การตรวจติดตามเชื้อไวรัสไข้หวัดนก H5N1 จากสัตว์ปีกในบริเวณชายแดนระหว่างประเทศไทย และประเทศเพื่อนบ้าน (ลาว และพม่า)

<mark>นายจิรเด</mark>ช ลาภขุนทด

### ศูนย์วิทยทรัพยากร

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

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### MONITORING OF INFLUENZA A H5N1 VIRUS FROM AVIAN SPECIES IN BORDER AREAS BETWEEN THAILAND AND NEIGHBORING COUNTRIES (LAOS AND MYANMAR)

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science Program in Veterinary Public Health Department of Veterinary Public Health Faculty of Veterinary Science Chulalongkorn University

Academic Year 2009

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Thesis Title	MONITORING OF INFLUENZA A H5N1 VIRUS FROM AVIAN		
	SPECIES IN BORDER AREAS BETWEEN THAILAND AND		
-2	NEIGHBORING COUNTRIES (LAOS AND MYANMAR)		
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การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อตรวจติดตามเชื้อไวรัสไข้หวัดนก H5N1 จากสัตว์ปีกใน บริเวณชายแดนระหว่างประเทศไทยและประเทศเพื่อนบ้าน (ลาว และพม่า) ระหว่างเดือนกันยายน พ.ศ. 2550 ถึง มิถนายน พ.ศ. 2551 โดยเก็บตัวอย่างจำนวน 2,175 ตัวอย่าง แบ่งเป็นตัวอย่างจาก สัตว์ปีกมีชีวิตจ<u>ำนวน</u> 2,1<mark>39 ตัวอย่าง</mark> แล<mark>ะอวัยวะภายในของสัตว์ปีกจำนวน 36</mark> ตัวอย่าง จากนั้น น้ำตัวอย่างทั้งหมดมาเพาะแยกเชื้อไวรัสไข้หวัดนก H5N1 ด้วยวิธีการจีดเข้าไข่ไก่ฟัก และตรวจ พิสูจน์ด้วยวิธี Hemagglutination test Multiplex RT-PCR Realtime RT-PCR และ PCR-ELISA จากนั้นศึกษาลักษณะทางพันธุศาสตร์ด้วยวิธีการถอดรหัสพันธุกรรมของยืนทั้งหมดของเชื้อไวรัส วิเคราะห์รหัสพันธุกรรมด้วยวิธี phylogenetic analysis และวิเคราะห์การเปลี่ยนแปลงของกรดอะ มิโนในตำแหน่งต่างๆ ที่มีความสำคัญบนยืนทั้ง 8 ยีน (PB2, PB1, PA, HA, NP, NA, M, และ NS) ผลการศึกษาพบอุบัติการณ์ของเชื้อไข้หวัดนกลายพันธุ์ H5N1 คิดเป็น 0.69% (15/2,175) โดยเชื้อ ไวรัสไข้หวัดนกที่พบเป็นเชื้อไวรัสไข้หวัดนกชนิดก่อโรครุนแรง (Highly Pathogenic Avian Influenza; HPAI) ซึ่งพบการเรียงตัวของกรดอะมิในชนิดเบสหลายตัวที่ HA cleavage site และ การลดจำนวนของกรดอะมิโน 20 ตัว ที่ NA stalk region รวมถึงไม่พบการเปลี่ยนแปลงของกรดอะ มิโนในตำแหน่งที่มีความสำคัญ การศึกษาทาง phylogenetic analysis พบว่าเชื้อไวรัสไข้หวัดนกที่ พบจัดอยู่ในกลุ่มเดียวกันกับที่แยกได้ในประเทศไทย อยู่ใน genotype Z หรือ clade 1 ดังนั้นไวรัส ไข้หวัด-นกที่พบในบริเวณซายแดนระหว่างประเทศไทยและประเทศเพื่อนบ้าน (ลาว และพม่า) มี ความใกล้เคียงกับไวรัสไข้หวัดนกที่แยกได้ในประเทศไทยในปี พ.ศ. 2547-2549 การศึกษาครั้งนี้ แสดงให้เห็นว่ามีการแพร่ระบาดของเชื้อไวรัสไข้หวัดนกในบริเวณชายแดนระหว่างประเทศไทยและ ประเทศเพื่อนบ้าน (ลาว และพม่า) ดังนั้นการเฝ้าระวังเชื้อไวรัสไข้หวัดนกในบริเวณชายแดน ระหว่างประเทศไทยและประเทศเพื่อนบ้าน (ลาว และพม่า) จะช่วยควบคุมและป้องกันการติดเชื้อ ไวรัสไข้หวัดนกลายพันธุ์ H5N1 ในคนได้

ภาควิชา สัตวแพทยสาธารณสุข ลายมือชื่อนิสิต <u>วิธรุง จางบระค</u>า สาขาวิชา <u>สัตวแพทยสาธารณสุข</u> ลายมือชื่ออาจารย์ที่ปรึกษาวิทยานิพน<del>อ์หลัก \_\_\_\_\_\_</del> ปีการศึกษา 2552 ลายมือชื่ออาจารย์ที่ปรึกษาวิทยานิพนธ์ร่วม \_\_\_\_\_\_*โก* // ##5075576031 : MAJOR VETERINARY PUBLIC HEALTH

KEYWORDS : AVIAN INFLUENZA VIRUS MONITORING cDNA SEQUENCING

JIRADEJ LAPKUNTOD: MONITORING OF INFLUENZA A H5N1 VIRUS FROM AVIAN SPECIES IN BORDER AREAS BETWEEN THAILAND AND NEIGHBORING COUNTRIES (LAOS AND MYANMAR). THESIS ADVISOR: ASSOC. PROF. ALONGKORN AMONSIN, D.V.M., Ph.D., THESIS CO-ADVISOR: ASST. PROF. RUNGTIP CHUANCHUEN, D.V.M., Ph.D., 100 pp.

The purpose of this study was to monitor Influenza A H5N1 virus from avian species in border areas between Thailand and neighboring countries (Laos and Myanmar) from September 2007 to June 2008. Two-thousand one hundred seventy five samples, including 2,139 live birds and 36 visceral organs, were collected. The H5N1 viruses were isolated and identified using embryonated egg inoculation, Hemagglutination test, Multiplex RT-PCR, Realtime RT-PCR, and PCR-ELISA. Then, the viruses were genetically characterized by using sequencing of whole genome avian influenza H5N1 viruses, phylogenetic analysis, and analysis of key determinant residue changes of 8 genes (PB2, PB1, PA, HA, NP, NA, M, and NS). The results revealed that the evidence of avian influenza H5N1 virus was 0.69% (15/2,175). The viruses had common genetic characteristics of Highly Pathogenic Avian Influenza (HPAI), with multiple basic amino acids in the HA cleavage site and a 20-amino acid deletion in NA stalk region. No point mutations were identified in the key determinant residues of those genes. Phylogenetic analysis of whole genes showed that the viruses clustered within the lineage of H5N1 avian isolates from Thailand-Vietnam lineage, genotype Z or clade 1. These indicate that avian influenza H5N1 virus circulating in border areas between Thailand, Laos and Myanmar were genetically related to avian influenza H5N1 virus in 2004-2006 in Thailand. In summary, this study presented the evidence of HPAI spreading in border area between Thailand and neighboring countries (Laos and Myanmar). Therefore, monitoring and surveillance of avian influenza virus along the border areas will be beneficial for prevention and control of H5N1 infection in humans.

Department: Veterinary Public Health Field of Study: Veterinary Public Health Academic Year: 2009 Student's Signature And Liebunted Advisor's Signature And And Co-Advisor's Signature Rugo thurschen

### ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to Associate Professor Dr. Alongkorn Amonsin, my advisor and Assistant Professor Dr. Rungtip Chuanchuen, my co-advisor. I am sincerely grateful for all they have advised and encouraged throughout the period of this study.

I would like to thank Assistant Professor Dr Suphachai nuanualsuwan, the chairman of thesis committee; Associate Professor Dr Thaveesak Songserm the member of thesis committee for their constructive criticisms and valuable suggestions.

I am thankful to Professor Dr. Roongroje Thanawongnuwech and Dr. Rachod Tantilertcharoen for all of their support, excellent instruction, and suggestion and kind assistance throughout my study period.

Lastly, I would like to thank my friends from the department of Veterinary Public Health, Faculty of Veterinary Science, Chulalongkorn University and Mr. Kamol Suwannakarn from Center of Excellence in Clinical Virology, Faculty of Medicine, Chulalongkorn University for their help, supports and encouragement throughout my study period.

Finally for my family, I am deeply grateful for their love, supports and encouragement.

### ศูนย์วิทยทรัพยากร จุฬาลงกรณ์มหาวิทยาลัย

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### LIST OF ABBREVIATIONS

AI	Avian Influenza
bp	base pair
°C	degree Celsius
cDNA	Complementary deoxyribonucleic acid
et al.	et alibi, and other
g	gram (s)
НА	Hemagglutinin
HPAI	Highly Pathogenic Avian influenza
h	hour (s)
М	Matrix
mg	milligram (s)
min	minutes (s)
μΙ	micro liter
μΜ	micro molar
NA	Neuraminidase
NP	Nucleoprotein
NS	Nonstructural protein
PA	Polymerase acidic protein
PCR	Polymerase Chain Reaction
PB1	Polymerase Basic protein 1
PB2	Polymerase Basic protein 2
RNA	Ribonucleic acid
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
sec	second (s)

### CHAPTER I

### INTRODUCTION

Avian influenza H5N1 virus is a Highly Pathogenic Avian Influenza (HPAI) virus. The virus is highly contagious and causes disease in several species of pet birds, wild birds, humans and food producing birds (e.g. chickens, ducks, turkeys, quails, and guinea fowls) (Abdel-Ghafar et al., 2008). World Organization for Animal Health or Office International des Epizooties (OIE) has included Avian Influenza (AI) in OIE listed diseases that are characterized by causing severe disease, fast spreading and promoting serious threats to economy and public health worldwide (OIE, 2000). The diseases in this category are required by law to be reported to government authorities. In Thailand, outbreaks of avian influenza H5N1 virus have caused both economical losses and the public health problems (Amonsin et al., 2008; Buranathai et al., 2007; Tiensin et al., 2005). In 2004, emergence of avian influenza H5N1 virus affected poultry production and exporting industries due to the eradication of poultry in the radius of outbreaks, banning exportation of chicken products to various countries and reduction of consumption within the country (Tiensin et al., 2005). From previous reports in Thailand, avian influenza H5N1 virus infected 17 persons with 12 deaths in 2004, infected 5 persons with 2 deaths in 2005, and infected 3 persons with 3 deaths in 2006. No human cases reported during 2007-2009. As of July 1, 2009, avian influenza H5N1 virus infected 25 persons with 17 deaths. During the same period of time, the virus infected 436 persons with 262 deaths in 15 countries worldwide (World Health Organization, 2008).

Based on nucleotide changes in hemagglutinin gene, avian influenza H5N1 viruses are classified into 10 main groups termed "clades" (WHO/OIE/FAO H5N1 Evolution Working Group, 2007). Clade 0 includes the ancestor of all avian influenza H5N1 viruses that spread in Hong Kong in 1997. Clade 1 comprises the viruses that expanded in Thailand, Southern Vietnam, Malaysia and Cambodia during 2003-2006 (Boltz et al., 2006; Li et al., 2004). Clade 2 contains 5 subclades including clade 2.1

covers the viruses that caused outbreaks in Indonesia in 2003-2007; clade 2.2 are those that spread in Europe and Africa in 2005-2007; clade 2.3 includes the viruses that spread in southern China and neighboring countries; clade 2.4 consists of the avian influenza H5N1 viruses distributed in China in 2002-2005 and clade 2.5 includes those detected in China and spread to Korea and Japan in 2003-2004. Clades 3 to 9 are avian influenza H5N1 viruses mostly detected in China (Webster and Govorkova, 2006).

Classification of avian influenza H5N1 virus genotypes can be performed by comparing genetic relatedness of their eight genes (Duan et al., 2008; Li et al., 2004)). This could be successfully accomplished when all genetic data of the genes are available. Genetic characteristics of avian influenza H5N1 viruses from Thailand were found to be similar to avian influenza viruses in Vietnam; therefore, the viruses have been classified as Thailand-Vietnam lineage or genotype Z (Amonsin et al., 2006a; Li et al., 2004; Viseshakul et al., 2004; Webster and Govorkova, 2006). A new genotype (genotype V or clade 2.3.4) of avian influenza H5N1 viruses "A/chicken/Thailand/NP-172/2006" was reported in Nakhon Phanom province during August 2006 - February 2007(Chutinimitkul et al., 2007). The virus was different from avian influenza H5N1 viruses recovered from most outbreaks in the country based on phylogenetic analysis of PA gene (NP-172). The virus was also classified into the same group of avian influenza viruses in southeastern China, Laos, and northern Vietnam (Chutinimitkul et al., 2007; Puthavathana et al., 2009). Currently, there are at least 2 clades (i.e. clade 1 and 2.3.4) or 2 genotypes (i.e. genotype Z and V) of avian influenza H5N1 viruses found to emerge and cause the avian influenza outbreaks in Thailand (Figure1) (Chutinimitkul et al., 2007). The genotype V was first detected in border areas and identified into the same group of viruses in neighbor countries (Laos and Myanmar), suggesting that the viruses may be disseminated from neighbor countries to Thailand (Chutinimitkul et al., 2007). The presence of two different clades/genotypes also indicates the multiple introductions of avian influenza H5N1virus into the country. Therefore, it is essential to conduct avian influenza virus surveillance and monitor for the emergence of novel viruses or genotypes along border areas of Thailand.

In this study, we isolated, identified and genetically characterized avian influenza H5N1 viruses from avian species around the border areas of Thailand between Thailand and neighboring countries (Laos and Myanmar). The results obtained from this study will provide insight information of the evolutionary history and the possible pathways of transmission of the viruses. Also, this study will demonstrate the avian influenza H5N1 evidence among avian Influenza A viruses isolates in the border areas of Thailand and distinguish clades/genotypes of avian influenza A viruses that have spread in Thailand.

### Objectives of Study.

- To collect samples, isolates, identify avian influenza H5N1 viruses from avian species from villages and fresh markets around border areas between Thailand and neighboring countries (Laos and Myanmar)
- To analyze genetic relatedness and identify genotypes of the avian influenza H5N1 viruses.

## ศูนย์วิทยทรัพยากร จุฬาลงกรณ์มหาวิทยาลัย



Figure1: A) Phylogenetic relationships of the polymerase acid protein gene comparing genotype Z, Z+, and V.

B) Hemagglutinin gene of avian influenza A H5N1 viruses in Thailand 2006 compared with several other strains worldwide

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(Chutinimitkul et al., 2007).

จุฬาลงกรณ์มหาวิทยาลัย

### CHAPTER II

### **REVIEW LITERATURES**

### A. Morphology of avian influenza H5N1 virus

Influenza virus belongs to the Orthomyxoviridae family. The virus can be divided into three types A, B and C. Influenza A virus can infect humans, mammals, and avian species. Influenza B virus can infect human only. Influenza C virus can infect human and rarely infect swine (Webster et al., 1992). Influenza A virus is an enveloped virus that has 2 glycoproteins, haemagglutinin (HA) protein and neuraminidase (NA) protein. Virions are spherical to pleomorphic and 80-120 nm in diameter. HA caries rod-shaped spike and NA contains mushroom-shaped spike on enveloped (De Jong et al., 2000)). Within enveloped, the virus has single-stranded RNA of negative polarity containing 8 segments with different molecular weight; i.e. Polymerase Basic protein 1 and 2 gene (PB1 and PB2), Polymerase gene (PA), Hemagglutinin gene (HA), Nucleoprotein gene (NP), Neuraminidase gene (NA), Matrix protein gene (M), and Nonstructural protein gene (NS), respectively (Figure2).



Figure 2. Diagram of avian influenza H5N1 virus structure.

RNA segments are contained in the viral core. Protein synthesized from 8 RNA segments have 10 types such as PB2, PB1, PA, HA, NP, NA, M1, M2, NS1 and NS2. Functions of these proteins are shown in Table1 (Lamb and Choppin, 1983). Influenza A viruses are classified based on their haemagglutinin (HA) and neuraminidase (NA) surface glycoproteins. At present, 16 HA (H1-16) and 9 NA (N1-9) subtypes have been identified (Fouchier et al., 2005).

