff is quaffed raw. *Gachhi* identify one or two date palms—older, taller, female trees—that produce the tastiest sap.

“It’s sweet. We used to drink it often when we went in the field, before Nipah,” says Sultana. Children love it mixed with puffed rice. “You have to drink the sap by 9 or 10 in the morning,” Sultana says. After that, it begins to ferment. Approximately 90% of the population in Bangladesh is Muslim, so consuming alcohol is forbidden. Some villagers imbibe fermented sap on the sly.

Drinking the stuff raw, it turns out, is a game of Russian roulette. During field investigations, IEDCR and ICDDR,B researchers discovered a common association with many Nipah cases not

explained by human-to-human transmission: The victims, both adults and children, had drunk raw date palm sap before getting sick.

Then the researchers caught bats in the act. In early 2008, a team led by ICDDR,B veterinarian Salah Uddin Khan trained motion sensor-tripped infrared cameras on tapped date palms. Over 20 nights, they made dozens of observations of *Pteropus* and other bats alighting on the shaved part of the date palms. Sometimes the bats would lick the sap running into the jug. Sap in the jugs, researchers surmise, can be contaminated with bat saliva and urine—and thus the virus. That may explain Nipah’s seasonality in Bangladesh. Cases here have occurred only between December and April, roughly coinciding with the time that palm sap is collected.

A second hypothesis is that seasonality is linked to the bat’s biological rhythms. Nipah virus and *Pteropus* bats appear to have



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—STEPHEN LUBY,
CDC EPIDEMIOLOGIST



coevolved over centuries or millennia. “Bats don’t seem to be bothered by it,” says Luby. “It’s like *E. coli* in our guts.” Bats breed in cycles, and females give birth around the same time. “Presumably, the mother’s antibodies protect newborn pups from Nipah. Then the antibody wanes and they become susceptible,” says Luby. During that period, more virus might be shed in the urine, heightening transmission risk.

With strong evidence that date palm sap is an important transmission pathway, scientists and health experts are looking for ways to break the transmission chain. “Getting people to stop drinking raw date palm sap is the only answer,” argues IEDCR’s Rahman. That will be devilishly hard to pull off. Drinking raw sap is a cherished Bengali custom, says Sultana.

Scientists have hit upon a measure that may stem infections in the short term. Villagers told anthropologist Nazmun Nahar, now a consultant to ICDDR,B, that years ago *gachhi* would protect the part of the date palm trunk stripped of bark with a covering made from bamboo slats. These bamboo skirts had fallen out of use.

In 2007, ICDDR,B persuaded *gachhi* to deploy bamboo skirts on a few trees. Infrared cameras indicated that the skirts are effective. “They keep the bats out, so the sap is cleaner,” says Luby. This year, with support from the U.S. Agency for International Development, they are scaling up the intervention and trying jute, which can be fashioned into skirts more cheaply and quickly, and plastic. ICDDR,B staffers have been going from village to village to hold community meetings to explain the risk of raw date palm sap and the importance of using skirts. They persuade village elders to put up warning posters. “This may

not be a basic science question, but it’s the cutting edge of emerging infections,” Luby says. “How do you come up with solutions that are scientifically sound, locally acceptable, and can be scaled up?” Crucially, date palm owners are warming to the concept. “I can stop drinking raw sap, but it’s hard to stop my children from drinking it,” says Abdus Sobhan Shaikh, who is now using jute skirts on two of his 20 trees.

More widespread awareness of the threat might have saved Begum and her daughter. Ali told investigators that his wife had drunk raw date palm sap at least three times in December, before she became ill. Each time she shared the ambrosia with several other women—none of whom came down with the disease. That the virus infected only Begum and Dilruba, and spared their companions, makes theirs a classic case.

Heaven sent?

In Rajbari, ripe brown sapodilla fruits are hanging from the boughs of trees and rotting on the ground. “Fruit bats love it,” says Khan. In a village on the outskirts of town, three veterinarians wearing face masks soothe a calf tethered to a tree. It lows plaintively. One guy jabs a syringe into its jugular. The team is drawing blood from as many animals as they can lay hands on: livestock, dogs and cats, and, of course, fruit bats, which are captured with nets strung near rooks. A handful of Nipah cases in the past have been linked to sick cows and goats, and a cluster in 2003 was traced to a pack of nomadic pigs.

Khan’s team came here within a few days after Anwara Begum’s death to draw animal blood. Then they were looking for immunoglobulin M (IgM) antibodies, which develop within a few days after exposure to an antigen.

Coming to grips. Responding to an outbreak in Rajbari last January, a team led by Salah Uddin Khan (*top right*) draws blood from a calf to look for anti-Nipah antibodies. Rebeca Sultana (*bottom right*) and colleagues are going village to village warning about the risk of raw date palm sap.

Results are not yet in. The researchers have come back in early February, a month after Begum’s death, to prospect for immunoglobulin G antibodies, which show up in the blood after a few weeks.

In contrast to the Malaysian outbreak, which was triggered by one or possibly two strains, Nipah outbreaks here have involved a number of strains. “We see quite a bit more variability,” says Luby. Not surprisingly, infected individuals in Bangladesh exhibit a range of symptoms, with mild cases called “Nipah fever” and, compared with Malaysia, more cases with pneumonia. If some strains are more apt to infect the lungs, Luby says, that could explain why many Nipah cases here are spread person to person. Epidemiology can’t shine a bright enough light on such questions: The number of cases is too small, says Luby. “So we are aggressively seeking strains,” he says.

In western Bangladesh, the scars from Nipah run deep. Some survivors are never the same. “People suffer personality changes and cognitive deficits. In a number of cases, these deficits have not gotten better,” says Luby. And because there is no vaccine or drug against Nipah, when an outbreak occurs, villagers feel helpless. “Many people think, ‘Medical care can’t do anything; it must be supernatural.’ It looks like a curse from Allah,” Luby says. If his team is right, simply preventing date palm sap contamination may be all it takes to banish the curse. —RICHARD STONE