CHAPTER 2

Literature Review

2.1 Avian influenza

2.1.1 Background of avian influenza

Avian Influenza (AI) is a highly contagious disease of poultry, which has become a threat to food security and public health in countries around the world. The disease is caused by influenza type A viruses of the Orthomyxovirideae family. Aquatic birds were revealed as the reservoir for all influenza viruses. Influenza type A viruses can be transmitted to various animal species (e.g. birds, horses, pigs, sea mammals, etc.) and humans. Therefore, AI type A viruses are pathogens important for both veterinary and human health in the world. (Horimoto & Kawaoka, 2001).

The epidemiology of AI in the last 13 years was complex and characterized by two main global panzootics. The first panzootic wave started in 2004, peaked in 2006, and ended in 2012. The ongoing second panzootic has been observed since 2013 with a maximal activity in 2015 (OIE, 2018). Data from the World Animal Health Information System (WAHIS – OIE) shows that, from January 2014 to November 2016, AI has been identified in 77 countries with 13 detected strains of AI viruses. Avian Influenza outbreaks continue to be a public health concern globally, due to the circulation of various AI virus strains (H5N1, H5N6, H5N8, H7N9, etc.) is ongoing.

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2.1.2 Structure of avian influenza viruses

Most of the AI viruses have a spherical shape, 80-120 nm in diameter, but polymorphological forms can also occur. An AI virus particle (virion) contains the genome (i.e. genetic material of the virus) with about 250 million daltons of molecular weight (Shaw & Palese, 2013). The viral genome consists of 8 segments of negative-sense single-stranded RNA, encoding for 15 proteins in overall among those,10 proteins including HA, NA, NP, PA, M1, M2, NS1, NS2, PB1, and PB2 have been well-described functionally while the 5 proteins PB1-F2, PB1-N40, PA-X, PA-N155, and PA-N182 just recently have been functionally identified (T. H. Nguyen et al., 2016). The outer structure of an AI virus consists of a membrane having 3 integral proteins, the HA (haemagglutinin), NA (neuraminidase), and M2 (matrix 2) proteins (Figure 2.1). Approximately 80 percent of the glycoprotein spikes embedded in the envelope are HA which enables the virus to attach to the surface of a host cell and undertakes its hemagglutinating activity. Importantly, the HA protein induces the host immune system to produce antibodies neutralizing the virus which are essential for the host protective immunity against AI virus infection. The NA facilitates the release of newly produced virus particles from the host cell. The two surface antigenic proteins HA and NA perform crucial functions in the host humoral immune response to AI viruses.

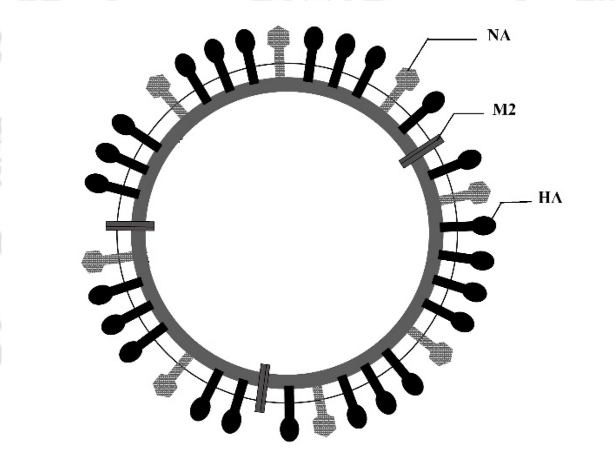

Figure 2.1 Outer structure of an avian influenza virus HA.

2.1.3 Classification of Avian Influenza viruses

As all AI viruses belong to influenza type A viruses, further classifying and subtyping is based on the antigenicity of two surface proteins, hemagglutinin (HA) and neuraminidase (NA). Up-to-date, 18 HA subtypes (H1–18) and 11 NA subtypes (N1–11) have been identified among influenza A viruses (Le & Nguyen, 2014). Numerous types of HA and NA enable genetic reassortment resulting different combinations of these genes for the emergence of new subtypes of AI virus (Lu et al., 2014).

Based on pathogenicity and the basis of severity, AI viruses in domestic poultry are classified into two distinguished forms: highly pathogenic avian influenza (HPAI) and low pathogenic avian influenza (LPAI). Low pathogenic AI is caused by influenza viruses

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of low virulence and appears as depression, mild respiratory diseases, ruffled feathers and decreased egg production in laying poultry. However, LPAI viruses of subtypes H5 and H7 can mutate into HPAI viruses. To date, all outbreaks of the HPAI have been caused by some viruses of subtypes H5 or H7 of AI (T. H. Nguyen et al., 2016). Highly pathogenic AI is a severe disease caused by highly virulent influenza viruses and characterized by severe to fatal infections in domestic poultry worldwide. The disease can have systemic spread to affect many internal organs. Mortality rate at 100% can be approached in infected poultry flocks within a very short duration.

According to the WHO/OIE/FAO H5N1 Evolution Working Group, H5N1 viruses can be classified into numerous virus "clades" based on the HA gene. Clades are defined by 3 criteria: (1) sharing of a common ancestral node in the phylogenetic tree (clade-defining); (2) monophyletic evolution with a bootstrap value of ≥60 at the clade-defining node (after at least 1000 neighbor-joining bootstrap replicates); and (3) average percentage pairwise nucleotide distances of <1.5% within clades (WHO/OIE/FAO H5N1 Evolution Working Group, 2014). Viruses within initial clades can continue to evolve, forming sublineages (i.e. subclades) periodically. Once these subclades meet the clade-defining criteria as their initial clades, they are designated as separate clades. Under the WHO/OIE/FAO classification system, 10 initial clades (0 - 9) and many subclades of Asian-lineage H5N1 have been recognised (Smith & Donis, 2015). When separate monophyletic groups emerge within a specific clade and meet the nucleotide divergence criteria (and having bootstrap values >60), they are split into second order clades but still a part of the initial first order clade. As a second order clade continues to evolve it may be split into third order clades and so on. The same clade designation criteria apply to first, second, and any higher order clade designations. Fifth-order groups are designated using an additional letter to the right of the fourth order clade (i.e., 2.3.2.1a) (Smith & Donis, 2015).

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2.1.4 Evolution of Avian Influenza viruses

Influenza type A viruses have high genetic variability as a result of molecular changes in the RNA segments that happen due to two most important mechanisms which are point mutation (referred to as antigenic drift) and reassortment (i.e antigenic shift). New virus strains develop through both mutation and reassortment events. Mutations in the antigenic structure of influenza viruses have formed numerous subtypes and strains. Point mutations are minor changes in the viral genome with amino acid changes in HA and/or NA and in turn, gradual changes in the antigenicity of the virus. RNA viruses like AI viruses are subject to point mutations because of lack of proofreading ability. In vaccinated poultry populations, immunological pressure, or more likely inadequate population immunity, may drive antigenic drift (David E. Swayne, 2013). Antigenic drift in H5 HA has given rise to the formation of numerous new strains and clades of the HPAI A/H5N1. Genetic reassortment (or referred to as ‘antigenic shift’), by contrast, occurs when a host cell is simultaneously infected with at least two different influenza viruses that gene segments from different viruses combine to produce a new hybrid virus. In theory, from the shuffling of the 8 genomic segments of the influenza virus, 256 combinations of RNA be possibly produced. In addition, mixed infections, which occur relatively frequently in nature, can lead to genetic reassortment. This mechanism results in drastic changes in the antigenicity of influenza viruses resulting in new HA and/or NA proteins and new subtypes of the virus. The broad susceptible host range of influenza viruses appears to facilitate this event. On the other hand, populations will have no immunity to the new emerging subtype due to its distinct proteins to the previously circulating strain while scientists are unable to prepare effective vaccines against them in advance (van den Berg et al., 2008). Multiple lineages of viruses can be quickly created by those frequent mutation and reassortment events. In the case of HPAI H5N1, 7 of the 8 gene segments (except H5 HA) originally present in the 1997 virus from Hong Kong have been replaced by reassortment events over the years with segments from different avian lineages. (Kelly et al., 2008). As a consequence of genetic reassortment, the emergence of a new virus subtype can cause an epidemic in a very short amount of time.

2.1.5 Pathogenicity of Avian Influenza viruses

Although only 2 pathotypes of AI viruses can be illustrated in the laboratory (i.e. highly and low pathogenic), natural infection by AI viruses results in a wide range of clinical outcomes as the 4 following categories: highly virulent, moderately virulent, mildly virulent, and avirulent. Categorization has been conducted based on mortality patterns, clinical signs and lesions observed in poultry in the field. Factors that can influence those outcomes include virus strain, host species, the age of the host, host immunity, and environmental factors, etc. The highly virulent infection by highly

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pathogenic H5 or H7 AI viruses usually results in severe, highly fatal systemic disease with possibly 100% morbidity and mortality rate, especially in chickens or closely related gallinaceous birds (David E. Swayne, 2013).

The ability of AI viruses to cause disease vary greatly by species (D. L. Suarez & Schultz-Cherry, 2000). For instance, viruses that are highly pathogenic for chickens may show no or mild clinical signs in ducks or other species (Alexander et al., 1978; Cooley et al., 1989). The primary determinant of the viral virulence and pathogenicity in birds is the haemagglutinin (HA). Briefly, the HA protein of an AI virus adsorbs to host cell receptors containing sialic acid. Cleavage of the HA protein into two subunits (i.e. HA1 and HA2) is important for viral infectivity that the virus becomes infectious to replicate inside the host cell (Stephenson et al., 2004). The HPAI viruses can result in multi-system infection because their HA can be cleaved by ubiquitous furin proteases that are present in a range of tissues throughout the host body. In contrast, the HA1 unit of LPAI viruses can be cleaved by only trypsin-like proteases limited to the respiratory tract or the intestinal tract of the host, which accounts for the restricted replication and lower virulence resulting in localized or mild infection. Another issue of an AI virus pathogenicity is the binding between the receptor-binding site of the HA and the receptor on the host cells. The binding avidity of AI viruses to avian cells varies greatly depending on the structure of the oligosaccharide which impacts host specificity and cell, tissue, and organ tropism within the host. Changes in the receptor-binding site of the HA have been shown to change the host range of an influenza virus (Naeve et al., 1984).