Segment	Encoded	Nucleotide	Function
	polypeptide	length (bp)	
1	PB2	2,341	Host-cell RNA cap binding: component
			of RNA transcriptase
2	PB1	2,341	Initiation of transcription: possibly
			endonuclease activity: component of
			RNA transcriptase
3	PA	2,233	Elongation of mRNA chains: component
			of RNA transcriptase
4	HA	1,778	Surface glycoprotein; major antigenic
			determinant
5	NP	1,565	Associated with RNA segment to form
			ribonucleoprotein: structural component
			of RNA transcriptase
6	NA	1,413	Surface glycoprotein: neuraminidase
			activity.
7	M1	1,027	Major protein component of virus:
			underlies lipid bilayer
	M2		Spliced mRNA, nonstructural protein:
			ion channel
8	NS1	890	Nonstructural protein: function unknown
	NS2		Spliced mRNA, nonstructural protein:
			function unknown

 Table 1: RNA segments, encoding protein and their function. (Lamb and Choppin, 1983)

### B. Genotype classification for H5N1

Influenza A virus subtype H5N1 (Avian Influenza H5N1 virus) is the important subtype that caused AI in many animal species and humans. In 2001 - 2004, avian influenza H5N1 virus can be further classified into genotypes A-E, V-Z and Z+ (Li et al., 2004). Since 2002, genotype Z viruses are the main virus that cause outbreaks in Asia (Guan et al., 2002). Avian Influenza H5N1 viruses outbreaking in China and eastern Asia in 2001-2006 have been classified in genotype Z. Furthermore, genotype V viruses were classified in southern China in 2003-2006 and have been detected in VietNam and Laos in 2006-2007 (Boltz et al., 2006; Duan et al., 2008; Li et al., 2004). The genotype classification of avian influenza H5N1 virus can be performed when genetic data of 8 genes of viruses are available for comparing the genetic relatedness in each gene. The genotype classification steps including preparation of complete nucleotide sequence of each avian influenza H5N1 virus gene, comparison of nucleotide sequence by using computer program to analyze genetic relationship which displays in phylogenetic tree, classification of lineage of each gene of avian influenza H5N1 virus, arrangement of the sequential combination of the lineage of each gene, and assignment the genotype of avian influenza H5N1 virus (Duan et al., 2008; Li et al., 2004).

### C. Clade nomenclature system for H5N1

The avian influenza H5N1 viruses have appeared at least in 60 countries and continued to evolve and be diversed. Dissimilar names have been used in publications to explain emerging lineages of highly pathogenic avian influenza A (H5N1) viruses (WHO/OIE/FAO H5N1 Evolution Working Group, 2007). This generates difficulty for discussion and comparison of the various lineages. Since 1996, avian influenza A H5N1 viruses have experienced reassortment into many different genotypes. It is only the hemagglutinin protein that has not been replaced in the variant isolates. Evolution of hemagglutinin protein has an initial constant that the strains may be efficiently assessed. WHO/OIE/FAO suggested to improve a clade nomenclature system based on the evolutions of hemagglutinin protein; for reason unify the system so that interpretation of sequence and surveillance data from different laboratory becomes easier, removing stigmatizing labelling of clades by geographical reference, providing clade for easy

future expansion of the phylogenetic tree, and providing a starting point for a more extensive system to follow antigenic variation and reassortment.

Clade descriptions (WHO/OIE/FAO H5N1 Evolution Working Group, 2007)

0 = early progenitors; predominately 1996-2002 from Hong Kong (HK) and China (mostly avian, few human)

1 = 2002/2003 progenitors from HK; 2003-2006 from Vietnam, Cambodia, Thai, Laos, Malaysia (mixed A/H)

2.1 = 2003-2007 from Indonesia (mixed avian/human)

2.2 = 2005 progenitors from Qinghai Lake outbreak and Mongolia; 2005-2007 isolates from Eastern and Western Europe, the Middle East, and Africa (mixed avian/human)

2.3 = 2003-2006 from China, HK, Vietnam, Thailand, Laos, and Malaysia (mixed avian/human)

2.4 = 2002-2005 from China (predominately Yunnan and Guangxi Provinces) (all avian)

2.5 = 2003/2004 from Korea, Japan, China; 2006 lineage from Shantou Prov. (all avian)

3 = 2000-2001 from HK, China, Vietnam (all avian)

4 = 2002/2003 lineage from HK and China; 2005/2006 from Guiyang Prov.

(all avian)

5 = 2000-2003 from China and Vietnam; 2004 lineage from Guangxi Province (all avian)

6 = 2002/2004 from China (all avian)

7 = 2002/2004 from China; 2005/2006 from Yunnan, Hebei, Shanxi Provinces (all avian)

8 = 2001-2004 from HK and China (all avian)

9 = 2003-2005 from China (all avian)

### D. Avian influenza (H5N1) outbreaks in Thailand

In Thailand, outbreaks of avian influenza H5N1 virus caused both economic losses and public health problems. Avian influenza H5N1 virus infected 17 persons with 12 deaths in 2004, infected 5 persons with 2 deaths in 2005, infected 3 persons with 3 deaths in 2006 and no human cases reported in 2007-2009. As of July 1, 2009, avian influenza H5N1 virus infected 25 persons with 17 deaths while avian influenza H5N1 virus infected 436 persons with 262 deaths in 15 countries worldwide (World Health Organization, 2008). In Thailand, outbreaks of avian influenza H5N1 virus in avian species since 2004 were reported at least 7 waves (Amonsin et al., 2006a; Buranathai et al., 2007; Suwannakarn et al., 2009; Tiensin et al., 2007; Viseshakul et al., 2004). For example, outbreaks in January-March 2004, July-December 2004 (Tiensin et al., 2005), October-December 2005, January-March 2006, November 2006–March 2007, January 2008 and November 2008 (Amonsin et al., 2008; Chaichoune et al., 2009) respectively.

One of the main causes of avian influenza H5N1 virus's outbreak is the migratory birds that excreted numerous viruses from intestine. Migratory birds received viruses from their reservoirs or somewhere in the migratory way (Liu et al., 2005). Backyard chicken and free-grazing ducks can play a role as H5N1 hosts (Tiensin et al., 2005). Other studies reported that avian influenza H5N1 virus infected other mammals such as tigers, leopards (Amonsin et al., 2006); Thanawongnuwech et al., 2005), cat (Amonsin et al., 2006), and dog (Amonsin et al., 2007; Songserm et al., 2006).

### F. Identification and diagnosis methods of avian influenza virus in laboratory

One of molecular methods used to detect H5N1 subtypes is polymerase chain reaction (PCR) assays. A reverse transcriptase polymerase chain reaction (RT-PCR) was developed to detect the avian influenza H5N1 virus using specific primers. The specificity and sensitivity of the assay was shown by testing with subtypes of influenza A virus (Payungporn et al., 2004). Another detection method World organization for animal health has recommended, is virus isolation by embryonated egg inoculation (high sensitive and standard method) (WHO, 2002). Avian influenza viruses were isolated using specific antibody negative, embryonated chicken eggs. The supernatant fluid from

viral transport media (VTM) suspension was inoculated into allantoic sacs of the eggs. Samples yielding positive hemagglutination activity (HA test) were tested for influenza genes (WHO, 2002). Total RNA was extracted and purified from the allantoic fluid. Then, viral RNA was reverse transcribed into cDNA. To identify the virus subtype, a RT-PCR was performed using the primers specific for avian influenza H5N1 virus (WHO, 2002). Antigen tests using PCR-ELISA technique was claimed greater than conventional PCR according to its specificity and sensitivity. In addition, it needs less expense equipment than real-time PCR (Chaharaein et al., 2009).

### G. Molecular characterization of avian influenza virus (H5N1) in Thailand

Molecular characteristics of avian influenza H5N1 virus in Thailand were previously observed such as determinant residues relating to virulence, oseltamivir resistance, amantadine resistance, typical characteristics of both avian-like and humanlike viruses etc.

In HA gene, the cleavage site has multiple basic amino acids of highly pathogenic characteristics. These basic amino acids affected hemagglutinin molecule that is sensitive to protease enzyme. Since protease enzyme can be found in all organs, the H5N1 virus can spread to every organs and causes human or animal death (Horimoto and Kawaoka, 2005). Receptor binding sites retain amino acid residues at 222Q (Glutamine) and 224G (Glycine). The viruses isolated from mammal and poultry in Thailand contain Glutamine in position 226 and Glycine in position 228. These positions indicate preferencial binding of the virus to receptor sialic acid  $\alpha$  2, 3-Gal- terminated saccharide more than sialic acid  $\alpha$  2, 6-Gal- terminated saccharide (Stevens et al., 2006).

Sequence of the NA gene contains 20-amino acid deletion from position 49 to 68. This deletion causes the adaptation of virions on cell membrane (Guan et al., 2002). The change of amino acids at NA stalk region was the result of the adaptation or evolution occuring from the infection in wild aquatic birds to domestic poultry (Matrosovich et al., 1999). The position 275 of NA gene contain amino acid Y (Tyrosine), indicating oseltamivir sensitive viruses (Gubareva et al., 2000).

M2 protein at position 31 (serine; S) can appear in amantadine resistance. Viruses have the S31N substitution (Serine; S to Asparagine; N) that might present amantadine or rimantadine resistance (Puthavathana et al., 2005).

Single amino acid substitution at position 627 of PB2 protein (Glutamic acid; E to Lysine; K) found in isolates from mammal or human. This substitution produces high violence in human (Shinya et al., 2004). However, this substitution can be found in avian species from Qinghai, Eurasia, and Africa.

C-terminal sequence in NS gene of HPAI H5N1 virus had substitution from Arg-Ser-Lys-Val (RSKV) to Glu-Ser-Glu-Val (ESEV) and had 5 amino acid deletions (position 80 to 84). These will increase the virulence and pathogenicity of the virus, interrupt immunity and cause extensive pathology in mammals (Jackson et al., 2008; Lipatov et al., 2005).

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### CHAPTER III

### MATERIALS AND METHODS

The aim of this study was to monitor of Influenza A H5N1 virus from avian species in border areas between Thailand and neighboring countries (Laos and Myanmar) from September 2007 to June 2008. The experimental study included 3 phases; phase 1, collection of samples from avian species in the border areas between Thailand and neighboring countries (Laos and Myanmar); phase 2, isolation and identification of avian influenza H5N1 viruses and phase 3, genetic characterization of avian influenza H5N1 viruses using cluster analysis and identification of virus genotypes. The conceptual framework of this study is shown in Figure 3.



Figure 3: The conceptual framework in this study

Phase 1: Collection of samples from avian species and visceral organs in provinces along the borders between Thailand and neighboring countries (Laos and Myanmar)

### Location and type of samples

Samples were collected in provinces along the borders of Thailand and neighboring countries, Myanmar and Laos. Selection of places for sample collection depended on history of outbreaks of avian influenza virus, mortality rate, and animal movement. The samples were collected from villages and fresh markets in Chiang Rai, Chiang Mai, Mae Hong Son, Tak, Kanchanaburi, Ratchaburi, Prachuap Khiri Khan, Ranong, Loei, Nong Khai, Nakhon Phanom, Mukdahan, and Ubon Ratchathani (Figure 4). Sample collections were carried out twice a month for 8 months (16 times).

Two thousands one hundreds and seventy five samples, including 2,139 live birds and 36 visceral organs, were collected from border areas between Thailand, Laos and Myanmar. The samples included feces, cloacal contents, tracheal exudates and internal organs e.g. trachea, lung, liver, spleen and heart. Sterile cotton swabs were used to obtain samples from choanal-slit, trachea and cloaca. Each collected sample was placed in a sterile plastic tube containing 2 ml-viral transport media (VTM) and kept on ice. Then, the samples were transferred to Department of Veterinary Public Health, Faculty of Veterinary Science, Chulalongkorn University. All samples were kept at -80°C immediately after arrival.

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Figure 4: Locations for sample collection (gray areas) along the border of Thailand, Laos and Myanmar in this study, including Chiang Rai, Chiang Mai, Mae Hong Son, Tak, Kanchanaburi, Ratchaburi, Prachuap Khiri Khan, Ranong, Loei, Nong Khai, Nakhon Phanom, Mukdahan, and Ubon Ratchathani.

### Phase 2: Isolation and identification of HPAI H5N1 virus

### Virus isolation

Avian Influenza viruses were isolated from samples using inoculation of embryonated chicken eggs (WHO, 2002). The specific antibody negative-embryonated chicken eggs at the age of 9-11 days were used. The supernatant fluid from VTM suspension were inoculated into allantoic sacs of the eggs and incubated at 37°C. After the incubation period of 24-96 hour, the inoculated-embryonated eggs showing infected lesions or death were collected and chilled at 4°C. The allantoic fluid were harvested and tested for Hemagglutination activity (HA test) (WHO, 2002). The samples yielding positive hemagglutination activities were frozen at -80°C until needed (David et al., 1998). Virus isolation by inoculated embryonated eggs were accomplished at Biosafety level 2+ laboratory, Veterinary Diagnostic Laboratory, Faculty of Veterinary Science, Chulalongkorn University.

### Virus identification

Total RNA were extracted from the allantoic fluid with positive HA test using QIAamp Viral RNA Mini Kit (Qiagen<sup>®</sup>, Hilden, Germany). At the beginning, buffer AVL containing carrier RNA was pipetted 560 µl into a 1.5 ml microcentrifuge tube. The 140 µl portion of allantoic fluid was added into the buffer AVL–carrier RNA in the microcentrifuge tube and mixed by pulse-vortexing for 15 sec. Then, the mixture was incubated at room temperature (15–25°C) for 10 min. 560 µl of ethanol (96–100%) was added to the sample, and the mixture was mixed by pulse-vortexing for 15 sec. The tube was further briefly centrifuged to remove drops from inside the lid. The 630 µl solution was applied carefully into the QIAamp Mini column (collection tube) without wetting the rim, and centrifuged at 8000 rpm for 1 min. The QIAamp Mini column was placed into a clean 2 ml collection tube, and the tube containing the filtrate was discarded. The QIAamp Mini column was opened carefully, and procedure was repeated with remainder solution. Then, the five hundred µl buffer AW1 was added and centrifuged at 8000 rpm for 1 min. The QIAamp Mini column was placed into a clean 2 ml collection. Then, the five hundred µl buffer AW1 was added and centrifuged at 8000 rpm for 1 min. The QIAamp Mini column was placed into a clean 2 ml collection.

and the tube containing the filtrate was discarded. Five hundred µl of Buffer AW2 was added and the column was centrifuged at full speed (14,000 rpm) for 3 min. The QIAamp Mini column was placed in a new 2 ml collection tube, and centrifuged at full speed for 1 min. The QIAamp Mini column was placed in a clean 1.5 ml microcentrifuge tube. Sixty µl of Buffer AVE equilibrated to room temperature was added and incubated at room temperature for 1 min. The column was finally centrifuged at 8000 rpm 1 min for viral RNA collection.

The viral RNA was reverse transcribed into cDNA as previously described by random primer (Promega, Madison, WI, USA) (Viseshakul et al., 2004). The 4 µI RNA was mixed with 0.1 µg Random primers and incubated at on 70°C for 15 min for combination between RNA and random primers. Then, 20 µI cDNA synthesis reaction mixture comprising 5.0 µI RNA was mixed with Random primers, 1x Improm-II<sup>™</sup> Reaction buffer (Promega<sup>®</sup>) 4.0 µI, 2.5 mM MgCl<sub>2</sub> 2.0 µI (Promega<sup>®</sup>), 0.5 mM dNTPs 2.0 µI (Fermentas<sup>®</sup>, Marryland, USA), 40 U/µI Ribonuclease Inhibitor 0.3 µI (Promega<sup>®</sup>), 1U Improm-II<sup>™</sup> Reverse Transcriptase 1.0 µI (Promega<sup>®</sup>), and RNase-free water to a final volume of 20 µI. The condition step included 25°C for 5 min, 42°C for 60 min, and 70°C for 15 min. cDNA from the reverse transcription reaction was collected and chilled at - 20°C before identified in the next step.

