



RESEARCH ARTICLE

Pathogenic viruses associated with bats of Malaysia

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ABSTRACT

Numerous human diseases, including those caused by viruses like Nipah virus and SARS-CoV, can be traced back to bats as their origin. Malaysia, notably Sabah and Sarawak in Borneo Island, is home to a rich diversity of bats that serve as hosts for various viruses. This comprehensive review represents the inaugural exploration of viruses found in Malaysian bats, as documented in scientific journals. It also encompasses documented instances of bat virus-related disease outbreaks in Malaysia up to the present day, along with an analysis of the risk factors associated with virus spillover events. Furthermore, this review offers insights into prospective research areas of significance and suggests potential mitigation strategies.

Keywords: Bats; zoonotic; viruses; ectoparasites; risk factors.

INTRODUCTION

The global rise in diseases caused by bat-related viruses has piqued the interest of researchers worldwide, prompting a comprehensive exploration of bat viruses. Despite the significance of bat-associated virus discovery, the available data remains largely insufficient. It has been estimated that over 1 million virus species are found in mammals, with about half a million posing a possible threat to human health (Carlson *et al.*, 2019). Currently, only a minute fraction of the predicted 320,000 total mammalian viruses has been documented (Carlson *et al.*, 2021; Anthony *et al.*, 2013). Among all mammals, bats are the second highest mammal group to pose the greatest threat of zoonotic disease risk (108/1100 of all bat species or 9.8% are zoonotic hosts) after rodents (244/2220 species are zoonotic host) (Allocati *et al.*, 2016; Han *et al.*, 2016).

In the context of Malaysia, the body of published research on bat viruses is quite limited. This review aims to consolidate our knowledge of bat-origin viruses in Malaysia to date, shedding light on the potential risk factors for virus spillover within the country. Additionally, we will discuss potential strategies for mitigating the risk of spillover events. This review will also encompass viral diseases of bat origin that have made their way into Malaysia.

MATERIALS AND METHODS

To compile this information, we conducted a systematic search for virus sequences using the National Center for Biotechnology Information (NCBI) Genbank and Database of bat-associated viruses Website (DBatVir) for cross-checking. (Chen *et al.*, 2014; Zhou *et al.*, 2022). We utilized keywords such as "Bat," "virus," and "Malaysia" to retrieve relevant data, which was subsequently subjected to meticulous filtering. Each viral sequence was scrutinized to confirm its Malaysian bat origin.

This review also included all bat-borne viruses detected in Malaysia even if their nucleotide sequences have not been published. Additionally, medically important bat-borne viruses (such as Rabies virus) that have been found in other countries but not yet detected from bats in Malaysia, are discussed in the context of Malaysia.

RESULTS & DISCUSSION

Bat viruses recorded in Malaysia

The total number of sequences of bat-associated viruses recorded in Asia, as of April 30, 2023, is 6,672, which represents the highest number of sequences gathered worldwide. Among these, 80 sequences were identified as from Malaysia. However, this number is comparatively lower than the counts in China (3,728), Vietnam (771), and Thailand (447) and Indonesia (93), but higher than the counts in The Philippines (24) and Singapore (61). The heightened level of research conducted in other regions may account for the greater prevalence of viral findings and publications. Increased surveillance efforts prompted by the emergence of viral diseases, such as the SARS-CoV-1 outbreak in China, has also contributed to the rise in virus sequence publications.

There are a total of 20 odd virus species (*Pteropine orthoreovirus* (PRV)-Pulau (PRV2P), PRV-Melaka (PRV3M), PRV-Kampar (PRV4K), Bat alphacoronavirus, Alphacoronavirus sp., Bat coronavirus Philippines/Diliman1525G2/2008, Bat betacoronavirus, Coronavirus PREDICT CoV, Betacoronavirus sp., SARS-CoV-2, Coronavirus CoV-104, Hipposideros bat coronavirus, Herpesviridae sp., West Nile virus, Batu cave virus, Carey Island virus, Nipah henipavirus, Tioman virus, Hipposideros bat paramyxovirus, and Sarawak mobatvirus) from six virus families (*Reoviridae*, *Coronaviridae*, *Herpesviridae*, *Flaviviridae*, *Paramyxoviridae*, and *Hantaviridae*) which have their origin in bats of Malaysia (Table 1). More virus sequence amplified

Table 1. Bat-related virus found in Malaysia (Virus sequences available in NCBI database)

