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REVIEW

Emerging and re-emerging viruses in Malaysia, 1997–2007

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Summary Over the past decade, a number of unique zoonotic and non-zoonotic viruses have emerged in Malaysia. Several of these viruses have resulted in significant morbidity and mortality to those affected and they have imposed a tremendous public health and economic burden on the state. Amongst the most devastating was the outbreak of Nipah virus encephalitis in 1998, which resulted in 109 deaths. The culling of more than a million pigs, identified as the amplifying host, ultimately brought the outbreak under control. A year prior to this, and subsequently again in 2000 and 2003, large outbreaks of hand-foot-and-mouth disease due to enterovirus 71, with rare cases of fatal neurological complications, were reported in young children. Three other new viruses – Tioman virus (1999), Pulau virus (1999), and Melaka virus (2006) – whose origins have all been linked to bats, have been added to the growing list of novel viruses being discovered in Malaysia. The highly pathogenic H5N1 avian influenza has also been detected in Malaysia with outbreaks in poultry in 2004, 2006, and 2007. Fortunately, no human infections were reported. Finally, the HIV/AIDS epidemic has seen the emergence of an HIV-1 recombinant form (CRF33_01B) in HIV-infected individuals from various risk groups, with evidence of ongoing and rapid expansion.

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Introduction

Malaysia (2°30'N, 112°30'E) is located in the heart of South-east Asia with a population of approximately 27 million as of 2007. It is made up of Peninsular Malaysia and the Malaysian Borneo, which are separated by the South China Sea, divided

into a federation of 13 states and three federal territories including the Kuala Lumpur Federal Territory. The majority of the population is concentrated in Peninsular Malaysia with approximately 20 million people living in this more developed area of the country. The local climate is tropical and characterized by annual monsoons from October to January.

Progressive development over the last 50 years since independence in 1957 has meant that infectious diseases have gradually ceased to be the leading cause of death, with chronic diseases such as cardiovascular disease, cancer, chronic respiratory diseases, and diabetes mellitus becoming

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more commonplace amongst the increasingly more affluent Malaysians. However over the last decade attention has once again been drawn to infectious diseases, with the emergence of new infections and the re-emergence of diseases previously well-controlled in the Asian region in general, and in Malaysia in particular.^{1,2} In Southeast Asia alone, the discovery of new human viruses that cause large and devastating epidemics, such as the severe acute respiratory syndrome (SARS) coronavirus and the highly pathogenic avian influenza (HPAI) H5N1 virus, have been reported. Unlike some countries in the region, Malaysia was spared from the outbreak of SARS coronavirus and has yet to identify the HPAI H5N1 infection in humans that adversely affected neighboring countries (although frequent outbreaks in poultry have been reported). Nevertheless, in the last decade, Malaysia has been battling different challenges caused by various known or novel zoonotic and non-zoonotic viruses identified for the first time in the country.

In this article we provide historical, epidemiological, clinical, and scientific insights into the emerging, re-emerging, and recombinant viruses identified in Malaysia between 1997 and 2007 (Figure 1). Although the discovery of novel viruses on some occasions may be explained partly as being an artifact of increased surveillance and reporting efforts in the country – the best example being the investigation of Nipah virus, which led to the incidental discovery of other novel zoonotic viruses – recent studies, taking into account various socio-economic, environmental, and ecological factors associated with the emergence of infectious diseases,^{3–6} show that lower-latitude developing countries including Malaysia are particularly at risk of emerging infectious disease events due primarily to zoonotic pathogens of wildlife origin and vector-borne patho-

gens.² Here, we discuss probable events that have been implicated in the appearance of these viruses, with the aim of strengthening the existing preventive measures and strategies for managing potentially new and unfamiliar diseases in the future.

Enterovirus 71

Human enterovirus 71 (EV-71) and 11 other group A coxsackieviruses (CV-A) are members of the *human enterovirus A* (HEV-A) species from the *Enterovirus* genus of the *Picornaviridae* family. Enteroviruses are distributed worldwide and can be transmitted effectively through the fecal–oral route and to a lesser extent, by respiratory transmission. The great majority of enterovirus infections are asymptomatic, but some can lead to serious illnesses particularly in infants and the immunocompromised. Hand-foot-and-mouth disease (HFMD) in children is usually caused by enteroviruses such as CV-A10, CV-A16, and EV-71.

An epidemic of HFMD was first reported in Sibü, Sarawak (Malaysian Borneo) in April 1997,^{7–10} followed by smaller outbreaks in Peninsular Malaysia in the same year (Figure 1).^{7,8,11} More than 2600 children were infected. Most presented with a febrile illness and characteristic lesions on the palms, soles, and oral mucosa, but a small proportion, mainly children <6 years of age, presented with severe neurological complications such as aseptic meningitis, poliomyelitis-like acute flaccid paralysis, or fatal encephalomyelitis. Cardiopulmonary symptoms such as neurogenic pulmonary edema with secondary myocardial dysfunction leading to rapid cardio-respiratory decompensation were also reported. Twenty-nine children eventually succumbed to the disease in Sarawak¹⁰ and 12 in Peninsular Malaysia.¹¹