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Avian influenza viruses exert cell pathology in the host by two mechanisms: necrosis or apoptosis (David L Suarez et al., 1998). In case of HPAI, the virus initially replicates in epithelial cells then disseminates through the vascular and lymphatic systems to various organs. When the virus replicates in the parenchymal cells of the organs, it results in necrotic and apoptotic cellular death, followed by inflammation and multi-organ failure. Conversely, the virus may instead replicate in the endothelial cells, resulting in damage to the blood vessels and increased permeability with oedema, haemorrhage, microthrombosis and multiorgan failure (David E Swayne, 2009).

2.2 Highly pathogenic avian influenza (HPAI)

2.2.1 Identification and significance of HPAI

According to the World Organization for Animal Health (OIE Terrestrial Manual, 2016), based on the pathogenicity in chickens, the following criteria have been adopted for the classification of an AI virus as highly pathogenic: (1) by an intravenous inoculation with 0.2 mL of a 1:10 dilution of a bacteria-free, infective allantoic fluid, a minimum of eight susceptible 4- to 8-week-old chickens must show >75% mortality within 10 days or (2) 10 susceptible 6-week-old chickens inoculated with the virus resulting in an intravenous pathogenicity index (IVPI) of > 1.2. The HPAI receives immense attention in the world and thus, for the purposes of the OIE Terrestrial Code, any infection of poultry caused by any HPAI virus or by H5 and H7 subtypes of LPAI must be notifiable to the authorities in countries and the OIE. HPAI in other birds rather than poultry, including wild birds, are also notifiable.

The HPAI is a disease of global interest due to the continued increase in the number of affected countries and subtypes. According to a recent report of the OIE, 14 HPAI virus strains found and 80 countries affected by the disease (Pavade, 2017). Occasionally, interspecies transmission of HPAI viruses has occurred. The disease has caused huge economic losses, given the high mortality, massive culling in poultry and the huge cost of control measures. According to the latest update of the OIE for HPAI, domestic poultry in all regions was affected by the disease outbreaks and more than 60 countries and territories reporting the disease occurrence at least once in the period of 2013 - 2017. The most affected regions were Europe and Asia (Figure 2.2). Until February 2018, 11 countries and territories including Cambodia, P. R. China, Chinese Taipei, India, Iraq, Korea, Mexico, Netherlands, Saudi Arabia, South Africa and Vietnam in 3 world regions (Africa, Americas and Asia) were still affected (OIE, 2018). More than 100 million poultry (mostly chickens) have been destroyed in an effort to contain the HPAI outbreaks during January 2013 – January 2018. More than half of reported losses occurred in Asia. Severe economic losses have been caused by the HPAI in terms of high poultry mortality and culling rate, reduced production and price of poultry products, ban on exports, especially the costs of control and preventing measures.

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HPAI has had great public health significance. Although AI viruses are highly species-specific, HPAI is a zoonosis disease which primarily affects animals. However, on rare occasions, the disease can cross the species barrier to infect humans. Indeed, the HPAI has been threatening human health due to sporadic cases of HPAI subtype H5N1 occurring in people since 1997. Transmission to humans has occurred via close contact with infected birds or heavily contaminated environments. However, the unknown possibility for transmission of H5N1 viruses between humans makes this a critical human health risk.

Among subtypes of the HPAI type A, the H5N1 viruses (especially those of the Asian lineage) have drawn much attention due to significant outbreaks and high mortality in both domestic and wild birds over the last few years. Birds infected with the H5N1 viruses show a big variety of disease ranging from subclinical to fatal systemic illness. The virus virulence in poultry and wild birds as well as the ability to infect mammalian species has raised global concerns. Moreover, the HPAI H5N1 viruses have also infected humans, while other AI viruses are generally highly species-specific.

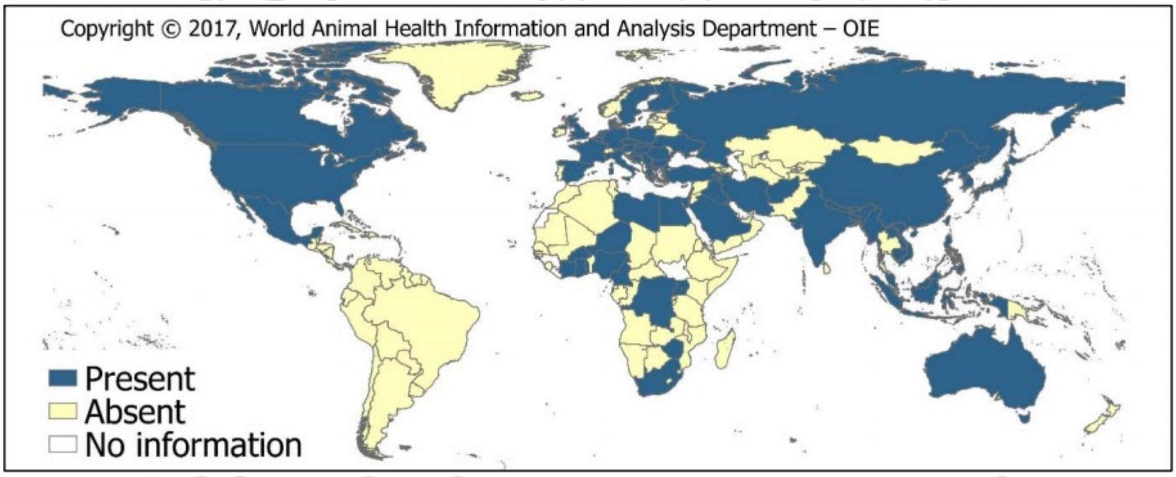

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Figure 2.2 Countries and territories affected at least once by HPAI outbreaks in domestic birds, January 2013- January 2018. (in the OIE Situation Report for Highly Pathogenic Avian Influenza, latest update: 28/02/2018)

2.2.2 Historical background and current situation of subtype H5N1 HPAI in the world

Early, HPAI was considered a rare disease in poultry. The disease was known as ‘fowl plague’ when it was identified in 1955 by Schäfer. Shortly afterwards, an ancient strain of HPAI virus of the H5N1 subtype was found in chickens in Scotland in 1959 (A/Chicken/Scotland/59) (Horimoto & Kawaoka, 2001), which was also known as the cause of the first HPAI outbreak due to a virus of H5 subtype (Alexander, 2007). Subsequently, the H5N1 subtype of HPAI was firstly isolated in Asia in 1996 from a goose farmed in Guangdong province, China. Outbreaks of a similar virus were reported in live animal markets and poultry farms in Hong Kong later in 1997. In 2003, HPAI type A H5N1 viruses were found in Vietnam and Indonesia then became enzootic in poultry in Asia (D. E. Swayne et al., 2015). None of the previous outbreaks had ever approached the size of the Asian outbreaks of H5N1 in late 2003 and early 2004. In terms of geographical distribution, the rate of spread and severity, HPAI A/H5N1 in poultry in Asia had been defined as unprecedented (WHO 2004/03/02).

In 2002, genotype Z of Asian lineage H5N1 HPAI viruses emerged. A series of genetic reassortment events gave rise to the dominance of H5N1 genotype Z in Asian domestic poultry which was responsible for the outbreaks in late 2003 and early 2004. All of the viruses that caused outbreaks in Indonesia, Vietnam and Thailand during that time were genotype Z viruses. Epidemics in Asia were caused by various viruses genotypically linked to the Goose/GD/96 lineage via the HA gene (Sims et al., 2005). A number of strains have been circulating in the region since the first outbreaks in 2003. Intragenic variation (antigenic drift) in the HA protein has resulted in numerous clades, typically marking the major timelines of the viral evolution and vaccine used in each period of time. Since the first outbreak, 10 distinct clades and many sub-clades of Asian-lineage of the HPAI subtype H5 viruses have been recognised to circulate in poultry population in Asia (WHO/OIE/FAO H5N1 Evolution Working Group, 2014). Earlier, the viruses isolated from Hong Kong in 1997 was of clade 0. Later, viruses isolated from outbreaks in Thailand, Vietnam, Laos, Cambodia and Malaysia were included in clade 1. Among the H5N1 clades, clade 2 showed significant genetic variation into numerous subclades (V. N. Bui et al., 2014) as the H5N1 viruses from Indonesia and Quinghai were clustered in clade 2.1 and 2.2, respectively, while those from Japan and Korea were of clade 2.5. The Chinese Fujian-liked viruses belonged to a separated clade 2.3.4. The antigenic properties of viruses in each clade are different to a certain extent and potentially influence the protective efficiency of H5N1 vaccines (Lekcharoensuk, 2008).

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The propagation of disease in the region has primarily resulted from the movement of domestic poultry or their products, and people (Eagles et al., 2009). The high density of ducks raising in Asia has been found to play a key role in the epidemiology and transmission of HPAI H5N1 viruses to other domestic birds (Gilbert et al., 2006). Virus dissemination has been potentially facilitated by the trade of live poultry, which has been very common in the region, as various susceptible poultry species coming from various locations being brought together and having direct contact to each other. This practice could facilitate the exchange and spread of viruses (Fournié et al., 2012). In addition, wild birds had a potential contribution to the maintenance and wide spread of H5N1 viruses in Asia (Li et al., 2004).

The occurrence of HPAI subtype H5N1 in poultry and humans has been particularly high in Southeast Asian and Egypt (Soares Magalhaes et al., 2010). Outbreaks in Asia have resulted in high mortality and depopulation of hundreds of millions of birds (especially domestic poultry) as well as fatal human cases. Since the emergence of HPAI H5N1 subtype in 1997, more than 60 countries in Asia, Europe and Africa have notified the disease outbreak at least once to the OIE (Amen et al., 2015) (OIE, 2016). The disease has caused huge economic losses to the poultry production, given not only death and culling of affected flocks, loss of markets but also costly prevention, management, or eradication the viruses and disease. Currently, there are six countries where H5N1 HPAI is considered endemic including China, India, Indonesia, Vietnam, Bangladesh, and Egypt. Vietnam has the highest number of reported H5N1 outbreaks in the world. From the end of 2003 to 28 November 2016, Vietnam had 2746 outbreaks of HPAI subtype H5N1 in poultry notified to the OIE (OIE, 2016).