Family	Genus	Virus	Host	Specimen	Reference
Reoviridae	Orthoreovirus	PRV-Pulau (PRV2P)	<i>Pteropus hypomelanus</i>	Faeces, urine	Voon et al., 2011; Pritchard et al., 2006
		PRV-Melaka (PRV3M)	Human (nucleotide sequence has close-relatedness to PRV from fruit bat in Malaysia)	Human respiratory specimens	Chua et al., 2007, 2008; Pritchard et al., 2006; Voon et al., 2011
		PRV-Kampar (PRV4K)	Human (nucleotide sequence has close-relatedness to PRV from fruit bat in Malaysia)	Human respiratory specimens	Chua et al., 2007, 2008; Pritchard et al., 2006; Voon et al., 2011
Coronaviridae	Alphacoronavirus	Bat alphacoronavirus	Chiropterans	Bat guano	Tan et al., 2021
		Alphacoronavirus sp.	<i>Penthetor lucasi</i>	Feces	Unpublished
	Betacoronavirus	Bat coronavirus	<i>Megaerops ecaudatus</i>	Oral, rectal, and	Anthony et al., 2017
		Philippines/Diliman1525G2/2008	<i>Cynopterus horsfieldii</i>	Urogenital swabs, urine	
			<i>Dyacopterus spadiceus</i>		
Herpesviridae	Unclassified	Bat betacoronavirus	Chiropterans	Bat guano	Tan et al., 2021
		Coronavirus PREDICT CoV	<i>Cynopterus brachyotis</i> , <i>Hipposideros bicolor</i> , <i>Rhinolophus creaghi</i> , <i>Hipposideros galeritus</i> , <i>Hipposideros diadema</i> ,	Oral swab, Rectal swab	Anthony et al., 2017
		Betacoronavirus sp.		Urine, Urogenital swab	
		SARS-CoV-2	<i>Penthetor lucasi</i>	Feces	Unpublished
		Coronavirus CoV-104	unclassified Chiroptera	Swabs	Unpublished
		Hipposideros bat coronavirus	<i>Tylonycteris pachypus</i>	Rectal swabs	Unpublished
		Herpesviridae sp.	<i>Hipposideros cervinus</i>	Rectal swab	Unpublished
		West Nile virus	<i>Eonycteris spelaea</i>	No data	Paskey et al., 2020
		Batu cave virus	<i>Emballonura monticola</i> , <i>Cynopterus brachyotis</i> , <i>Rhinolophus siamensis</i>	Throat and rectal swab	Ain-Najwa et al., 2020
		Carey Island virus	<i>Cynopterus brachyotis</i>	Culture	Moureau et al., 2015
Paramyxoviridae	Henipavirus	Nipah henipavirus	No data	No data	Kuno et al., 1998
			<i>Pteropus hypomelanus</i> , <i>Pteropus vampyrus</i> ,	Urine	Chua et al., 2002b; Rahman et al., 2010; Sharifah et al., 2009
		Tioman parubulavirus	<i>Pteropus hypomelanus</i>	Urine	Chua et al., 2001b
Hantaviridae	Unclassified	Hipposideros bat paramyxovirus	<i>Hipposideros galeritus</i>	Urine	Unpublished
		Sarawak mobatvirus	<i>Murina aenea</i>	Lung tissue	Zana et al., 2019

have been recorded from Coronaviridae than the other virus families in Malaysia. Twenty bat species have been positively identified, although several Chiropterans were not identified up to species nor genus level. Most of the viruses were named according to the place where they were first found, example Melaka virus, Batu Caves virus.

The first virus documented in Malaysia was Carey Island virus in 1970, followed by Batu Cave virus in 1971. From 1998 onwards, Malaysia has documented cases of bat virus diseases, including Nipah virus, Melaka virus, Tioman virus, Pulau virus, and SARS-CoV virus diseases (Chua *et al.*, 1999; Guan *et al.*, 2003; Pritchard *et al.*, 2006; Chua *et al.*, 2007; Yaiw *et al.*, 2007a, 2008) (Figure 1).

The timeline of viruses' sample collection is illustrated in Figure 1 showing publication starting in 1970 and having an exponential growth starting in the year 2008 until 2021. We provided a visual representation of geographic distribution of viruses within Malaysia in Figure 2, which shows a relatively higher number of viruses found from different locations within Peninsular Malaysia in comparison to East Malaysia. Higher sampling effort in Peninsular Malaysia may be one of the reasons for the different numbers of location as most outbreak started in the Peninsular hence the outbreak-motivated surveillance effort relatively extensively done in the Peninsular.