To identify the virus subtype, a multiplex reverse transcriptase polymerase chain reaction (multiplex RT-PCR) was performed using the previously-published primers specific for M, HA, and NA genes of Influenza A (Payungporn et al., 2004). The 25 µl multiplex PCR amplification reaction mixture comprised 1x Master mix 10 µl (Eppendorf<sup>®</sup>, Hamburg, Germany), 0.5 µM of each primer 1.5 µl, 1.0 µl of cDNA from the previous reverse transcription reaction, 1.0 mM MgCl<sub>2</sub> 1 µl (Promega<sup>®</sup>) and RNase-free water to a final volume of 25 µl. The amplification cycles included an initial denaturation step at 94°C for 3 min, followed by 40 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, extension at 72°C for 30 sec, and a final extension step at 72°C for 7 min (Payungporn et al., 2004).

A 5 µl volume of the PCR products was mixed with 2 µl of loading buffer (0.2% Orange G in 50% glycerol) and loaded into 0.2% agarose gel. The mixture was exposed to electrophoresis on a 2% agarose gel stained with ethidium bromide (0.5µg/ml). The gel was photographed under UV light with a gel documentation system (Vilber Lourmat<sup>®</sup>, Lavalle Cedex, France). The expected size of the multiplex PCR products for the M, H5 and N1 genes was 125, 148 and 110 bp, respectively. Also cDNA sequencing of whole genome avian influenza H5N1 viruses were performed.

To identify the virus subtype, one step multiplex real-time RT-PCR was performed using the previously-published specific primers and probes for M, HA, and NA genes of Influenza A (Payungporn et al., 2006). The 10  $\mu$ l one step multiplex real-time RT- PCR reaction mixture comprised 2x Reaction mix 5  $\mu$ l (Invitrogen<sup>®</sup>, USA) and 0.2  $\mu$ l SuperScript. III RT/Platinum<sup>®</sup> *Taq* Mix, 0.5  $\mu$ M of each primer in 0.25  $\mu$ l, 1.0  $\mu$ l of RNA sample, 0.25  $\mu$ l Probe for M, HA, and NA genes (0.5  $\mu$ M) and RNase-free water to a final volume of 10  $\mu$ l. The PCR reaction included a reverse transcription step at 50°C for 30 min, followed by initial denaturation at 95°C for 10 min, 40 cycles of denaturation at 95°C for 15 sec, annealing and extension at 60°C for 30 sec (Payungporn et al., 2004).

Polymerase chain reaction enzyme linked immunoassays (PCR-ELISA) technique was performed using the previously-published specific primers and probes for M, HA, and NA genes of Influenza A (Chaharaein et al., 2009). PCR-ELISA contained 2 steps including multiplex PCR and ELISA step. The viral RNA was reverse transcribed into cDNA. Multiplex PCR was performed using specific primers for M, HA, and NA genes. The 50 µl Multiplex PCR reaction mixture comprised Taq DNA polymerase 0.5 µl, 50 µM of each primer in 0.5 µl, 2.0 µl of cDNA from the previous reverse transcription reaction, 5 µl PCR DIG labeling mix, 5 µl 10x buffers MgCl<sub>2</sub> and RNase-free water to a final volume of 50 µl. The amplification reaction included an initial denaturation step at 95°C for 15 min, followed by 35 cycles of denaturation at 95°C for 45 sec, annealing at 55°C for 5 min (Chaharaein et al., 2009). And the ELISA step, the samples were submitted to avian influenza researcher, faculty of veterinary science, Chulalongkorn University who

developed this method. This step was preceded with PCR ELISA for DIG detection (Roche<sup>®</sup>, Switzerland).

Phase 3: Genetic characterization of H5N1 virus and identification of virus genotypes

### Genetic characterization of H5N1 viruses

After RNA extraction and cDNA preparation in phase 2, PCR amplification using specific primers for PB2, PB1, PA, HA, NP, NA, M, and NS was performed. The 50  $\mu$ l multiplex PCR amplification reaction mixture comprised 1x Master mix 20  $\mu$ l (Eppendorf<sup>®</sup>), 0.2  $\mu$ M of each primer in 1.0  $\mu$ l, 2.0  $\mu$ l of cDNA from the previous reverse transcription reaction, 1.0 mM MgCl<sub>2</sub> 2  $\mu$ l (Promega<sup>®</sup>) and RNase-free water to a final volume of 50  $\mu$ l. The amplification cycles included an initial denaturation step at 94°C for 3 min, followed by 40 cycles of denaturation at 94°C for 30 sec, annealing at 50-55°C for 30 sec and extension at 72°C for 90 sec, and concluded by a final extension step at 72°C for 7 min

PCR products were examined by agarose gel electrophoresis. A 25 µl volume of the PCR products was mixed with 2 µl of loading buffer. The products were further gelpurified using QIAquick gel extraction kit (QIAGEN<sup>®</sup>). The DNA fragment was excised from the agarose gel and placed into an eppendorf tube. These volumes of Binding buffer was added 1 volume of gel. The mixture was incubated at 50°C for 10 min and mixed by vortex every 2–3 min. One gel volume of isopropanol was added to the sample and mixed. The liquid was applied to the QIAquick column, and centrifuged for 1 min at 13,000 rpm. The 0.5 ml binding buffer was added to QIAquick column and centrifuged for 1 min at 13,000 rpm. To wash, The 0.75 ml Wash buffer was added to QIAquick column for 1 min at 13,000 rpm.

Purified PCR products were sent to Molecular Informatrix Laboratory Limited, Shatin N.T. in Hong Kong for DNA sequencing. The DNA sequence data were validated by using Chromas V1.45 (Griffith University, Queensland, Australia) and aligned using BioEdit program (Carlsbad, CA, USA). The phylogenetic analysis was performed by the clustal analysis using the MEGA 3.1 program (Tempe, AZ, USA).

### Identification of viral genotypes

Steps of genotype classification included 1) preparation of complete nucleotide sequence of each genes of avian influenza virus, 2) comparison of nucleotide sequences using computer MEGA 3.1 program (Tempe, AZ, USA) to analyze genetic relationship, 3) classification of lineage of each gene of avian influenza virus, 4) arrangement of the sequential combination of the lineage of each gene, and 5) assignment the genotype of avian influenza H5N1 virus (Duan et al., 2008; (Li et al., 2004)). Bootstrapping support for tree topologies was operated using NJ methods with 1000 replicates performed by MEGA 3.1 program. A distinct phylogenetic lineage was then identified based on NJ bootstrap support of  $\geq$ 70% or Bayesian posterior probability of  $\geq$ 95%. A genotype was assigned using the 8 gene sequential combinations.

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### Instruments and chemical substances

### PCR assay

- 2,3-dideoxynucleoside triphosphate (dNTPs), 5mM (Fermentas®, USA)
- Improm-II<sup>™</sup> Reverse Transcriptase (Promega<sup>®</sup>, Madison, WI, USA)
- Improm-II<sup>™</sup> 5x Reaction buffer (Promega<sup>®</sup>, Madison, WI, USA)
- MgCl<sub>2</sub>, 25 mM (Promega<sup>®</sup>, Madison, WI, USA)
- Random primers, 0.5 μg (Promega<sup>®</sup>, Madison, WI, USA)
- Recombinant RNAsin<sup>®</sup> Ribonuclease Inhibitor, 40 u/ μl (Promega<sup>®</sup>, Madison, WI, USA)
- Ultrapure<sup>TM</sup> Distilled water DNase, RNase free (GIBCO<sup>®</sup>, USA)
- 2.5x Master Mix (Eppendorf<sup>®</sup>, Hamburg, Germany)
- Mg<sup>2+</sup> solution, 25 mM (Eppendorf<sup>®</sup>, Hamburg, Germany)
- GeneRuler<sup>™</sup> 100 bp DNA ladder (Fermentas<sup>®</sup>, USA)
- Agarose gel (Molecular grade)
- Ethidium Bromide 10 mg/ml (Sigma Aldrich Inc., USA)
- 0.2% Orange G loading dye in 50% glycerol (Carlo Ebra Reagent<sup>®</sup>, USA)
- 40x Tris-boric acid –EDTA (TBE) powder (Bio Basic Inc<sup>®</sup>, USA)
- 2x Reaction mix 5 µl (Invitrogen<sup>®</sup>, USA)
- SuperScript. III RT/Platinum<sup>®</sup> Taq (Invitrogen<sup>®</sup>, USA)

Viral Transport Media (VTM)

QIAamp<sup>®</sup> Viral RNA mini kit (Qiagen<sup>®</sup>, Hilden, Germany)

QIAquick Gel Extraction Kit (Qiagen<sup>®</sup>, Hilden, Germany)

PCR tube 0.2 ml (Axygen Scientific<sup>®</sup>, CA, USA)

Microcentrifuge tube 2 ml

Micropipette 0.5-2, 2-20, and 100-1000 µl (Gilson<sup>®</sup>, France)

Micropipette tip 2, 200 and 1,000 µl

Thermo cycler (Thermo electron corporation<sup>®</sup>, Cambridge, UK) Gel electrophoresis system (OWL Scientific Inc<sup>®</sup>, USA) UV transilluminator (Vilber Lourmat<sup>®</sup>, Lavalle Cedex, France)

Centrifuge (Denville Scientific Inc<sup>®</sup>, USA)

Centindge (Derivine Scientific Inc., 00

Refrigerator -20°C and -80°C

### CHAPTER IV

### RESULTS

In this study, we monitored Influenza A H5N1 virus from avian species in border areas between Thailand and neighboring countries (Laos and Myanmar) from September 2007 to June 2008. Two thousands one hundreds and seventy five samples were collected from the border areas. The samples were subjected to influenza A isolation, identification, and genetically characterization using embryonated egg inoculation, hemagglutination assay, multiplex RT-PCR, realtime RT-PCR, PCR-ELISA, nucleotide sequencing and phylogenetic analysis.

The 2,175 samples (2,139 samples from live poultry and 36 samples from visceral organs) were collected from 42 districts of 13 provinces along the borders of Thailand and neighboring countries, Myanmar and Laos. The samples were collected from villages and fresh markets of Chiang Rai, Chiang Mai, Mae Hong Son, Tak, Kanchanaburi, Ratchaburi, Prachuap Khiri Khan, Ranong, Loei, Nong Khai, Nakhon Phanom, Mukdahan, and Ubon Ratchathani provinces (Table 2). Selection criteria of locations for sample collection depended on history of outbreaks of avian influenza, mortality rate, and animal movements.

 Table 2: List of locations, number of samples and date of sample collection in this study.

Province	District	No. samples	total	Date of sample collection
	Muang	37		
Chiene Dei	Mae Sai	20	0.4	Sep 07
Chiang Rai	Chiang Saen	24	04	Sep 07
	Phan	3		

	Muang	12	60	Opt 07	
LUEI	Chiang Khan	57	09		
Nong Khoi	Sangkhom	1	57	Opt 07	
Nong Khai	Si Chiang <mark>Ma</mark> i	56	57		
	Pai	1			
Mae Hong Son	Pang Ma Pha	6	165	Dec 07	
	Khun Yuam	158			
	Muang	81			
Nakhon Phanom	Tha Phanom	92	196	Dec 07	
_	Tha Uthen	23			
Ratchaburi	Suan Phueng	87	87	Dec 07	
Ubon	Khong Chiam	225	220	Feb 08	
Ratchathani	Warin Chamrap	4	229 Feb		
	Sai <mark>Y</mark> ok	67			
Kanchanaburi	Thong Pha Phum	12	104	Mar 08	
	Sangkhla Buri	25			
	Mae Sot	132			
Tak	Mae Ramat	152	328	Mar 08	
	Ban Tak	44			
	Muang	58			
Mukdahan	Don Tan	66	196	April 08	
P1 75	Nikom	72		3	
Drachuan Khiri	Than Sakae	45			
Khan	Kui Buri	11	201	April 08	
	Muang	145			
	Muang	57			
Ranong	La Un	46	176	May 08	

Kra Buri	73		
Mae Rim	1		June 08
Chiang Dao	45		
Hang Dong	61	283	
Saraphi	102	203	
San Sai	50		
San Kamphaeng	25		
		2175	
	Kra Buri Mae Rim Chiang Dao Hang Dong Saraphi San Sai San Kamphaeng	Kra Buri73Mae Rim1Chiang Dao45Hang Dong61Saraphi102San Sai50San Kamphaeng25	Kra Buri73Mae Rim1Chiang Dao45Hang Dong61Saraphi102San Sai50San Kamphaeng252175

List of samples collected from different avian species is shown in Table 3. Of the 2,175 samples, 2,139 live birds (Chicken n= 1,907, Duck n= 179, quail n= 31, Pigeon n= 6, Baya weaver n= 5, Turkey n= 3, Red-whiskered bulbul n= 3, Spotted dove n= 2, each kind of peafowl, white-rumped sham, and hill myna n= 1) and 36 visceral organs (Chicken n= 22, Duck n= 1, Watercock n= 3, and Moorhen n= 10) were collected from 42 districts of 13 provinces in border areas between Thailand and neighboring countries (Laos and Myanmar).

Hest	No. samples		
HOSI -	Live birds	Food market	
Chicken	1,907	22	
Duck	179	1	
Quail	31	ากร	
Watercock	UND	3	
Moorhen		10	
Pigeon	6	เยาลย	
Baya weaver	5		
Turkey	3	-	
Red-whiskered Bulbul	3	-	

Table 3: List of samples collected from different avian species. (n=2,175)
Spotted Dove	2	-
other	3	-
Total	2,139	36

Other : peafowl, white-rumped sham, and hill myna

### Isolation and identification of Influenza A H5N1 virus

In this study, the 2,175 samples were subjected for virus isolation using egg inoculation at the Biosafety level 2+ laboratory. Of 2,175 samples, 68 (3.13 %) were positive for Hemagglutination activity. Among these positive samples, 60 samples were from chicken (88.2%) and 8 samples were from Duck (11.8%). In partition, provinces that have most positive HA samples were Prachuap Khiri Khan (35.3%) and Mukdahan (16.2%) (Table 4).

 Table 4: List of hemagglutination activity (HA) positive samples by species and location.

 (n=68)

Province	Chicken	Duck	Total	Percentage %
Loei	2	2	4	5.88
Nong Khai	1	1	2	2.94
Chiang Rai	5	- 5		7.35
Nakhon Phanom	2	-	2	2.94
Kanchanaburi	9	-	9	13.23
Tak	8	2	10	14.71
Ubon Ratchathani	1	ทรท	1	1.47
Mukdahan	10	1	11	16.17
Prachuap Khiri Khan	22	2	24	35.29
Total	60	8	68	100

The HA-positive samples were then examined by multiplex RT-PCR , realtime RT-PCR, and PCR-ELISA for influenza A (M gene) and subtype H5N1 (H5 and N1 genes) Of

68 HA positive samples, 15 samples were positive for M, H5, and N1 by multiplex RT-PCR (Table 5). The samples were recovered from chicken (n=13) and duck (n=2). These 15 samples were confirmed as H5N1 viruses by realtime RT-PCR and PCR-ELISA. Subtype identification of HPAI H5N1 virus by realtime RT-PCR and PCR-ELISA is now shown in Table 6.

-		Sample				
Province	Host	Influenza A	Subtype H5	Subtype N1		
		(M gene)	(HA gene)	(NA gene)		
Loci	chicken	2	2	2		
Loei	duck	2	2	2		
Chiang Rai	c <mark>h</mark> icken	1	1	1		
Prachuap Khiri	Chicken	10	2	7		
Khan	Chicken	10	2	1		
Total		15	7*	12*		

 Table 5:
 List of H5N1 viruses identify by multiplex RT-PCR

\*The samples were tested positive for H5N1 subtype either by Realtime RT-PCR or PCR ELISA.

 Table 6: Subtype identification of HPAI H5N1 virus by realtime RT-PCR and PCR-ELISA

	R	Results			
ID <mark>sa</mark> mple	HA gene (H5)	NA gene (N1)			
177	H5	N1			
205	H5	N1			
212	Н5	N1			
219	H5	N1			
31	H5	N1			
13/6	H5 <sup>ª</sup>	N1			
15/6	H5 <sup>ª</sup>	N1			
23/6	Н5	N1			

	27/6	H5	N1
2	45/6	H5 <sup>ª</sup>	N1
2	48/6	H5 <sup>ª</sup>	N1
Q	97/6	H5 <sup>°</sup>	N1
Q	98/6	H5 <sup>°</sup>	N1 <sup>a</sup>
1	00/6	H5 <sup>°</sup>	N1 <sup>a</sup>
1	01/6	H5 <sup>a</sup>	N1 <sup>a</sup>

<sup>a</sup> Positive samples by PCR-ELISA only

The results of influenza A virus identification (positive matrix gene) by RT-PCR is shown in Figure 5. Positive Matrix gene samples yielded PCR product with 125 bp insize. These results confirmed the identification of 15 samples as influenza A virus. The results of subtype H5 identification (Hemagglutinin gene positive) by RT-PCR is shown in Figure 6. Positive Hemagglutinin gene samples generated 148 bp- PCR product. Again, these results were in agreement with the result of the identification of those Influenza A virus subtype H5.