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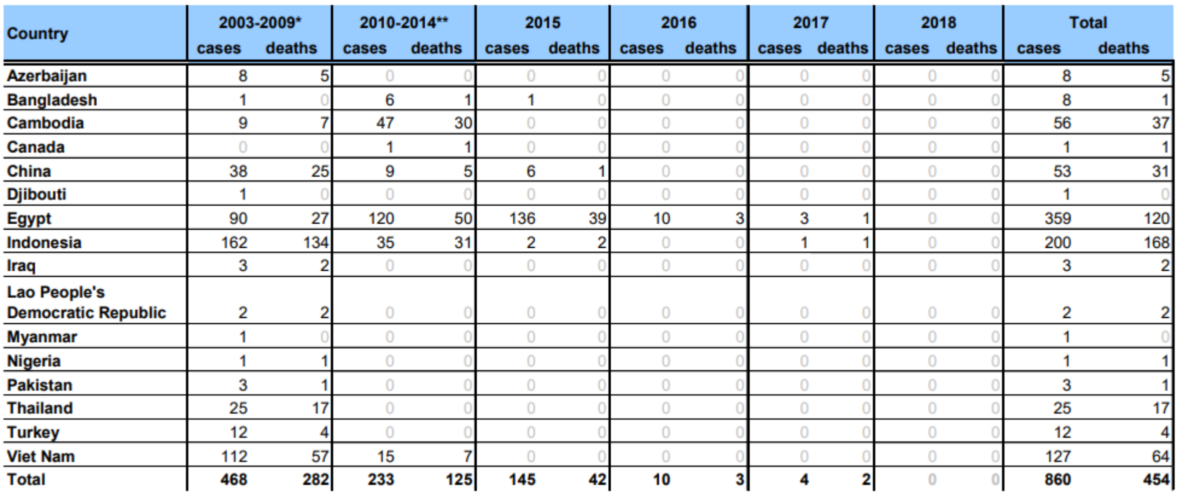
In 1997, the HPAI virus was known to cross the species barrier to infect humans as there were no reports of human death due to the disease before. With the Hong Kong outbreak, which caused 18 cases (6 fatal), it was obvious that these viruses are potentially virulent to humans. Subsequently, hundreds confirmed human cases of HPAI A/H5N1 had been reported to the WHO. From 2003 to 2018, 860 human cases (458 death) of type A H5N1 virus infection have been reported from 16 countries (see in Figure 2.3). Exposure to birds was involved in most of the cases of human infection with H5N1 viruses, which includes exposure via slaughtering and processing of bird meat, bird death next door, visiting or trading in live-bird markets, consumption of sick or dead poultry, etc. Viruses isolated from humans were found as in the same clades of those circulating in local poultry (Hammond et al., 2017).



Country	2003-2009*		2010-2014**		2015		2016		2017		2018		Total	
	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths
Azerbaijan	8	5	0	0	0	0	0	0	0	0	0	0	8	5
Bangladesh	1	0	6	1	1	0	0	0	0	0	0	0	8	1
Cambodia	9	7	47	30	0	0	0	0	0	0	0	0	56	37
Canada	0	0	1	1	0	0	0	0	0	0	0	0	1	1
China	38	25	9	5	6	1	0	0	0	0	0	0	53	31
Djibouti	1	0	0	0	0	0	0	0	0	0	0	0	1	0
Egypt	90	27	120	50	136	39	10	3	3	1	0	0	359	120
Indonesia	162	134	35	31	2	2	0	0	1	1	0	0	200	168
Iraq	3	2	0	0	0	0	0	0	0	0	0	0	3	2
Lao People's Democratic Republic	2	2	0	0	0	0	0	0	0	0	0	0	2	2
Myanmar	1	0	0	0	0	0	0	0	0	0	0	0	1	0
Nigeria	1	1	0	0	0	0	0	0	0	0	0	0	1	1
Pakistan	3	1	0	0	0	0	0	0	0	0	0	0	3	1
Thailand	25	17	0	0	0	0	0	0	0	0	0	0	25	17
Turkey	12	4	0	0	0	0	0	0	0	0	0	0	12	4
Viet Nam	112	57	15	7	0	0	0	0	0	0	0	0	127	64
Total	**468**	**282**	**233**	**125**	**145**	**42**	**10**	**3**	**4**	**2**	0	0	**860**	**454**

Figure 2.3 Cumulative number of confirmed human cases of avian influenza A H5N1 reported to the WHO. (From WHO, 2 March 2018).

2.2.3 H5N1 HPAI situation in Vietnam

2.2.3.1 Background

Vietnam is a country that is located in Southeast Asia, where agriculture contributes much to the national economy. Food animal production contributes around 32% of the total agricultural gross domestic product (T. H. Nguyen et al., 2016). Since the re-emergence and epidemic of the HPAI subtype H5N1 in Asia in 2003, Vietnam has been within the most severely affected countries and has become one of 6 countries in which H5N1 is endemic in poultry (other 5 countries including China, Indonesia, India, Bangladesh and Egypt). The disease has threatened both poultry producers (especially smallholders who depend on husbandry for their livelihood) and public health. H5N1 HPAI has had serious, detrimental effects on the economy since its first reported outbreaks in 2003. Millions of poultry were culled, poultry production was reduced during disease occurrences. Market demand and price were also decreased causing further benefit losses to poultry producers that smallholders were heavily affected. In late 2003, the first known cases of human infection with the HPAI H5N1 viruses in Vietnam occurred. Until now, the country has a total of 127 confirmed HPAI-H5N1 human cases (64 of which were fatal) reported to the WHO (see in Figure 2.3). Since 2014, there have been no new cases. Human infections in the country have been related to close contact

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with infected (sick or dead) poultry and consumption of undercooked poultry meat or blood (Songserm et al., 2006).

2.2.3.2 Epidemic waves

Vietnam experienced 5 large epidemic waves of HPAI subtype H5N1 (C. C. Tran et al., 2016). The first wave in December 2003 - February 2004 involving 2506 outbreaks and 38,830,535 culled birds. The second wave commenced on early December 2004 and finished on early April 2005 when it included 1511 reported outbreaks and 2,171,312 birds culled. The third epidemic wave started on late October 2005 and lasted until the middle of December 2005 including 457 outbreaks, and 891,915 destroyed birds. The fourth wave was reported in from December 2006 to January 2007 when 99,040 poultry was culled. In the fifth wave reported from May to September 2007, 169,188 domestic birds were culled. The first 3 waves occurred around the Vietnamese New Year holiday period (D. U. Pfeiffer et al., 2007). The fifth wave mainly affected 22 provinces in the North while the fourth wave mainly affected 12 provinces in southern Vietnam. Subsequently, sporadic outbreaks since October 2007 resulted in the culling of 75,421 poultry around the country (FAO, 2010). According to the newest official report of the DAH, H5N1 HPAI outbreaks were reported from 27 out of 63 provinces and main cities in Vietnam in 2017 (Dispatch No.2904/TY-DT, 2017). Until recently, most of the outbreaks have been located in the 2 high-risk areas Red River Delta and Mekong Delta in the country (see in Figure 2.4).

2.2.3.3 Circulating virus clades

Although the emergence of AI in Vietnam was not until late 2003, viruses of subtypes H5N1 of HPAI and some subtypes of LPAI viruses were isolated from domestic aquatic birds during live-bird market surveillance in Vietnam in 2001 and 2003 (Jadhao et al., 2009). Various clades of the H5 subtype HPAI viruses have been detected in Vietnam since 2003 (see in Table 2.1). Early, most poultry infections were caused by viruses that belonged to clade 1 in both high-risk regions. Subsequently, numerous subclades of clade 2 have emerged in the country. Firstly, clade 2.3.4 viruses gradually replaced clade 1 viruses in northern and central regions between 2005 and 2010. After that, subgroups of clade 2.3.4 viruses were identified in poultry in 2014. Clade 2.3.2.1 has been replacing the previously dominating clade 2.3.4 in North and Middle of Vietnam

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since 2011. This clade subsequently evolved into its variants (2.3.2.1A/B/C), and became widespread in the country (Cuong et al., 2016). Despite the emergence of new virus strains in the North and Middle of Vietnam, including clade 2.3.2.1 in 2010, clade 1 viruses (and its 1.1 subclade in 2014) were dominant in southern Vietnam until 2014 (Creanga et al., 2013). Clade 2.3.2.1C emerged in the Mekong Delta at the end of 2013 and replaced the dominance of clade 1 in the South until recently (Cuong et al., 2016). Virus isolates in Vietnam were found to be highly pathogenic and deadly mostly in chickens. However, more severe clinical lesions and increased death rate caused by HPAI infection have been reported in ducks (Rhyoo et al., 2015). Fatal cases in ducks have been found since 2005. Notably, in 2007, HPAI H5N1 outbreaks occurred in free-grazing ducks in 13 Vietnamese provinces in the Mekong Delta which resulted in the massive culling of more than 10 million of poultry.