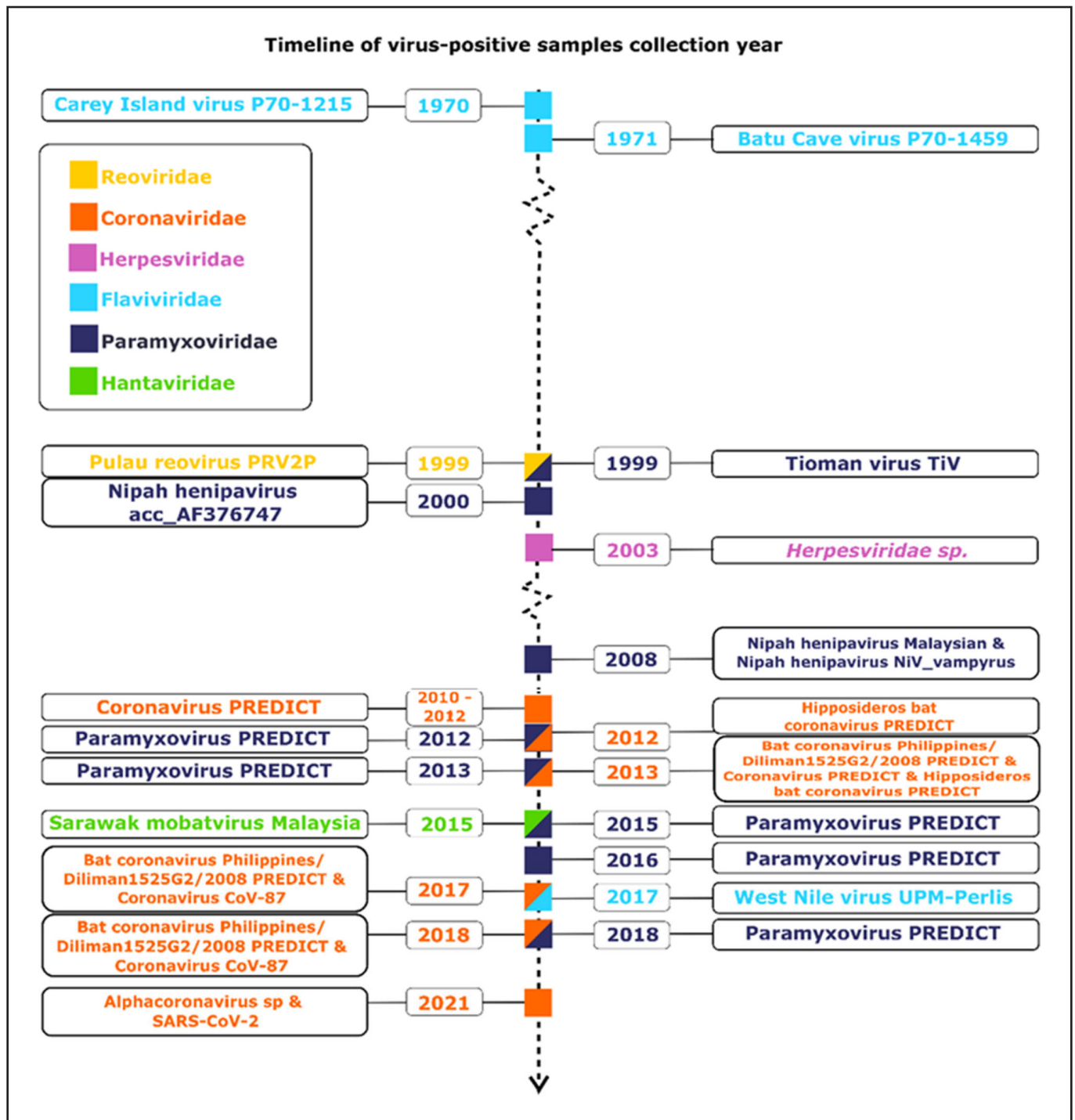


Figure 1. Timeline of collection year of bat-related-virus positive samples from various samples in Malaysia.

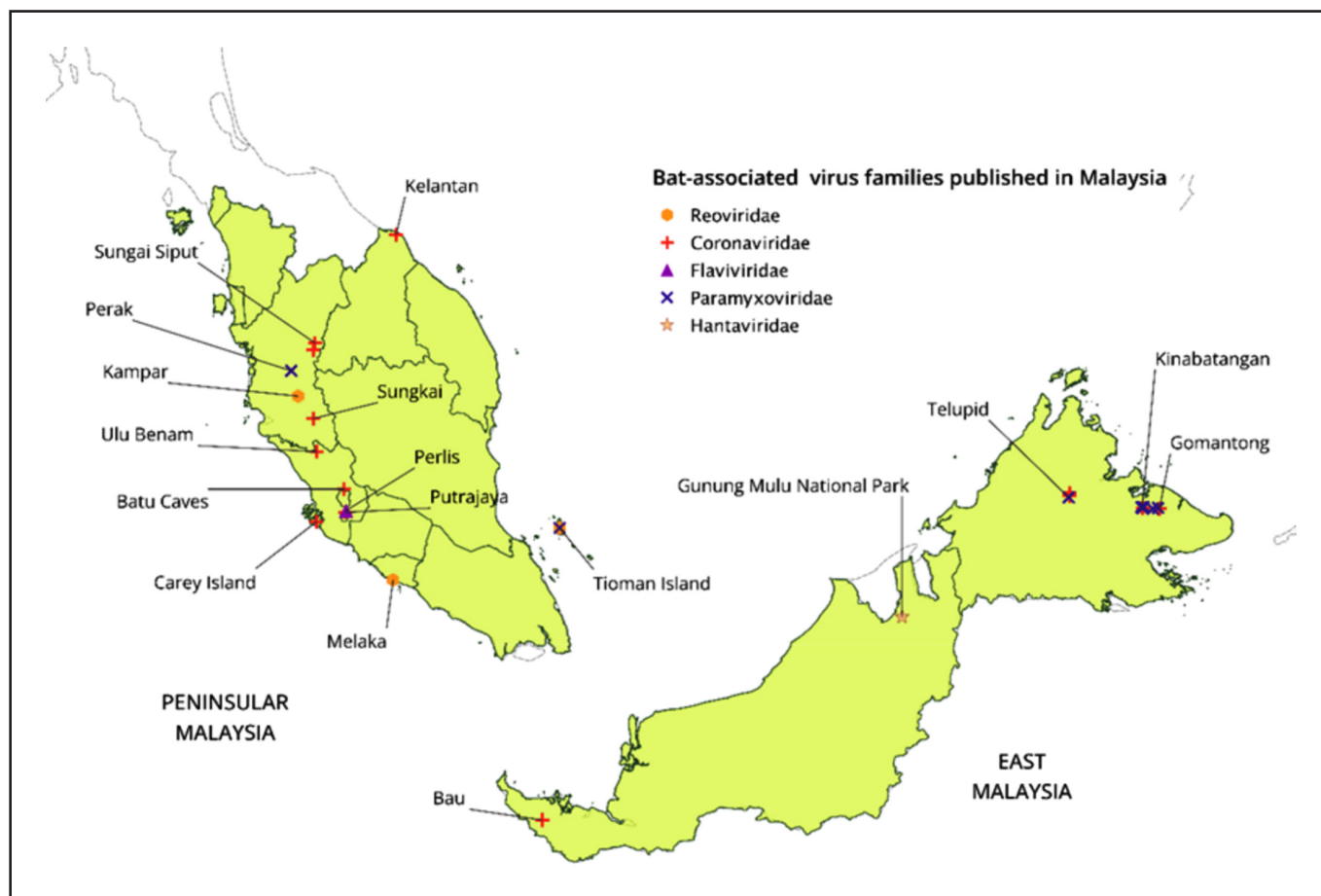


Figure 2. Mapping of published bat-associated virus families in Malaysia.

