

# Avian influenza outbreaks in Malaysia, 1980 – 2017

Nur Adibah M<sup>1</sup>, Zailina H<sup>1,2</sup>, Arshad, S.S<sup>3</sup>

<sup>1</sup>*Department of Environmental and Occupational Health, Faculty of Medicine & Health Sciences,*

<sup>2</sup>*Centre of Excellence EOH, Faculty of Medicine & Health Sciences*

<sup>3</sup>*Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine,  
Universiti Putra Malaysia, 43400 UPM Serdang. Selangor Darul Ehsan, Malaysia*

Corresponding author: Zailina Hashim; [zailina@upm.edu.my](mailto:zailina@upm.edu.my). Department of Environmental and Occupational Health, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor; Tel: +603-89472406; Fax: +603-89472395

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## ABSTRACT

Malaysia has experienced avian influenza virus (AIV) outbreaks over the past three decades. Four waves of H5N1 high pathogenicity avian influenza (HPAI) took place in 2004, 2006, 2007, and 2017. The first epidemics of HPAI H5N1 subtype occurred in Kelantan, Kuala Lumpur, and Perak in 2004. At that time, the outbreak was caused by 1, 2.3, and 2.3.4 clades. Molecular analysis of AIV of the HA gene found that the H5N1 strain was highly homologous and grouped together in a Vietnam/Thailand/Malaysia (VTM) sublineage to the previously H5N1 isolates from domestic and migratory birds in Thailand, Vietnam, Cambodia, and Hong Kong during 2003 – 2005. Genetically differ of these clades with 2.1 clade from Indonesia suggested that the virus was contained within poultry rather than through the repeated reintroduction of viruses from external sources. In 2006, H5N1 virus was detected in Kuala Lumpur and the states of Perak and Pulau Pinang. The outbreak at that time was genetically similar to the previous outbreaks of the Fujian-like sublineage (clade 2.3.4) in 2004. H5N1 isolates were detected in Selangor, Kelantan, and Kuala Lumpur in 2007 and only in Kelantan in 2017. During 1980 – 2017, outbreaks of low pathogenicity avian influenza (LPAI) includes H4N3, H4N6, H3N6, H5N6, H2N9, H4N1, H7N1, H2N9, H3N8, H9N2, H10N5, H3N2, and H5N2 subtypes. The outbreaks in Malaysia suggests that culling has played an important role in the prevention of poultry from AIV infection, the reduction of virus containment in the environment, and the prevention of virus transmission from poultry to humans.

**Keywords:** *avian influenza, H5N1, clade, control, Malaysia*

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## 1. Introduction

Over the past three decades, re-emergence of infectious diseases has become a major worldwide issue, particularly in the Asian region. As epidemics escalate, identification of new human viruses including severe acute respiratory syndrome (SARS) coronavirus, Enterovirus 71, Nipah virus, and the AIV has drawn attention in Malaysia. Nevertheless, in the nearly 10 years, the virus has been re-introduced in Malaysia which remains as a challenge in controlling the outbreak. Until now, H5N1 infection in humans has yet

to be reported although it has gradually affected the neighbouring countries causing the death (Ministry of Health Malaysia, 2017). It was commonly believed that the viruses were not able to transmit to humans because of the absence of required human cellular receptors (Ninpan et al., 2017).

A study conducted by Rahman et al. (2014a) found that the three isolated H5N1 strains showed mild and moderate clinical manifestations only. Human pathogenic avian influenza A (H5N1) has been suspected to be prevalent in Malaysia, but without regard to patients' residence or history of infection and severity of an outbreak of the disease, the source of

infection is yet to discover which need further investigations. Detection of H5N1 in Malaysia creates awareness about the possibility of the prevalence of deadly AIV in Malaysia (Rahman et al., 2014b). To date, lacks of studies investigate the source of infection of the virus. Detection of H5N1 in Malaysia creates awareness about the possibility of the prevalence of deadly virus avian influenza in Malaysia (Rahim et al., 2014a).

AIV is an infectious and contagious disease of birds. The aetiological agent is a negative- stranded. The RNA virus is segmented with a genome that belongs to the type A genus of Orthomyxoviridae family (Zhu et al., 2017), enveloped and is pleomorphic with a size ranging from 80 – 120 nm (Wibowo et al., 2017). Influenza A virus and other group Influenza A subtypes are members of the species influenza A virus from the influenzavirus A genus. To date based on the primary viral surface glycoproteins, haemagglutinin (HA) and neuraminidase (NA) have been recognized into 16 HA subtypes and 9 NA subtypes. The “HA” and “NA” subtypes can replicate into different sort of possible combinations in natural host reservoirs (Kang et al., 2017). The infection caused by LPAIV can either cause only mild clinical signs such as reduced body weight (Lin et al., 2017), ruffled feathers or associated with respiratory, digestive tract or nervous systems disorders and in breeder flocks causing with a reduce in production of eggs (Pantin-Jackwood et al., 2017, Dulo et al., 2017). The viruses commonly found in the wild bird reservoir are in most cases a virulent. Wild birds shed the LPAIV mostly through faeces and infection via faecal-oral route among water birds by conjunctivitis or mild respiratory transmission that infects poultry (Malo et al., 2017; Dale et al., 2017). The great majority of orthomyxoviridae infections are asymptomatic, but some can lead to severe illnesses particularly in mammals, including humans. Some of LPAIV infections are restricted to single or a few outbreaks and then fade away, while other (e.g. avian influenza H9N2 in the Middle East and Far-East Asia) spread efficiently and become endemic in susceptible populations (Ducatez et al., 2017, Kraus et al., 2016).