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Table 2.1 Summary of clades of HPAI viruses in Vietnam.
(From Division of Epidemiology, MARD-DAH, 2016)

Years	**Northern Vietnam**	**Southern Vietnam**
2003 – 2005	First outbreaks of HPAI in Vietnam, Clade 1	
2007 – 2008	Clade 2.3.4 Clade 7 (in illegal imported chickens)	Clade 1
2009	Clade 2.3.4	
2010	Clade 2.3.2	Clade 1 and 2.3.2/2.3.4
2011 – 2013	Clade 2.3.2.1	
2014	Clade 2.3.2.1C Clade 2.3.4.4 (H5N6)	Clade 2.3.2.1C Clade 1.1
2015	Clade 2.3.4.4 (H5N6)	Clade 2.3.2.1C
2016	Clade 2.3.4.4 (H5N6) Clade 2.3.2.1C	Clade 2.3.2.1C
2017	Clade 2.3.4.4 (H5N6) Clade 2.3.2.1C	Clade 2.3.2.1C

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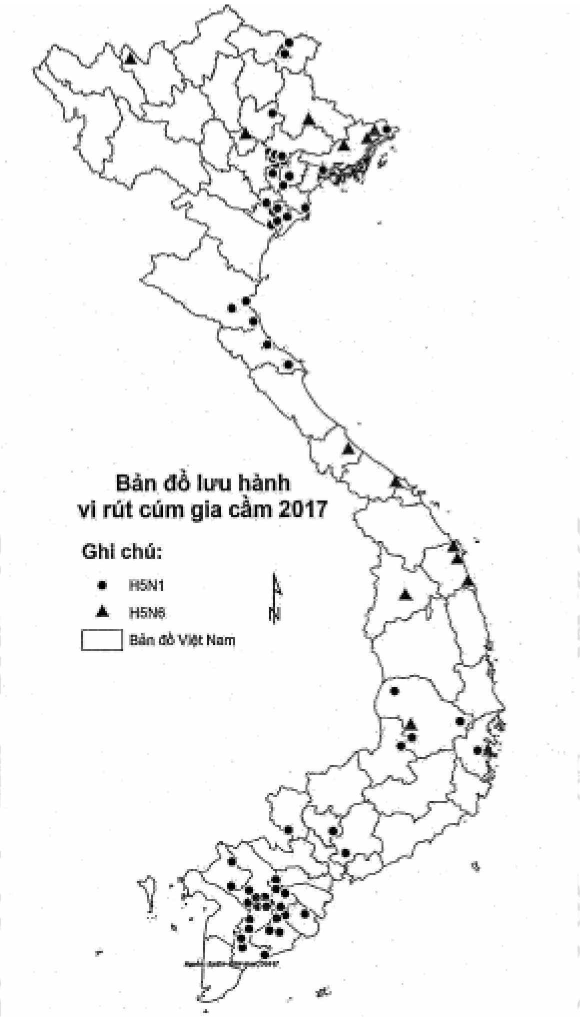

Figure 2.4 Map of HPAI outbreaks in Vietnam in 2017.
(From Division of Epidemiology, Department of Animal Health, Ministry of Agriculture and Rural Development (MARD-DAH)
(Dispatch No.2904/TY-DT, 2017)

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2.2.3.4 Risk factors of HPAI

Vietnam has a high density and population of domestic ducks which has been considered as the main factor in the spread and persistence of H5N1 HPAI in the country (Cha et al., 2013). Outbreaks of H5N1 HPAI in Vietnam in late 2006 mainly related to ducks. It was suspected that the management of free-grazing ducks and ducks in enclosed farms may influence HPAI H5N1 transmission dynamics among poultry in the country (Phan Q. Minh, 2010). Most of the domestic ducks have been raising mainly in the Mekong Delta and the Red River Delta which are also the areas most affected by H5N1 HPAI (Figure 2.4).

Many foci of infection have been particularly in the Mekong Delta. Those two HPAI H5N1 high-risk areas not only coincide with irrigated rice paddy fields but also with areas of live-bird market and high live poultry movement frequency (D. U. Pfeiffer et al., 2007). In addition, a large proportion of smallholder farming keeps chickens and water birds together and many smallholder farmers have rice growing activity where domestic waterfowls are grazed on post-harvest spilt rice to utilize the natural feed source. These movements make it difficult or impossible for the farmers to prevent their birds from becoming infected and then infecting other poultry that they come into contact with (X. M. Bui, 2010). The popularity of free-grazing, backyard duck farms, especially those with low-biosecurity, was recognized as a risk factor for H5N1 HPAI outbreaks in the country (Sonnberg et al., 2013). Reports from the national veterinary authorities suggested that small commercial farms and the village/household farms are more susceptible to AI infection when compared to larger commercial farms. Those farming and husbandry practices could facilitate interaction of susceptible poultry with potentially infected birds and the interspecies transmission of the viruses (Gilbert et al., 2006). On the other hand, Vietnamese consumers prefer fresh poultry meat that birds likely to be slaughtered after purchase at the market or at home (D. U. Pfeiffer et al., 2007). This custom can create a potential for the AI viruses transmission to humans.

2.2.3.5 Interventions

Since a range of control measures implemented especially mass routine vaccination campaigns in poultry in late 2005, disease occurrence and outbreaks have been reduced significantly. The cost of the vaccination program has been beared by the Vietnamese government with additional financial support as well as free vaccine doses from many international organizations, including the OIE, the FAO, the World Bank, and others, helping to contain the disease (C. C. Tran et al., 2016). The national mass vaccination program has been observed to offer certain protection to the national poultry population that circulation of the HPAI H5N1 virus and disease outbreaks were found mostly in unvaccinated poultry especially ducks (P. Q. Minh et al., 2009; Taylor & Dung, 2007). It is critical to run the vaccination program in the two high-risk regions (i.e. Red River Delta and Mekong Delta) in the country. Indeed, vaccination has been implemented in all communes in all provinces in the Deltas under the national poultry vaccination program. Until now, vaccination likely to be involved in every production cycle following

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the instruction of vaccines' manufactures and the national regulation (C. C. Tran et al., 2016).

Other general measures taken in Vietnam for the prevention and control of AI include massive depopulation during outbreaks occurrence, poultry movement controls, closure of unlicensed live poultry markets, surveillance and rapid diagnosis, public sensitization and awareness about the disease, compensation, disinfection of equipment and facilities, decontamination or disposal of contaminated materials (Soares Magalhaes et al., 2010). In 2007, the DAH launched a document instructing on farming practice for domestic duck production, which stipulate confinement needed or registration of every free-grazing duck flock. Measures applied in response to outbreak occurrences including no treatment of affected animals, stamping out, disinfection, vaccination in response to the outbreak(s), movement control, surveillance outside containment and/or protection zone. Those actions were believed to contribute to the containment of H5N1 outbreaks (D. U. Pfeiffer et al., 2007). However, despite control efforts and a significant decrease in the number of H5 AI virus poultry outbreaks in the country in recent years, eradication has not been achieved (Bertran et al., 2015) partly due to the emergence of H5N1 HPAI antigenic variants with their distinct antigenic properties and virulence that enable them to escape immunity with some of the currently used vaccines (Cha et al., 2013). Therefore, it is necessary for continuous alterations of the vaccine strains to be antigenically matched with the currently circulating virus strains along with the strict application of other control measures to limit virus transmission within the poultry population and to prevent sporadic human infections. Surveillance and characterization of AI viruses remain extremely important to implement effective animal health and public health control and preventive measures in Vietnam (Creanga et al., 2017).

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2.3 Epidemiology and disease manifestation of HPAI subtype H5N1

2.3.1 Epidemiology

2.3.1.1 Host range of H5N1 HPAI

Influenza A viruses exhibit host species adaptation that they have been found in a wide range of host species including poultry, wild birds, pigs, humans, cats, tigers, horses, cattle, seals, whales, etc. (T. D. Nguyen, 2011). Briefly, AI viruses have been isolated from not lower than 90 known free-living bird species of 13 different orders but the actual number of naturally infected species is likely much greater. However, most AI infections have not produced clinical disease in those species, excepting H5N1 HPAI viruses (Guangdong lineage). AI infections have been reported in the interface between human and birds (e.g. agriculture, husbandry, caged, exhibition, etc.) especially most domesticated Galliformes and Anseriformes (David E. Swayne, 2013). In general, some specific subtypes of the AI virus appear only in certain species. However, wild waterfowl, particularly the two orders Anseriformes (ducks, geese and swans) and Charadriiformes (e.g. gulls, shorebirds), are natural reservoirs and carriers of all virus subtypes of type A influenza (Cagle et al., 2012; Webster et al., 1992). Usually, AI viruses are nonpathogenic in these species that infected natural hosts usually show none or few signs of disease (David E Swayne, 2009).

By 2001, the host range of Asian lineage H5N1 viruses had extended to domestic ducks, which is believed to be a significant factor in the spread of the disease in Southeast Asian (Eagles et al., 2009). Previously, ducks infected with H5 and H7 HPAI viruses developed only subclinical to mild disease. However, the pathobiology of HPAI viruses has changed, as many Asian lineage H5N1 viruses can cause systemic infection and death in ducks (Cagle et al., 2012).

2.3.1.2 Virus transmission

Modes of transmission and the location of viral shedding may differ the by host species and by the infected virus strain (Kelly et al., 2008). It can be either direct or indirect transmission. By direct contact between infected host and a susceptible host, the disease is transmitted horizontally. Transmission occurring most frequently and easily among individuals of the same species or species with close relation (David E. Swayne, 2013). Infected birds can excrete large amounts of the influenza viruses from their intestinal tract into their faeces. However, transmission from respiratory route via secretions (could be from the nares, mouth or conjunctiva) has also appeared in poultry and in aquatic bird species that several studies on H5N1 strains found that the virus was present in higher titres in the trachea than the cloaca in mallards and Pekin ducks, both in inoculated and in-contact birds (Eagles et al., 2009; Sturm-Ramirez et al., 2005). The indirect transmission includes contact with aerosol droplets and other virus-contaminated equipment, fomites and materials (e.g., feed, water, cages, clothing, shoes, crates,

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vehicles etc.) which contributes to the dissemination of viruses. In addition, water-borne transmission can provide a mechanism for the maintenance of AI viruses in natural aquatic bird habitats. Because influenza viruses replicate preferentially in the intestinal tract of waterfowl, virus transmission through the fecally contaminated water-oral route is an important mechanism of virus dissemination among the bird population (Horimoto & Kawaoka, 2001). Vertical transmission remains unclear however, eggshell surfaces can be contaminated with the virus (Potima, 2007). Movement of humans and live poultry (either free-grazing or trading) is a critical mechanism for the further spread of avian influenza viruses (Webster et al., 2006). According to Webster, the spread of AI viruses in previous outbreaks of H5 and H7 HPAI in multiple countries was directly attributable to humans moving live poultry and poultry products. Movement of domestic waterfowl and trade through live-bird markets were strongly believed to play a critical role in the transmission of the disease. As an example, the rising incidence and widespread of HPAI in late 2003 and early 2004 were probably because of the increasing spread of the viruses from domestic waterfowl and live bird markets as reservoirs of infection, resulting in greater environmental contamination (Sims et al., 2005).