**Figure 5**: Identification of influenza A viruses (matrix gene positive) by multiplex RT-PCR. The size of expected PCR product is 125 bp.



Figure 6: Identification of influenza A subtype H5 (hemagglutinin gene positive) by multiplex RT-PCR. The expected product size is 148 bp.

In this study, we also confirmed the HA positive samples using realtime RT-PCR and PCR-ELISA. The results were consistented with those from multiplex RT-PCR. The viruses were identified as avian influenza H5N1 virus by Realtime RT-PCR. Identification of M gene (Matrix gene), H5 (Hemagglutinin gene), and N1 (Neuraminidase gene) by realtime RT-PCR is shown in Figure 7, 8, and 9, respectively.



**Figure 7**: Identification of M gene of influenza A virus by realtime RT-RCR. Positive results of PCR amplification were Ct value of < 40 Ct.



**Figure 8**: Identification of HA gene of influenza A virus by realtime RT-RCR. Positive results of PCR amplification were Ct value of < 40 Ct.



**Figure 9**: Identification of NA gene of influenza A virus by realtime RT-RCR. Positive results of PCR amplification were Ct value of < 40 Ct.

Influenza A H5N1 virus was also confirmed by PCR-ELISA. This method was developed by a researcher from the faculty of Veterinary Science, Chulalongkorn University. PCR-ELISA technique is considered to be a high specificity and sensitivity method. The technique also provides consistent result with multiplex RT-PCR and realtime PCR technique (Chaharaein et al., 2009). The results of PCR-ELISA are shown in Figure 10.

### H5 N1 M H5 N1 M

13/6 (H5N1, chicken) 15/6 (H5N1, chicken) 23/6 (H5N1, chicken) 27/6 (H5N1, chicken) 45/6 (H5N1, chicken) 97/6 (H5N1, chicken) 98/6 (H5N1, chicken)



100/6 (H5N1, chicken) 101/6 (H5N1, chicken) Positive control 101/6 (H5N1, chicken) 101/6 (H5N1, chicken) Positive control Positive control Negative control

212 (H5N1, chicken) Negative control Positive control 205 (H5N1, duck) 177 (H5N1, duck) 219 (H5N1, chicken) Positive control Negative control

H5 N1

Figure 10: Identification of influenza A virus subtype H5N1 by PCR-ELISA. Positive M, H5, and N1 genes were identified as increasing of color intensity ( $O.D._{405}$ - $O.D._{492}$ > 0.3).

### DNA sequencing of avian influenza H5N1 viruses

In this study, we have selected 3 avian influenza H5N1 viruses which were the representatives from each province of Loey, Chiang Rai, and Prachuap Khiri Khan for whole gene sequencing. We have sequenced whole genome of 3 avian influenza H5N1 viruses, CU-345 (ID- sample: 219), CU-346 (ID- sample: 31) and CU-347 (ID- sample: 23/6). The description of avian influenza H5N1 virus samples characterized in this study is shown in Table 7. Nucleotide sequences of 8 genes (PB2, PB1, PA, HA, NP, NA, M, and NS) of 3 avian influenza H5N1 viruses is shown in Table 8.

 Table 7: Host, location, and year of avian influenza H5N1 virus samples sequenced in this study.

CU-ID	Description	Llast	Leastian	
samples	Description	HOSI	Location	year
CU345	A/duck/Loei/Thailand/CU-345/07	Duck	Loei	2550
CU346	A/chicken/Chiang Rai/Thailand/CU-346/07	Chicken	Chiang Rai	2550
CU347	A/ck/Prachuap Khiri Khan/Thailand/CU-	Chicken	Prachuap Khiri	2551
	347/08		Khan	

<sup>\*</sup>CU-345 (ID- sample: 219), CU-346 (ID- sample: 31) and CU-347 (ID- sample: 23/6)

Table 8: Available nucleotide sequence of 8 genes (PB2, PB1, PA, HA, NP, NA, M, andNS) of 3 avian influenza H5N1 viruses characterized in this study.

	Nucleotide sequence (bp)								
samples	PB2	PB1	PA	HA	NP	NA	М	NS	
campico	gene	gene	gene	gene	gene	gene	gene	gene	
CU345	_*	_*	517	456	555	1353	_*	825	
CU346	-*	-*	-*	755	555	1381	-*	-*	
CU347	2282	2294	2221	1717	1523	1382	985	854	
-* : N/A not available									

### Genetic relatedness and genotype analysis of avian influenza H5N1 virus

Genetic relatedness of avian influenza H5N1 virus can be analyzed by sequence comparison of these viruses with the viruses isolated from Thailand and foreign countries. Results of genetic relatedness of 3 avian influenza H5N1 viruses are shown in phylogenetic tree (Figure 12- 31). The polymorphisms of key determinant residues of 8 genes of 3 avian influenza H5N1 viruses were analyzed and compared with the original avian influenza H5N1 virus "Goose/Guangdong/1/96" and other avian influenza viruses from in Thailand and other countries (Table 9-16).

### Genotype analysis

The genotype of avian influenza H5N1 virus can be classified when genetic data of 8 genes of viruses are available for comparing the genetic relatedness. Genotype analysis of avian influenza H5N1 virus in this study was performed in only 1 sample (CU-347) with the sequences of the 8 genes. The nucleotide sequences from each gene segment analyzed were as follows: PB2 1-2277 bp, PB1 1-2271 bp, PA 2151 bp, HA 1-1676 bp, NP 1-1481 bp,NA 1-1386 bp, M 1-960 bp, and NS 1-690 bp. A distinct phylogenetic lineage was recognized based on NJ bootstrap support of  $\geq$ 70% or Bayesian posterior probability of  $\geq$ 95% as indicating the origin. A genotype was assigned when the 8 genes lineage resulted in a unique gene constellation. Nevertheless, genotypes were also named in cases where viruses had the same gene lineage but diverged by some key determinant, for example, an amino-acid deletion, e.g. genotypes Z and Z+ diverge only in the presence or absence of a 20-aa deletion in the neuraminidase (NA) protein (Guan et al., 2002).

Previous studies have shown that 2 clades (clade 1 and 2.3.4) or 2 genotypes (genotype Z and V) of avian influenza H5N1 viruses were found to emerge and cause the avian influenza outbreaks in Thailand (Chutinimitkul et al., 2007). Genotypes of avian influenza H5N1 viruses in Thailand, 2004-2008 were analyzed and compared with the original avian influenza H5N1 virus "Goose/Guangdong/1/96" and other avian influenza viruses from other countries and are shown in Figure 11. In this study, phylogenetic

analysis of whole genome (CU-347) showed that the viruses clustered within the lineage of H5N1 avian isolates from Thailand-Vietnam lineage, genotype Z or clade 1. In contrast, the viruses isolated in Indonesia and China formed a separate lineage. In Thailand 2004-2008, genotypes were detected including genotype Z and genotype V. Genotypes Z and V have only HA and NA genes origin from Goose/Guangdong/1/96lingeage (Figure 11). Genotypes Z and V have seven segmented genes from a common source whereas their PA genes have unlike origins (Chen et al., 2006). Results of genetic analysis of 3 avian influenza H5N1 viruses are shown in phylogenetic tree (Figure 12-31).

Currently, the classification of clade system of avian influenza H5N1 virus was developed based on the evaluation of the HA gene. This classification system was developed by the group of scientists and relevant international organizations such as World Health Organization (WHO), World Animal Health Organization (OIE) and the Food and Agriculture Organization (FAO). In this study, the 3 avian influenza H5N1 viruses (CU-345, CU-346 and CU-347) belonged to clade 1 that was considered as an important clade and the cause's outbreaks of avian influenza in Thailand and Vietnam as well as Cambodia, Laos and Malaysia. The results of clade classification can infer the evolution of virus from the same original virus group (same ancestor).

In summary, avian influenza H5N1 viruses isolated from border areas of Thailand, Laos and Myanmar were classified as genotype Z or clade 1. The viruses belonged to the common avian influenza H5N1 genotype or clade that has caused the outbreaks of avian influenza in Thailand.

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Figure 11: Genotype of avian influenza 1996 H5N1 viruses in Thailand, 2004-2008. The eight gene segments, represented by Gs/GD horizontal bars are, from top to bottom, polymerase (PB2, PB1, PA), hemagglutinin 2004 (HA), nucleoprotein (NP), neuraminidase (NA), matrix (M), and nonstructural (NS) genes. A different color represents each 2005 2006 2007 2008

### Hemagglutinin gene

Avian influenza H5N1 viruses from this study (CU-345, CU-346, and CU-347) are grouped in Clade 1 which is a group related to the avian influenza outbreaks in the last 4-5 years in Thailand (Figure 12). Connecting peptide sequences or HA cleavage site of those viruses harbor multiple basic amino acids (RERRRKK), similar to the majority of HPAI H5N1 isolates outbreaks. CU-346 and CU-347 isolates had a glutamine at position 222 and a glycine at position 224, which are related to receptor binding sites for avian species (Matrosovich et al., 1999; Shinya et al., 2006; Webster et al., 1997). The polymorphisms of Hemagglutinin protein of avian influenza viruses at key determinant residues such as HA cleavage site, receptor binding site, glycosylation, and antigenic sites are shown in Table 9, 10, and Figure 13, 14. In this study, avian influenza H5N1 viruses (CU-345, CU 346, and CU347) have no mutations in any key determinants of Hemagglutinin gene.

### HA phylogenetic analysis



**Figure 12**: Phylogenetic analysis of HA gene of H5N1 viruses isolated in this study comparing with other H5N1 viruses isolated from Thailand and other countries. The analysis was based on nucleotides 1-1676 of the HA gene.

 Table 9: Genetic analysis of deduced amino acid of HA gene at key determinant

 residues in cleavage sites and receptor binding sites.

	HA gene					
Virus	Connecting peptide Sequences		Receptor b	inding site		
	323-329 <sup>ª</sup>	129 <sup>b</sup>	175 <sup>⊳</sup>	222°	224 <sup>°</sup>	
A/chicken/Thailand/CU-347/2008	RERRRKK	L	L	Q	G	
A/duck/Thailand/CU-345/2007	RERRRKK	_*	-*	-*	_*	
A/chicken/Thailand/CU-346/2007	RERRRKK	_*	L	Q	G	
A/Thailand/16/2004	RERRRKK	L	L	Q	G	
A/chicken/Thailand/CU-K2/2004	RERRRKK	L	L	Q	G	
A/Thailand/676/2005	REKRRKK	V	L	Q	G	
A/chicken/Thailand/ <mark>CK-1</mark> 62/2005	REKRRKK	L	L	Q	G	
A/chicken/Thailand/NP-172/2006	RERRRK-	S	L	Q	G	
A/chicken/Thailand/ <mark>PC-16</mark> 8/200 <mark>6</mark>	RERRRKK	L	L	Q	G	
A/chicken/Thailand/PC-170/2006	REKRRKK	L	L	Q	G	
A/duck/Thailand/CU-328/2007	RERRRKK	L	L	Q	G	
A/duck/Thailand/KU- <mark>56</mark> /2007	REKRRK-	S	L	Q	G	
A/chicken/Thailand/ST-351/2008	RERRRKK	L	L	Q	G	
A/chicken/NIAH114843/2008	RERRRKK	L	L	Q	G	
A/Viet Nam/1194/2004	RERRRKK	L	L	Q	G	
A/Viet Nam/1203/2004	RERRRKK	L	L	Q	G	
A/duck/Vietnam/1228/2005	RERRRKK	Μ	Μ	Q	G	
A/duck/Vietnam/5/2007	REGRRKK	М	Μ	Q	G	
A/chicken/Indonesia/4/2004	RERRRKK	S	L	Q	G	
A/duck/Indonesia/MS/2004	RERRRKK	S	50	Q	G	
A/Indonesia/CDC596/2006	RERRRKK	L	L	Q	G	
A/Indonesia/CDC599/2006	RERRRKK	L	L	Q	G	
A/Indonesia/CDC88 <mark>7/20</mark> 06	RESRRKK	S	L	Q	G	
A/Indonesia/CDC938/2006	RESRRKK	S	L	Q	G	
A/turkey/Turkey/1/2005	GERRRKK	А	L	Q	G	
A/whooper swan/Mongolia/3/2005	GERRRK	S	CLC	Q	G	
A/goose/Guangdong/1/1996	RERRRKK	S	L	Q	G	
A/bar-headed goose/Qinghai/5/2005	GERRRKK	S	L	Q	G	
A/duck/Guangxi/150/2006	RERRRK-	S	L	Q	G	
A/duck/Fujian/668/2006	RERRRK-	S	L	Q	G	

<sup>a</sup> Avian influenza viruses in this study have connecting peptide sequences that are RERRKK <sup>b</sup> Amino acid at position 129 and 175 of HA gene: S (Serine)/L: (Leucine) and L (Leucine) respectively.

<sup>c</sup> Amino acid at position 222 and 224 of HA gene: Q (Glutamine), G (Glycine) respectively.

**Table 10**: Genetic analysis of deduced amino acid of HA gene at key determinantresidues in glycosylation sites.

Virus	Glycosylation sites <sup>®</sup>						
	10-12	11-13	23-25	154-	165-	193-	286-
A/chicken/Thailand/CU-347/2008	N-S	N-T	N-T	N-T	N-T	N-T	N-S
A/duck/Thailand/CU-345/2007	_*	-*	_*	_*	-*	-*	N-S
A/chicken/Thailand/CU-346/2007	_*	-*	_*	N-T	N-T	N-T	N-S
A/Thailand/16/2004	N-S	N-T	N-T	N-T	N-T	N-T	N-S
A/chicken/Thailand/CU-K2/2004	N-S	N-T	N-T	N-T	N-T	N-T	N-S
A/Thailand/676/2005	N-S	N-T	N-T	N-T	N-T	N-T	N-S
A/chicken/Thailand/CK-162/2005	N-S	N-T	N-T	N-T	N-T	N-T	N-S
A/chicken/Thailand/NP-172/2006	N-S	N-T	N-T	N-T	N-T	N-T	N-S
A/chicken/Thailand/PC-168/2006	N-S	N-T	N-T	N-T	N-T	N-T	N-S
A/chicken/Thailand/PC-170/2006	N-S	N-T	N-T	N-T	N-T	N-T	N-S
A/duck/Thailand/CU-328/2007	N-S	N-T	N-T	N-T	N-T	N-T	N-S
A/duck/Thailand/ <mark>KU-</mark> 56/2007	N-S	N-T	N-T	N-T	N-T	N-T	N-S
A/chicken/Thailand/ST-351/2008	N-S	N-T	N-T	N-T	N-T	N-T	N-S
A/chicken/NIAH114843/2008	N-S	N-T	N-T	N-T	N-T	N-T	N-S
A/Viet Nam/1194/2 <mark>00</mark> 4	N-S	N-T	N-T	N-T	N-T	N-T	N-S
A/Viet Nam/1203/2004	N-S	N-T	N-T	N-T	N-T	N-T	N-S
A/duck/Vietnam/1228/2005	N-S	N-T	N-T	N-T	N-T	N-T	N-S
A/duck/Vietnam/5/2007	N-S	N-T	N-T	N-T	N-T	N-T	N-S
A/chicken/Indonesia/4/2004	N-S	N-T	N-T	N-T	N-T	N-T	N-S
A/duck/Indonesia/MS/2004	N-S	N-T	N-T	(N-A) <sup>b</sup>	N-T	N-T	N-S
A/Indonesia/CDC596/2006	N-S	N-T	N-T	N-T	N-T	N-T	N-S
A/Indonesia/CDC599/2006	N-S	N-T	N-T	N-T	N-T	N-T	N-S
A/Indonesia/CDC887/2006	N-S	N-T	N-T	N-T	N-T	N-T	N-S
A/Indonesia/CDC938/2006	N-S	N-T	N-T	N-T	N-T	N-T	N-S
A/turkey/Turkey/1/2005	N-S	N-T	N-T	(D-A) <sup>b</sup>	N-T	N-T	N-S
A/whooper	N-S	N-T	N-T	(D-A) <sup>b</sup>	N-T	N-T	N-S
swan/Mongolia/3/2005							
A/goose/Guangdong/1/1996	N-S	N-T	N-T	(N-A) <sup>b</sup>	N-T	N-T	N-S
A/bar-headed	N-S	N-T	N-T	N-A	N-T	N-T	N-S
goose/Qinghai/5/2005							
A/duck/Guangxi/150/2006	N-S	N-T	N-T	N-T	N-T	N-T	N-S
A/duck/Fujian/668/2006	N-S	N-T	N-T	N-T	N-T	N-T	N-S

<sup>a</sup> Amino acid at glycosylation site of HA gene: N (Asparagine), S (Serine), T (Threonine)

 $^{\scriptscriptstyle \rm b}$  Amino acid at position 154-156 (N-A) and (D-A) do not have glycosylation properties.