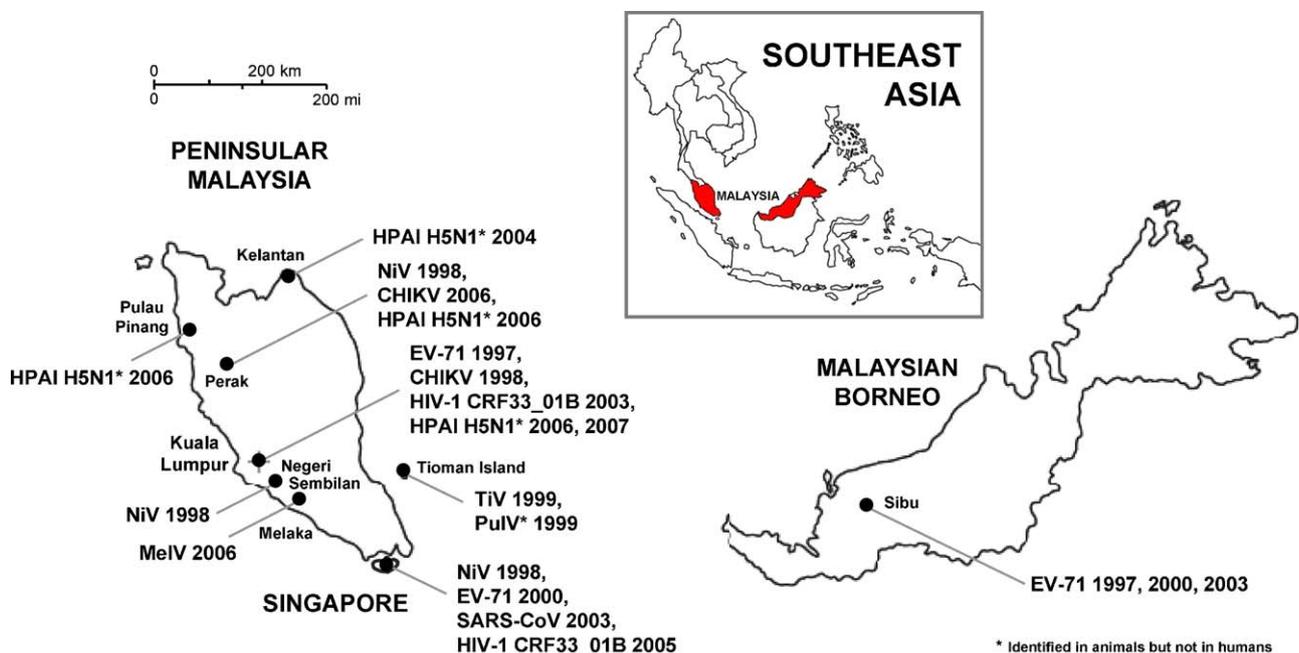


Figure 1 Zoonotic, non-zoonotic, and vector-borne viruses emerged in Malaysia between 1997 and 2007. The abbreviations for the viruses and their year(s) of emergence at various locations in Malaysia are shown. Viral outbreaks in Singapore during the same period are also indicated. HPAI H5N1, highly pathogenic avian influenza subtype H5N1; NiV, Nipah virus; CHIKV, chikungunya virus; EV-71, enterovirus 71; HIV-1 CRF33_01B, HIV type 1 circulating recombinant form (CRF33_01B); MeIV, Melaka virus; TiV, Tioman virus; PuIV, Pulau virus; SARS-CoV, severe acute respiratory syndrome coronavirus.

EV-71 was isolated from neuronal or non-neuronal samples from most of the infected children, including those who succumbed to the infection,^{7–9,11} suggesting its role as the etiologic agent. Different lineages of EV-71 subgenotypes C1 and C2 and two previously undefined subgenotypes B3 and B4 were identified in these outbreaks, with novel subgenotype B3 being the most prevalent strain in Sarawak in 1997, particularly among patients with the milder form of HFMD.^{7,12–14} Further outbreaks occurred in 2000 and 2003 in Sarawak where newly identified subgenotypes B4 and B5 were predominant.^{12,15,16} Interestingly, phylogenetic and recombination analyses revealed that some EV-71 strains isolated in the 1997 outbreak were intertypic recombinant involving EV-71 subgenotype B3, CV-A16, and various HEV-A species.^{17,18} These recombinant strains were isolated from uncomplicated HFMD cases and were thought to have reduced viral fitness and adaptation to host immunity compared to the parental strains. The pathogenic potential and clinical attributes of these recombinants, however, are not well understood.

Subsequent to the first outbreaks in Malaysia, several large EV-71-associated HFMD outbreaks were also reported across the Asia Pacific region: in Taiwan in 1998;¹⁹ in Western Australia in 1999;²⁰ in Singapore,^{21,22} Japan,²³ Korea,¹² and Taiwan in 2000;²⁴ in Vietnam in 2005;²⁵ and in China in 2008.²⁶ Despite the occurrence of numerous HFMD and EV-71 outbreaks worldwide since 1969, no outbreaks involving fatal cases of brainstem encephalitis and neurogenic pulmonary edema have been reported,²⁷ except for those in Malaysia, Taiwan (which claimed 78 lives¹⁹), and recently in Vietnam and China. This shows that over the past decades EV-71 may have evolved to become more pathogenic than its ancestral strains. In the meantime, the synergistic potential exerted by other infectious agents in the pathogenesis of the disease cannot be ruled out. This includes the description of acute flaccid paralysis caused by adenoviruses^{9,28} and echovirus 7-associated encephalomyelitis²⁹ during these outbreaks. Furthermore, a prospective study conducted during the 2000 and 2003 HFMD outbreaks in Sarawak showed that co-infection with another enterovirus or adenovirus among children with EV-71 was common.¹⁶