2.3.2 Disease manifestation of the HPAI subtype H5N1: clinical signs and lesions

Differences in pathogenicity were observed among different HPAI H5N1 virus-infected species. The course of the disease, clinical signs and severity of infection in domestic poultry are extremely variable and inconsistent from an asymptomatic or unapparent form with few or no signs to overwhelming systemic illness (Bertran et al., 2015; Cagle et al., 2012; Pantin-Jackwood et al., 2012). The extent to which the severity of illness depends on many factors such as the virulence of the virus, the infected species (e.g. duck, chicken, Muscovy duck etc.) with their age, immune and health status, their husbandry management, etc. However, in general, two forms of illness can be seen as the followings: (1) Mild form: illness may be displayed by anorexia, mild effects on the respiratory system or reduced egg production (2) Severe form: systemic infection of the virus can affect the respiratory tract, invades multiple internal organs which can result in massive haemorrhaging. Many of the following clinical signs were seen in poultry infected with HPAI virus strains (including H5N1 strains): quietness and extreme depression; sudden drop in production of eggs or abnormally-formed eggs; swelling or

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cyanosis of wattles and comb or skin under the eyes; nostrils' discharge or respiratory signs such as coughing, sneezing; nervous signs, incoordination or convulsion; diarrhoea; deaths occur followed by rapid spread with mortality rate can approach up to 100% over short time (Bertran et al., 2015; J. Pfeiffer et al., 2009).

Lesions may include straw-colour fluid in the subcutaneous tissues, congestion and oedema, hyperemia or haemorrhage, necrotic foci and inflammatory lesions in various internal organs including lungs, trachea, proventriculus, intestines, ovaries and reproductive tract (Amen et al., 2015; Kelly et al., 2008). Histological changes appear in organs coincide with gross lesions found. In general, viral antigen staining correlated with histopathological findings (Bertran et al., 2015).

2.4 Domestic ducks and H5N1 Highly Pathogenic Avian Influenza

2.4.1 Background

Domestic ducks are the second most abundant poultry species in many Asian countries. The species is considered a natural reservoir for HPAI H5N1 viruses, while infected ducks can remain clinically normal while excreting the viruses (Hulse-Post et al., 2005). The role of domestic ducks was reported either in the emergence of new genotypes, in the propagation and biological evolution of AI viruses or in the maintenance and spread of the viruses to other terrestrial poultry in many studies (Barman et al., 2017; Hulse-Post et al., 2005; Pabilonia, 2012). Infection of ducks with H5N1 HPAI viruses is a risk for poultry production (both commercial and backyard system). A laboratory study of the WHO on H5N1 viruses in domestic ducks proved that the infected birds can cause high levels of environmental contamination and be a significant source of infection for chickens and other birds as they could asymptomatically shed the virus for more than 2 weeks (WHO, 2004). Ducks were recognized to be able to excrete H5N1 viruses from the intestinal tract and primarily from upper respiratory tract (Hulse-Post et al, 2005). In addition, an experimental study revealed the virus in feather epithelium of call ducks infected with H5N1 HPAI virus, suggesting that shed feathers could be a potential source of virus transmission (Yamamoto et al., 2007).

Before the emergence of H5N1 HPAI viruses of the Asian lineage, there were very small numbers of report on HPAI natural infections in ducks. By 2000, domestic ducks were included in the host range of Asian lineage H5N1 HPAI viruses that multiple

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genotypes emerged in the species which were closely related to the original goose genotype (A/goose/Guangdong/96) (Martin et al., 2006; Sims et al., 2005). The carriage of H5N1 viruses by asymptomatic domestic waterfowl is a unique feature contributing to making the disease epidemic in 2004 became unprecedented. The first report of H5N1 HPAI virus isolates in ducks in Asia were from live-bird markets in 1997 in Hong Kong, which presumably resulted from virus transmission from chickens. However, a virulent challenge experience with the HK/97 strains of H5N1 HPAI virus shown no morbidity or mortality in ducks, although the virus was recovered from oropharyngeal swabs and from lungs, while the virus strain was highly mortal in all of the gallinaceous species experimentally inoculated (Shortridge, 1999). The first H5N1 virus detection in ducks in P.R. China was from a healthy one on a farm in Guangxi province (Martin et al., 2006). Infected but apparently healthy ducks potentially spread the viruses even across territorial boundaries that healthy ducks transported from mainland China to Hong Kong in 2000 were found to be infected with H5N1 HPAI viruses (Guan et al., 2002). In another case, an H5N1 infection was detected in duck meat imported from China to the Republic of Korea in 2001 indicating that healthy ducks presented for slaughter could be systemically infected, showing that long-distance transport of virus potentially happens via poultry meat trading. An experiment recorded that virus was able to replicate and multiply to high titres in many tissues of inoculated ducks (Tumpey et al., 2002).

Historically, HPAI viruses rarely caused disease in ducks (order Anseriformes) (Alexander et al., 1978; Cooley et al., 1989; Shortridge, 1999). However, starting in late 2002, increased mortality and higher pathogenicity of H5N1 HPAI viruses was observed in ducks (Hulse-Post et al., 2005; J. Pfeiffer et al., 2009). The first fatal H5N1 HPAI infections in ducks were reported in 2002 in Hong Kong when wild migratory birds and captive waterfowl, including ducks, were found dead in 2 parks. Virus isolates from those cases were assessed in the laboratory for their replication, transmission and pathogenicity potential in ducks. The result showed that these 2002 isolates caused systemic infection with high virus titres and severe lesions in multiple organs, especially in the brain of the inoculated ducks. Severe neurological dysfunction and death were observed. In addition, high virus titres could be detected from the ducks' drinking water and from other birds in close-contact, which demonstrates an efficient transmission of the virus from infected ducks (Sturm-Ramirez et al., 2004). Subsequently, many emerging H5N1 HPAI viruses

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of the Asian lineage were found to cause high mortality in ducks (Cha et al., 2013). High infection rates were also revealed in clinically normal domestic ducks (Hulse-Post et al., 2005). Not all infected ducks displayed clinical signs in the field and the outcome of infection in experimentally inoculated ducks depended on the strain of virus or the age of susceptible host (Pantin-Jackwood & Swayne, 2007; Songserm et al., 2006).

In Southeast Asia, ducks were believed to play a key role in the perpetuation and spread of H5N1 in the region (Hulse-Post et al., 2005; Sturm-Ramirez et al., 2005). Free-grazing ducks, which scavenge on rice paddies post-harvest and account for a considerable proportion of the duck population in Vietnam and other Southeast Asian countries, has been identified as a major risk factor for HPAI outbreaks in chickens in the region (Gilbert et al., 2006; Meyer et al., 2017). Authorities in Thailand had discovered that the distribution of free-grazing ducks correlated with the transmission of H5N1 influenza viruses to chickens (Songserm et al., 2006). Similarly, in Vietnam, the huge population of domestic ducks, particularly free-grazing ducks feeding in rice fields, has been recognized as a risk factor for the persistence and spread of the viruses (Gilbert et al., 2008). Therefore, measures imposed on this species (especially vaccination) is part of the HPAI control strategy in many countries (Cagle et al., 2011).

2.4.2 Pathologic features of H5N1 infection in ducks

Virulence of the HPAI H5N1 viruses has increased in waterfowl over time. Isolates of Asian H5N1 HPAI virus have been found to cause illness and mortality in ducks (Kwon et al., 2005; Pantin-Jackwood & Swayne, 2007; WHO, 2004). The pathogenicity in domestic ducks of different viruses of the HPAI subtype H5N1 varies significantly and is impacted by ducks' age as the disease displayed by younger ducks more severely (Pantin-Jackwood et al., 2012). A study of H5N1 infections of ducks in different raising systems reported clinical signs including high fever, depression, dyspnea, ocular and nasal discharge accompanied by conjunctivitis, diarrhoea, nervous signs and convulsion (Songserm et al., 2006). Another study observed reluctance to eat, wry neck, incoordination and death in experimentally infected Pekin ducks with an H5N1 virus strain (Londt et al., 2008). However, none or mild clinical signs with no mortality in older ducks were also observed (Londt et al., 2010; Songserm et al., 2006). The virus was found to have a wide dissemination and systemic spread to various organ including brain,

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trachea, heart, lung, liver, spleen, pancreas, kidney and bursa (Chua et al., 2010; Songserm et al., 2006). Death was preceded by the highest viral loads detected in the brain and heart (Londt et al., 2008).

Gross lesions were detected at postmortem necropsy including haemorrhage in leg and footpad, congestion and haemorrhage in or serous fluid surrounding multiple affected organs including the heart, pancreas, spleen, liver, lungs, intestines, kidneys, bursa, etc. Congested blood vessels in the brain were observed in some cases (Chua et al., 2010; Songserm et al., 2006).

Infected ducks showed varying combinations of histopathologic changes. Prominent lesions were extensive pneumonia, severe pulmonary oedema and necrotizing pancreatitis. Focal hepatitis, splenic lymphoid depletion or necrosis, enteritis and tubulonephritis were also exhibited. Ducks displaying nervous signs had nonsuppurative encephalitis and gliosis in their brains while necrosis and hyaline degeneration of myocardium were predominantly detected in dead ducks (Songserm et al., 2006). Another study of H5N1 pathogenesis in call duck showed necrosis and/or viral antigens in the feather epithelium of birds in all age groups. Nonpurulent encephalitis and focal necrosis of the pancreas and heart also were commonly seen in inoculated birds (Yamamoto et al., 2007).