Of the 16 HA subtypes of AIV detected in wild birds, only a fraction of H5 and H7 subtypes have the potential for mutation of LPAI phenotype into a HPAI — a systemic disorder with a violent clinical course with up to 100% mortality in domestic poultry (Taye et al., 2017). It was shown by the acquisition of multiple basic amino acids residues (arginine and lysine) in the HA cleavage site at the region between the two HA1 and HA2 (Nao et al., 2017). The irrespective virulence that restricted to subtypes H5 and H7 are reported to animal health authorities as notifiable avian influenza (OIE, 2017). The actions taken upon detection of H5 or H7 LPAI in poultry are to prevent introduction of avian influenza, since measures (e.g. culling) often pertain to clinically

healthy flocks, but the rationale for such procedures is to minimize the risk of mutation from LPAIV into HPAIV. It must be stressed that HPAIV can cause sub-clinical infections in certain species of birds, mostly waterfowl as the main reservoirs of the virus. Avian influenza raises public attention due to the zoonotic potential of selected viruses of H5, H7, H9 and, more recently, H10 subtypes (Turner et al., 2017; Fong, 2017, Su et al., 2016; Munoz et al., 2016).

First reported outbreak of HPAI in Asian country was found in Hong Kong, China during 1997, which the direct transmission of AIV subtype H5N1 from infected birds to humans that led to death of 18 people (Bui et al., 2016; Peng et al., 2017; Verhagen et al., 2015a). Human - to - human transmission of this virus appeared to be rare; isolates from this strain contained only avian genes, which might have been one reason for the ineffective transmission to humans. The HA and receptor-binding specificity of this highly pathogenic H5N1 avian influenza virus was reported to be due to an avian type (Sia $\alpha$ 2-3Gal-specific) gene (Xu et al., 2016). Since December 2003, the AIV virus strain has emerge throughout many Asian countries, including China, South Korea, Japan, Thailand, Vietnam, Indonesia, Malaysia, Cambodia and Laos (Lipatov et al., 2004).

The sporadic outbreaks from 2004 - 2007 indicated that the Malaysian samples changed to the sub-branch that included China samples, reflecting fluctuation of virus regional characteristics in different time periods. The composition of the phylogenetic tree and mutations of key site amino acids in HA proteins reflected the fact that the majority of strains are regional and long term, and virus diffusion exist between countries including between China, Laos, Malaysia, Indonesia, Azerbaijan, Turkey and Iraq composing its own regional virus pedigree and the same origin (Zhang et al., 2010a). Guan and others' evolution study on the H5N1 virus in Asia also shows that lineages with ostensible inheritance and antigen differences have emerged and that viruses become constant in one regional, with characteristics of endemicity (Harfoot et al., 2017; Tian et al., 2015; Muzyka et al., 2016).

The amino acid mutation at HA protein key sites, indicating a “low–high–highest–high–low” pattern of Malaysian strains (WHO, 2007). Virus strains in Asia are from different origins: strains from Southeast Asia and East Asia are of the same origin, whereas those from West Asia, South Asia and Africa descend from one ancestor. The virus has managed to continuously circulate in domestic birds, with frequent transmission to wild birds. During the circulation and spread of the H5N1 viruses, the HA genes diversified into multiple genetic lineages (“clades”), without evidence of gene exchange between the influenza viruses (Shepard et al., 2014). However, this changed from 2009 onward, when HPAI viruses of subtypes H5N2, H5N5, H5N6, and

H5N8 were found to contain the H5 gene of the GsGd lineage, together with NA and various other genes of LPAI virus origin (Verhagen et al., 2015b; Richard et al., 2015).

In this article we give an overview of historical, epidemiological, clinical, and scientific insights into the AIV identified in Malaysia from 1980 - 2017 (Figure 1). Discovery of AIV may be explained as a result of the death infected birds that transmitted the virus to poultry population in the country. Recent studies, highlighting the factors appear to be contributing to the emergence of avian influenza disease including environmental, ecological factors and socio-economic (Bui et al., 2017; Artois et al., 2016; Ortiz-Rodriguez et al., 2013). Here, we discuss probable events that have been involved in the occurrence of the virus, with the purpose of strengthening the existing prevention and control strategies for managing the outbreak of potential probable future avian influenza disease with new and unfamiliar subtypes.