Diverse EV-71 subgenotypes determined from different genomic regions indicate that multiple EV-71 lineages have been circulating in the Asia Pacific region since 1997, with each subgenotype causing distinct outbreaks of varying clinical manifestations and neurovirulence. Driven by such complexity, it has not been possible to identify a single neurovirulent genotype nor its pathogenic mechanisms associated with the severe neurological outcomes – although recent studies have suggested that subgenotype B5¹⁶ and particular subgenotypes from the genogroup C^{14,30} are linked with neurological complications. Continued molecular epidemiological surveillance is essential to delineate the temporal trends of EV-71 transmission, the neuropathogenic potential of its subgenotypes, and to plan for effective clinical interventions plus preventive measures.^{12,15}

Nipah virus

In September 1998, an outbreak of respiratory illness and encephalitis with low morbidity and mortality rates occurred among pigs in commercial farms (initially presumed as porcine

respiratory and encephalitis syndrome) in the Kinta district of Ipoh, Perak state in Peninsular Malaysia.³¹ Later in the month, a group of workers linked with pig farming presented with acute febrile encephalitis that was associated with a high case fatality rate.^{32–34} The disease affecting humans was initially diagnosed as Japanese encephalitis (JE), which is endemic in Malaysia,³⁵ prompting health authorities to attempt to contain the outbreak based on preventive measures against JE in the Kinta district and also throughout the country. The infected pigs in the Kinta district were presumed to be disease-free and eventually transported to pig farms and abattoirs in other states in Malaysia, and also to neighboring Singapore.

Two months later in December 1998, a similar outbreak was detected in Sikamat town, and then by February 1999 in Sungai Nipah village and the town of Bukit Pelandok, all within the city of Seremban in the state of Negeri Sembilan located in central Peninsular Malaysia.³⁴ Approximately 70% of the infected patients were of Chinese ethnicity and were directly involved in pig farming activities (as pig farmers and workers). Those affected had had direct contact with pigs in the 2 weeks before the onset of illness, suggesting direct viral transmission from pigs to humans with a short incubation period. These patients presented with an acute illness with fever, headache, dizziness, vomiting, and reduced levels of consciousness, before rapidly progressing to severe encephalitis that was associated with a high mortality rate.^{36,37} Up to 25% of cases had concomitant respiratory syndromes.^{34,38} A similar outbreak was then reported in Singapore among abattoir workers who had handled infected pigs imported from Malaysia.³⁹

In early March 1999, a novel paramyxovirus responsible for the outbreak was isolated from the cerebrospinal fluid of an encephalitic patient from Sungai Nipah village, and was later named the Nipah virus (NiV).^{34,40} The discovery of NiV played a critical role and was an important turning point in controlling the outbreak. Strong evidence showed that transmission of NiV to humans was through close contact with infected pigs,^{41,42} and affected those directly involved in pig farming activities such as assisting in pig breeding and birthing of piglets, those administering injections or medications to pigs, and those handling dead pigs. This led to an immediate halt in direct handling and transportation of pigs within the country and subsequently the culling of over a million pigs.⁴⁰ Human-to-human transmission of NiV was also described among healthcare workers,⁴³ although this was uncommon.⁴⁴ The overall case fatality rate for the outbreak reported by the Ministry of Health was 38.5%; 109 fatalities from 283 cases of viral encephalitis.⁴⁵

Genetic characterization revealed that NiV is closely related to the Hendra virus (HeV), a paramyxovirus species causing severe respiratory disease in horses and humans in Queensland, Australia, which first emerged in 1994.⁴⁶ The full-length genome sequences of NiV and HeV are approximately 18 kb,⁴⁷ significantly longer than the average size of 15.5 kb of other viral genomes from the *Paramyxovirinae* subfamily. NiV and HeV also share similar genomic organization, both having increased nucleotide length of the 3' non-coding region in most of the genes. Taking together the close phylogenetic relationship of NiV and HeV^{40,48} plus its distinct divergence and little immunological cross-reactivity with other established genera within the *Paramyxoviridae*, the two geographically remote viruses were classified as belonging to a new genus – *Henipavirus*^{49,50} (Figure 2).

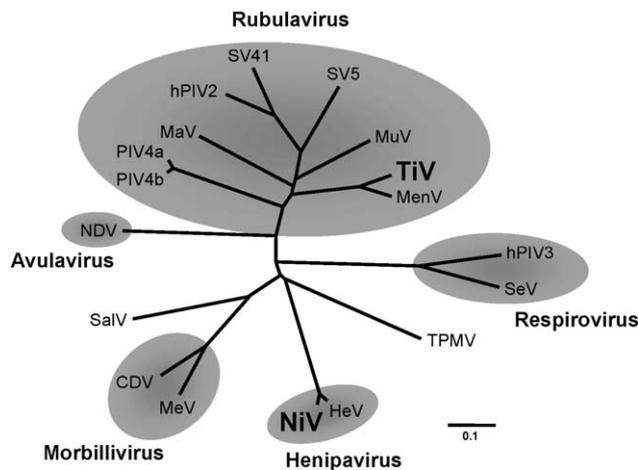


Figure 2 Phylogenetic reconstruction of the deduced amino acid sequence of the complete N gene of select *Paramyxovirinae* subfamily members. Nipah virus and Tioman virus, two newly emergent paramyxoviruses in Malaysia, are clustered within the *Henipavirus* and *Rubulavirus* genus, respectively. *Rubulavirus* genus: MuV, mumps virus; MenV, Menangle virus; TiV, Tioman virus; MaV, Mapuera virus; SV5, simian parainfluenza virus 5; SV41, simian parainfluenza virus 41; hPIV2, human parainfluenza virus 2; hPIV4a, human parainfluenza virus 4a; hPIV4b, human parainfluenza virus 4b. *Respirivirus* genus: SeV, Sendai virus; hPIV3, human parainfluenza virus 3. *Henipavirus* genus: NiV, Nipah virus; HeV, Hendra virus. *Morbillivirus* genus: MeV, measles virus; CDV, canine distemper virus. *Avulavirus* genus: NDV, Newcastle disease virus. Unclassified viruses: SalV, Salem virus; TPMV, Tupaia paramyxovirus.