The publication of bat-associated viruses has experienced exponential growth since the 1990s, as depicted in Figure 3. *Coronaviridae*, accounting for 7.5% ($n=38$) of publications in Malaysia, is the most frequently studied group, followed by *Paramyxoviridae* viruses ($n=29$) and *Flaviviridae* viruses ($n=8$).

The earliest virus publication available dates back to 1998, focusing on *Flaviviridae* viruses collected in 1970, specifically Carey Island virus and Batu Caves virus (Kuno *et al.*, 1998). Another sequence, Pnomh Phen virus, mentioned in the same paper, lacked a clear origin and is excluded from this review. During that period, the prevalence of flaviviruses, including Dengue virus, was on the rise, leading to research efforts aimed at constructing a comprehensive phylogeny for flaviviruses (WHO, 1997). Flavivirus research did not

resurface until 2017 when West Nile virus sequences were detected in several species of bats in Perlis, Malaysia (Ain-Najwa *et al.*, 2020).

In 2003, a single *Herpesviridae* virus partial sequence from an *Eonycteris spelaea* bat in unspecified location in Malaysia was submitted to the NCBI database by researchers from the National Institute of Animal Health in Japan (NCBI, n.d.).

The cumulative number of research studies on Malaysian bat viruses increased from 12 virus sequences collected since 1971 to 26 in 2017, reflecting a substantial growth rate in virus research. This surge in interest among scientists to explore bats and the pathogens they carry can be attributed to advances in technology, new methodologies, and instruments in the field.

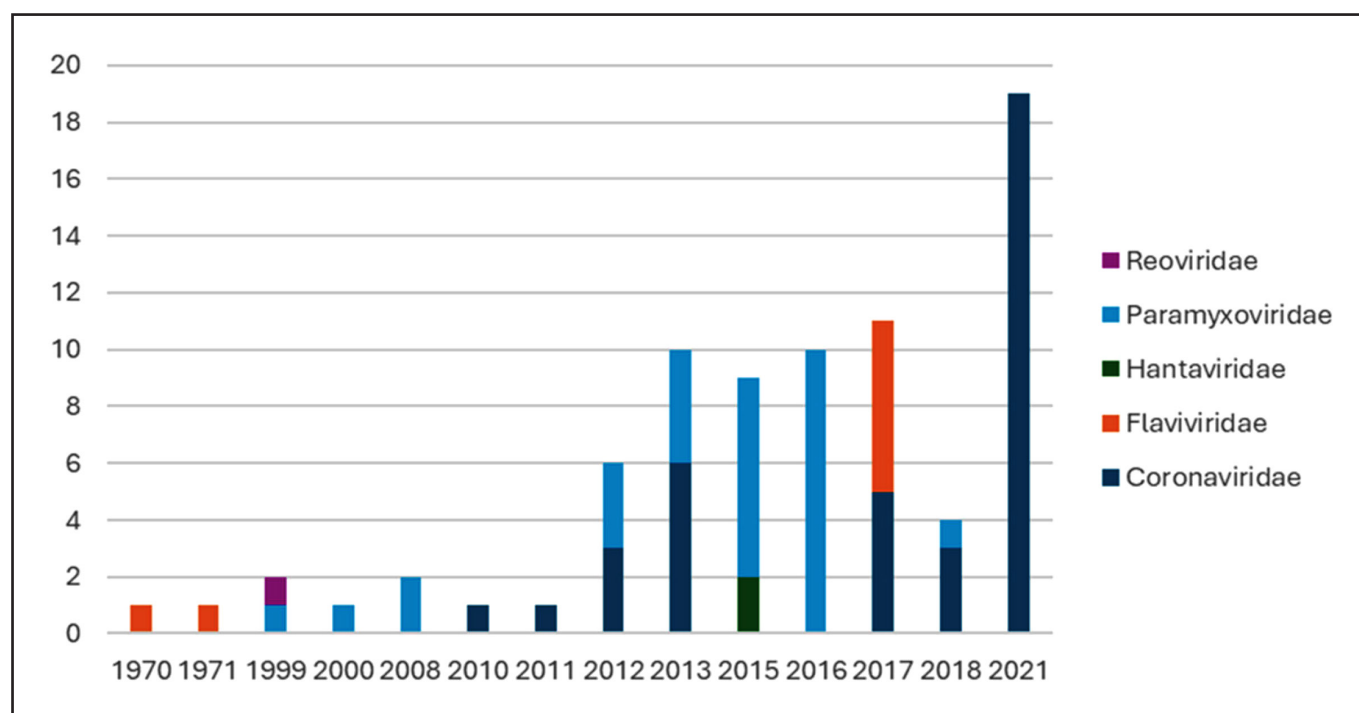


Figure 3. The number of bat virus sequence publications by year of publication and their respective families.

In Malaysia, virus detection in recent studies primarily relied on reverse transcription polymerase chain reaction (RT-PCR), with additional methods such as virus isolation and electron microscopic observation of infected cells. RT-PCR is recognized as a highly reliable and cost-effective approach for screening RNA viruses. However, it's worth noting that isolation and cultivation methods are considered the gold standard for virus screening (Guliy et al., 2019).