## 2. Methodology

### 2.1. Data extraction and inclusion criteria

The review included all serological and molecular research on avian influenza in Malaysia as the main inclusion criteria. The articles were collected from the MedLine/PubMed(<http://www.ncbi.nlm.nih.gov/PubMed>), Scopus(<https://www.scopus.com/search/form.uri?zone=TopNavBar&origin=sbrowse>), Sciencedirect(<http://www.sciencedirect.com/>), and Elsevier (<https://www.elsevier.com/>) databases. For the systematization of information, a database was built that included the references of all selected publications, as well as the title, author, year of publication, country or countries where the study was conducted, collaborating countries, and language of publication. From the results, the study searched for diagnostic techniques (serological or molecular), percentage of positive samples, viral subtypes diagnosed, and relationship of the local outbreak with neighbouring countries. The peer-reviewed articles on avian influenza in Malaysia published from January 1989 to July 2017.

### 2.2. Search strategy

The processes for searching, selecting, extracting and compiling the publications in the databases were conducted using Medical Subject Headings (MeSH) keywords and Boolean connectors. The articles were selected using a two-stage approach. During the first stage, the publications were selected based on their 'Title, Abstract, Keywords' function to define the specific disease and 'All Fields' function at country level. During the second stage, the full text of the articles that

were previously selected in the first phase was reviewed. At this stage, the search engines had excluded the publications that did not meet the established criteria for the study.

One example search is: (<sup>a</sup>influenza [MeSH Terms] OR (<sup>a</sup>influenza<sup>o</sup>[All Fields] AND <sup>a</sup>birds<sup>o</sup>[All Fields]) OR <sup>a</sup>influenza in birds<sup>o</sup>[All Fields] OR (<sup>a</sup>avian<sup>o</sup>[All Fields] AND <sup>a</sup>influenza<sup>o</sup>[All Fields]) OR <sup>a</sup>avian influenza<sup>o</sup>[All Fields]) OR <sup>a</sup>avian flu<sup>o</sup>[All Fields]) AND (<sup>a</sup>Malaysia[All Fields]).

Data were quality-checked using filtration to remove duplicates and non-validated data. A single case in poultry was considered an outbreak if a single or group of poultry was kept together in a single location at a unit time.

## 3. Highly pathogenic avian influenza (HPAI) viruses

This review is including the H5N1 outbreak in Malaysia in four different periods during 2004, 2006, 2007 and 2017. Migration of dead wild birds, legal and illegal poultry trade, production ecology, smuggling of exposed fighting cocks activities, entrepreneurial nature of small commercial producers and traders, different ways that commercial and smallholder systems manage the outbreak, commercial movements of birds and tourism activities were highlighted as the risk factors along the outbreak., The risk factor during 2017 outbreak is still under investigations.

### 3.1. H5N1 outbreak in 2004

After detection of HPAI H5N1 in the Republic of Korea in December 2003 (Lee et al., 2005), similar outbreaks by antigenically related strains were subsequently detected among poultry in Asia. Simultaneously, the cause for this apparent continuing spread of H5N1 in this region remains unclear (Li et al., 2004b). Starting from the mid of 2004, the emergence of H5N1 virus has expanded to several countries in Southeast Asia resulting outbreaks in backyard and domestic poultry in Vietnam, Thailand, South Korea, Japan, Cambodia, and Indonesia, and Malaysia. The generation of viruses undergo subtle genetic changes through mutation, recombination, and reassortment showed the mechanism of virus genetic variation that increased the host range forming a pandemic.

In August 7, 2004, Malaysia reported the first outbreak of H5N1 that adversely affect the high density of free-range commercial ducks and village poultry in backyard farm (Alexander et al., 2007a). Other 15 outbreaks were occurred after a few weeks until following the onset of symptom index of the initial outbreak that last on November 19, 2004 (Sabirovic et al., 2005) around the index case in the same area (Alexander, 2007b; OIE, 2008; Smallman-Raynor and Cliff, 2008). The 35 HPAI viruses were detected from at

least 12 localities in the state of Kelantan, near the Thailand border. The isolated nine viruses were from ducks and 26 others were from chickens (Sharifah et al., 2005a). The other positive cases of the virus also been detected in a village named Setapak, near Kuala Lumpur and later in Changkat Tualang and Bukit Merah lake town resort in Perak state (Suraini et al. 2004a).