This unique relationship between NiV and HeV played an important role in identifying the natural reservoir for NiV in Malaysia. Based on findings that fruit bat species of the genus *Pteropus* are natural hosts of HeV,^{51,52} extensive serological surveillance within and outside the outbreak epicenters was conducted involving various species of wild fruit bats plus other wildlife and domestic animals. Neutralizing antibodies against NiV were detected among the variable flying foxes (previously known as the Island flying foxes; *Pteropus hypomelanus*), Malayan flying foxes (*Pteropus vampyrus*), and other species of the order Chiroptera, suggesting widespread infection of NiV in the bat population in Peninsular Malaysia.^{53,54} A novel method of collecting free-living fruit bat urine samples using plastic sheets was then adopted, and eventually NiV was isolated from urine samples and swabs from fruits partially eaten by the variable flying foxes,⁵⁵ corroborating the serological evidence that fruit bats are the natural hosts of NiV.⁵⁶ Following this feat, serological and/or genetic evidence of NiV circulating among the expansive species of flying foxes was also reported in Thailand,⁵⁷ Indonesia,⁵⁸ Cambodia,^{59,60} Bangladesh,^{61,62} and India⁶³ indicating wide dissemination of NiV across Southeast and South Asia, especially in countries where populations of the *Pteropus* species are overlapping. Recent studies have also shown NiV circulating in Madagascar located in the southeastern part of Africa⁶⁴ and Ghana in West Africa.⁶⁵

The discovery of NiV as the causative agent of severe febrile encephalitis in humans and the identification of

pteropid fruit bats as the natural hosts has helped regional as well as international scientific communities to be better prepared with management strategies for similar emerging zoonotic diseases, as witnessed in the recent NiV-associated encephalitis outbreaks in Bangladesh.^{61,62,66,67} During the Bangladesh outbreaks between 2001 and 2005, the transmission pattern of NiV, however, seemed different from that in Malaysia because there was no involvement of pigs, and many of the patients who presented largely with neurological symptoms were young males who may have had direct contact with fruits eaten by bats or had consumed contaminated date palm sap.⁶⁸ In addition, NiV spread occurred over a wide area in the country complicated by cases of human-to-human transmission.⁶⁹ In some areas, the case fatality rate was as high as 75%.⁶⁶ In India, a similar viral encephalitis outbreak was also reported in 2001 in the city of Siliguri, West Bengal not far from Bangladesh.⁷⁰ Anthropogenic factors such as unrestrained deforestation and human population expansion in Bangladesh and India could possibly have been linked to these outbreaks. The high virulence of NiV and HeV and the wide range of susceptible hosts plus the absence of therapeutic and prophylactic interventions have led to the classification of these viruses as Biosafety Level 4 pathogens.⁷¹

Tioman virus

The pursuit of the natural host of NiV led to the unexpected discovery of another novel zoonotic virus in 1999. On an island about 25 km off the eastern coast of Peninsular Malaysia, a new virus of the *Paramyxoviridae* family called the Tioman virus (TiV) was identified.^{72,73} TiV was isolated from pooled urine samples of free-living variable flying foxes (*P. hypomelanus*) roosting on tree branches along the coastal region of the island. TiV displayed characteristic microscopic features similar to those of other viruses in the *Paramyxoviridae* family: spherical, pleiomorphic, and enveloped viral particles with glycoprotein spikes.⁷² Serological tests revealed cross-reactivity with the Menangle virus (MenV), another newly described *Paramyxoviridae* member that caused infections among pigs and humans in New South Wales, Australia in 1997.⁷⁴ Similar to NiV, evidence of TiV circulating among bats in wide geographical proximity has been reported.⁶⁴

Phylogenetic analyses show that TiV and MenV are clustered in the *Rubulavirus* genus with up to 85% amino acid similarity in the nucleocapsid (N) gene between the two viruses (Figure 2). The N protein shows significant sequence homologies with other *Rubulavirus* members, including the mumps virus (approximately 50%). Human disease caused by TiV has not been documented thus far, although possible human infections, as determined by the presence of antibodies against TiV among the island inhabitants, have been observed.⁷⁵ Animal experiments have demonstrated that TiV is neurotropic causing necrosis in the cerebrum and hypothalamus in intracerebrally-inoculated mice⁷⁶ and is capable of causing mild disease in pigs, mainly by affecting the lymphoid tissues.⁷⁷

Chikungunya virus

The first sporadic outbreak of chikungunya virus (CHIKV) infection occurred between late 1998 and early 1999 in Port Klang, Kuala Lumpur.⁷⁸ Fifty-one patients living in low-cost

and squatter estates presented with fever, polyarthritides of the small joints of the hands and feet, transient maculopapular rashes, myalgia, and arthralgia. The clinical symptoms resembled those of the more ubiquitous classical dengue fever; this posed an initial challenge in diagnosing the disease since dengue virus is endemic in the country.⁷⁹

Chikungunya virus (chikungunya, from the root verb kunyanya that means 'to dry up or become contorted' in Makonde language in Tanzania⁸⁰) is related to group A arboviruses. CHIKV is an *Alphavirus* from the *Togaviridae* family that shares the same vectors responsible for spreading dengue virus, namely *Aedes aegypti* and, to a lesser extent, *Aedes albopictus*, which are the common peridomestic mosquito species in the Southeast Asia region. Studies conducted in the 1970s detected antibody against CHIKV among the rural populations in Malaysia, indicating the presence of CHIKV infection.^{81,82} However, until recently, outbreaks causing human diseases have not been reported.