**Table 11:** Genetic analysis of deduced amino acid of HA gene at key determinantresidues in antigenic sites.

VirusAntigenic #FNetworks #8386138140141Achicken/Thailand/CU-345/2007A'chicken/Thailand/CU-345/2007A'chicken/Thailand/CU-346/2007A'chicken/Thailand/CU-346/2007 <t< th=""><th></th><th colspan="7">Antigenic sites<sup>®</sup></th></t<>		Antigenic sites <sup>®</sup>						
8386138140141Achicken/Thailand/CU-345/2007 <th>Virus</th> <th>Antigenie</th> <th>c site E</th> <th>Ar</th> <th>A</th>	Virus	Antigenie	c site E	Ar	A			
A/chicken/Thailand/CU-345/2007       -       <		83	86	138	140	141		
A/duck/Thailand/CU-345/2007       -*       -*       -*       -*       -*       L         A/chicken/Thailand/CU-346/2007       -*       -*       -*       -*       L         A/thailand/16/2004       A       V       Q       K       S         A/chicken/Thailand/CU-K2/2004       A       V       Q       K       S         A/thailand/Co/6/2005       P       A       Q       K       S         A/chicken/Thailand/CK-162/2005       A       A       Q       K       S         A/chicken/Thailand/PC-170/2006       A       A       Q       K       S         A/chicken/Thailand/PC-170/2006       P       A       Q       K       S         A/chicken/Thailand/FC-170/2006       P       A       Q       K       S         A/duck/Thailand/FC-170/2006       P       A       Q       K       S         A/duck/Thailand/FC-170/2006       A       Q       K       S         A/duck/Thailand/SU-56/2007       A       A       Q       K       S         A/duck/Thailand/SU-56/2007       A       V       Q       K       S         A/duck/Uetham/1228/2005       A       V       Q       K	A/chicken/Thailand/CU-347/2008	A	V	Q	К	S		
A/chicken/Thailand/CU-346/2007LA/Thailand/16/2004AVQKSA/chicken/Thailand/CU-K2/2004AVQKSA/chicken/Thailand/CK-162/2005AAQKSA/chicken/Thailand/CK-162/2006AAQKSA/chicken/Thailand/PC-170/2006AAQKSA/chicken/Thailand/CU-328/2007AAQKSA/chicken/Thailand/CU-328/2007AAQKSA/chicken/Thailand/ST-351/2008AVQKSA/chicken/Thailand/ST-351/2008AVQKSA/chicken/Thailand/ST-351/2008AVQKSA/chicken/Thailand/ST-351/2008AVQKSA/chicken/Ithailand/ST-351/2008AVQKSA/chicken/Ithailand/ST-351/2008AVQKSA/chicken/Ithailand/ST-351/2008AVQKSA/chicken/Ithailand/ST-351/2008AVQKSA/chicken/Ithailand/ST-351/2008AVQKSA/chicken/Ithailand/SC-32/2005AVQKSA/chicken/Ithailand/SC-32/2005AQKSSA/chicken/Ithdonesia/MS/2004AAQKSA/indonesia/CDC599/2006AALRS <td< td=""><td>A/duck/Thailand/CU-345/2007</td><td>_*</td><td>_*</td><td>_*</td><td>_*</td><td>-*</td></td<>	A/duck/Thailand/CU-345/2007	_*	_*	_*	_*	-*		
A/Thailand/16/2004AVQKSA/chicken/Thailand/CU-K2/2004AVQKSA/chicken/Thailand/CK-162/2005AAQTPA/chicken/Thailand/CK-162/2006AAQTPA/chicken/Thailand/PC-170/2006AAQKSA/chicken/Thailand/PC-170/2006PAQKSA/chicken/Thailand/PC-170/2006PAQKSA/chicken/Thailand/PC-150/2007AAQKSA/chicken/Thailand/ST-351/2008AVQKSA/chicken/Thailand/ST-351/2008AVQKSA/chicken/Thailand/ST-351/2008AVQKSA/chicken/Thailand/ST-351/2008AVQKSA/chicken/Thailand/ST-351/2008AVQKSA/chicken/Indunesia/MS/2004AVQKSA/chicken/Indonesia/MS/2005AAQKSA/chicken/Indonesia/MS/2004AAQKSA/indonesia/MS/2005AALRSA/indonesia/MS/2006AALRSA/indonesia/MS/2005IAQRSA/indonesia/MS/2005IAQRSA/indonesia/MS/2005IAQRSA/indonesia/MS/2005IAQ	A/chicken/Thailand/CU-346/2007	_*	_*	_*	_*	L		
A/chicken/Thailand/CU-K2/2004AVQKSA/Thailand/676/2005PAQKSA/chicken/Thailand/CK-162/2006AAQTPA/chicken/Thailand/PC-168/2006AVLKSA/chicken/Thailand/PC-170/2006PAQKSA/chicken/Thailand/PC-170/2006PAQKSA/chicken/Thailand/PC-170/2006AAQKSA/chicken/Thailand/ST-351/2008AVQKSA/chicken/Thailand/ST-351/2008AVQKSA/chicken/Thailand/ST-351/2008AVQKSA/chicken/Thailand/ST-351/2008AVQKSA/chicken/Thailand/ST-351/2008AVQKSA/chicken/Indunesia/J2009AVQKSA/chicken/Indunesia/J2009AVQKSA/chicken/Indonesia/MS/2004AQKSA/indonesia/MS/2004AAQKSA/indonesia/MS/2005AALRSA/indonesia/CDC599/2006AALSPA/indonesia/CDC599/2006AALSPA/indonesia/CDC599/2006AALSPA/indonesia/CDC599/2006AALSPA/indonesia/CDC599/2006AAQ <td>A/Thailand/16/2004</td> <td>А</td> <td>V</td> <td>Q</td> <td>К</td> <td>S</td>	A/Thailand/16/2004	А	V	Q	К	S		
AThailand/676/2005PAQKSA/chicken/Thailand/CK-162/2005AAQTPA/chicken/Thailand/PC-170/2006AVLKSA/chicken/Thailand/PC-170/2006PAQKSA/chicken/Thailand/PC-170/2006PAQKSA/duck/Thailand/CU-328/2007AAQKSA/duck/Thailand/CU-328/2007AAQKSA/duck/Thailand/ST-351/2008AVQKSA/chicken/Thailand/ST-351/2008AVQKSA/chicken/Inlailand/ST-351/2008AVQKSA/duck/Thailand/ST-351/2008AVQKSA/duck/Metnam/129/2004AVQKSA/duck/Vietnam/129/2004AVQKSA/duck/Vietnam/1228/2005AAQKSA/duck/Indonesia/MS/2004AAQKSA/ludonesia/CDCS96/2006AALRSA/ludonesia/CDC938/2006AALSPA/ludnesia/CDC938/2006AAQKSA/ludnesia/CDC938/2006AAQRSA/ludnesia/CDC938/2005IAQRSA/ludnesia/CDC938/2006AAQRSA/ludnesia/CDC938/2005IAQR<	A/chicken/Thailand/CU-K2/2004	A	V	Q	К	S		
A/chicken/Thailand/CK-162/2005AAQKSA/chicken/Thailand/PC-168/2006AVLKSA/chicken/Thailand/PC-170/2006PAQKSA/duck/Thailand/CU-328/2007AAQKSA/duck/Thailand/CU-328/2007AAQKSA/duck/Thailand/CU-328/2007AAQKSA/duck/Thailand/KU-56/2007AAQKSA/chicken/Thailand/ST-351/2008AVQKSA/chicken/Thailand/ST-351/2008AVQKSA/chicken/IL14843/2008AVQKSA/duck/Vietnam/12904AVQKSA/duck/Vietnam/1292/2004AVQKSA/duck/Vietnam/5/2007AAQKSA/duck/Indonesia/MS/2004AAQKSA/ludonesia/CDCS96/2006AALRSA/ludonesia/CDC387/2006AALSPA/ludonesia/CDC388/2006AALSSA/ludonesia/CDC388/2006AAAQSSA/ludoesia/CDC388/2006AAAGSSA/ludoesia/CDC388/2006AAAGSSA/ludoesia/CDC388/2006AAAGSSA/ludoesia/CDC388/2006A <td>A/Thailand/676/2005</td> <td>Р</td> <td>А</td> <td>Q</td> <td>К</td> <td>S</td>	A/Thailand/676/2005	Р	А	Q	К	S		
A/chicken/Thailand/NP-172/2006AQTPA/chicken/Thailand/PC-168/2006PAQKSA/chicken/Thailand/PC-170/2006PAQKSA/duck/Thailand/CU-328/2007AAQTPA/duck/Thailand/KU-56/2007AAQKSA/duck/Thailand/KU-56/2007AAQKSA/chicken/Thailand/KU-56/2007AVQKSA/chicken/Thailand/KU-56/2008AVQKSA/chicken/ILaH114843/2008AVQKSA/viet Nam/1194/2004AVQKSA/duck/Vietnam/1228/2005AVQKSA/duck/Vietnam/5/2007AVQKSA/duck/Vietnam/5/2007AAQKSA/duck/Vietnam/1208/2005AALRSA/duck/Indonesia/MS/2004AAQKSA/lndonesia/CDC599/2006AALRSA/Indonesia/CDC338/2006ATLSPA/undonesia/CDC338/2005IAQRSA/goose/Guangdong/1/1996AAQRSA/goose/Guangdong/1/1996AAQRSA/duck/Fujan/668/2006AAQTPA/duck/Fujan/668/2006AAQTP <td>A/chicken/Thailand/CK-162/2005</td> <td>А</td> <td>А</td> <td>Q</td> <td>К</td> <td>S</td>	A/chicken/Thailand/CK-162/2005	А	А	Q	К	S		
A/chicken/Thailand/PC-168/2006AVLKSA/chicken/Thailand/PC-170/2006PAQKSA/duck/Thailand/CU-328/2007AAQTPA/duck/Thailand/KU-56/2007AAQKSA/chicken/Thailand/KU-56/2007AVQKSA/chicken/Thailand/KU-56/2007AVQKSA/chicken/Italiand/ST-351/2008AVLRSA/chicken/NIAH114843/2008AVQKSA/viet Nam/1194/2004AVQKSA/duck/Vietnam/1228/2005AVQKSA/duck/Vietnam/1228/2005AVQKSA/duck/Indonesia/MS/2007AVQKSA/duck/Indonesia/MS/2004AALRSA/lndonesia/CDC599/2006AALRSA/Indonesia/CDC938/2006ATLSPA/Indonesia/CDC938/2006AAQRSA/Indonesia/Mongolia/3/2005IAQRSA/goose/Guangdong/1/1996AAQRSA/goose/Guangdong/1/1996AAQRSA/duck/Fujian/668/2006AAQTPA/uduck/Fujian/668/2006AAQTP	A/chicken/Thailand/NP-172/2006	A	А	Q	Т	Р		
Achcicken/Thailand/PC-170/2006       P       A       Q       K       S         A/duck/Thailand/CU-328/2007       A       A       Q       T       P         A/duck/Thailand/KU-56/2007       A       A       Q       K       S         A/duck/Thailand/KU-56/2007       A       A       Q       K       S         A/chicken/Thailand/KU-56/2007       A       V       Q       K       S         A/chicken/Thailand/ST-351/2008       A       V       Q       K       S         A/chicken/INIAH114843/2008       A       V       Q       K       S         A/Viet Nam/1194/2004       A       V       Q       K       S         A/duck/Vietnam/1228/2005       A       V       Q       K       S         A/duck/Vietnam/5/2007       A       A       Q       K       S         A/duck/Indonesia/MS/2004       A       A       Q       K       S         A/Indonesia/CDC599/2006       A       A       L       R       S         A/Indonesia/CDC598/2006       A       T       L       S       P         A/Indonesia/CDC598/2006       A       A       Q       R       S	A/chicken/Thailand/PC-168/2006	A	V	L	К	S		
A/duck/Thailand/CU-328/2007AAQKSA/duck/Thailand/KU-56/2007AVQKSA/chicken/Thailand/ST-351/2008AVQKSA/chicken/INIAH114843/2008AVQKSA/viet Nam/1194/2004AVQKSA/viet Nam/1203/2004AVQKSA/duck/Vietnam/1228/2005AVQKSA/duck/Vietnam/5/2007AVQKSA/duck/Vietnam/5/2007AAQKSA/duck/Indonesia/MS/2004AAQKSA/ludonesia/CDC596/2006AALRSA/Indonesia/CDC599/2006AALSPA/Indonesia/CDC598/2006ATLSPA/Indonesia/CDC598/2006AAQRSA/Indonesia/CDC598/2006AAQRSA/Indonesia/CDC938/2005IAQRSA/undoper swan/Mongolia/3/2005IAQRSA/bar-headed goose/Qinghai/5/2005IAQRSA/uck/Guangxi/150/2006AAQTPA/uck/Fujian/668/2006AAQTP	A/chicken/Thailand/PC-170/2006	Р	А	Q	К	S		
A/duck/Thailand/KU-56/2007AAQTPA/chicken/Thailand/ST-351/2008AVQKSA/chicken/NIAH114843/2008AVQKSA/Viet Nam/1194/2004AVQKSA/Viet Nam/1203/2004AVQKSA/duck/Vietnam/1228/2005AVQKSA/duck/Vietnam/5/2007AVQKSA/duck/Vietnam/5/2007AAQKSA/duck/Indonesia/MS/2004AAQKSA/lndonesia/MS/2004AAQKSA/Indonesia/CDC596/2006AALRSA/Indonesia/CDC593/2006AALSPA/Indonesia/CDC338/2006ATLSPA/Indonesia/Monogolia/3/2005IAQRSA/undoper swan/Monogolia/3/2005IAQRSA/bar-headed goose/Qinghai/5/2005IAQRSA/duck/Guangxi/150/2006AAQTPA/duck/Fujian/668/2006AAQTP	A/duck/Thailand/CU-328/2007	A	А	Q	К	S		
A/chicken/Thalland/ST-351/2008       A       V       Q       K       S         A/chicken/NIAH114843/2008       A       V       Q       K       S         A/Viet Nam/1194/2004       A       V       Q       K       S         A/Viet Nam/1203/2004       A       V       Q       K       S         A/Viet Nam/1203/2004       A       V       Q       K       S         A/duck/Vietnam/1228/2005       A       V       Q       K       S         A/duck/Vietnam/5/2007       A       V       Q       K       S         A/duck/Indonesia/MS/2004       A       A       Q       K       S         A/Indonesia/CDC596/2006       A       A       L       R       S         A/Indonesia/CDC599/2006       A       A       L       R       S         A/Indonesia/CDC938/2006       A       T       L       S       P         A/Indonesia/CDC938/2005       I       A       Q       R       S         A/Indonesia/CDC938/2005       I       A       Q       R       S         A/Indonesia/CDC938/2005       I       A       Q       R       S         A/Indonesia/G	A/duck/Thailand/KU-56/2007	А	А	Q	Т	Р		
A/chicken/NIAH114843/2008AVLRSA/Viet Nam/1194/2004AVQKSA/Viet Nam/1203/2004AVQKSA/duck/Vietnam/1228/2005AVQKSA/duck/Vietnam/5/2007AVQRSA/duck/Vietnam/S/2004AAQKSA/duck/Indonesia/MS/2004AAQKSA/duck/Indonesia/MS/2004AALRSA/Indonesia/CDC596/2006AALRSA/Indonesia/CDC599/2006ATLSPA/Indonesia/CDC938/2006ATLSPA/Indonesia/CDC938/2006ATLSPA/Indonesia/CDC938/2005IAQRSA/undonesia/CDC938/2005IAQRSA/undonesia/CDC938/2005IAQRSA/undonesia/CDC938/2005IAQRSA/undonesia/CD0938/2005IAQRSA/undonesia/CD0938/2005IAQRSA/undonesia/CD0938/2005IAQRSA/undocyclunghai/5/2005IAQRSA/undocyclunghai/5/2006AAQTPA/undocyclunghai/5/2006AAQTPA/undocyclunghai/5/2006A	A/chicken/Thailand/ST <mark>-35</mark> 1/2008	А	V	Q	К	S		
AViet Nam/1194/2004       A       V       Q       K       S         AViet Nam/1203/2004       A       V       Q       K       S         A/duck/Vietnam/1228/2005       A       V       Q       K       S         A/duck/Vietnam/5/2007       A       V       Q       R       S         A/duck/Vietnam/5/2007       A       V       Q       K       S         A/duck/Indonesia/MS/2004       A       A       Q       K       S         A/ludonesia/CDC596/2006       A       A       Q       K       S         A/Indonesia/CDC599/2006       A       A       L       R       S         A/Indonesia/CDC599/2006       A       A       L       S       P         A/Indonesia/CDC598/2006       A       T       L       S       P         A/Indonesia/CDC938/2006       A       T       L       S       P         A/Indonesia/CDC938/2005       I       A       Q       R       S         A/Indonesia/CDC938/2005       I       A       Q       R       S         A/Indonesia/CDC938/2005       I       A       Q       R       S         A/goose/Guangdong/1/199	A/chicken/NIAH114843/2008	А	V	L	R	S		
AViet Nam/1203/2004AVQKSA/duck/Vietnam/1228/2005AVQKSA/duck/Vietnam/5/2007AVQRSA/chicken/Indonesia/4/2004AAQKSA/duck/Indonesia/MS/2004AAQKSA/lndonesia/CDC596/2006AALRSA/Indonesia/CDC599/2006AALSPA/Indonesia/CDC938/2006ATLSPA/Indonesia/CDC938/2006IAQRSA/Indonesia/CDC938/2006IAQRSA/Indonesia/CDC938/2006IAQRSA/Indonesia/CDC938/2006IAQRSA/Indonesia/CDC938/2005IAQRSA/Indonesia/CDC938/2005IAQRSA/Indonesia/CDC938/2005IAQRSA/Indonesia/CDC938/2005IAQRSA/Indonesia/CDC938/2005IAQRSA/Indonesia/CDC938/2005IAQRSA/Indonesia/CDC938/2005IAQRSA/Indonesia/CDC938/2006AAHRSA/Indonesia/CDC938/2006AAQRSA/Indonesia/CDC938/2006AAAHRSA/Indonesia/CDC938/	A/Viet Nam/1194/2 <mark>0</mark> 04	A	V	Q	К	S		
A/duck/Vietnam/1228/2005AVQKSA/duck/Vietnam/5/2007AVQRSA/chicken/Indonesia/4/2004AAQKSA/duck/Indonesia/MS/2004AAQKSA/Indonesia/CDC596/2006AALRSA/Indonesia/CDC599/2006AALRSA/Indonesia/CDC387/2006ATLSPA/Indonesia/CDC938/2006ATLSPA/Indonesia/CDC938/2006IAQRSA/undonesia/CDC938/2005IAQRSA/undonesia/CDC938/2005IAQRSA/undonesia/CDC938/2005IAQRSA/undonesia/CDC938/2005IAQRSA/undonesia/CDC938/2005IAQRSA/undonesia/CDC938/2005IAQRSA/undonesia/CDC938/2005IAQRSA/undonesia/CDC938/2005IAQRSA/undonesia/CDC938/2006AAQRSA/undonesia/CDC938/2005IAQRSA/undonesia/CDC938/2006AAQRSA/undonesia/CDC938/2006AAQRSA/undonesia/CDC938/2006AAQRSA/undonesia/CDC938/2006 </td <td>A/Viet Nam/1203/2004</td> <td>А</td> <td>V</td> <td>Q</td> <td>К</td> <td>S</td>	A/Viet Nam/1203/2004	А	V	Q	К	S		
A/duck/Vietnam/5/2007AVQRSA/chicken/Indonesia/4/2004AAQKSA/duck/Indonesia/MS/2004AAQKSA/Indonesia/CDC596/2006AALRSA/Indonesia/CDC599/2006AALRSA/Indonesia/CDC887/2006ATLSPA/Indonesia/CDC938/2006ATLSPA/Indonesia/CDC938/2006ATLSPA/Indonesia/CDC938/2005IAQRSA/Indonesia/CDC938/2005IAQRSA/Indonesia/CDC938/2005IAQRSA/Indonesia/CDC938/2005IAQRSA/Indonesia/CDC938/2005IAQRSA/Indonesia/CDC938/2005IAQRSA/Indonesia/CDC938/2005IAQRSA/Indonesia/CDC938/2005IAQRSA/Indonesia/CDC938/2006AAQRSA/Indonesia/CDC938/2006AAQRSA/Indonesia/CDC938/2006AAQTPA/Indonesia/CDC938/2006AAQTPA/Indonesia/CDC938/2006AAQTPA/Indonesia/CDC938/2006AAQTPA/Indonesia/CDC938/2006 <td>A/duck/Vietnam/1228/2005</td> <td>А</td> <td>V</td> <td>Q</td> <td>К</td> <td>S</td>	A/duck/Vietnam/1228/2005	А	V	Q	К	S		
A/chicken/Indonesia/4/2004AAQKSA/duck/Indonesia/MS/2004AAQKSA/Indonesia/CDC596/2006AALRSA/Indonesia/CDC599/2006AALSPA/Indonesia/CDC887/2006ATLSPA/Indonesia/CDC938/2006ATLSPA/Indonesia/CDC938/2006ATLSPA/Indonesia/CDC938/2005IAQRSA/undoper swan/Mongolia/22005IAQRSA/goose/Guangdong/1/1996AAQRSA/duck/Guangxi/150/2006AAQTPA/duck/Fujian/668/2006AAQTP	A/duck/Vietnam/5/2007	А	V	Q	R	S		
A/duck/Indonesia/MS/2004AAQKSA/Indonesia/CDC596/2006AALRSA/Indonesia/CDC599/2006ATLSPA/Indonesia/CDC938/2006ATLSPA/Indonesia/CDC938/2006ATLSPA/Indonesia/CDC938/2006IAQRSA/Indonesia/CDC938/2005IAQRSA/unkey/Turkey/1/2005IAQRSA/unkey/Turkey/1/2005IAQRSA/unkey/I/2005IAQRSA/unkey/I/2005IAQRSA/unkey/I/2005IAQTPA/unkey/I/2006AAQTPA/unkey/I/2006AAQTPA/unkey/I/2006AAQTP	A/chicken/Indonesia/4/2004	А	А	Q	К	S		
A/Indonesia/CDC596/2006AALRSA/Indonesia/CDC599/2006AALRSA/Indonesia/CDC887/2006ATLSPA/Indonesia/CDC938/2006ATLSPA/Indonesia/CDC938/2006IAQRSA/turkey/Turkey/1/2005IAQRSA/whooper swan/Mongolia/3/2005IAAQRSA/goose/Guangdong/1/1996AAHRSA/duck/Guangxi/150/2006AAQTPA/duck/Fujian/668/2006AAQTP	A/duck/Indonesia/MS/2004	А	А	Q	К	S		
A/Indonesia/CDC599/2006AALRSA/Indonesia/CDC938/2006ATLSPA/Indonesia/CDC938/2006ATLSPA/turkey/Turkey/1/2005IAQRSA/whooper swan/Mongolia/3/2005IAQRSA/goose/Guangdong/1/1996AAHRSA/bar-headed goose/Qinghai/5/2005IAQRSA/duck/Guangxi/150/2006AAQTPA/duck/Fujian/668/2006AAQTP	A/Indonesia/CDC596/2006	А	А	- 152	R	S		
A/Indonesia/CDC887/2006ATLSPA/Indonesia/CDC938/2006ATLSPA/turkey/Turkey/1/2005IAQRSA/whooper swan/Mongolia/3/2005IAQRSA/goose/Guangdong/1/1996AAHRSA/bar-headed goose/Qinghai/5/2005IAQRSA/duck/Guangxi/150/2006AAQTPA/duck/Fujian/668/2006AAQTP	A/Indonesia/CDC599/2006	А	А	L	R	S		
A/Indonesia/CDC938/2006ATLSPA/turkey/Turkey/1/2005IAQRSA/whooper swan/Mongolia/3/2005IAQRSA/goose/Guangdong/1/1996AAHRSA/bar-headed goose/Qinghai/5/2005IAQRSA/duck/Guangxi/150/2006AAQTPA/duck/Fujian/668/2006AAQTP	A/Indonesia/CDC887/2006	А	Т	L	S	Р		
A/turkey/Turkey/1/2005IAQRSA/whooper swan/Mongolia/3/2005IAQRSA/goose/Guangdong/1/1996AAHRSA/bar-headed goose/Qinghai/5/2005IAQRSA/duck/Guangxi/150/2006AAQTPA/duck/Fujian/668/2006AAQTP	A/Indonesia/CDC938/2006	А	Т	L	S	Р		
A/whooper swan/Mongolia/3/2005IAQRSA/goose/Guangdong/1/1996AAHRSA/bar-headed goose/Qinghai/5/2005IAQRSA/duck/Guangxi/150/2006AAQTPA/duck/Fujian/668/2006AAQTP	A/turkey/Turkey/1/2005	I	А	Q	R	S		
A/goose/Guangdong/1/1996         A         A         H         R         S           A/bar-headed goose/Qinghai/5/2005         I         A         Q         R         S           A/duck/Guangxi/150/2006         A         A         Q         T         P           A/duck/Fujian/668/2006         A         A         Q         T         P	A/whooper swan/Mongolia/3/2005	- I Q	A	Q	R	S		
A/bar-headed goose/Qinghai/5/2005         I         A         Q         R         S           A/duck/Guangxi/150/2006         A         A         Q         T         P           A/duck/Fujian/668/2006         A         A         Q         T         P	A/goose/Guangdong/1/1996	А	А	Н	R	S		
A/duck/Guangxi/150/2006         A         A         Q         T         P           A/duck/Fujian/668/2006         A         A         Q         T         P	A/bar-headed goose/Qinghai/5/2005	1 1	А	Q	R	S		
A/duck/Fujian/668/2006 A A Q T P	A/duck/Guangxi/150/2006	А	А	Q	Т	Р		
	A/duck/Fujian/668/2006	А	А	Q	Т	Р		