The neuraminidase inhibitors oseltamivir carboxylate, zanamivir, and peramivir were found to be fully sensitive by the vast majority of those H5N1 strains. However, some of the strains have increase in IC50 values. Sequence analysis of these strains revealed mutations in the normally highly conserved residues 116 and 117 of the N1 neuraminidase. Sequence analysis of the Matrix protein (M2) gene showed that all of the Malaysian isolate of H5N1 contained resistant dual mutations (L26I and S31N) associated with adamantane-based drugs, rimantadine and amantadine. The virus had an intravenous pathogenicity index (IVPI) in 4 weeks old chickens. An index of 3.0 means all chickens inoculated intravenously from this virus died within 24 hour (determined by AAHL, Geelong, Australia, the OIE Reference Centre for Avian Influenza). The presence of multiple basic amino-acids adjacent to the HA cleavage site sequence of TIGECKYVKSRLVLTGLRNSPQRERRRKKRGLF and intravenous IVPI index of the isolated virus indicated in high pathogenicity (Sharifah et al., 2013a).

The origin of the epidemic was not established and unclear (Sims et al., 2007). Strikingly, the sublineage of genotype Z H5N1 viruses prevalent in 2004 invariably showed an adamantane-resistance conferring amino acid substitution at position Asn (31) of the M2 gene protein, while this mutations was mostly not present in sublineages of H5N1 viruses isolated in other geographic regions (Li et al., 2004a; Puthavathana et al., 2005; Cheung et al., 2006). Since the emergence of HPAI H5N1 viruses of the A/Goose/Guangdong/1/96 (GsGd) lineage in Southeast Asia, the virus has managed to continuously circulate in domestic birds, with frequent transmission to wild birds. During the circulation and spread of the H5N1 viruses, the HA genes diversified into multiple genetic lineages (“clades”), without evidence of gene exchange between the influenza viruses (Verhagen et al., 2016a). The clade entering Southeast Asia from China as a lineage susceptible to adamantanes. Then, a S31N mutation occurs in a lineage in the Red River Delta in northern Vietnam (called adamantane resistant Vietnamese lineage spread) in 2003 and subsequently the lineage isolated from different hosts in 2004 and 2005 from different countries in continental South East Asia such as Thailand, Laos, Cambodia, Vietnam and Malaysia (Sorn et al., 2013; Hill et al., 2009). The explanation for the similarity of viruses from these geographically separate areas including Malaysia and Thailand remains obscure, although there is known to be legal

and illegal animal trade between these regions via Laos (Smith, 2006a).

H5N1 virus at this time was referred to Z genotype that belonged to clade 1 (Figure 1) that likely generated among poultry from 2002 – 2003 in Yunnan province, Southern China (Pua et al., 2017, Sornerg et al., 2013). Phylogenetic analysis of AIV of the HA gene found in clade 1 was highly homologous and grouped together in a sublineage (VTM sublineage) to the previously isolates from domestic and migratory birds in Thailand, Vietnam, Cambodia and Hong Kong during 2003 – 2005 causing outbreaks in poultry infections in Malaysia (Lekcharoensuk et al., 2012; Suraini et al. 2004b).

The third sublineage of clade 2 (Figure 1) has been found in southern China, Hong Kong, Laos, Malaysia, and Thailand (Pappaioanou, 2009). The clade 2.3 that was derived from viruses previously identified in Yunnan province (Sturm-Ramirez et al., 2005; Yen et al., 2008a) and clade 2.3.4 (Fournié et al., 2012a). A new H5N1 variant (Fujian-like lineage) also has emerged during this period that belongs to Fujian-like variant (clade 2.3.4), related to genotype V (Fournié et al., 2012b). In contrast, viruses circulating in Indonesia belonged to clade 2.1 that correspond to differences in levels of resistance in their isolated viruses. The clade was believed to have originated in Hunan province, China (Guan et al. 2009; Wang et al. 2008). This suggested that the viruses responsible for the outbreaks spread amongst poultry in Indochina including Malaysia on one hand and in Indonesia on the other were each common-source introductions. The perpetuation of H5N1 viruses in Malaysia were maintained within poultry rather than through the repeated reintroduction of viruses from external sources (Peiris et al., 2007). China thus appears to have been the epicentre of disease spread, with countries in Indochina acting as a sink (Wallace et al., 2007). However, within these countries, geographic diversity was already emerging with viruses circulating in northern Vietnam more closely related to those in Thailand, and viruses in the Mekong region related to those in Cambodia (Smith, 2006a). As the protection immunity of cross-clade against H5N1 virus remains questionable, high mutation rate within surface glycoproteins HA and wide antigenic diversity means unclear justification on which H5N1 subtype will cause the next potential pandemic strain.

In ASEAN countries, the disease spread progressively from “source country” to ASEAN Member’s states that are sharing land border and subsequently to other ASEAN member countries. The disease is also capable of spreading from “source country” to other country separated by sea. Epidemiological data on HPAI H5N1 virus clades supported these phenomena. Transmission of the

disease in Malaysia has been stipulated by the other mode via poultry related activities (Lim et al., 2013). Changes of Malaysian isolates to the sub-branch that included China isolates, has indicate the fluctuation of virus regional characteristics in different time period of cases. The virus not only has regional characteristics but has the tendency to spread the infection into neighbouring countries if it has the same origin (Zhang et al., 2010b). Dead wild migratory birds have been associated with the spread of the virus from Asia (Li et al., 2004b), suggesting that the virus in apparently healthy migratory birds can be carried over long distances.