Furthermore, unpublished research work has also identified viruses in bats (Tan et al., 2022). These newly discovered viruses include previously undescribed betacoronaviruses, alphacoronaviruses, and specific strains known as Borneo Alpha-1, Borneo Alpha-2, Borneo Beta-1, and Borneo Beta-2 BatCovs (Mahyudin et al., 2021; Tan et al., 2022).

The first detection of Hantavirus in Malaysia occurred in Sarawak and belongs to the Mobatvirus genus. This virus was found in an insectivorous bat known as *Murina aenea*, commonly referred to as the bronze tube-nosed bat (Zana et al., 2019).

Typically, the screening of viruses in bats involves collecting samples such as feces, rectal swabs, and salivary swabs. However, in a particular study, researchers also examined lung tissue samples from bats, leading to the discovery of viruses like Hantavirus (Zana et al., 2019).

Bat virus screening and diseases recorded in Malaysia

Bat Paramyxovirus

Nipah virus

The importance of surveillance in Malaysia was vividly underscored by the emergence of the Nipah virus pandemic. In September 1998, this pandemic claimed as many as 105 lives, resulting in a distressing fatality rate of 39.6%, all caused by an initially mysterious virus (Lam & Chua, 2002). To contain the outbreak, over 1 million pigs were culled, inflicting a substantial economic blow to the nation (WHO & FAO, 2002; Lam & Chua, 2002).

At the outset, it was determined that pigs were the primary carriers of this deadly virus, which then spread to workers on infected pig farms (Lam & Chua, 2002). However, further investigation prompted the initiation of surveillance on bats due to the virus's close genetic association with the Hendra virus (Yob et al., 2001).

Serology tests were conducted on both megachiroptera and microchiroptera, leading to the identification of five species that tested positive for the Nipah virus. These were *Pteropus hypomelanus*, *Pteropus vampyrus*, *Rhinolophus creaghi*, *R. archuatus*, *R. trifolius*, *Hipposideros diadema*, *H. cervinus*, and *H. galeatus* (Rahman et al., 2010; Wells et al., 2022).

Additionally, Nipah virus isolates were retrieved from the urine and salivary swabs of partially consumed fruits by bats. This strongly suggested that fruit bats serve as the reservoir hosts for the virus (Chua et al., 2002b).

Tioman Virus (TioV)

The Tioman virus, classified as a Rubulavirus within the *Paramyxovirus* genus, derives its name from Tioman Island, where it was originally discovered during a search for the Nipah virus in 1999 (Chua et al., 1999). This virus was isolated from urine samples obtained from pteropodid bats, specifically *Pteropus hypomelanus*. The samples yielded three distinct viruses: the newly identified Tioman virus (TioV), Nipah virus (NiV), and Pulau virus (PuV).

Ongoing research continues to explore the medical significance of the Tioman virus. Notably, its genetic resemblance to the Menangle virus (MenV), which caused a disease outbreak in pigs in Australia in 1997 (Philbey et al., 1998), suggests the potential susceptibility of pigs to TioV (Chua et al., 2002a). Infection studies conducted by Yaiw et al. in 2007 and 2008 involved inoculating animals with the virus, resulting in neuronal infections accompanied by necrosis in mice (Yaiw et al., 2007b) and thyroid infections in pigs (Yaiw et al., 2008).

In 2007, a study on the inhabitants of Tioman Island revealed evidence of prior exposure to the Tioman virus. Serological analysis identified five individuals out of 169 who tested positive for Tioman virus antibodies (Yaiw et al., 2007a).

Bat coronavirus

Severe Acute Respiratory Syndrome (SARS)-CoV

The initial appearance of the SARS virus occurred in Guangdong, China, between 2002 and 2003 (Guan et al., 2003). Subsequently, in 2003, the first cases of SARS were reported in Malaysia, resulting in two fatalities and the recovery of three individuals, as reported by Ibrahim and Fakhrul-Razi (2006). Initial investigations revealed that the source of SARS in China could be traced back to Himalayan palm civets (*Paguma larvata*) found in a live-animal market, prompting a widescale culling of civets to prevent further outbreaks (Guan et al., 2003).

However, subsequent experiments involving the inoculation of human SARS-CoV into civets produced only overt clinical symptoms of the disease, indicating that civets were not the true reservoir hosts (Li et al., 2005). Later research confirmed that the natural host of the virus was the cave dwelling fruit bat (*Rousettus leschenaultia*) (Li et al., 2005).

SARS-CoV has been identified in bats in various regions, including China, Kenya, and North America (Wang et al., 2006; Dominguez et al., 2007; Tong et al., 2009).

However, in Malaysia, other types of coronaviruses, including betacoronaviruses and alphacoronaviruses, have also been detected from *Penthetor lucasi*, *Megaerops ecaudatus*, *Cynopterus horsfieldii*, *Dyacopterus spadiceus*, *Cynopterus brachyotis*, *Hipposideros bicolor*, *Rhinolophus creaghi*, *Hipposideros galeritus*, *Hipposideros diadema*, and other unspecified fruit bats (Anthony et al., 2017; Zover, 2023).

SARS-Cov-2 (COVID-19)

SARS-CoV-2 belongs to the *Sarbecovirus* subgenus within the *Coronavirus* genus, part of the *Coronaviridae* family. It shares significant genetic similarities with the SARS-CoV virus, which emerged in China in 2002-2003 (Guan et al., 2003). This novel coronavirus was declared a global pandemic by the World Health Organization in March 11, 2020 (WHO, n.d.). The initial cases were identified in Wuhan city, Hubei province, China, in December 2019 (Lu et al., 2020). It was imported to Malaysia in January 2020 by a Chinese nationality from Singapore traveled into Johor, Malaysia (Hashim et al., 2021). Subsequently, during the second wave of the outbreak in February 2020, Malaysia experienced about five big fatal clusters along with other imported cases traveling into Malaysia (Hashim et al., 2021).

