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Nipah virus—a potential agent of bioterrorism?

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Abstract

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

Keywords: Nipah virus; Zoonotic paramyxovirus; Ribavirin; Bioterrorism

1. Introduction

The event of bioterrorism-related inhalation of anthrax in the United States towards the end of 2001 has brought into stark reality the possibility of biological weapons as a tool of mass destruction and renewed fear of the re-introduction of biological warfare. It was in 1969 that the United States discontinued its offensive biological research program (Department of the Army, 1997) and the former Soviet Union spent years working on pathogens which had potential capability as biological weapons (Vorobjev et al., 1997). Iraq has been reported to produce anthrax for use in Scud missiles and conducted research on camelpox virus as a substitute for smallpox as a biological weapon (Zilinskas, 1997).

Pathogens such as smallpox, plague, anthrax, botulinum toxin, Marburg and equine encephalitis viruses are considered to have the right attributes and characteristics to make good biological weapons. The global interest in emerging infectious diseases has led to the discovery of new highly virulent agents that can serve as potential agents of bioterrorism.

The outbreak of viral encephalitis in Malaysia invoked scenes of widespread panic for many months before the virus was identified to be Nipah virus, a recently emerging deadly paramyxovirus (MMWR, 1999a,b). Although the outbreak was a natural phenomenon, it could have been a scenario of bioterrorism because it produced fear, disease, disabilities, death, social disruption and severe economic loss to the country.

This review covers many of the facets of the outbreak caused by this new virus, with emphasis of its effects on human and animal health, the economic impact on a developing nation, and the social disruption it created. All these are factors that argue for Nipah virus to have the potential to be an agent of bioterrorism.

2. Nipah virus—historical background

An outbreak of viral encephalitis occurred over a 35-week period from 29 September 1998 to 31 May 1999 among pig farmers, creating considerable anxiety and fear throughout the country (Parashar et al., 2000). The outbreak started in the Kinta district of Perak and spread to three other localities, namely Sikamat and Bukit Pelanduk (the largest pig-rearing area in Southeast Asia) in Negeri Sembilan and Sungei Buloh in Selangor where the last case was reported. Of the 265 cases reported to the Ministry of Health in Malaysia, 105 were fatal, and thus the case fatality rate was 39.6%.

Initially the outbreak in the Kinta district was thought to be due to Japanese encephalitis (JE), a mosquito-borne disease, endemic in Malaysia with an average of 53 cases and 3 deaths annually. However, despite mosquito control measures and JE vaccination, the cases did not subside and

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the disease spread to Negeri Sembilan through the sale and movement of infected pigs. In Malaysia, active trading and movement of pigs between farms belonging to family members is a usual practice. The outbreak also spread to neighboring Singapore where 11 workers in an abattoir developed the disease with one fatality (Paton et al., 1999; Chew et al., 2000).

In Malaysia, 93% of the reported cases involved those directly in the pig farming industries, namely pig farm workers, pig farm proprietors, housewives and family members assisting in the farms. Others infected were those involved in the trading and transporting of pigs. A small number of cases (1.9%) were seen in those involved in the culling operations and there was one case in an abattoir worker. Twelve cases occurred in those who were not involved in the pig industry but had worked or lived in close vicinity to pig farms.

The majority of the cases occurred in those aged 20 and above (92.4%) and actively engaged in the pig industry. Only 6.4% of cases occurred among the age group of 10–19 years and 1.2% among children below 10 years. The majority of the cases (82.6%) involved males with a racial breakdown of 70.6% Chinese, 17.0% Indians and 11.3% among others who were mainly foreign workers. There were three cases (1.1%) involving Malays.

The outbreak in Singapore occurred between 10 and 19 March 1999 among abattoir workers handling pigs from infected farms in Malaysia. Those who had direct contact with urine and feces of live pigs were at higher risk of infection. In addition to the 11 cases, there were two apparently asymptomatic case patients among workers in this abattoir.