The isolates were more closely related to those already present in Thailand while viruses in the Mekong region related to those in Cambodia (Smith et al., 2006a). This showed that geographic diversity was already emerging with viruses circulating in northern Vietnam that more closely related to those in Thailand. Commercial movements of birds has expand between this region that is thought to be causes of the close similarity between the viruses isolated in northern Viet Nam, Thailand, Cambodia, and Malaysia. Although the quickly precise route of introduction and spread of the viruses to the country was not determined, the initial outbreak was likely to be linked to popular albeit illegal trade poultry activity among villagers. Smuggling of exposed fighting cocks from neighbouring countries such as Thailand and Indonesia had spread the virus (Tee et al., 2009a; Leibler et al., 2009a) to local village poultry in Peninsular Malaysia (Leong et al., 2008a). With increasing reports of HPAI infecting humans in Thailand and Vietnam, the findings highlight the fact that these viruses gain high possibility to enter Malaysia through infected humans (Sharifah et al., 2005b).

According to WHO (2006), production ecology (e.g. ducks in wetlands, backyard systems where multiple species scavenge together and have contact with wild birds, proximity of birds and people, intensification of production, where densely packed commercial units may create a heavy virus load for workers and nearby farms), the entrepreneurial nature of small commercial producers and traders, the different ways that commercial, and smallholder systems management were found as the key factors affecting the spread of the disease in Malaysia. The emergence of the disease was effectively well-controlled and prevented by depopulation and quarantine / clinical surveillance of poultry and birds within a 1-km and 10-km radius of the index case and movement restrictions on birds and their products to other states (OIE, 2008).

During the H5N1 outbreak, about 14,3000 birds were infected. To control the outbreak, culling of infected and exposed or in-contact poultry, active surveillance on poultry is restricted within a 20 km radius of the focus of infection and continuous visual surveillance for signs

of disease progression and mortalities were conducted in the threatened area (Sharifah et al., 2005). Multiple species of poultry were affected during these outbreaks, including domestic fowl, chickens, ducks and wild birds (Sharifah et al., 2013b).

In September 2004, Agri-Food and Veterinary Authority of Singapore has suspended all imports of poultry, eggs and ornamental birds from Malaysia. The disruption of poultry and eggs led to shortage of Singapore's food supply. To ensure resilient supply of safe poultry and egg at source and prevent frequent disruptions to the supply, AVA worked closely with Malaysian counterpart, the Department of Veterinary Services to create Disease-Free Zones (DFZs) in the states of Johor, Perak, Selangor, Malacca, and Negeri Sembilan. To increase the level of product quality and safety, all accredited poultry farms are located in DFZs. The DFZs, together with the introduction of enhanced disease control and surveillance programmes, enabled exports to resume in January 2005 without compromising animal or public safety (Leong et al., 2008b). The rationale for establishing and maintaining the DFZs is to allow the export of poultry and eggs from the DFZs to continue should there be another HPAI outbreak in Malaysia outside the DFZs (Poovorawan et al., 2013).

No human cases were reported during this period. This is due to the Malaysian viruses, possessed Glu (^27) on the PB2 gene (Sharifah et al., 2005c) found that the viruses have the affinity for avian rather than human cell receptors.

### 3.2 H5N1 outbreak in 2006

Genetic and antigenic analyses revealed the emergence and predominance of a H5N1 virus sublineage (Fujian-like) in poultry during late 2005. Serological evidence of H5N1 suggests the seroconversion of the virus in market poultry is low and the vaccination may have facilitated the selection of the Fujian-like sublineage. Emergence and predominance of H5N1 variant had replaced most of the previously established circulating multiple regional distinct sublineages in different regions of southern China. These viruses were subsequently transmitted to Malaysia, resulting in a new outbreak wave in Southeast Asia (Smith, 2006b).

On February 7 - March, 2006, a domestic fowl and poultry sample detected as containing highly pathogenic H5N1 subtype at a free-range poultry farm. The emergence of new variant, called a Fujian-like sublineage (clade 2.3.4) (Figure 1) has also reported during this period, related to genotype V (WHO-OIE-FAO, 2008, Kwon et al., 2011). Origins of the avian

influenza outbreaks largely confined to a wider geographical area compared the previous outbreaks in 2004. The incidence of the disease involved villages in Kuala Lumpur and the states of Perak and Pulau Pinang along the more industrialized west coast of Peninsular Malaysia (OIE, 2008). The outbreak occurred over a wider geographical area originated from villages in Kuala Lumpur and the states of Perak and Pulau Pinang along the more industrialized western coast of Peninsular Malaysia (OIE, 2008).