<sup>a</sup> Amino acid at position 83, 86, 138, 140, 141 of HA gene: A (Alanine), V (Valine), H (Histidine), Q (Glutamine), L (Leucine), R (Arginine), K (Lysine), N (Asparagine), S (Serine), P (Proline)

### HA analysis

	HA cleavage site	e Receptor binding site
Consensus	LATGLENSPORERREKKEGLFGAIAGE	IATRSKVNGQSGRMEFFWTILKPN
30 Sequences	320 330 3	220 230
CU345HA	LATGLENSPORERREKKEGLEGALAGE	*****
CU346HA	LATGLENSPORERREKKEGLEGAIAGE	IATRSKVNGQSGRMEFFWTILKPN
CU347HA	LATGLENSPORERREKKEGLFGAIAGF	IATRSKVNGQSGRMEFFWTILKPN
A/Thailand/16/2004	LATGLENSPORERREKKEGLEGAIAGE	IATRSKVNGQSGRMEFFWTILKPN
A/chicken/Thailand/CU-K2/2004	LATGLENSPORERREKKEGLFGAIAGF	IATRSKVNGQSGRMEFFWTILKPN
A/Thailand/676/2005	LATGLENSPOREKERKKEGLEGAIAGE	IATRSKVNGQSGRMEFFWTILKPN
A/chicken/Thailand/CK-162/2005	LATGLENSPOREKREKKEGLEGALAGE	IATRSKVNGQSGRMEFFWTILKPN
A/chicken/Thailand/NP-172/2006	LATGLENSPLREERRKXXGLEGAIAGE	IHTRSKVNGQSGRMDFFWTMLKPN
A/chicken/Thailand/PC-168/2006	LATGLENSPORERREKKRGLEGAIAGE	IATRSKVNGQSGRMEFFWTILKPN
A/chicken/Thailand/PC-170/2006	LATGLENSPOREKREKKEGLEGAIAGE	IATRSKVNGQSGRMEFFWTILKPN
A/duck/Thailand/CU-328/2007	LATGLENSPORERREKKRGLEGAIAGE	IATRSKVNGQSGRMEFFWTILKPN
A/duck/Thailand/KU-56/2007	LATGLENSPLEERREKXXGLEGAIAGE	IATRSKVNGQSGRMDFFWTMLKPN
A/chicken/Thailand/ST-351/2008	LATGLRNSPORERRRKKRGLFGAIAGF	IATRSKVNGQSGRMEFFWTILKPN
A/chicken/Sukhothai/NIAH114843/200	LATGLENNPORERREKKRGLEGAIAGE	IATRSKVNGQSGRMEFFWTILKPN
A/Viet Nam/1194/2004	LATGLENSPORERREKKRGLEGALAGE	IATRSKVNGQSGRMEFFWTILKPN
A/Viet Nam/1203/2004	LATGLENSPORERREKKRGLEGALAGE	IATRSKVNGQSGRMEFFWTILKPN
A/duck/Vietnam/1228/2005	LATGLENSPORERREKKRGLEGAIAGE	IATRSKVNGQSGRMEFFWTILKPN
A/duck/Vietnam/5/2007	LATGLENSPOREGREKKEGLEGALAGE	IATRSKVNGQSGRMEFFWTILKPN
A/chicken/Indonesia/4/2004	LATGLENSPORERREKKRGLEGALAGE	IATRSKVNGQSGRMEFFWTILKPN
A/duck/Indonesia/MS/2004	LATGLENSPORERREKKRGLEGAIAGE	IATRSKVNGQSGRMEFFWTILKPN
A/Indonesia/CDC596/2006	LATGLENSPORERREKKEGLEGAIAGE	IATRSKVNGQSGRMEFFWTILKPN
A/Indonesia/CDC599/2006	LATGLENSPORERREKKEGLEGAIAGE	IATRSKVNGQSGRMEFFWTILKPN
A/Indonesia/CDC887/2006	LATGLENSPORESERKKEGLEGALAGE	IATRSKVNGQSGRMEFFWTILKPN
A/Indonesia/CDC938/2006	LATGLENSPORESERKKEGLEGALAGE	IATRSKVNGQSGRMEFFWTILKPN
A/turkey/Turkey/1/2005	LATGLENSPOGERREKKEGLEGAIAGE	IATRSKVNGQSGRMEFFWTILKPN
A/whooper swan/Mongolia/3/2005	LATGLENSPOGERRERKEGLEGAIAGE	IATRSKVNGQSGRMEFFWTILKPN
A/goose/Guangdong/1/1996	LATGLENTPORERREKKEGLEGAIAGE	IATRPKVNGQSGRMEFFWTILKPN
A/duck/Fujian/668/2006	LATGLENSPLEERREKXXGLEGAIAGE	IATRSKVNGQSGRMDFFWTILKPN
A/duck/Guangxi/150/2006	LATGLENSPIRERREKXIGLEGAIAGE	IATRSKVNGQSGRMDFFWTILKPN
A/bar-headed goose/Qinghai/5/2005	LATGLENSPOGERREKKEGLEGAIAGE	IATRSKVNGQSGRMEFFWTILKPN
		Ĩ Â A

**Figure 13**: Comparison of deduced amino acid of HA protein at HA cleavage site at position 323-329 and receptor binding site at position 222-224



HA1 DQICIGYHAN TIMEKNVTVTHAQDILEKTHNGKLCDLDGVKPL Majority 10 20 30 40 50 CU347HA A/Thailand/16/2004 A/chicken/Thailand/CU-K2/2004 ..... A/Thailand/676/2005 A/chicken/Thailand/CK-162/2005 A/chicken/Thailand/NP-172/2006 ..... A/chicken/Thailand/PC-168/2006 . . . . . . . . . . . . . . . ..... A/duck/Thailand/CU-328/2007 A/duck/Thailand/KU-56/2007 A/chicken/Thailand/ST-351/2008 A/chicken/Sukhothai/NIAH114843/2008 A/Viet Nam/1194/2004 A/Viet Nam/1203/2004 A/duck/Vietnam/1228/2005 A/duck/Vietnam/5/2007 A/chicken/Indonesia/4/2004 A/duck/Indonesia/MS/2004 A/Indonesia/CDC596/2006 A/Indonesia/CDC599/2006 A/Indonesia/CDC887/2006 A/Indonesia/CDC938/2006 A/turkey/Turkey/1/2005 A/whooper swan/Mongolia/3/2005 A/goose/Guangdong/1/1996 A/duck/Fujian/668/2006 A/duck/Guangxi/150/2006 .... A/bar-headed goose/Qinghai/5/2005 ILRDCSVAGWLLGNPMCDEFINVPEWSYIVEKANPANDLCYPGDFNDYEE Majority 60 70 80 90 100 ..... CU347HA .....K...... ..... A/Thailand/676/2005 .... A/chicken/Thailand/CK-162/2005 ....N..... A/chicken/Thailand/NP-172/2006 ...V...... A/chicken/Thailand/PC-168/2006 .....P......P./chicken/Thailand/PC-170/2006 ..... A/duck/Thailand/CU-328/2007 .....N..... A/duck/Thailand/KU-56/2007 A/chicken/Thailand/ST-351/2008 .v... ..V..... A/chicken/Sukhothai/NIAH114843/2008 A/Viet Nam/1194/2004 . .V. . . . . . . . . . . . . . . . ..... A/Viet Nam/1203/2004 A/duck/Vietnam/1228/2005 .v.... A/duck/Vietnam/5/2007 .....N.....A/chicken/Indonesia/4/2004 .....G.....N..... A/Indonesia/CDC596/2006 ....T......S..... A/Indonesia/CDC938/2006 ....L.....I.....N.....N.A/whooper swan/Mongolia/3/2005 .....S..... A/goose/Guangdong/1/1996 . . . . . .....N... A/duck/Fujian/668/2006 .....N..... A/duck/Guangxi/150/2006 A/bar-headed goose/Qinghai/5/2005 ...N.....