3. Clinical aspects

When the outbreak started in Negeri Sembilan 5 months after Perak, three pig farmers were admitted to the University of Malaya Medical Center, Kuala Lumpur. The patients presented with fever, headache, and altered level of consciousness (Chua et al., 1999). Myoclonus was present in two of the three patients and there were signs of brainstem dysfunction with hypertension and tachycardia. Rapid deterioration led to irreversible hypotension and death. A larger series of 94 patients seen in the same hospital confirmed the original clinical findings (Goh et al., 2000). Besides the features mentioned, it was noted that the disease predominantly affected the nervous system. Fifty-two patients (55%) had a reduced level of consciousness and they had prominent signs of brainstem dysfunction, including abnormal doll's eye reflex, pinpoint pupils with variable reactivity, and prominent vasomotor changes consisting of hypertension and tachycardia, which suggested involvement of the medullary vasomotor center. Segmental myoclonus characterized by focal, rhythmic jerking of the muscles was present in 32%.

In another series of 103 patients who attended the Seremban Hospital, the clinical features were also similar with a mortality of 41% (Chong et al., 2000). Complete recovery was reported in 40% but 19% had mild residual neurological signs. In Singapore, although the symptoms were generally the same, there were four patients who had respiratory symptoms with two presenting with atypical pneumonia (Paton et al., 1999).

The presence of Nipah virus in respiratory and urine of patients was demonstrated and this posed a danger for nosocomial transmission (Chua et al., 2001). In addition, the presence of virus in cerebrospinal fluid of encephalitic patients was associated with high mortality (Chua et al., 2000a).

4. Clinical relapse

Patients who had recovered from clinical illness and some who had only serological evidence of infection but were not ill were monitored for up to 2 years. Goh et al. (2000) reported four patients who had late neurologic dysfunction, with three of them having a relapse 13–39 days after an initial mild illness. To date, the Ministry of Health of Malaysia has on record of twelve patients who had clinical relapses of Nipah infection. These patients had either neurological symptoms after an initial illness without re-exposure to pigs, or long latency from the initial exposure to the virus. The onset of symptoms was acute with fever, headache, focal neurological signs, seizure, dizziness, reduced consciousness, and myoclonus. Two of these patients died during follow-up.

5. Use of ribavirin

Following the discovery of Nipah virus as the etiologic agent of the outbreak, ribavirin was used in an open-label trial (Chong et al., 2001a). Ribavirin was the drug of choice because it has broad spectrum activity against both RNA and DNA viruses (Huggins, 1989; Lin and Keeffe, 2001). It has also been reported to have clinical efficacy against hemorrhagic fever with renal syndrome caused by Hantaan virus as well as Lassa fever (McCormick et al., 1986; Huggins et al., 1991). Rodriguez et al. (1994) reported that ribavirin had a positive effect on respiratory syncytial virus infection. Furthermore, this drug has been demonstrated to cross the blood–brain barrier following oral administration, making it useful for the treatment of viral encephalitis (Connor et al., 1993).

A total of 140 patients from the Seremban Hospital and the University Malaya Medical Center, Kuala Lumpur were given ribavirin. Fifty-two patients were managed prior to the availability of ribavirin and these served as the control group. Oral ribavirin was given to 128 patients whilst 12 patients received intravenous ribavirin. There were 45 deaths (32%) in the treated group and 29 (54%) in the controls.

This preliminary study showed that ribavirin treatment in acute Nipah encephalitis was associated with a 36% reduction in mortality and an increased survival without neurological deficits, although the latter did not achieve statistical significance.

6. Laboratory investigations

Thrombocytopenia with platelet counts of <140,000 mm^{-3} was found in 30% of 94 patients and leukopenia (white blood cell counts of <4000 mm^{-3}) in 11% (Goh et al., 2000). Blood urea, creatinine and electrolyte levels were normal in all patients. Elevated levels of alanine aminotransferase were found in 33% of patients and aspartate aminotransferase in 42%. In 6% of patients, the chest radiographs were abnormal, with increased focal markings over the lung fields. All of these patients had primarily neurologic features at presentation, and only one had cough.

Cerebrospinal fluid examination in 92 patients was abnormal with elevated white blood cell counts and elevated protein levels. The results of computed tomography of the brain were normal. Twenty-seven patients underwent magnetic resonance imaging (MRI) of the brain during the acute phase of illness and all had widespread focal lesions in the subcortical and deep white matter and, to a lesser extent, in the gray matter on T2-weighted sequences and fluid-attenuated inversion recovery sequences. Further studies of MRI scans of the brain of Nipah patients with neurological manifestations in Malaysia and in Singapore provided similar findings (Lee et al., 1999; Lim et al., 1999; Sarji et al., 2000). It was interesting to note that abnormal cerebral MRI imaging was seen in 16% of subjects with asymptomatic Nipah infection, one of whom was a nurse, indicating that the infection could be spread nosocomially (Tan et al., 2000).