Phylogenetic analysis of the H5 and polymerase basic 2 (PB2) genes identified that the H5N1 strain was different from the 2004 outbreak but highly similar to the strain from Indonesia and China, suggesting the repeated introduction of variety H5N1 lineages into the country, possibly by legal and illegal trade in poultry and its by-products, legal or illegal and commercial movements of birds rather than by migratory birds (Kilpatrick et al., 2006a; Smith et al. 2006b). The Indonesian officials suspected that the introduction H5N1 from Indonesia to Malaysia by boating activities (Leibler et al., 2009b). Although the variant also been isolated with other Southeast Asian countries such as Laos, Vietnam, and Thailand (Smith, 2006b; Saito et al. 2008), the H5N1 isolates from Malaysia did not grouped together in a VTM sublineage with the viruses from Vietnam, Thailand, Laos, and Cambodia as occurred in 2004.

One dominant sub-lineage (clade 2.3) has replaced those previously identified H5 sublineages in affected poultry from distinct geographical region (Smith et al., 2006c). This could be driven by continuous mutation process occurring in HA gene of H5N1 viruses during its circulation in birds that enhanced evolution of new sublineages from poultry in 2006 (Chen et al., 2006). The viruses from clade 2.3 isolated from human cases from China also belonged to this same sublineage from poultry in Laos and from wild birds in Hong Kong. This suggests the emergence and predominance of this sublineage in Southeast Asia since 2006 (Smith, 2006c). After the critical period in 2006, no subsequent case has been reported in Malaysia which suggests that there is no current potential for an outbreak risk which considers no local transmission among the local bird populations.

Detection by Reverse transcription polymerase chain reaction (RT PCR) for H5, H6, H7, and H9 strains of wild forest birds and a few shore birds from 2004 - 2006 were tested negative (Rahim et al., 2015b). This probably suggests that birds in Malaysia are considered free from the virus. Since the transmission between birds and from birds to human is low, it is expected that the strains introduced on both the

domestic poultry and game bird in Malaysia were mostly likely from migratory birds or poultry trade. Malaysia form part of high potential for flyways migration route for wild birds, giving much attention. Hence, eradicating the introduced birds of the infected fowl and the seizure the farm help to control the transmission of AIV in Malaysia. This could further justify the report by the MOHM and WHO which reported that Malaysia is virus-free for the disease for human infection although the geographical location is bordered by countries that contained the virus.

However, the infection of wild birds by the virus is still a major concern because birds are free-moving organism whose interact between birds and other fauna. Multiple species of poultry were affected during these outbreaks, including infected free-range chickens, ducks, and quails (Kilpatrick et al., 2006b).