2.4.3 Immune response of ducks to HPAI

Higgins reviewed that antibody production was found to be greatest in chicken and respectively followed by pheasant, turkey, quail, and duck with the use of various antigen in several pieces of research. Both experimental infections or vaccination using killed AI viruses showed a similar immunologic response (Higgins, 1996). It was found that ducks develop poor antibody responses and produce less HI antibodies to natural and experimental AI infections (Kida et al., 1980; Toth & Norcross, 1981). The incompetence of ducks to produce HA-specific antibody is probably related to lack of duck antibody, which was discussed by Suarez & Schultz-Cherry (2000). In general, ducks' immune response to infection or vaccination is quite similar to that in other poultry species. Protection against AI is mostly conferred by the production of antibodies against the viral HA protein (details are provided in the next sections).

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The susceptibility to infection, disease and immune response to vaccination of domestic ducks including different species and breeds, can vary depending on the species and age of the bird. However, the association between serological titres and protection against challenge is not so clear for vaccines in ducks (Pantin-Jackwood & Suarez, 2013). Nevertheless, antibody levels above a certain threshold can be predictive of protection (E. Spackman & Swayne, 2013). On the other hand, the duration of the vaccine-induced immunity in ducks, which is an important factor when vaccination implemented in the field, has not been fully addressed.

2.5 Vaccination in prevention and control of HPAI

2.5.1 Background and immunological basis of vaccine protection against HPAI

Vaccination is one of the efforts to manage the risk of HPAI in many countries, which is targeted at the flock level of their poultry population. Depending on conditions in each country, vaccines can be used in programs to prevent, manage, or eradicate the AI viruses and disease (D. E. Swayne, 2006). When a vaccination program is implemented, there are several issues under consideration. One of those issues is the immunogenicity of a vaccine, which refers to its ability to induce an immune response in a vaccinated animal (Hannoun et al., 2004). The host immune response to vaccination varies greatly by species (D. L. Suarez & Schultz-Cherry, 2000).

A protective immune response in poultry induced by vaccination may be due to the humoral immunity (i.e. antibody production of B lymphocytes), the cell-mediated immunity (i.e. the action of T lymphocytes, or a combination of both (van den Berg et al., 2008). However, the primary determinant of the protective immunity of a host against AI viruses is an antibody response because the study of Chamber et al. proved that when the birds have cloacal bursectomy, which removes their ability to mount a humoral response, they have no protection against HPAI virus challenge (Chambers et al., 1988). After vaccination (or natural infection), antibodies are produced in the body of the host. Only neutralizing antibodies produced to the surface proteins HA, NA, and M2 provide a protective effect to the host. Antibodies produced against the NA protein can provide partial protection against homologous NA subtypes but not as much as that by the HA protein. Similarly, antibodies to the M2 protein can provide minimal but insufficient

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protection in poultry against HPAI. Antibodies against the other (internal) proteins do not confer any protection. Importantly, the HA protein is the major antigen that elicits antibodies protecting the host against illness and mortality. Antibodies to the HA protein are subtype-specific that they only neutralize influenza virus of the homologous HA subtype. This has major implications for vaccination because vaccines are limited to providing only subtype protection (Lee & Suarez, 2005). Produced HA-specific neutralizing antibodies can block viral attachment to host cells and can persist for extended periods of time. Such neutralizing anti-HA antibodies can be measured using a variety of methods however, the easier-to-perform hemagglutinin-inhibition (HI) test is recommended (OIE, 2015). The level of protection against infection and subsequent shedding of the virus is influenced by the degree of antigenic similarity between HA of vaccine and infected virus. Therefore, vaccines used for any vaccination program have to be chosen to have a close antigenic match with the HA antigen of the circulating virus strain in the field because it will be optimal for the protective immunity of poultry. As there is the continuous evolution of HPAI viruses which leads to antigenic variation, countries implementing HPAI vaccination (Bhatia et al., 2016).

2.5.2 Vaccination in the world

Epizootics of HPAI subtype H5N1 were eradicated in many countries using a four-component stamping-out strategy including biosecurity, rapid diagnostics and surveillance, massive culling of infected and susceptible birds in close-contact, and public education (David E Swayne, 2012). However, the disease control and eradication were unachievable in some countries using traditional methods, which lead to the use of vaccination as a component of their control program against HPAI in poultry. The rationale for using vaccine is to reduce and prevent poultry morbidity and death, reduce virus shedding and environmental contamination thereby reducing transmission to both other birds and humans, maintain their poultry production, maintain rural livelihoods and food security (D. E. Swayne, 2011).

Previously, vaccination had been used against H5 and H7 low pathogenic notifiable AI in poultry in the USA, Italy, Mexico, Guatemala and El Salvador from 1970s (started with an inactivated vaccine in oil emulsion) because those 2 LPAI virus subtypes (H5 and H7) were able to mutate and become HPAI viruses. Vaccination of poultry against HPAI

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H5N1 viruses was implemented for the first time in Hong Kong during 2002 and has been practised during the last decade in several other countries (D. E. Swayne, 2011) although the effectiveness of vaccination under field conditions still remains mostly unquantified. However, effective HPAI vaccinations are always necessary for preventing disease occurrences, subclinical infections and emergence of new virus antigenic variants (Tarigan et al., 2018). Between 2002 and 2010, AI vaccines were used to protect at-risk poultry population of 14 countries and were comprised of more than 95% inactivated vaccines administered to individual birds and nearly 5% live recombinant vectored vaccines administered by massive spraying (primarily recombinant Newcastle disease vectored vaccine with H5 influenza gene insert). Top 4 countries using AI vaccine includes China (including Hong Kong Special Administrative Region), Vietnam, Indonesia and Egypt, which implemented nationwide routine vaccination programs and accounted for more than 99% of vaccine use in the world (D. E. Swayne, 2012). Vaccination program began after H5N1 HPAI became endemic in poultry populations in these countries where poultry production is characterized by small commercial farms (sector 3, following the FAO grouping of poultry production consisting of 4 operational levels) or village/backyard farms (sector 4), which have logistical problems about implementing vaccination to a huge number of premises and households while farmers are probably less educated on the disease and vaccination issues. Vaccination coverage rates have been found to be low in the 2 mentioned sectors of poultry production in these countries and consequently, the overall vaccination coverage rate needed to achieve their national poultry population immunity has not been consistently attained (D. E. Swayne, 2012; David E Swayne, 2012). Hong Kong Special Administrative Region, with a small number of poultry compared to the production figures of other countries, had the highest vaccination coverage rate at nearly 100% after full implementation of its vaccination programme in 2004. However, clinical illness and mortality rate in poultry in these countries and regions have been reduced along with human cases. (D. E. Swayne, 2011). The remaining 10 countries used less vaccine and targeted only high-risk poultry either to prevent or to manage HPAI in an effort to eradicate the disease (D. E. Swayne, 2012).

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2.5.3 Vaccination for the prevention and control of H5N1 HPAI in Vietnam

2.5.3.1 Overview

Prevention and control of H5 HPAI in Vietnam have been challenging due to the diverse and complex poultry and domestic duck production systems, as well as the veterinary infrastructure and financial ability, have not been adequate to effectively stamp out the disease all around the country. In addition, the stamping-out measures also caused numerous ethical and environmental concerns (T. D. Nguyen, 2011). To tackle the disease, vaccination has been implemented for the purpose of decreasing bird susceptibility to infection, reducing the number of infected birds and their mortality, and decreasing environmental contamination by viruses shed, which in turn reducing viral transmission to both poultry and humans, allowed maintenance of farmer livelihoods and food security of the country. This strategy helps in providing time for restructuring veterinary services and poultry production systems, which works for a long-term goal of H5N1 HPAI eradication (David E Swayne, 2012).

After the failure of a massive culling strategy to control virus spread and eradicate H5N1 HPAI, a nationwide vaccination program of both commercial and backyard poultry against the disease was implemented in Vietnam in late 2005, after 2 trials in Tien Giang and Nam Dinh province. Chickens and ducks more than 14 days old had been vaccinated using inactivated H5N1 vaccine (for both ducks and chickens) and H5N2 vaccine (for only chickens) from China (P. Q. Minh et al., 2009; C. C. Tran et al., 2016). The biannual vaccination campaign (in April-May and in September-November) targeted high-risk areas (i.e. Red River Delta and Mekong Delta) for their disease situation, high human and poultry densities as well as high risk of extensive virus circulation. Along with that, culling was limited to affected flocks only (T. D. Nguyen, 2011). Following that very first vaccination campaign, no AI outbreaks were reported in poultry or humans from December 2005 until August 2006. The virus appeared again later might due to the introduction of new circulating viruses that the vaccine used at that time no longer had a protective effect. However, there were no new human cases reported until June 2007 (Gutierrez et al., 2009).

In Vietnam, vaccination of domestic ducks is part of the national HPAI control strategy. For the period of 2005-2009, the country mass vaccination campaign

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achieved higher vaccination rates in breeder and meat-type ducks (90% of 322 million population) compared to that in breeder and meat-type chickens (50% of 1.3 billion population) (D. E. Swayne, 2012). Certainly, the veterinary services intensively vaccinated domestic ducks due to the big population of this species was recognized by the National Center for Veterinary Diagnosis (2005) as an asymptomatic reservoir and a primary factor in the persistence and spread of H5N1 HPAI viruses in the country (Stéphanie Desvaux et al., 2011). A high vaccination coverage rate in domestic ducks is expected to assist in reducing environmental contamination with the virus, which in turn, can result in a lower exposure and outbreak rate in other poultry species (Gilbert et al., 2008).

Currently, the mass vaccination program is implemented nationally widely, however, most seriously in the two high-risk regions (i.e. Red River Delta and Mekong Delta) in Vietnam. HPAI vaccines have been given to the poultry population across all geographical areas in all provinces in the Deltas under the national poultry vaccination program. To date, thanks to the public knowledge about the disease and the importance of vaccination in disease containment and prevention, vaccination likely to be involved in every production cycle at the recommended poultry age following the instruction of vaccines' manufactures and the national regulation. The mass vaccination program is intensively run village/household farms where most of the human cases had occurred. Vaccination can be performed by either commune animal health workers or by farmers, or both. The country government provides strong support for HPAI prevention at commune level. Vietnamese authorities have been sponsoring vaccine costs for smallholders (chicken and duck farms with less than 2000 poultry) since 2005 intensively in high-risk areas including the Mekong Delta provinces (FAO, 2011), while large commercial farms purchase and apply own vaccine following recommendations from vaccines' manufactures.