Figure 14: Comparison of deduced amino acid of HA protein at glycosylation sites and antigenic sites (underlines represent glycosylation sites and triangles represent antigenic sites).

LKHLLSRINHFEKIQIIPKSSWSSHEASLGVSSACPYQGKSSFFRNVVWL Majority

-					
	110	120	130	140	150
XXXX	******	XXXXXXXXXX	xxxxxxxx	xxxxxxxxx	XXXXXX CU345HA
XXXX	XXXXXXXXXXX	XXXXXXXXXX	XXXXXXXXX	XXXXXXXXXX	CU346HA
					CU347HA
			.v		A/Thailand/16/2004
					A/chicken/Thailand/CU-K2/2004
			vv		A/Thailand/676/2005
					A/chicken/Thailand/CK-162/2005
		D.	s	TP	A/chicken/Thailand/NP-172/2006
				L	A/chicken/Thailand/PC-168/2006
					A/chicken/Thailand/PC-170/2006
		N			A/duck/Thailand/CU-328/2007
		D.	s	TP	A/duck/Thailand/KU-56/2007
					A/chicken/Thailand/ST-351/2008
		R		L.R	A/chicken/Sukhothai/NIAH114843/2008
					A/Viet Nam/1194/2004
	<mark></mark> .				A/Viet Nam/1203/2004
		G	A.		A/duck/Vietnam/1228/2005
		P	A.	R	A/duck/Vietnam/5/2007
		D.	s	s	A/chicken/Indonesia/4/2004
		D.	s		A/duck/Indonesia/MS/2004
	L.	D.		L.R	A/Indonesia/CDC596/2006
	L.	D.		L.R	A/Indonesia/CDC599/2006
		GD.	s	LRSP	A/Indonesia/CDC887/2006
		D.	s	L.SP	A/Indonesia/CDC938/2006
		D.	A	R	A/turkey/Turkey/1/2005
		D.	s	R	A/whooper swan/Mongolia/3/2005
	T	N.	D	H.R	A/goose/Guangdong/1/1996
		D.	s	TP	A A/duck/Fujian/668/2006
		D.	s	TP	A/duck/Guangxi/150/2006
		D.	s	R	A/bar-headed goose/Qinghai/5/2005

IKKNSTYPTIKRSYNNTNQEDLLVLWGIHHPNDAAEQTKLYQNPTTYISV Majority

160	170	180	190	200	
*****	XXXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXX CU345HA	
v	. <mark></mark>			CU346HA	
				CU347HA	
				A/Thailand/16/2004	
				A/chicken/Thailand/CU-K2/2004	
				A/Thailand/676/2005	
I				A/chicken/Thailand/CK-162/2005	
N	I	S	R.	A/chicken/Thailand/NP-172/2006	
				A/chicken/Thailand/PC-168/2006	
				A/chicken/Thailand/PC-170/2006	
				A/duck/Thailand/CU-328/2007	
N	I	s		A/duck/Thailand/KU-56/2007	
				A/chicken/Thailand/ST-351/2008	
				A/chicken/Sukhothai/NIAH114843/200	08
				A/Viet Nam/1194/2004	
				A/Viet Nam/1203/2004	
	1	м		A/duck/Vietnam/1228/2005	
	1	мм		A/duck/Vietnam/5/2007	
			R	A/chicken/Indonesia/4/2004	
A			R	A/duck/Indonesia/MS/2004	
N			R	A/Indonesia/CDC596/2006	
N			R	A/Indonesia/CDC599/2006	
K		NE	R	I A/Indonesia/CDC887/2006	
TK		NE	R	I A/Indonesia/CDC938/2006	
DNA			R	A/turkey/Turkey/1/2005	
DNA			R	A/whooper swan/Mongolia/3/2005	
A				A/goose/Guangdong/1/1996	
N	I	s	I	A/duck/Fujian/668/2006	
N	I	s	I	A/duck/Guangxi/150/2006	
NA			R	A/bar-headed goose/Qinghai/5/2005	

Figure 14 (Cont): Comparison of decuded amino acid of HA protein at glycosylation sites and antigenic sites (underlines represent glycosylation sites and triangles represent antigenic sites).

EYAYKI	VKKGDSTIM	KSELEYGNCN	TKCQTPMGAI	NSSMPFHNIH	HPLTIG Majority
	260	270	280	290	300
					CU345HA
					CU346HA
					CU347HA
					A/Thailand/16/2004
					A/chicken/Thailand/CU-K2/2004
					A/Thailand/676/2005
					A/chicken/Thailand/CK-162/2005
	A	v	I		A/chicken/Thailand/NP-172/2006
					A/chicken/Thailand/PC-168/2006
					A/chicken/Thailand/PC-170/2006
			.R		A/duck/Thailand/CU-328/2007
	A	v	I		A/duck/Thailand/KU-56/2007
					A/chicken/Thailand/ST-351/2008
					A/chicken/Sukhothai/NIAH114843/2008
					A/Viet Nam/1194/2004
					A/Viet Nam/1203/2004
					A/duck/Vietnam/1228/2005
					A/duck/Vietnam/5/2007
	A				A/chicken/Indonesia/4/2004
	A				A/duck/Indonesia/MS/2004
	A	D			A/Indonesia/CDC596/2006
	A	D			A/Indonesia/CDC599/2006
	A		<mark></mark>		A/Indonesia/CDC887/2006
	A	S			A/Indonesia/CDC938/2006
.N			I		A/turkey/Turkey/1/2005
.N			I		A/whooper swan/Mongolia/3/2005
	A				A/goose/Guangdong/1/1996
	A	V	I		A/duck/Fujian/668/2006
	A	v	I		A/duck/Guangxi/150/2006
.N	•••••		I		A/bar-headed goose/Qinghai/5/2005

Figure 14 (Cont): Comparison of deduced amino acid of HA protein at glycosylation sites and antigenic sites (underlines represent glycosylation sites and triangles represent antigenic sites). (Cont)

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### Neuraminidase gene

Genetic relatedness of NA gene of avian influenza H5N1 viruses was determined (Figure 15). Avian influenza H5N1 viruses from this study (CU-345, CU 346, and CU347) are grouped into Genotype Z. Sequence analysis of those viruses revealed that all three isolates contained a 20 amino acid deletion in the NA stalk (position 49-68). On the other hand, no amino acid deletion was detected in the Goose/Guangdong/1/96 isolate. Genetic analysis of NA protein at key determinant residues including NA stalk region, and Oseltamivir resistance residues are shown in Table 12 and Figure 16, 17. Avian influenza H5N1 viruses (CU-345, CU 346, and CU347) were found to have no mutation at NA stalk region and were sensitive to Oseltamivir.



**Figure 15:** Phylogenetic analysis of NA gene of H5N1 viruses isolated in this study compared to that of other H5N1 viruses from Thailand and other countries. The analysis was based on nucleotides 1-1386 of the NA gene.

 Table 12: Genetic analysis of NA protein at key determinant residues in NA stalks region

 and Oseltamivir resistance residues.

	330	NA ger	ne		
Virus	NA stalk region	Os	eltaivir resi	stant residu	es
	49-68 <sup>ª</sup>	119 <sup>⁵</sup>	275°	293 <sup>d</sup>	295 <sup>°</sup>
A/chicken/Thailand/CU-347/2008	20 aa deletion	E	Н	R	Ν
A/duck/Thailand/CU-345/2007	20 aa deletion	E	Н	R	Ν
A/chicken/Thailand/CU-346/2007	20 aa deleti <mark>on</mark>	E	н	R	Ν
A/Thailand/16/2004	20 aa deletion	E	Н	R	Ν
A/chicken/Thailand/CU-K2/2004	20 aa deletion	E	Н	R	Ν
A/Thailand/676/2005	2 <mark>0</mark> aa deletion	Е	Н	R	Ν
A/chicken/Thailand/CK-162/2005	20 aa deletion	Е	Н	R	Ν
A/chicken/Thailand/NP-172/2006	20 aa deletion	E	Н	R	Ν
A/chicken/Thailand/PC-168/2006	20 aa deletion	E	Н	R	Ν
A/chicken/Thailand/PC-170/2006	20 aa deletion	E	н	R	Ν
A/duck/Thailand/CU <mark>-32</mark> 8/20 <mark>07</mark>	20 aa deletion	Е	Н	R	Ν
A/duck/Thailand/KU-56/2007	20 aa deletion	Е	н	R	Ν
A/chicken/Thailand/ST-351/2008	20 aa deletion	E	н	R	Ν
A/chicken/NIAH114843/2008	20 aa deletion	Е	н	R	Ν
A/Viet Nam/1194/2 <mark>00</mark> 4	20 aa deletion	Е	н	R	Ν
A/Viet Nam/1203/2004	20 aa deletion	Е	н	R	Ν
A/duck/Vietnam/1228/2005	20 aa deletion	Е	Н	R	Ν
A/duck/Vietnam/5/2007	20 aa deletion	Е	Н	R	Ν
A/chicken/Indonesia/4/2004	20 aa deletion	E	Н	R	Ν
A/duck/Indonesia/MS/2004	20 aa deletion	Е	н	R	Ν
A/Indonesia/CDC596/2006	20 aa deletion	Е	н	R	Ν
A/Indonesia/CDC599/2006	20 aa deletion	E	н	R	Ν
A/Indonesia/CDC887/2006	20 aa deletion	E	Н	R	Ν
A/Indonesia/CDC938/2006	20 aa deletion	E	н	R	Ν
A/turkey/Turkey/1/2005	20 aa deletion	Е	Н	R	Ν
A/whooper swan/Mongolia/3/2005	20 aa deletion	Е	Н	R	Ν
A/goose/Guangdong/1/1996	no deletion	E	Н	R	N
A/bar-headed goose/Qinghai/5/2005	20 aa deletion	Е	Н	R	Ν
A/duck/Guangxi/150/2006	20 aa deletion	E	Н	R	N
A/duck/Fujian/668/2006	20 aa deletion	Е	Н	R	Ν

<sup>a</sup> Amino acid at position 49-68 of NA gene

<sup>b</sup> Amino acid at position 119 can result in Oseltamivir resistance when contains E119V mutation <sup>c</sup> Amino acid at position 275 can result in Oseltamivir resistance when contains H275Y mutation <sup>d</sup> Amino acid at position 293 can result in Oseltamivir resistance when contains R293K mutation <sup>e</sup> Amino acid at position 295 can result in Oseltamivir resistance when contains N295S mutation

### NA stalk region

					S	
Consensus	NMIS:	IWVSHSIHTGNQHQAE	?		-1SI	NTNFLTEKAVASVKLAGNS
30 Sequences	30	40	50	60	70	80 9
CU345NA	NLIS:	IWVSHSIHTGNQHKAE	?		IS	NTNFLTEKAVASVKLAGNS
CU346NA	NLIS:	IWVSHSIHTGNQHKAE			IS	NTNFLIEKAVASVKLAGNS
CU347NA	NLIS:	IWVSHSIHTGNQHKAE	·		IS	NTNFLTEKAVASVKLAGNS
A/Thailand/16/2004	NLIS:	IWVSHSIHTGNQHKAE	P		-ISI	NTNFLTEKAVASVKLAGNS
A/chicken/Thailand/CU-K2/2004	NLIS:	IWVSHSIHTGNQHKAE			151	NTNFLTEKAVASVKLAGNS
A/Thailand/676/2005	NLIS:	IWVSHSIHTGNQQKAE	2		IS	NTNFLTEKAVASVKLAGNS
A/chicken/Thailand/CK-162/2005	NLIS	IWVSRSIHTGNQQKAE	P		- ISI	NTNFLTEKAVASAKLAGNS
A/chicken/Thailand/NP-172/2006	NMIS:	IWVSHSIQTGNQHQAE			IR	NTNFLTENAVASVTLAGNS
A/chicken/Thailand/PC-168/2006	NLIS	IWVSHSIHTGNQHKAE	P		IS	NTNFLTEKAVASVKLAGNS
A/chicken/Thailand/PC-170/2006	NLIS	IWVSHSIHTGNQQKAE			-ISI	NTNFLTEKAVASVKLAGNS
A/duck/Thailand/CU-328/2007	NLIS:	IWVSRSIHTGNQQKAE	?		- I SI	NTNFLTEKAVASLKLAGNS
A/duck/Thailand/KU-56/2007	NMIS:	IWVSHSIQTGNQHQAE	P		IRI	NTNSLTENAVASVTLAGNS
A/chicken/Thailand/ST-351/2008	NLIS:	IWVSHSIHTGDQHKAE			-ISI	NTNFLTEKAVASVKLAGNS
A/chicken/Sukhothai/NIAH114843/2008	NLIST	IWVSHSTHTGNQQKAE	?		IS	NTNFLTEKAGASVKLVGNS
A/Viet Nam/1194/2004	NMIS	IWVSHSIHTGNQHQSE	P		-ISI	NTNLLTEKAVASVKLAGNS
A/Viet Nam/1203/2004	NMIS:	IWVSHSIHTGNQHQSE			-ISI	NTNFLTEKAVASVKLAGNS
A/duck/Vietnam/1228/2005	NMIS	IWVSHSIHTGNQHQAE	P		IS	NTNFLTEKAVASVKLAGNS
A/duck/Vietnam/5/2007	NMVS:	IWFSHSIHTGNQHQAE	P		-ISI	NTNFLTEKAVASVKLAGNS
A/chicken/Indonesia/4/2004	NMIS:	IWVSHSIQTGNQHQAE	XXXXXXXXXX	XXXXXXXXXXX	(ISI	NTNPLTEKAVASVTLAGNS
A/duck/Indonesia/MS/2004	NMIS:	IWVSHSIQTGNQHQSE	5		-ISI	NTNPLTEKAVASVTLAGNS
A/Indonesia/CDC596/2006	NMIS:	IWVSHSIQTGNQHQAE	5		-1SI	NTNPLTEKAVASVTLAGNS
A/Indonesia/CDC599/2006	NMIS:	IWVSHSIQTGNQHQAE	5		IS	NTNPLTEKAVASVTLAGNS
A/Indonesia/CDC887/2006	NMIS:	IWVSHSIQKGNQHQAE	5		IS	NTNPLTEKAVASVTLAGNS
A/Indonesia/CDC938/2006	NMIS:	IWVSHSIQKGNQHQAE	b		IS	NTNPLTEKAVASITLAGNS
A/turkey/Turkey/1/2005	NMIS:	IWVSHSIQTGNQCQAE	P		IS	NTKFLTEKAVASVTLAGNS
A/whooper swan/Mongolia/3/2005	NMIS:	IWVSHSIQTGNQRQAE	P		IS	NTKFLTEKAVASVTLAGNS
A/goose/Guangdong/1/1996	NIIS:	IWVSHSIQTGNQHQAE	CNQSIITYE	NNTWVNQTYVN	11SI	NTNFLTEKAVASVTLAGNS
A/duck/Fujian/668/2006	NMIS:	IWVSHSIQTGNQHQAE	P		IR	NANFLTENAVASVTLAGNS
A/duck/Guangxi/150/2006	NMIS:	IWVSHSIQTGNQHQAD			IR	NTNFLTENAVASVTLAGNS
A/bar-headed goose/Qinghai/5/2005	NMIS:	IWVSHSIQTGNQRQAE			-ISI	NTKFLTEKAVASVTLAGNS

Note: 20 amino acid deletions at NA stalk regions is the position at 49-68.