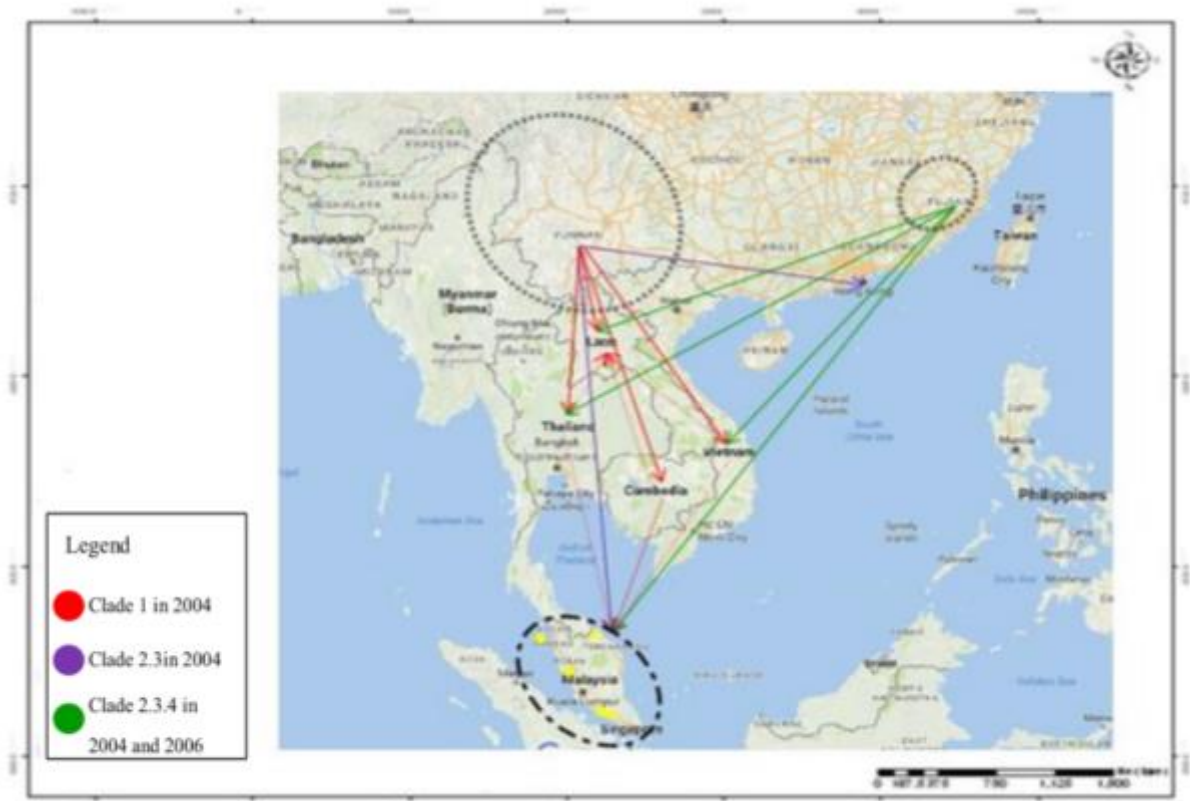
### 3.3. H5N1 outbreak in 2007

In June 2, 2007, there were 10 outbreaks detected in a poultry village in Sungei Buloh, Selangor state (OIE, 2008). Through effective prevention and control measures, the cases were resolved within few months. At this time, a recombinant gene encoding of peptide epitopes, consisting fragments of HA1, HA2 and a polybasic cleavage site of H5N1 strain Malaysia that was amplified and cloned into pET-47b(+) bacterial expression vector (Chee et al., 2014).

As AIV are known to occur in the neighbouring countries such as Indonesia and Thailand, it is not impossible for the virus to be re-introduced to Malaysia. A study by Ramji (2009) adhered to the following finding as well. The early occurrence of infection in Malaysia was in Kota Bahru, Kelantan and prompt further detection in Setapak, Kuala Lumpur. The domestic fowl import from neighbouring countries and tourism activities are the risk contributor to the spread of virus to Malaysia. Kalimantan, Indonesia has reported serious cases of H5N1 infection (WHO, 2007). Although Kalimantan is the Sabah's and Sarawak's nearest neighbours, no case has been found in both regions.

### 3.4. H5N1 outbreak in 2017

Since 2007, no other case of AIV has been detected in Malaysia until 2017. The H5N1 outbreak in 2017 has been speculated previously that there is a tendency for the re-occurrence of HPAI H5N1 among poultry (Tee et al., 2009b). As compared with other countries that have temperate climates, there is no seasonal pattern of the virus circulation in Malaysia because the areas lying close to the equator approx. 0–10°N latitude (Malaysia and Singapore) (Saha et al., 016)



**Figure 1.** Local dispersion of HPAI A (H5N1) clades together with cumulative spatial distribution HPAI A (H5N1) outbreaks in poultry in 2004 and 2006 (location of outbreaks shaded between yellow dot).

However, the large epidemic period from the last epidemic in nearly 10 years has aroused speculation that spread and infection of the virus can be associated with certain seasons and perhaps some other climatic factors including rainfall and temperature. The virus is compatible at the specified temperature, pH, salinity, and other physical environmental parameters. The viruses strains have the tendency to die off if the temperature in an introduced environment is not similar and ecological condition or not suitable to the avian biology. In local climate, the higher tendency for transmission of virus at frequency at relative low humidity of about 20 % to 35 % and at temperature between in 5°C as compared to 20°C. Average temperature for all the surveyed habitats ranged from 25°C - 28°C which may be too extreme for virus transmission (Rahim et al., 2015c).

After September 2007, the outbreak re-occurred in Kota Baharu, Kelantan and declared as a state disaster by the Kelantan Government. Until now, no human infection by avian influenza virus was reported in Malaysia. However, widespread circulation of AIV among susceptible avian populations has created the significant number of mortality and morbidity. This

raised a great concern over the high mutation rates and frequent genetic reassortment of the RNA into different segment or shifting between avian and human viruses in mammals, which could enhance to the generation of potentially influenza pandemic of novel strains that mitigate transmissibility of AIV among human.

#### 4. Low pathogenic avian influenza (HPAI) viruses

The early case of a laboratory-confirmed AIV in Malaysia is H4N3, H4N6, and H3N6 in 1989, isolation from infected passerines and domestic birds (Ibrahim et al. 1986, Ibrahim et al., 1990a). The influenza isolates are from a magpie robin (*Copsychus saularis musicus*) which had died at a transit aviary in Selangor that designated A/magpie robin/Malaysia/23/89 (H4N3) and the other from a yellow vented bulbul (*Pycnonotus goiaver personatus*) which had been found sick and unable to fly was designated A/bulbul/Malaysia/41/89 (H4N3) (Ibrahim et al., 1990b). The subsequent isolates of LPAI subtypes is H5N6 in 1989 (Ibrahim et al., 1990; Aini and Ibrahim, 1986), H2N9 in 1992 (Verhagen et al., 2016b), H4N1 in 1992 (He et al,

2013a), H7N1 in 1994, H2N9 in 1996 (Verhagen et al., 2016c), H10N5 in 1998, H3N2 in 2002 (Alexander et al., 2007c), H3N8 in 2004 (Ng et al. 2006), and H9N2 in 1998 (He et al., 2013b). The circulation of H3N2 is reported in 2010 WHO. In addition, H5N2 has been detected during 2004 and 2014 (Rasoli et al., 2010; Hakim et al., 2013).