Seven years after its first appearance, new CHIKV infections re-emerged in Bagan Panchor (about 50 km from Ipoh), Perak between March and April 2006.^{83,84} More than 200 villagers in a fishing community were infected with CHIKV strains derived from a common ancestral lineage from the 1998/99 outbreak, but this recent outbreak had a notably higher number and rate of infections. This outbreak coincided with the largest ever CHIKV epidemics affecting the Indian Ocean territories between 2005 and 2006, where in Reunion alone more than 266 000 people, about a third of the total population, were infected. In India, an estimated 1.4 million human cases were reported during 2006. In these outbreaks, a substantial proportion of patients also showed unusual clinical manifestations, including severe neurological symptoms and fulminant hepatitis.^{85,86} Although unclear, plausible explanations for the re-emergence of CHIKV in this region include tourism and migration labor (Malaysia is greatly dependent on migrant workers from neighboring countries where CHIKV is endemic⁷⁸), viral mutation, and CHIKV introduction into a naïve population.⁸⁷ Phylogenetic evidence has shown that the contemporary Malaysian strains isolated in 2006 were, however, distinct from the epidemic strains reported in the Indian subcontinent (2005–2006), suggesting that CHIKV is indeed endemic in Malaysia.⁸³ Since the concurrent re-emergence of CHIKV in the Indian Ocean region and Malaysia seem to be unrelated, it is possible that other factors could have played an important role in driving these outbreaks. Climate anomalies, for instance, may have favored the mosquito vectors and consequently facilitated CHIKV emergence in these areas.^{88,89}

Pulau virus and Melaka virus

During the search for NiV in fruit bats on Tioman Island in 1999, another novel virus, initially thought to be a bat paramyxovirus, was identified. While serological tests excluded the presence of a paramyxovirus, further electron microscopic, serologic, and phylogenetic investigations established the fusogenic agent as a reovirus.⁹⁰ Designated as Pulau virus (PuLV) (pulau denotes 'island' in the Malay language), PuLV is a dsRNA virus displaying the typical ultrastructural morphology of a reovirus. PuLV formed large syncytium in Vero cells and showed serologic reactivity against Nelson Bay virus (NBV), another known bat *Orthoreovirus*

isolated from the Australian flying foxes (*Pteropus alecto*).⁹¹ To date, PuLV has not been associated with any human diseases, and very little is known about the host range, pathogenesis, and epidemiology of this newly recognized virus.

More recently in 2006, another novel *Orthoreovirus* named Melaka virus (MeLV) associated with acute respiratory disease in the human was identified.⁹² MeLV was isolated from an adult male who developed a high fever and acute respiratory symptoms. Icosahedral viruses similar to those of the *Orthoreovirus* genus were noted on microscopic examination of mammalian cell lines infected with MeLV, and serological studies of the patient serum showed neutralization activity against PuLV. Nucleotide analysis of the small (S) segments revealed a close genetic relationship of MeLV to PuLV and NBV, and clustering within the *Orthoreovirus* genus subgroup III of the *Reoviridae* family. In the meantime, two adolescent children of the index patient also developed fever (without respiratory symptoms) approximately 6 days after the onset of his illness. Interestingly, epidemiological investigations of the index case revealed that about one week before he developed the illness, he had a bizarre exposure to a bat that flew into his living room for a short period. Subsequently, antibodies against MeLV were detected from the family members including his wife who was asymptomatic. Although direct evidence of bat-to-human transmission of MeLV has not been documented, the role of the bat as a possible reservoir of MeLV has not been ruled out. MeLV is believed to be the first *Orthoreovirus* associated with acute respiratory disease in the human.⁹²

HIV type 1 (clade CRF33_01B)

Since the first cases of AIDS reported in 1986,⁹³ the rise in HIV/AIDS in Malaysia has continued unabated. As of December 2007, a total of 80 938 HIV type 1 (HIV-1) infections have been identified, while 10 334 people have died of AIDS-related illnesses nationwide. Of these, 72% were injecting drug users (IDUs), followed by 16% transmission via heterosexual route (Figure 3a). It has been suggested that the early epidemic was a spillover from Thailand, located at Malaysia's northern border. Malaysia is currently defined as a country with a 'concentrated' HIV-1 epidemic, based on relatively low rates of infection in the general population as measured by a prevalence of less than 0.1% among women attending government antenatal clinics, and seemingly isolated high prevalence rates among high-risk groups such as IDUs and female sex workers. In 2003 it was reported that Malaysia had the fifth fastest growing HIV infection rate in the Asia Pacific region, with the infection rate doubling every three years (<http://www.unicef.org/aids/>).

HIV-1 exhibits tremendous genetic diversity that is driven by high rates of mutation and recombination, coupled with high viral turnover and the persistent nature of infection.^{94–97} By these mechanisms, HIV-1 group M, which is largely responsible for the global pandemic, diversified into 11 subtypes and subsubtypes (A1, A2, B, C, D, F1, F2, G, H, J, and K) and various types of recombinants.^{98,99} HIV-1 recombinants with epidemic spread are known as circulating recombinant forms (CRFs). Five strains of CRFs have been reported so far in Asia: CRF01_AE, CRF15_01B, and CRF34_01B in Thailand and CRF07_BC and CRF08_BC in China. In addition to CRFs,