No cases (or less, if any) of the disease in humans had been reported since vaccination was fully implemented. Notably, there were no reports of disease in fully vaccinated poultry. Almost all H5N1 HPAI outbreaks in domestic birds occurred in non-vaccinated flocks or improperly vaccinated flocks thus not having protective immunity. However, despite efforts to control H5N1 HPAI through vaccination, Vietnamese have not eradicated the disease totally, partly due to the emergence of the virus antigenic

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variants that are able to escape immunity with some of the currently used vaccines and the inability to obtain population immunity in susceptible poultry species (Cha et al., 2013; David E Swayne, 2012). Therefore, it is necessary for continuous alterations of the vaccine strains and each new vaccine involved in the mass vaccination program in the country should be tested against newly emerging and circulating viruses in terms of both efficacy of vaccines and field effectiveness of vaccination regimes.

About the vaccination policy in Vietnam, the main reason for less effectiveness of vaccination in the field is the low vaccine coverage rate which does not allow the flock immunity in susceptible poultry population (D. E. Swayne, 2012), the Vietnamese government issued a regulation stipulating that "to achieve herd level immunity after vaccination, 70% of the poultry in each flock must demonstrate a seroprotection (antibody titres of ≥ 4 log2 measured by haemagglutination inhibition (IH) test) to be considered protected, and 80% of the poultry flocks in each province or region must show flock level immunity". (MARD – DAH, Circular No. 487/TY-DT and Circular No. 07/2016/TT-BNNPTNT).

2.5.3.2 Vaccines used in Vietnam

Since late 2005, there have been various vaccine types (e.g. live attenuated, inactivated, reverse genetic) with different vaccine virus strains used for the vaccination program in poultry in Vietnam. Most of them are imported from China due to the vaccines' antigenic match with the virus strains circulating in Vietnam. Inactivated, HPAI H5N1 strain vaccines have been most commonly used in Vietnam since the first day of vaccination implementation. The virus strains of HPAI subtype H5N1 that have been used in inactivated vaccines for the mass vaccination program in the country belongs to: clades 0 ('Re-1' vaccine, by Zhaoqing Dahuanong, China; and 'Vifluvac', a vaccine domestically manufactured by Navetco, Vietnam), clade 2.3.4 ('Re-5', by Zhaoqing Dahuanong, China) and clade 2.3.2.1 ('Re-6', again from Zhaoqing Dahuanong, China) (Cuong et al., 2016). Vaccine strains have been chosen to be antigenically-matching with the circulating viruses in the field for specific periods of time, depending on the evolutionary status of circulating HPAI viruses (e.g. intragenic variation (antigenic drift) of the HA protein (especially H5)).

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Because the vaccines used have to be sufficiently matched antigenically with the prevailing viruses (see in Table 2.1), currently, three commercially available vaccines are used in certain regions of the country to fight with H5 HPAI, including: Navet-Vifluvac mainly against clade 2.3.4.4 (H5N6) with some protection (60%) against 2.3.2.1C (H5N1), Re-5 against 2.3.2.1C (H5N1) and Re-6 mainly against 2.3.2.1C (H5N1) with some protection (60%) against 2.3.4.4 (H5N6) (MARD - DAH, Dispatch No. 2905/TY-DT). Surveillance of the country authorities shown that the inactivated vaccines used can provide certain protection to not only chickens and but also ducks and other domestic poultry species as no outbreaks have occurred in strictly vaccinated flocks. According to the Vietnamese veterinary authorities (DAH, 2014), the dominance of clade 2.3.2.1C since 2014 has urged the country to import the Re-6 vaccine from China for use up to date (Le & Nguyen, 2014). The Re-6 inactivated H5N1 HPAI vaccine carries the HA and NA genes from the strain A/duck/Guangdong/S1322/10 (DK/GD/S1322/10) of clade 2.3.2.1, which has close antigenic similarity to the subclade 2.3.2.1C circulating widely in southern Vietnamese provinces. Before, the vaccine was used in domestic poultry in China and other Southeast Asian countries since 2012 (Zeng et al., 2016). With the update on the H5N1 vaccine virus strain in 2014, no new human cases have been reported until the current time (see in Figure 2.4). It is expected that the vaccine will continue to be used in the foreseeable future as it provides a certain protective effect against the disease in the poultry population.

2.5.3.3 Issues of vaccination implementation in Vietnam

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Although the vaccines in nationwide use have shown reasonable efficacy for protection against different clades of H5N1 viruses (Zeng et al., 2016), the real protective effect of vaccination may differ greatly from the laboratory assessment to the field evaluation. Results from experiments cannot be directly extrapolated to the complexity of the field because of differences in their circumstances (J. Pfeiffer et al., 2010). Numerous factors that can influence vaccination effectiveness in the field includes endogenous factors of individual vaccinated birds (age, health and immune status, concurrent disease or infection, genetic factors, etc.), maternal immunity, farm management practice and husbandry conditions (e.g. water, nutrition, stress), and also vaccine administration skill, etc. In addition, difficulties in massive vaccinations in semi-commercial farms and village/household farms are well known. (Pantin-Jackwood &

Suarez, 2013). As an example, keeping vaccine cold during transport and storage and the proper vaccine administration are also factors of vaccination success (van den Berg et al., 2008).

Limited field trial data on the effectiveness of vaccination in poultry is available. Furthermore, due to the overwhelming number of chickens compared to other poultry species, most of the available studies were performed in chickens while there are not many field studies on vaccine efficacy and vaccination effectiveness in ducks. Due to differences in the immune responses between these two species, results in chickens should not be assumed to ducks (Pantin-Jackwood & Suarez, 2013; J. Pfeiffer et al., 2010). Furthermore, the immune response to H5N1 HPAI virus vaccination was found to be different in various domestic poultry species even when they were vaccinated with the same vaccine. Higgins reported that antibody production was greatest in chicken, respectively followed by pheasant, turkey, quail, and duck with the use of various antigen in several pieces of research (Higgins, 1996). As a result, their vaccine-induced protection against infection was also variable. On the other hand, a single vaccination was recognized to be not sufficient for all poultry species, all ages of birds and all production systems in the field. It may provide protective immunity for broiler chickens, but long-lived layer and breeder chickens, ducks, meat turkeys, geese may need more vaccinations for long-term protection (David E Swayne & Darrell R Kapczynski, 2008). This information should be considered to run effective H5N1 HPAI vaccination programs for different poultry species targeted (Cagle et al., 2011; Cagle et al., 2012). The importance of vaccination protocols specifically designed for domestic ducks was appreciated by a study conducted in Vietnam previously, in which authors mentioned the need for more vaccine doses to induce seroprotection in this species (S. Desvaux et al., 2012). In overall, vaccination protocols should be developed to ensure both individual and population immunity (David E Swayne & Darrell R Kapczynski, 2008).

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With the used inactivated vaccines in Vietnam, booster doses have been often recommended by vaccines' manufactures for long-term protection and preventing virus transmission, which is necessary for groups of mix ages and species of poultry (Peyre et al., 2009). Importantly, 1-dose regime vaccination of poultry against HPAI has not been recommended in endemic areas (Tarigan, 2018). Finding from a previous study indicated that a single dose of an inactivated vaccine in oil emulsion containing H5 seed strains may be optimized for chickens but could not provide sufficient protective effect for ducks against a HPAI virus (Eggert & Swayne, 2010). However, vaccination coverage rate with two shots in ducks was found to be low in a study conducted in a province in Mekong Delta region that only around one-fifth of vaccinated ducks received a 2-dose regime vaccination (Cuong et al., 2016). Indeed, currently, single-dose vaccination in meat-type ducks is quite popular in Mekong Delta (SDAH Ben Tre province, personal communication). This may be due to the fact that the recommended 2-dose vaccination requires time-consuming and labour-intensive efforts and also results in stress for the ducks just prior to selling time. In addition, vaccine manufacturer recommended a 28 day withholding period before slaughter for the two-dose vaccination regime. That does not fit well with the early finishing for meat duck production which is commonly carried out at around 63 days (i.e., 21 days for the first vaccine, 21 days for the booster, and 28 more days withholding equals 70 days, would mean a 10% increase in the length of the production cycle). Rumours concerning adverse reactions to the vaccine also can deter meat duck farm owners from presenting their flocks for a booster vaccination (Soares Magalhaes et al., 2010). These facts suggest an inquiry into whether a single vaccination can induce the desired protective immune response in ducks for a certain period of time as well confirmation of the benefits of the 2-dose H5N1 vaccination regime for ducks.

2.6 Surveillance of HPAI in Vietnam

2.6.1 Background

The success of prevention and control of HPAI requires an effective surveillance and monitoring system following a vaccination program in any country (Peyre et al., 2009). Indeed, in efforts to prevent and control H5N1 HPAI in the poultry population as well as to prevent human exposure, the Vietnamese government has associated a HPAI surveillance program with the vaccination program in poultry since the first mass vaccination campaign commenced in 2005 (Bertran et al., 2015). That national routine surveillance program, which is commonly biannual, includes 3 sections: (1) ‘clinical surveillance’, (2) ‘virus circulation surveillance’ to identify and characterize the currently circulating HPAI viruses and (3) ‘post-vaccination sero-surveillance’ after mass vaccination courses in domestic poultry (MARD - DAH, Dispatch No. 487/TY-DT in 2009).

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‘Clinical surveillance’ is carried out frequently by SDAH of every province, especially for repopulated poultry flocks, newly-opened farms, flocks in regions currently suffering from AI outbreaks, high-risk areas. For ‘virus circulation surveillance’, oropharyngeal and/or cloacal swabs and carcass samples are collected for real-time reverse transcriptase polymerase chain reaction (RRT-PCR) to detect virus circulation both in non-vaccinated and vaccinated flocks and to observe virus evolution. Last but not least, ‘post-vaccination sero-surveillance’ is conducted to evaluate antibody response of vaccinated flocks and thus, to evaluate the effectiveness of the vaccination program in each province or region of the country.