H9N2 outbreak in Malaysia occurred in mid 1900s and then re-emerged in 2004. The virus was tested positive for H5N2 in a poultry farm at Perak state, which exports its products to Singapore. The high prevalence of H9N2 subtype throughout Asia, along with their demonstrated ability to infect mammals including human, puts them high on the list of influenza viruses with pandemic potential (Chaharaein et al., 2006).

## 5. Conclusion

Clade 2.3 viruses might spread over an extensive area, similar to 2.3.4, because clade 2.3.4 viruses are widespread from Fujian province, China that continuously evolving in the regions where H5N1 virus are endemic. Clade 2.3 viruses have been circulated in Yunnan province, China. Clade 2.3 subtype H5N1 viruses have been circulating in Southeast Asia and have accumulated continuous mutation process occurring in HA gene of H5N1 viruses during its circulation in birds that enhanced evolution of new sublineages in 2004 and 2006. New clade 2.3.4 with reassortants that possessed a seroconversion of the virus from most of the previously circulate multiple sublineages in different regions of southern China which probably transmit the virus to Malaysia. Although Clade 2.3.4 circulated in Malaysia in 2004 and 2006, but outbreaks of a wider geographical area was observed in 2004 compared to 2006. The legal and illegal trade in poultry and its by-products, legal or illegal and commercial movements of birds might be related to these outbreaks, rather than by migratory birds.

The detection of H5N1 virus in poultry might forecast a poultry outbreak if surveillance and control measures in poultry is inadequate. However, after 2007, DVS reported H5N1 virus infections without concurrent poultry outbreaks until 2017. The long period of the subsequent outbreak cases suggest that the H5N1 was spread from farm to farm by poultry movement and inefficient biosecurity so as so that the virus strains of poultry were grouped in sublineages within similar region. The study can conclude that early detection of avian influenza outbreaks and a rapid response are essential in controlling the introduction of virus from migratory birds to poultry and in preventing farm-to-farm spread.

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Table 1. Avian influenza outbreaks in Malaysia from 1980 – 2017

AIVs Subtype	Clade	Pathognicity	Province/ autonomous region	Date of report	Outbreaks	Possible risk factors
H5N1	1	HPAI	Kelantan Setapak,	August 7, -	16	i. Migration of dead wild birds from Asia (Li et al., 2004)
H5N1	2.3 2.3.4	HPAI	Kuala Lumpur Changkat Tualang, Perak Bukit Merah lake town resort, Perak	November 19, 2004		ii. Legal and illegal trade within the region of Viet Nam, Thailand, Cambodia, and Malaysia (Smith, 2006c) iii. Smuggling of exposed fighting cocks activities from neighbouring countries such as Thailand and Indonesia (Tee et al., 2009c; Leibler et al., 2009c) iv. Production ecology (e.g. ducks in wetlands, backyard systems where multiple species scavenge together and have contact with wild birds, proximity of birds and people, intensification of production, where densely packed commercial units may create a heavy virus load for workers and nearby farms); v. Entrepreneurial nature of small commercial producers and traders; vi. Different ways that commercial and smallholder systems manage (OIE, 2006)
H5N1 H5N1	2.3.4	HPAI HPAI	Kuala Lumpur Perak Pulau Pinang	February 7 - March, 2006	N/A	i. Poultry legal and illegal trade and commercial movements of birds from China and Indonesia (Kilpatrick et al., 2006c) ii. Migration of dead wild birds from Asia (Rahim et al., 2015d).
H5N1	N/A	N/A	Sungei Buloh, Selangor Kota Bahru, Kelantan Setapak, Kuala Lumpur. Petaling, Selangor	June 2, 2007	10	i. Import of domestic fowl from the neighbouring countries; ii. Tourism activities (WHO, 2007)

H5N1	N/A	HPAI	28 locations in six districts that involved 43 premises in Kelantan	February – March, 2017	N/A	N/A
H5N2	N/A	LPAI	Perak	2004 and 2014	N/A	N/A
H3N2	N/A	LPAI	N/A	2010	N/A	N/A
H3N8	N/A	LPAI	N/A	2004	N/A	N/A
H3N2	N/A	LPAI	N/A	2002	N/A	N/A
H9N2	N/A	LPAI	N/A	1998	N/A	N/A
H10N5	N/A	LPAI	N/A	1998	N/A	N/A
H2N9	N/A	LPAI	N/A	1996	N/A	N/A
H7N1	N/A	LPAI	N/A	1994	N/A	N/A
H2N9	N/A	LPAI	N/A	1992	N/A	N/A
H4N1	N/A	LPAI	N/A	1992	N/A	N/A
H4N3	N/A	LPAI	Selangor	1989	N/A	N/A

N/A = Information are not available

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