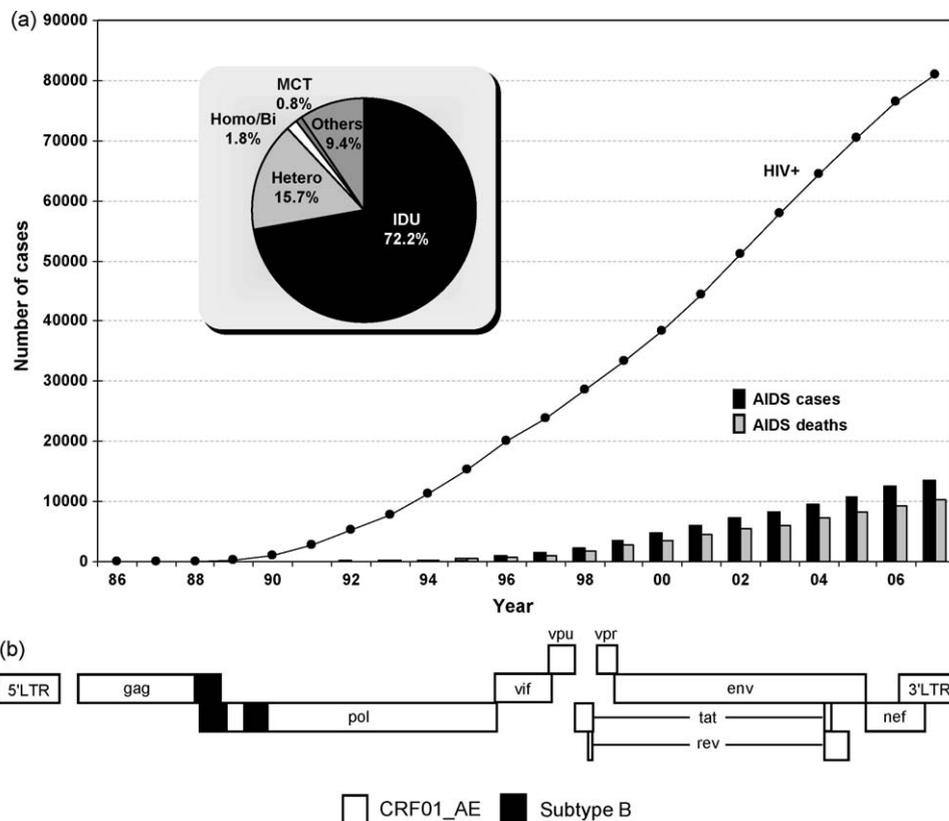


Figure 3 (a) Cumulative numbers of HIV-1 infections, AIDS cases, and AIDS-related deaths reported by the Ministry of Health in Malaysia (1986–2007). Inset: Distribution of HIV-1 infections in different risk categories. Abbreviations: IDU, injecting drug user; Hetero, heterosexual; Homo/Bi, male homo-/bisexual; MCT, mother-to-child transmission. (b) Structural representation of the novel HIV-1 CRF33_01B identified in Malaysia. Full-length genetic sequence revealed short subtype B fragments are recombined into the *gag* and *pol* gene in the backbone of CRF01_AE. The recombinant structures of all HIV-1 CRFs are available at the HIV Sequence Database, Los Alamos National Library.⁹⁸

various types of unique recombinant forms (URFs) that are detected in a single individual or a single epidemiologically-linked cluster have been identified in the region, where multiple lineages of HIV-1 strains co-circulate in the same population.

The evolution of the HIV-1 epidemic in Malaysia produced a unique lineage when a distinctive recombinant strain emerged in 2003.¹⁰⁰ Phylogenetic analyses of the HIV-1 protease and reverse transcriptase genes found 19% CRF01_AE/B intersubtype recombinants (a recombinant involving CRF01_AE and subtype B of Thai origin) amongst antiretroviral-naïve patients in Kuala Lumpur, all having a recombination profile different from other previously described CRFs. Designated as HIV-1 CRF33_01B from near full-length genome analyses (Figure 3b), this novel CRF was disseminating at a significant proportion among various high-risk populations, especially among the IDUs. Wide distribution of CRF33_01B involving all major ethnic and risk groups has provided evidence that extensive bridging of HIV-1 transmission between different risk groups has occurred in Malaysia.^{101,102}

We recently observed a unique parallelism of the transition in molecular epidemiological features of HIV-1 epidemics between Thailand and Malaysia.¹⁰¹ In the early phase of the Thai epidemic, two HIV-1 strains, CRF01_AE and subtype B, were circulating relatively independently among different risk populations. CRF01_AE was distributed among persons at risk

of sexual exposure, while subtype B was distributed mainly among IDUs.^{103,104} Co-circulation of CRF01_AE and subtype B in Thailand presented opportunities for inter-clade recombination to take place (a common feature for HIV-1), and has led to the generation of various forms of CRF01_AE/B recombinants.^{105–107} A similar molecular epidemiological trend has been observed in Malaysia. Studies conducted in 1992–1997 showed that CRF01_AE and subtype B were prevalent among 81% of heterosexuals and 55–92% of IDUs, respectively.^{108–110} However, recent studies have documented the gradual dilution of non-recombinant pure subtypes and the establishment of CRF33_01B as the emerging epidemic strain in Malaysia.¹⁰¹ Besides expanding rapidly within the country,¹¹¹ further molecular epidemiological surveillance has shown that CRF33_01B spread across the border to Singapore in 2005 (Paton NI and Sun YJ, personal communication). Such genetic complexity and dynamicity of HIV-1 provides opportunities for new recombinants to develop and spread within Malaysia and also in the region, thus presenting new challenges to disease diagnosis and treatment, particularly in the development of antivirals and vaccine candidates.