By August 9, 2022, Malaysia has reported more than 4.71 million confirmed cases and over 36,000 deaths due to COVID-19 (JHU CSSE COVID-19 Data). The disease symptoms range from mild to severe respiratory illness, depending on the individual's health prior to infection, and it can affect individuals of all ages (WHO, n.d.).

While the molecular sequence of SARS-CoV-2 closely resembles sequences found in SARS-CoV and SARS-related coronaviruses (SARSr-CoVs) in horseshoe bats (Lau et al., 2020), the exact origin of SARS-CoV-2 remains unconfirmed. However, its genomic sequence shows close relationships with viruses collected from *Rhinolophus pusillus* bats in 2015, such as SARSr-Rp-BatCoV-ZXC21/ZC45, SARSr-Ra-BatCoV RaTG13, and Pangolin-SARSr-CoVs, which were discovered in different regions of China (Hu et al., 2018).

Mobatvirus

The genus Mobatvirus, a member of the *Hantaviridae* family (Laenen et al., 2019), primarily resides in rodents but is also detected in bats, posing a potential threat to human infection (Forbes et al., 2018). Notably, in Sarawak, Malaysia, a new strain of Mobatvirus was recently discovered and designated as Sarawak mobatvirus (SARV)

(Zana et al., 2019). Researchers employed Hanta-virus specific RT-PCR to analyze bat lung tissues collected in Sarawak, Malaysia, and identified two positive samples out of the 116 tested. Remarkably, one positive sample was obtained from a female bat *Murina aenea*, while the other came from a male *M. aenea* (Zana et al., 2019). This discovery marks the first known instance of a *Hantaviridae* virus found in bats within Malaysia's borders.

Rabies virus

The Rabies virus poses a significant public health concern in Malaysia due to its high fatality rate (Banyard & Fooks, 2020; Izani et al., 2022). In 2022, Malaysia witnessed a total of 32 human fatalities from Rabies in Sarawak, along with two reported cases of Rabies infection in Selangor (Izani et al., 2022; News Desk, 2022). Rabies virus belongs to the *Rhabdoviridae* family and the *Lyssavirus* genus. Reservoir hosts, primarily domesticated dogs, and other mammals like bats, are associated with *Lyssaviruses*. However, to date, there have been no documented detections of the Rabies virus nor any *Rhabdovirus* in bats within Malaysia. In contrast, *Rhabdoviruses* from bats have been reported from other countries such as United States, Canada, Brazil and China (Davis et al., 2013; Nadin-Davis et al., 2017; Hu et al., 2018; Cargnelli et al., 2021; Condori et al., 2022; Dettinger et al., 2022).

Flavivirus

Flaviviruses belong to the *Flaviviridae* family and are associated with vector hosts such as mosquitoes and ticks (Thomas et al., 2014). In Malaysia, three types of bat-associated flaviviruses have been identified: West Nile virus, Batu Caves virus, and Carey Island virus. West Nile Virus was recorded in Carey Island (Kuno et al., 1998) while Batu Caves virus and Carey Island virus were the first bat-associated flaviviruses reported in Malaysia in 1998 (Kuno et al., 1998). This discovery occurred during a period of increasing flaviviral diseases outbreaks, including Dengue virus, prompting extensive research to construct a comprehensive phylogeny of flaviviruses (WHO, 1997).

Nearly two decades later, in 2017, another strain of flavivirus, West Nile virus, was detected and documented (Ain-Najwa et al., 2020). This virus was found in 12.2% of bats from the *Emballonuridae*, *Pteropodidae*, and *Rhinolophidae* families captured in Perlis, Malaysia. The presence of West Nile virus RNA in these bats, confirmed through PCR testing, suggests that Malaysian bats are hosts to this virus (Ain-Najwa et al., 2020).

Orthoreovirus

Melaka virus (MeIV)

The Melaka virus belongs to the *Orthoreovirus* genus, specifically categorized as a *Reovirus*. It was initially discovered in 2006 when it was isolated from an adult human residing in Melaka who presented symptoms of acute respiratory disease (Chua et al., 2007). Although there is no direct confirmation of the virus's presence in bats, the fact that a bat had been in the man's living room shortly before his illness strongly suggests a possible association of the virus with bats. Furthermore, the genetic resemblance between the Melaka virus and other bat-related viruses like Pulau virus and Nelson Bay virus (NBV) from Australia further supports this hypothesis (Pritchard et al., 2006; Chua et al., 2007, 2008; Voon et al., 2011).

The virus was initially identified when a throat swab was collected from the afflicted patient who was suffering from high fever, sore throat, and severe weakness. Subsequent laboratory tests confirmed the presence of the virus. It exhibited a syncytial cytopathic effect (CPE) in various mammalian cells and mosquito-derived cells (Chua et al., 2007). Notably, Melaka virus did not react to antisera, and PCR results ruled out its identity as adenovirus, influenza A and B, parainfluenza 1, 2, and 3, as well as respiratory syncytial virus (Chua et al., 2007).

Phylogenetic analysis conducted by Chua and colleagues in 2007 revealed a close relationship between Pulau virus and Melaka virus, placing them within the same group as NBV (Chua *et al.*, 2007). An epidemiological investigation highlighted that about a week before the patient displayed symptoms, a bat had entered and exited the patient's living room, where he was watching television, fluttering about "frantically" for 2-3 minutes (Chua *et al.*, 2007).