7. Histological findings

Histological findings showed severe endothelial damage and vasculitis, mainly in the arterioles, capillaries, and venules (Chua et al., 1999, Goh et al., 2000, Wong, 2000). The most severely affected organ was the brain, but other organs including the lung, heart and kidney were also not spared. Vasculitic vessels were characterized by vessel-wall necrosis, thrombosis, and inflammatory-cell infiltration of neutrophils and mononuclear cells. Syncytial-cell formation was seen in the endothelium of affected blood vessels in the brain and lung, and in the Bowman's capsule of the glomerulus. Zones of microinfarction and ischaemia were commonly found around or adjacent to vasculitic blood vessels, affecting both the gray and white matter of the cerebrum, basal ganglion, cerebellum, brainstem and spinal cord. Immunostaining for Nipah viral antigens using hyperimmune anti-Hendra and anti-Nipah sera showed intense staining of endothelial cells of dead and dying parenchymal cells in the central nervous system consistent with a severe encephalitis (Chua et al., 2000b). Less intense immunostaining was also seen in other tissues, including the lung, heart, spleen, and kidney.

8. Virology findings

A virus causing rapid syncytial formation in Vero cells (ATCC, CCL81) was isolated from the CSF of two patients in March 1999 (Chua et al., 1999). The infected cells stained strongly positive with Hendra virus antibodies by indirect immunofluorescence but not with monoclonal antibodies against other paramyxoviruses including measles, respiratory syncytial virus and parainfluenzavirus 1 and 3, enteroviruses, human herpesviruses, JE and other flaviviruses. Cross neutralization studies between Nipah and Hendra viruses resulted in an 8- to 16-fold difference in neutralizing antibodies, indicating that the viruses, though related, were not identical.

Electron microscopic (EM) studies of the virus demonstrated features characteristic of viruses belonging to the family *Paramyxoviridae* (Chua et al., 2000b; Goldsmith et al., 2000). Members of this family typically possess a single-stranded non-segmented RNA genome of negative polarity that is fully encapsidated by protein. Virus particles vary in size from 120 to 500 nm. Thin-section EM studies of infected cells revealed filamentous nucleocapsids within cytoplasmic inclusions incorporated into virions budding from the plasma membrane. Typical "herringbone" nucleocapsid structures were observed in infected cells by means of negative stain preparations. Pleomorphic extracellular virus particles, with an average diameter of 500 nm, and fine surface projections, were sporadically seen.

Sequence data of the Nipah virus genome placed it in the family *Paramyxoviridae* (Chua et al., 2000b; Harcourt et al., 2000), confirming that Nipah and Hendra viruses are genetically related. Phylogenetic analysis demonstrated that although Hendra and Nipah viruses are closely related, they are clearly distinct from any of the established genera within the *Paramyxoviridae* family. A new genus, *Henipavirus*, has been proposed for Hendra and Nipah viruses. Complete nucleotide sequences of two Nipah virus isolates, one from the throat secretion and the other from the CSF of a surviving encephalitic patient, showed that they differed by only 4 out of 18,246 nucleotides (Chan et al., 2001).

9. Nipah virus infection in pigs

The pig-breeding industry in Malaysia is very important and the national statistics in 1998 showed that there were over 2.3 million animals in the country, managed by 2100 households (The facts finding report on the encephalitis outbreak in Malaysia 1998–1999, 2000). The total value of annual national output was estimated at about US$ 400 million and the total export value at US$ 100 million.