Highly pathogenic avian influenza (H5N1)

The highly pathogenic avian influenza (HPAI) H5N1 virus that originated in southern China in the mid-1990s,¹¹² was respon-

sible for large disease outbreaks in poultry in China and other countries in the region in 2003–2004. Affected countries faced severe economic loss due to mortality in poultry and the loss of domestic and international trade of poultry and its products. Widespread circulation among avian populations and the significant number of human infections and deaths caused by HPAI H5N1 (as of June 19, 2008, 385 human cases including 243 deaths have been reported; www.who.int/csr/disease/avian_influenza/country/en) have raised concern over the plausible genetic mutation event (genetic reassortment) that could lead to the generation of an influenza strain with enhanced transmissibility among humans, possibly triggering a pandemic.¹¹³

In Malaysia, the first ever outbreak of HPAI caused by subtype H5N1 was reported in August 2004 in a village in the state of Kelantan located approximately 22 km from the Thai border in northeastern Peninsular Malaysia.^{114,115} The virus, discovered in fighting cocks smuggled from a neighboring country, was transmitted mainly among the local village chickens. Molecular analysis showed that the H5N1 strain was highly homologous to the H5N1 strains previously isolated from Thailand and Vietnam. A few weeks following the first outbreak, eight other outbreaks were reported, largely affecting poultry in villages located around the index case. In these outbreaks, no human cases or deaths were reported and the disease was brought under control by depopulation and quarantine/clinical surveillance of poultry and birds within a 1-km and 10-km radius of the index case, respectively, coupled with restrictions on the movement of birds and their products to other states.¹¹⁵

In February 2006, fresh outbreaks of HPAI H5N1 emerged over a wider geographical area involving villages in Kuala Lumpur and the states of Perak and Pulau Pinang along the more industrialized western coast of Peninsular Malaysia.¹¹⁵ Phylogenetic analysis of the *H5* and *PB2* genes revealed that the H5N1 strain isolated from infected village chickens, ducks, and quails was different from that of the 2004 outbreak and was highly similar to the H5N1 isolates from Indonesia and China, suggesting different H5N1 lineages were introduced into the country, possibly by the poultry trade rather than through migratory birds.¹¹⁶ Although these outbreaks were brought under control through effective disease control and preventive efforts, including the culling of about 60 000 birds, a similar outbreak re-occurred in another village in Selangor state not far from Kuala Lumpur in June 2007.¹¹⁵ Through effective control measures, the 2007 outbreak was resolved several months later.

In Malaysia, cock fighting is a popular albeit illegal activity among village folks. Fighting cocks smuggled from neighboring countries (i.e., Thailand and Indonesia) have been implicated in the introduction and spread of HPAI H5N1 in Peninsular Malaysia. Such public health threats – that increase the risk of infection in humans – could be avoided by implementing a stricter ban by the authorities on cock fighting and smuggling activities in the country. In addition, continued surveillance of avian populations including domestic ducks (thought to be the potential reservoir and source of H5N1 persistence¹¹⁷) is important to counter possible re-occurrence or re-introduction of HPAI H5N1, a phenomenon that is not uncommon in the Southeast Asia region.¹¹⁸

Discussion

Over the last eleven years, between 1997 and 2007, Malaysia has witnessed the unprecedented appearance of eight novel viruses that have never been documented in the country before (Table 1). The majority of these viruses, such as EV-71, NiV, CHIKV, and HIV-1 recombinant, have caused serious human infections and/or deaths of varying magnitudes and are of epidemiological importance, whereas others have yet to be known to cause widespread human diseases (e.g., MeIV and HPAI H5N1) or cross into a wider range of susceptible hosts (e.g., TiV and PulV). Factors leading to this emergence are not entirely understood but various features associated with such a trend – including socio-economic, environmental, and ecological factors^{3–6} – have been hypothesized.

Unlike enterovirus infections that usually occur in densely populated areas, where hygiene levels are poor and food or water supplies may be contaminated, conditions that favor the fecal–oral route of transmission, more complex attributes, be it of the environment, viral hosts/vectors, or humans, have been associated with the emergence of the other novel viruses in Malaysia. For instance, early studies implicated the effects of the El Niño/Southern Oscillation (ENSO) phenomenon in 1997/98 in the emergence of NiV.¹¹⁹ Directly preceding these outbreaks, Malaysia experienced a severe drought resulting from the El Niño conditions (the largest and warmest to develop in the Pacific Ocean in the past century). The situation was aggravated by the excessive haze produced by the aggressive slash-and-burn deforestation activities in Indonesia. This series of environmental and human events may have affected the natural habitat of the pteropid bats, forcing their migration and subsequent encroachment into fruit orchards surrounding the pig-farming area, resulting in the unanticipated introduction of NiV from its natural host to pigs as the amplifying host. Recent investigations, however, refuted the effect of ENSO and showed evidence that NiV is frequently present in fruit bats in Malaysia, and that its spillover – from bats to pigs and subsequent transmission to human – was largely a chance event that might have occurred as a result of increased habitat encroachment and agricultural expansion by humans, confounded by the temporal and spatial dynamicity of both the virus and hosts, and the hosts (pigs) immunity against NiV infection.^{119–121} In fact, similar anthropogenic drivers have been linked to outbreaks in Malaysia, Bangladesh, and India, raising concerns that these countries, which are currently experiencing promising economic growth, are still at risk of NiV re-occurrence should unrestrained deforestation and agricultural intensification activities not be closely regulated.