Additionally, two other family members, an 11-year-old daughter and a 6-year-old son, exhibited symptoms of the virus infection and tested positive for it. However, the patient's wife, despite testing positive for the virus, did not manifest any symptoms of the illness (Chua *et al.*, 2007).

Pulau Virus (PuV)

Pulau Virus (PuV) was serendipitously discovered during an exploration for the Nipah virus reservoir on Tioman Island in 2001 (Pritchard *et al.*, 2006). PuV was successfully isolated from the fruit bats, *Pteropus poliocephalus*. A comprehensive phylogenetic analysis of inferred protein sequences has indicated a genetic kinship between PuV and Nelson Bay virus (NBV), both belonging to the *Orthoreovirus* genus (Gard & Compans, 1970; Pritchard *et al.*, 2006).

Despite these intriguing findings, numerous aspects of PuV remain enigmatic. Its host range, pathogenesis, and epidemiology have yet to be fully elucidated (Tee *et al.*, 2009). Furthermore, no documented cases of human disease attributed to Pulau Virus have been recorded to date.

Kampar virus

The Kampar virus, named after the location of its initial discovery, exhibits a significant degree of genetic similarity and cell line susceptibility pattern resemblance to the Melaka Virus. This similarity has been confirmed through both molecular and serological evidence. As an orthoreovirus, it is highly likely that the Kampar virus has its origins in bats (Lorusso *et al.*, 2015).

Notably, the patient who contracted the Kampar virus had fruit trees growing near their residence, with partially consumed fruits found just outside their living room window. The first isolation of the Kampar virus was from this patient, who displayed symptoms including fever, respiratory issues, and vomiting (Chua *et al.*, 2008).

Considering the closely related PuV virus has been identified in two different species of fruit bats namely *Pteropus poliocephalus* which was previously detected in Australia (Pritchard *et al.*, 2006), and *Pteropus hypomelanus* from Tioman Island (Pritchard *et al.*, 2006), there is a strong possibility that the Kampar Virus may also be present in multiple species of fruit bats (Chua *et al.*, 2008).

Herpesvirus

There is only one record of Herpesvirus in Malaysia. The amplified partial DNA polymerase sequence of an unclassified Herpesvirus from an *Eonycteris spelaea* from Malaysia was deposited in NCBI in 2003 (Imada *et al.*, unpublished; GenBank accession number: AB125970.1, Nucleotide).

Herpesvirus was found in bats in other countries such as China and Philippines from samples of bats' faeces and spleen respectively, and various range of species of insectivorous bats and a species of fruit bat (Watanabe *et al.*, 2009; Zheng *et al.*, 2016). It is also recorded from *Eonycteris spelaea* of Singapore (Mendenhall *et al.*, 2019).

In Malaysia, Herpesvirus had also been detected from other animals such as macaques, turtles, and elephants (Lee *et al.*, 2015; Loganathan *et al.*, 2021; Lee *et al.*, 2021). The record on Herpesvirus from bats in Malaysia is very scarce which demands further investigation.

Risk factors of virus spill over from bats

In the quest to establish effective measures for preventing the emergence of viruses, it becomes imperative to grasp the mechanics of viral spillover from bats to humans, which can subsequently become virulent. The transmission of viruses from mammals necessitates the alignment of various factors and the surmounting of multiple barriers to enable spillover into humans (Plowright *et al.*, 2017).

There exist three primary avenues that create opportunities for such spillovers: through excretion, slaughter, and vectors. These pathways consider the dynamics of disease within the reservoir hosts themselves, the modes of human exposure to these viruses, and the susceptibility of exposed humans (Plowright *et al.*, 2017). A proposed framework by a researcher succinctly describes the mechanisms involved in animal-to-human transmission. However, it's important to note that not all viruses, even if they manage to breach these barriers, can cause diseases in humans; this occurrence should be relatively rare. Consequently, comprehending how these barriers interconnect and evolve over time and space holds the key to enhancing predictions and preventive measures for zoonotic diseases (Plowright *et al.*, 2017).

While studies concerning viruses from bats frequently categorize them based on their family or species, one study argues that taxonomic identity is not the predominant factor influencing zoonotic potential (Mollentze & Streicker, 2020). Instead, several other crucial factors can increase a virus's capacity to transmit diseases.

One of the most significant factors identified by scientists is the roosting behavior of bats, particularly among those residing in larger communities or populations. Such bats exhibit a higher probability of sharing viruses, which enables the maintenance of viral infections and re-infections within the bat community (Luis *et al.*, 2013, 2015). Insectivorous bats that roost in caves and cohabit with other bat species share more viruses compared to rodents that share viruses across species (Luis *et al.*, 2015).

Frugivorous bats also serve as important reservoir hosts for medically significant viruses like Nipah virus, Hendra virus, and possibly Ebola virus (Chua *et al.*, 2002b; Peel *et al.*, 2022; Leroy *et al.*, 2005). In fact, frugivorous bats harbor more virus species than insectivorous, nectivorous, and sanguivorous bats (Luis *et al.*, 2015). Their feeding habits, which involve partially consuming fruits that may also be consumed by other animals, make it easier for viruses to be transmitted to different species and ultimately to humans, as observed in the case of the Nipah virus in Malaysia (Chua *et al.*, 2002b; Amman *et al.*, 2021). This suggests that frugivorous bats have a higher likelihood of leaving their viruses in the environment, potentially exposing other animals over time, thereby increasing the virulence of these viruses.