Pig diseases such as classical swine fever, Aujeszky's disease and porcine reproductive and respiratory syndrome are prevalent in Malaysia and their spread is aided by poor animal husbandry and overcrowding in the farms. However, between late 1998 and 1999, a new pig disease characterized by a pronounced respiratory and neurological syndrome, sometimes accompanied by sudden deaths of sows and boars, was recorded in peninsular Malaysia (Mohd Nor et al., 2000). The clinical features appeared to vary according to the age of the pigs. Pigs from 4 weeks to 6 months of age usually presented with acute febrile illness with respiratory signs ranging from rapid and labored respiration to a harsh non-productive cough (loud barking cough). Severe cases might present with hemoptysis and less severe cases, open mouth breathing. Neurological signs varied from trembling and neurological twitches, muscle spasms and myoclonus, rear leg weakness and varying degrees of spastic paresis or lameness and uncoordinated gait. Boars and sows displayed similar symptoms and infection had resulted in sudden death. Mortality was low, from less than 1–5%, but the infection rate approached 100% with the majority of the infections being asymptomatic.

Histologically, the principal lesion was a moderate to severe interstitial pneumonia with widespread hemorrhages and syncytial-cell formations in the endothelial cells of blood vessels of the lung (Mohd Nor et al., 2000; Chua et al., 2000b). Generalized vasculitis with fibrinoid necrosis, hemorrhages, and infiltration of mononuclear cells sometimes associated with thrombosis, were observed notably in the lung, kidney and brain tissue. Immunohistology showed a high concentration of the viral antigens in the endothelium of the blood vessels, particularly in the lung. A lesser number of pigs showed prominent meningeal inflammatory infiltrates. This picture suggested that respiratory secretions from infected pigs were likely to be a rich source of infectious virus.

10. Control and eradication

Japanese encephalitis (JE), an endemic mosquito-borne disease with seasonal occurrence in Malaysia, was thought to be responsible for the outbreak. However, there were features in this outbreak that were different from JE, suggesting the possibility of a different etiology. JE is predominantly an infection of children and there should be no clustering of cases in members of the same household, implying a much higher attack rate than usually seen with JE. The majority of the cases occurred in male adults who had direct contact with pigs, sparing those living in the same neighborhood but without direct contact with the animals, thus arguing against a mosquito-borne transmission. In addition, there was a history of illness in pigs, quite uncharacteristic of JE. What was even more frightening was the fact that cases occurred in farmers who had been previously immunized against JE. Although the official view still maintains that the outbreak was simultaneously caused by JE and Nipah viruses, a recent paper by Chong et al. (2001b) questioned the role of JE in this outbreak.

With the discovery of the new etiology of the pig disease, mosquito control activities and JE vaccination were stopped. An immediate "stamping-out" policy was instigated to cull all pigs in the outbreak areas (Mohd Nor et al., 2000). Initially, the process of culling was frustratingly slow due to lack of manpower, equipment and know-how, failing to bring the disease under control. Pig farmers took it upon themselves to help in the culling exercise, using batons to club the animals to death. This was not only inhuman but also highly dangerous to those involved in the exercise. After much delay and with the help of police, soldiers and the Veterinary Services Department, a total of 1.1 million pigs from nearly 900 farms were destroyed in the infected area, thus bringing the outbreak to an end.

Twelve experts from the Centers for Disease Control and Prevention (CDC), Atlanta, two from the Commonwealth Scientific and Industrial Research Organization (CSIRO), Geelong, and one from the Animal Research Institute, Queensland, assisted in the outbreak investigation. Other local measures to control the outbreak have been highlighted (Lam and Chua, 2001). Active case detection was initiated among farm workers and their families and risk factors associated with Nipah virus transmission were identified (Amal et al., 2000; Premalatha et al., 2000; Chong et al., 2001c; Tan and Tan, 2001).

In order to monitor the pig farms in the country, a surveillance program involving the testing of pigs in farms and in abattoirs using the Nipah IgG ELISA was initiated. Educational programs to highlight the danger of the new pig disease as well as to practice good animal husbandry for the farmers, including the wearing of appropriate attire, are on-going.

11. Compensation

Restrictions were imposed to prevent movement of pigs from infected farms. To prevent the smuggling of pigs, the government offered compensation for each animal surrendered for culling. However, the amount offered was less than half the market value of the animals, leading to rampant smuggling of pigs from infected farms to other states. Two humanitarian funds were set up to help with the compensation and elevate the economic loss of the farmers. To this day, there is still considerable acrimony over the issue of appropriate compensation to the farmers.