2.6.2 Sero-surveillance

‘Post-vaccination sero-surveillance’ plays a critical role in prevention and control of HPAI in Vietnam. The surveillance has been conducted to (1) detect infection or virus circulation in poultry, and (2) to define seroprotection of vaccinated flocks (or a bigger poultry population of a province or region) by measuring the antibody levels specific to the H5 antigen of HPAI viruses. The importance of the sero-surveillance is to determine seroprotection rate of individual vaccinated flocks and broader of the overall poultry population of one province or region. Seroprotection rate is the proportion of vaccinated poultry in a specific population (can be individual flock) demonstrating seropositivity (i.e. showing a protective antibody titre). Seroprotection rate determines whether flock and population immunity have been achieved after a vaccination program or not. Seroprotection rate is calculated using the cut-off level HI titre at 4 $\log_2$ by the haemagglutination inhibition (HI) test using an H5 antigen. The Vietnamese national regulations stipulate that to achieve flock level immunity after vaccination, 70% of the poultry in each flock must demonstrate a seroprotection (HI titres of $\geq$ 4 log2) to be considered protected, and 80% of the poultry flocks in each province or region must show flock level immunity because in theory there are not enough susceptible individuals to propagate an epidemic (MARD – DAH, Circular No. 07/2016/TT-BNNPTNT).

2.6.3 Serological assay for AI virus and antibody diagnosis

Serological assays refer to the detection of antibodies against a specific virus. During outbreaks of AI, this is particularly important for epidemiological investigations of viral infections. Serological tests are also used to determine the antibody responses to

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influenza virus vaccination which are reflected by rises in titre to the type or subtype of vaccine virus (Prince & Leber, 2003). The serological diagnosis of antibodies against type A of AI can be performed using different techniques such as haemagglutination inhibition (HI), Enzyme-linked immunoassay (ELISA), agar gel immunodiffusion (AGID), etc. (Capua & Alexander, 2009). However, for the AI virus, the HI assay is a laboratory procedure that has been employed routinely in diagnostic serology. The assay can be used to define the hemagglutinin (HA) subtype of an unknown AI virus isolate (virus subtyping). On the other hand, given its high sensitivity and specificity, the HI test is considered the ‘gold standard’ for AI antibody subtyping (Comin et al., 2013) which is used to detect and quantitate hemagglutination (HA) subtype-specific antibodies in serum or other body fluids by using a HA subtype-specific reference antigen. Influenza vaccines must induce protective immunity and the HI test is most commonly used for the detection of antibody to influenza since it is correlated with immune protection. The HI assay is the most recommended method for evaluating the immune status in individual animals or populations in response to vaccination, which is documented in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals – Chapter 2.3.4 (OIE, 2017). The assay is a less technical, relatively inexpensive and quick procedure utilizing standard laboratory equipment (Pedersen, 2008).

The basis of the HI test is the inhibition of HA due to the interaction between subtype-specific antibodies and homologous viral antigen. Hemagglutination is the result of the hemagglutinins (HA) on the surface of influenza virus binding to receptors on the membrane of avian or mammalian red blood cells (RBCs). When being together, if the influenza virus is present in an adequate concentration, erythrocytes are agglutinated, linking together to form clumping. This clumping is known as haemagglutination (Killian, 2010). Inhibition of HA occurs in the presence of subtype-specific antibodies. Antibody-positive samples will inhibit the HA activity of the antigen, while seronegative samples do not have that effect. Inhibition is evidenced by a distinct button of cells forming in the bottom of the well which will flow and form a “tear-drop” when the plate is tilted. The titre of subtype-specific antibodies present in the serum is calculated as the reciprocal of the highest dilution that produces a complete HI (Pedersen, 2008).

HA and HI test procedure can be variable in different laboratories. Each procedure may use different volumes of reagents and a different concentration of red blood cells,

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and the steps may be performed in a slightly different order. However, results from those procedures can be equivalent (OIE, 2016).

2.7 Research on vaccination effectiveness in Vietnam

In Vietnam, data on vaccine-induced seroprotection against HPAI under field conditions remain limited in ducks. Most of the available studies on HPAI vaccines and vaccinations have focused on chickens because of their high mortality rates and the potentially huge economic loss caused if disease outbreaks occur in their population. On the other hand, studies on ducks are mostly experimental under laboratory setting (Cagle et al., 2011; Cagle et al., 2012; Ferreira et al., 2012; Yuk et al., 2015). International publications on HPAI in domestic ducks in Vietnam is mainly about the disease epidemiology (Cuong et al., 2016; J. Pfeiffer et al., 2009), disease occurrence with related risk factors (Stéphanie Desvaux et al., 2011; McLaws et al., 2015), virus pathogenicity observed in laboratory experiments (Cagle et al., 2012; Cha et al., 2013), seroprevalence and circulation of HPAI viruses, their evolution (Le & Nguyen, 2014), etc. However, to the author's knowledge, there are not much (internationally) published research about the effectiveness of HPAI vaccination under field conditions in terms of antibody response of ducks, especially for the currently used vaccines (e.g. the Re-6 vaccine). Although the DAH annually has a report about their evaluation on vaccine-provided protection in poultry against the circulating HPAI viruses, however, their reports are kindly general without details in specific species. Some pieces of research relating to the humoral immune response of domestic poultry species to various H5N1 vaccines used have been published (most internally).

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Previously, a cross-sectional study was conducted in the Mekong River Delta in Vietnam in January – December 2009 identifying the probability of poultry flocks showing seroprotection against HPAI H5N1 following vaccination with the Re-1 vaccine (Gs/GD/96 virus strain) (T. D. Nguyen, 2011). More than 13,000 serum samples from vaccinated poultry (chickens and ducks) at different ages were collected from 7 provinces to be analyzed by the HI test for the presence of H5-specific antibodies. Criteria for the individual- and flock- level of seroprotection were in accordance with the national regulation mention above. Finding of the study shown that vaccinated chicken flocks were more than 2 times more likely to be protected against H5N1 HPAI than vaccinated flocks of ducks. A relatively similar field study using the same Re-1 vaccine conducted in 2008 - 2010 in 2 provinces in the Red River Delta was aimed to evaluate the immunogenicity of the vaccine in domestic poultry (S. Desvaux et al., 2013). The result of this study showed that 50% – 70% of the vaccinated poultry showed a serological evidence of protection (anti-H5 antibody titre above the defined positive titre measured by HI assay). The overall seroprevalence of vaccinated birds was only 36.9%. Ducks vaccinated after 20 days old with two injections had a higher mean HI titre compared to those vaccinated with only one injection, which suggested that vaccination protocol has an effect on the antibody response of ducks. In addition, larger flocks of ducks were found to have a higher HI-positive proportion but a lower mean HI titre compared to smaller flocks.

More recently, there are some investigations on the humoral immune response of ducks to vaccination with the currently used Re-6 vaccines conducted in some provinces in Mekong Delta in Vietnam. An experimental study on the humoral immune response of ducks against the Re-6 vaccine and the Navet-vifluvac (NIBRG-14 virus seed strain) vaccine in 2 different broiler breeds was conducted in Hau Giang province in Mekong Delta from 2013 January to 2014 October (Phan & Tran, 2016). It was shown that flock protection rates against the H5N1 virus by a maternal antibody of 14-day ducklings were insufficient (lower than 70% of the national regulation). However, there was a considerable difference in that maternal antibody protection levels of the 2 duck breeds (53% vs 13%). Two weeks after a single vaccine injection with both vaccines, there were small proportions of ducks (33% – 40%) obtaining the targeted antibody level for protection with mean HI titres at around 1.5 $\log_2$. However, a booster vaccine injection resulted in protection rates higher than 90% in all duck breeds vaccinated with both vaccines, with corresponding mean HI titre values between 4.93 and 6.87 $\log_2$. Besides that, a study on serological response induced by the same vaccine took place in a duck flock (unclear breed and purpose of production) and a Muscovy duck flock under farm conditions in a district of Kien Giang province (Pham, 2015). There was no or less evidence of maternal antibody against HPAI H5N1 virus in both species at 14 days of age (HI titres $< 4 \log_2$, mean HI titre and protective level were equal to zero). Three weeks after vaccination with a single dose of the Re-6 vaccine, around 68% of duck samples showed seroprotection (mean HI titre = 3.32 $\log_2$).

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For a different poultry species, a study was conducted in Tre chickens (a local breed) of households in Tien Giang province in Mekong Delta to evaluate antibody production of the Re-6 vaccine (V. T. Tran, 2016). This study showed that maternal antibody of chicks reduced to very low levels after 14 days old which was insufficient for protection against AI virus or to confound the vaccine effect afterwards. At the day 15th after vaccination, both mean HI titre and protective level (1.7 $\log_2$ and 35%, respectively) were insufficient to be assumed as protective against H5N1 HPAI according to the current national regulation. Expected protective levels were only shown after 75 days from vaccination. This finding was corresponding to result of another study conducted in various chicken breeds with another vaccine strain (Tran T. H. Lieu, 2013).

Results from those mentioned studies are consistent with a judgement that a waning in maternal antibody titre occurs over the succeeding 1-3 weeks (Simon, 1997) (Gharaibeh & Mahmoud, 2013). This finding reinforces the correctness of the current recommended schedule for an AI immunization in poultry at minimum 14 days of age. Concerning maternal antibody is essential because there has been evidence indicating that maternal antibody may decrease the efficacy of the inactivated vaccine against AI virus (Abdelwhab et al., 2012; De Vriese et al., 2010). On the other hand, findings of some mentioned studies indicated that the proportion of ducks gaining the targeted protective antibody level after a single injection with the Re-6 vaccine was not high. In addition, immune responses of poultry to the same vaccine may vary significantly. It can be even bigger variation when mass vaccination is implemented in different regions under various field conditions.

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However, there is still a lack of studies demonstrating in detail the distribution and variation of antibody titre and seroprotection rate induced by vaccine injections for domestic ducks in the field, especially in village/household farms where vaccination is likely to be more difficult compared to other poultry production systems. That information can be useful for explaining the effectiveness of vaccination program in the national poultry population.