Overlapping the NiV epidemic was the first sporadic outbreak of *Aedes* mosquito-borne CHIKV infections in Kuala Lumpur in 1998. The arbovirus then re-emerged seven years later in northern Malaysia, coinciding with the drought-associated CHIKV outbreaks affecting countries cresting the Indian Ocean,⁸⁸ by far the largest CHIKV epidemic on record. The cause of the disease outbreaks remains multifactorial. Previous lessons have suggested that increased tourism, viral adaptation, and host immunity,⁸⁷ plus importation of migrant workers from countries where CHIKV is endemic,⁷⁸ may play a substantial role in the spread of the virus to humans, although the effect of a warmer climate has also been reported.⁸⁸ As a whole, such a climate abnormality of prolonged drier-than-average conditions may be critical in introducing new viruses

Table 1 Emerging and re-emerging viruses in Malaysia, 1997–2007.

Virus	Family	Genus	Properties	Year of emergence	Locations in Malaysia	Remarks
Enterovirus 71	<i>Picornaviridae</i>	<i>Enterovirus</i>	+ssRNA, non-enveloped	1997, 2000, 2003	Sibu, Sarawak; Peninsular Malaysia	Fatal cases of brainstem encephalitis reported Novel subgenotypes B3, B4, and B5 emerged Recombinant involving coxsackievirus and other human enteroviruses identified
Chikungunya virus	<i>Togaviridae</i>	<i>Alphavirus</i>	+ssRNA, enveloped	1998 2006	Port Klang, Kuala Lumpur Bagan Panchar, Perak	Sporadic outbreak causing febrile illness with polyarthritits Endemic virus re-emerged 7 years later <i>Aedes aegypti</i> as vector
Nipah virus	<i>Paramyxoviridae</i>	<i>Henipavirus</i>	–ssRNA, enveloped	1998	Ipoh, Perak; Seremban, Negeri Sembilan	Novel bat paramyxovirus, closely related to Hendra virus Febrile illness and encephalitis with high mortality rate Acquired via close contact with infected pigs
Tioman virus	<i>Paramyxoviridae</i>	<i>Rubulavirus</i>	–ssRNA, enveloped	1999	Tioman Island	Novel bat paramyxovirus, closely related to Menangle virus Human infections and diseases in experimentally infected animals have been reported
Pulau virus	<i>Reoviridae</i>	<i>Orthoreovirus</i>	dsRNA, non-enveloped	1999	Tioman Island	Novel bat <i>Orthoreovirus</i> No human/animal disease reported
HIV type 1 (clade CRF33_01B)	<i>Retroviridae</i>	<i>Lentivirus</i>	Reverse-transcribing ssRNA, enveloped	2003	Kuala Lumpur	Novel recombinant descended from subtypes B and CRF01_AE Widespread among all major risk groups
Highly pathogenic avian influenza H5N1	<i>Orthomyxoviridae</i>	<i>Influenzavirus A</i>	–ssRNA, enveloped	2004 2006 2007	Kelantan Kuala Lumpur; Perak; Pulau Pinang Kuala Lumpur	Outbreaks confined to poultry and have been linked to fighting cocks smuggled from neighboring countries 60 000 birds culled to control outbreak
Melaka virus	<i>Reoviridae</i>	<i>Orthoreovirus</i>	dsRNA, non-enveloped	2006	Melaka	Novel bat <i>Orthoreovirus</i> associated with acute respiratory disease in the human

into the country, although more research is certainly needed to validate this relationship.¹²²

The discovery of bat-associated NiV (a *Henipavirus*), TiV (*Rubulavirus*), and PulV and MelV (both within the *Orthorovirus* genus) has highlighted the significant role that bats play as important reservoir hosts of emerging viruses.^{123,124} Although yet to cause severe, widespread human outbreaks, medical and research institutions should adopt a proactive approach in understanding and managing the potential threats posed by these viruses. This includes conducting large-scale surveillance to determine the prevalence, both in bats and humans of these viruses (as in Bangladesh, for example, during the recent NiV outbreaks^{62,68,69}), establishing diagnostic systems in public health laboratories that include the diagnosis of these viruses, and reviewing the traditional/medicinal practice of drinking fresh bat blood or eating improperly cooked bat products among certain rural communities.¹²⁵

HIV is among the most genetically diverse human pathogens. In addition, co-circulation of two or more HIV-1 subtypes in any population can lead to co-infection in individuals and consequently generate recombinant strains,^{106,107,126} as occurred with clade CRF33_01B in Malaysia, a fact that further increases the genetic plasticity of HIV-1. The possible phenotypic advantage inherited from recombination among different HIV-1 clades remains uncertain. However, it has been reported that HIV recombinants may have enhanced replicative capacity and transmissibility or higher risk of disease progression compared to its parental strains,^{127,128} as reflected by the high prevalence of such recombinants in particular populations with risk practices. Further genetic diversification of HIV-1, which will add further to the problems in the development of antivirals and vaccines, could be reduced by limiting the incidence of infections among the high-risk groups and among those who are already infected.

As Malaysia progresses towards becoming a developed country in the new millennium, a number of zoonotic, non-zoonotic, and vector-borne viruses have been reported for the first time in the country. Although a decade has past, the potential health threats faced by the population are far from over. In fact, the momentum with which these diseases are spreading has intensified over time, as seen by the exponential increase in the annual HIV/AIDS incidence in Malaysia and elsewhere in the world, the temporal persistence of EV-71 infections, the sporadic expansion of CHIKV worldwide, the broad regional distribution and aggressive NiV outbreaks in Bangladesh and India, and the re-occurrence of highly pathogenic H5N1 avian influenza in poultry and humans. Taken together, indispensable lessons learnt from the past plus current understanding of the probable circumstances leading to disease emergence suggest calls for better preparedness to deal with impending infectious disease risks.

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