12. Reservoir

Although Nipah virus primarily infects pigs, other animals such as dogs, cats, and horses have been similarly affected when they came into contact with infected pigs (Chua et al., 2000b). The similarity between Nipah virus to Hendra led to the speculation that Nipah virus could also infect bats. Johara et al. (2001) demonstrated neutralizing antibodies in 21% of 237 wild-caught bats, with the highest numbers in *Pteropus hypomelanus* (Island flying fox) and *P. vampyrus* (Malayan flying fox). However, they were not able to isolate Nipah virus or detect its presence by polymerase chain reaction from the tissues of these bats.

Chua et al. (2002) successfully isolated Nipah virus from the urine of *P. hypomelanus*, using a simple method of urine collection. Plastic sheets were placed underneath the colony of the bats to catch the urine drops and sterile cotton swabs were used to soak up the urine, 4–5 drops per swab. The virus isolated from the urine specimens was identical to the human strain. This finding, however, does not exclude the possibility of other bat or wildlife species as reservoirs of Nipah virus.

It has been speculated that these migratory fruit bats were forced away from their natural habitat in 1998 because of forest fires prevalent at that time in the region and attracted by the fruit trees in pig farms. Once pigs became infected from infective urine and other secretions, the virus spread rapidly among the densely populated animals in the farm and then to their handlers.

13. Economic aspects

The Nipah outbreak involved a major industry among the Chinese in the country, leading to considerable loss of revenue to the pig farmers and those working in related industries such as animal feed suppliers, lorry transport companies, abattoir workers, pork retailing, food vendors, etc. Greater loss came about when traditional importers of live pigs from neighboring countries stopped importing the animals.

In Malaysia, pig-breeding activities have always been family-based and many small farms are unlicensed. During the tragic outbreak, many deaths occurred among members of the same family. The outbreak has been estimated to affect some 8500 workers directly involved in the pig-breeding activities nationwide, with another 9400 workers engaged in supporting industries and 300,000 workers in other related industries. Although the outbreak is over and the pig farms declared free of Nipah virus, many still remain unemployed and find it difficult and expensive to re-start the pig industry. The government is encouraging the farmers to embark on centralized pig farming remote from townships and fruit orchards, with good farming practice, waste management, sludge disposal, and adequate buffer zones. However, such land is difficult and expensive to come by, and the whole process of resettling these farmers is exceedingly slow.

14. Conclusion

There are many potential human biological pathogens which can be considered as suitable for bioterrorism and the North Atlantic Treaty Organization handbook lists 39 agents, including bacteria, viruses, rickettsiae and toxins (Departments of the Army, Navy and Air Force, 1996). Such biological agents are considered to be more destructive than even chemical weapons and in certain circumstances, biological weapons can be as devastating as nuclear ones.

The global interest in emerging infectious diseases has led to the discovery of new viruses that have the potential to be agents of bioterrorism. Nipah virus, a recently emergent deadly zoonotic paramyxovirus, has a number of important attributes that make it a potential agent of bioterrorism. It is an extremely pathogenic organism with a case mortality in humans close to 40% and has been classified as a Biosafety Level 4 organism. Besides causing acute infection, it can also give rise to clinical relapse months and years after infection. Other than ribavirin, which is expensive and has undesirable side effects, there are no specific antiviral drugs to combat the virus and no vaccine will be available in the foreseeable future. Diagnostic capability is limited to very few laboratories around the world although this will improve once a non-infectious, specific test becomes available.

Nipah virus can be easily produced in large quantities in cell culture, an important criteria for consideration as a biologic weapon. It should be possible to stabilize it as an aerosol with the capacity for widespread dispersal. Besides infecting humans, the virus can also infect live stock, domestic animals and wildlife, and is likely to cause additional panic to the population. Since the complete virus genome has been characterized, genetic manipulation of the virus can be easily achieved if desired.

The outbreak of Nipah encephalitis in Malaysia has created economic chaos, and the recovery has been slow. In a multiracial country such as Malaysia, the handling of the outbreak is a delicate and sensitive problem and could easily be turned into a serious political, religious and racial issue.

Since the discovery of Nipah virus, only a handful of laboratories have access to the virus. However, because of the natural reservoir, it will not be difficult to isolate the virus from wildlife, making it readily available to any country. It is, therefore, not too far-fetched to think that Nipah virus can be considered a potential agent for bioterrorism.

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