Another influential factor in spillover events is the migration of bats (Luis *et al.*, 2015). Bats that migrate within a region have a greater potential to transmit viruses compared to those undertaking long-distance journeys, as the latter are more likely to perish during migration (Luis *et al.*, 2015).

Habitat loss represents another factor that amplifies the risk of viral spillover from bats. Interestingly, intermediate levels of habitat loss have been found to be more efficient in facilitating spillover compared to higher levels of habitat loss (Faust *et al.*, 2018).

Bats are frequently infested with ectoparasites, which are suspected to serve as potential vectors for spillover events. These ectoparasites encompass ticks, mites, and bat-flies, all known to host various pathogens, including Dengue virus, a novel Rhabdovirus, and Kaeng Khoi Virus (Feng *et al.*, 2017; Goldberg *et al.*, 2017; Abundes-Gallegos *et al.*, 2018; Xu *et al.*, 2019; Tendu *et al.*, 2022). A recent

study conducted in Malaysia on bat ectoparasites identified a total of 15 species of bat flies associated with 24 species of bats (Azhar et al., 2015). Furthermore, 15 species of mesostigmatid mites and six species of chiggers were found parasitizing bats in Malaysia (Ahmad et al., 2013). Unfortunately, information regarding the viruses carried by these ectoparasites in Malaysian bats is nearly non-existent.

Bats harbour numerous viruses and are asymptomatic

Bats exhibit remarkable characteristics that set them apart in their ability to host a greater variety of viruses within a single species, surpassing all other mammals. What makes this even more intriguing is that bats do so without displaying noticeable clinical symptoms, unlike other virus-carrying mammals (Olival et al., 2017).

There are several distinctive features that differentiate bats from their mammalian counterparts. For instance, bats possess a metabolic rate that is 2.5 to three times higher than similarly sized mammals engaged in physical activity. Their heartbeats exceed a staggering 1000 beats per minute, and they can endure body temperatures rising up to 41°C (Irving et al., 2021). While no direct research has conclusively linked these traits to their capacity to resist viral infections, it is clear that bats have adapted remarkably to thrive in these unique physiological conditions.

In terms of immune defense, bats employ highly effective and well-balanced mechanisms to combat viral infections and other pathogens, without succumbing to excessive immune responses (Banerjee et al., 2020; Irving et al., 2021). Bats possess elevated basal levels of certain immune defense proteins, including interferons (IFNs), interferons-stimulated genes (ISGs), heat-shock proteins (HSPs), ATP-binding cassette type subfamily B member 1 (ABCB1) and autophagy, in comparison to humans and other mammals like mice (Zhou et al., 2016; Chionh et al., 2019; Koh et al., 2019; Kacprzyk et al., 2021). These heightened levels of immune defense proteins significantly enhance their ability to fend off viral threats (Banerjee et al., 2020).

Additionally, bats have downregulated the expression of specific genes such as STING (Stimulator of Interferon Genes), an essential inflammasome sensor, NLRP3 (nucleotide-binding domain, leucine-rich repeat family pyrin domain-containing 3), and PYHIN (Pyrin and hematopoietic interferon-inducible nuclear domain) family genes (Ahn et al., 2016; Xie et al., 2018; Ahn et al., 2019). This fine-tuning of gene expression promotes a more balanced immune response, preventing excessive reactions (Xie et al., 2018; Banerjee et al., 2020).

CONCLUSION AND RECOMMENDATIONS

The study and discovery of bat viruses is still in its early stages (Guliy et al., 2019). In Malaysia, research on bat-borne viruses remains relatively limited. Nevertheless, it is crucial to note that bats in Malaysia do carry medically significant viruses, such as paramyxovirus and coronaviruses, which have tragically claimed Malaysian lives (Chua et al., 2001a; Hashim et al., 2021). Therefore, it is imperative to conduct ongoing surveillance of bat viruses in Malaysia to enhance preparedness for potential new virus outbreaks.

We strongly recommend increasing active surveillance of bat viruses in Malaysia to provide up-to-date data critical for public health. Utilizing modern technologies such as next-generation sequencing, multiplex assays, electrophysical analysis, biosensor technologies, PCR, and virus cell culture (Watzinger et al., 2006; Leland & Ginocchio, 2007; Boonham et al., 2014; Mehrotra, 2016) is paramount. Research into effective techniques and methodologies is vital to ensure accurate virus detection and minimize false positives and negatives. In addition to nucleic acid-based detection methods, immunological approaches offer an efficient means of understanding

virus infections in bats. The detection of viral antigens has the advantage of not requiring the isolation and culture of viruses from samples and allows for the screening of a higher number of samples simultaneously.

Beyond screening for viruses in bat samples, it is essential to investigate potential vectors for bat virus transmission to humans. Ectoparasites of bats, including bat flies, mites, ticks, chiggers, fleas, and others, are potential vectors, yet their role in virus transmission remains a subject of relatively limited research.

Multidisciplinary collaboration is pivotal in managing the risk factors associated with bat virus diseases and emergence. For instance, virologists should collaborate with experts in social sciences and government bodies to actively implement habitat restoration and wildlife conservation efforts. The conservation of wildlife habitats is essential to maintaining the delicate balance between humans and wildlife, which, when disrupted, can lead to virus spillovers from stressed bat populations.

While zoonotic spillovers may be rare, mitigation efforts at every level are crucial to minimizing the risk factors associated with bat-borne viruses to the fullest extent possible.

Conflict of Interests

The authors have no conflict of interests related to this publication.

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