Figure 16: Comparison of deduced amino acid of NA protein at NA stalk region.

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### Oseltamivir resistance amino acids

Consensus	IGSKGD	VFVIREPF	ISCSHLEC	LDAPNY	IYEECSCYL	PDAGEITCV	CRDNWHGSN
30 Sequences	110	120	1	270	280	290	30
CU345NA	IGSKGD	VFVIREPF	ISCSHLEC	LDAPNYH	IYEECSCY	PDAGEITCV	CRDNWHGSN
CU346NA	IGSKGD	VEVIREPE	ISCSHLEC	LDAPNYE	IYEECSCY	PDAGEITCV	CRDNWHGSN
CU347NA	IGSKGD	VEVIREPE	ISCSHLEC	LDAPNYE	IYEECSCY	PDAGEITCV	CRDNWHGSN
A/Thailand/16/2004	IGSKGD	VEVIREPE	ISCSHLEC	LDAPNYE	IYEECSCY	PDAGEITCV	CRDNWHGSN
A/chicken/Thailand/CU-K2/2004	IGSKGD	VEVIREPE	ISCSHLEC	LDAPNYE	IYEECSCY	PDAGEITCV	CRDNWHGSN
A/Thailand/676/2005	IGSKGD	VEVIREPE	ISCSHLEC	LDAPNYE	IYEECSCY	PDAGEITCV	CRDNWHGSN
A/chicken/Thailand/CK-162/2005	IGSKGD	VEVIREPE	ISCSHLEC	LDAPNYE	IYEECSCY	PDAGEITCV	CRDNWHGSN
A/chicken/Thailand/NP-172/2006	IGSKGD	VEVIREPE	ISCSHLEC	LNAPNYF	IYEECSCY	PDAGEIICV	CRDNWHGSN
A/chicken/Thailand/PC-168/2006	IGSKGD	VEVIREPE	ISCSHLEC	LDAPNYF	IYEECSCYE	PDAGEITCV	CRDNWHGSN
A/chicken/Thailand/PC-170/2006	IGSKGD	VEVIREPE	ISCSHLEC	LDAPNYE	IYEECSCYE	PDAGEITCV	CRDNWHGSN
A/duck/Thailand/CU-328/2007	IGSKGD	VEVIREPE	ISCSHLEC	LDAPNYE	IYEECSCYE	PDAGEITCV	CRDNWHGSN
A/duck/Thailand/KU-56/2007	IGSKGD	VEVIREPE	ISCSHLEC	LNAPNYF	IYEECSCYE	PDAGEIICV	CRDNWHGSN
A/chicken/Thailand/ST-351/2008	IGSKGD	VEVIREPE	ISCSHLEC	LDAPNYF	IYEECSCYE	PDAGEITCV	CRDNWHGSN
A/chicken/Sukhothai/NIAH114843/2008	IGSKGD	VEVIREPE	ISCSHLEC	LDAPDYF	IYEECSCY	PDAGEITCV	CRDNWHGSN
A/Viet Nam/1194/2004	IGSKGD	VEVIREPE	ISCSHLEC	LDAPNYF	IYEECSCY	PDAGEITCV	CRDNWHGSN
A/Viet Nam/1203/2004	IGSKGD	VEVIREPE	ISCSHLEC	LDAPNYE	IYEECSCY	PNAGEITCV	CRDNWHGSN
A/duck/Vietnam/1228/2005	IGSKGD	VEVIREPE	ISCSHLEC	LDAPNYF	IYEECSCYE	PDAGEITCV	CRDNWHGSN
A/duck/Vietnam/5/2007	IGSKGD	VFVIREPE	ISCSHLEC	LDAPNYE	IYEECSCYE	PDAGEITCV	CRDNWHGSN
A/chicken/Indonesia/4/2004	IGSKGD	VEVIREPE	ISCSHLEC	LDAPNYF	IYEECSCY	PDAGEITCV	CRDNWHGSN
A/duck/Indonesia/MS/2004	IGSKGD	VEVIREPE	ISCSHLEC	LDAPNYF	IYEECSCY	PDAGEITCV	CRDNWHGSN
A/Indonesia/CDC596/2006	IGSKGD	VEVIREPE	ISCSHSEC	LDAPNYE	IYEECSCYE	PDAGEITCV	CRDNWHGSN
A/Indonesia/CDC599/2006	IGSKGD	VEVIREPE	ISCSHSEC	LDAPNYE	IYEECSCY	PDAGEITCV	CRDNWHGSN
A/Indonesia/CDC887/2006	IGSKGD	VEVIREPE	ISCSHLEC	LDAPNYE	IYEECSCYE	PDAGEITCV	CRDNWHGSN
A/Indonesia/CDC938/2006	IGSKGD	VEVIREPE	ISCSHLEC	LDAPNYF	IYEECSCYE	PDAGEITCV	CRDNWHGSN
A/turkey/Turkey/1/2005	IGSRGD	VEVIREPE	ISCSHLEC	LDAPNYE	IYEECSCYE	PDAGEITCV	CRDNWHGSN
A/whooper swan/Mongolia/3/2005	IGSRGD	VEVIREPE	ISCSHLEC	LDAPNYE	IYEECSCYE	PDAGEITCV	CRDNGHGSN
A/goose/Guangdong/1/1996	IGSKGD	VEVIREPE	ISCSHLEC	LNAPNYE	IYEECSCY	PDAGEITCV	CRDNWHGSN
A/duck/Fujian/668/2006	IGSKGD	VEVIREPE	ISCSHLEC	LNAPNYE	IYEECSCYE	PDAGEITCV	CRDNWHGSN
A/duck/Guangxi/150/2006	IGSKGD	VFVIREPE	ISCSHLEC	LNAPNYE	IYEECSCYE	PDAGEIICV	CRDNWHGSN
A/bar-headed goose/Qinghai/5/2005	IGSRGD	VFVIREPE	ISCSHLEC	LDAPNYH	IYEECSCYE	PDAGEITCV	CRDNWHGSN

Note: N2 numbering system, amino acids at position 119, 275, 293, and 295 are related to Oseltamivir resistant/sensitive (Moscona, 2005)

Figure 17: Comparison of deduced amino acid of NA protein at position 119, 275, 293, and 295 relating to Oseltamivir resistance.

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### Matrix gene

Genetic relatedness of Matrix gene of avian influenza H5N1 viruses isolated was investigated (Figure 18). Avian influenza H5N1 virus (CU-347) was grouped into the group related to the outbreaks in Thailand and separated from China and Indonesia lineage. Matrix gene at key determinant residues such as amantadine resistance residues and characterization of avian-like and human-like amino acids are shown in Table 13 and Figure 19. In this study, avian influenza H5N1 virus (CU347) was found to have mutation at position L26I (Leucine to Isoleucine) and S31N (Serine to Asparagine) which have been shown to be involved in resistance to amantadine (Cheung et al., 2006). In addition, avian influenza H5N1 viruses (CU347) had typical characteristics of both avian-like and human-like viruses at E16, L55, and V28, respectively.

### M phylogenetic analysis



**Figure 18**: Phylogenetic analysis of M gene of H5N1 viruses isolated in this study compared to other H5N1 viruses from Thailand and other countries. The analysis was based on nucleotides 1-960 of the M gene.

					M gen	е			
Virus		Ama	ntadine	e resist	ance			Human	/
	h			b		b	a	ivian lik	e
	26	27	30	31	64 <sup>°</sup>	66 <sup>°</sup>	16 °	28 "	55ິ
A/chicken/Thailand/CU-347/2008	I	V	A	Ν	А	А	Е	V	L
A/duck/Thailand/CU-345/2007	-*	-*	_*	_*	_*	-*	-*	-*	-*
A/chicken/Thailand/CU-346/2007	_*	-*	-*	_*	_*	-*	-*	-*	-*
A/Thailand/16/2004	1	V	А	Ν	А	А	Е	V	L
A/chicken/Thailand/CU-K2/2004	1	V	А	Ν	А	А	Е	V	L
A/Thailand/676/2005	T	V	А	Ν	А	А	E	V	L
A/chicken/Thailand/CK-162/2005	I	V	А	Ν	А	А	Е	V	L
A/chicken/Thailand/NP-172/2006	L	V	А	S	S	Е	Е	V	L
A/chicken/Thailand/PC-168/2006	-	V	А	Ν	А	А	Е	V	L
A/chicken/Thailand/PC-170/2006	-	V	А	Ν	А	А	Е	V	L
A/duck/Thailand/CU-328/2007	L	V	А	Ν	А	А	Е	V	L
A/duck/Thailand/KU-56/2007	L	V	А	S	S	Е	Е	V	L
A/chicken/Thailand/ST-351/2008	-	V	А	N	А	А	Е	V	L
A/chicken/NIAH114843/2008	+	V	А	N	А	А	Е	V	L
A/Viet Nam/1194/ <mark>20</mark> 04	1	V	А	Ν	А	А	Е	V	L
A/Viet Nam/120 <mark>3/2</mark> 004	T	V	А	Ν	А	А	Е	V	L
A/duck/Vietnam/1228/2005	1	V	А	Ν	А	А	Е	V	L
A/duck/Vietnam/5/2007	Т	V	А	Ν	А	А	Е	V	L
A/chicken/Indonesia/4/2004	L	V	А	S	S	А	Е	V	L
A/duck/Indonesia/MS/2004	L	V	А	S	S	А	E	V	L
A/Indonesia/CDC596/2006	L	V	А	Ν	S	А	Е	V	L
A/Indonesia/CDC599/2006	L	V	А	Ν	S	А	E	V	L
A/Indonesia/CDC887/2006	L	А	А	S	S	А	Е	V	L
A/Indonesia/CDC938/2006	L	А	А	S	S	А	Е	V	L
A/turkey/Turkey/1/2005	L	V	А	S	S	Е	Е	V	L
A/whooper swan/Mongolia/3/2005	L	V	А	S	S	Е	Е	V	L
A/goose/Guangdong/1/1996	L	V	А	S	S	Е	Е	V	L
A/bar-headed goose/Qinghai/5/2005	L	V	А	S	S	Е	Е	V	L
A/duck/Guangxi/150/2006	L	V	А	S	S	E	E	V	L
A/duck/Fujian/668/2006	L	V	А	S	S	Е	Е	F	L

 Table 13: Genetic analysis of M2 protein at amantadine resistance residues and avian 

 like and human-like amino acids.

genetic analysis is based on M2 protein numbering system

<sup>b</sup> antiviral resistance when contain mutation at L26I, V27A, A30, S31N, A64S, and A66E

 $^\circ$  characteristics of avian-like amino acid at E16 and L55

<sup>d</sup> characteristics of human-like amino acid at V28

-\*: N/A not available

а

### M2 analysis

Consensus	MSLLTEVET	PTRNEWECH	RCSDSSDPI	LVVAANII	ILHLILWILD	RLFFKCIYRF	LKYGLKRGPAT	AGVPESMREEYRQEQ
28 Sequences	1	0	20	30	40	50	60	70 80
CU347M2	MSLLTEVET	PTRNEWECH	RCSDSSDP	IIVAANII	ILHLILWILD	RLFFKCIYRF	LKYGLKRGPAT	TAGVPESMREEYRQEQ
A/Thailand/16/2004	MSLLTEVET	PTRNEWECH	RCSDSSDP:	IVVAANIIO	ILHLILWILD	RLFFKCIYRF	LKYGLKRGPAT	TAGVPESMREEYRQEQ
A/chicken/Thailand/CU-K2/2004	MSLLTEVET	PTRNEWECH	RCSDSSDP	IVVAANIIO	ILHLILWILD	RLFFKCIYRF	LKYGLKRGPAT	AGVPESMREEYRQEQ
A/Thailand/676/2005	MSLLTEVET	PTRNEWECH	RCSDSSDP	IIVVAANIIO	ILHLILWILD	RLFFKCIYRF	LKYGLKRGPAT	AGVPESMREEYRQEQ
A/chicken/Thailand/CK-162/2005	MSLLTEVET	PTRNEWECH	RCSDSSDP	IVVAANIIO	ILHLILWILD	RLFFKCIYRF	LKYGLKGGPAT	TAGVPESMREEYRQEQ
A/chicken/Thailand/NP-172/2006	MSLLTEVET	PTRNEWECH	RCSDSSDPI	LVVAASIIO	ILHLILWILD	RLFFKCIYRF	LKYGLKRGPSI	TEGVPESMREEYRQEQ
A/chicken/Thailand/PC-168/2006	MSLLTEVET	PTRNEWECH	RCSDSSDP	IVVAANII	ILHLILWILD	RLFFKCIYRF	LKYGLKRGPAT	TAGVPESMREEYRQEQ
A/chicken/Thailand/PC-170/2006	MSLLTEVET	PTRNEWECH	RCSDSSDP	IVVAANII	ILHLILWILD	RLFFKCIYRF	LKYGLKRGPAT	TAGVPESMREEYRQEQ
A/duck/Thailand/CU-328/2007	MSLLTEVET	PTRNEWECH	RCSDSSDP	IVVAANII	ILHLILWILD	RLFFKCIYRF	LKYGLKGGPAT	TAGVPESMREEYRQEQ
A/duck/Thailand/KU-56/2007	MSLLTEVET	PTRNEWECH	RCSDSSDPI	LVVAASIIO	ILHLILWILD	RLFFKCIYRF	LKYGLKRGPSI	TEGVPESMREEYRQEQ
A/chicken/Thailand/ST-351/2008	MSLLTEVET	PTRNEWECH	RCSDSSDP	IVVAANIIO	ILHLILWILD	RLFFKCIYRF	LKYGLKRGPAT	TAGVPESMREEYRQEQ
A/chicken/Sukhothai/NIAH114843/2008	MSLLTEVET	PTRNEWECH	RCSDSSDP	IVVAANIIO	ILHLILWILD	RLFFKCIYRF	LKYGLKRGPAT	TAGVPESMREEYRQEQ
A/Viet Nam/1194/2004	MSLLTEVET	PTRNEWECH	RCSDSSDP	IVVAANIIG	ILHLILWILD	RLFFKCIYRF	LKYGLKRGPAT	TAGVPESMREEYRQEQ
A/Viet Nam/1203/2004	MSLLTEVET	PTRNEWECH	RCSDSSDP	IVVAANIIO	ILHLILWILD	RLFFKCIYRF	LKYGLKRGPAT	TAGVPESMREEYRQEQ
A/duck/Vietnam/1228/2005	MSLLTEVET	PTRNEWECH	RCSDSSDP:	IVVAANIIO	ILHLILWILD	RLFFKCIYRF	LKYGLKRGPAT	TAGVPESMREEYRQEQ
A/duck/Vietnam/5/2007	KSLLTEVET	PTRNEWECH	RCSDSSDP	IVVAANIIO	ILHLILWILD	RLFFKCIYRF	LKYGLKRGPAR	RAGVPESMREEYRQEQ
A/chicken/Indonesia/4/2004	MSLLTEVET	PTRNEWECH	RCSDSSDPI	LVVAASIIO	ILHLILWILD	RLFFKCIYRF	LKYDLKRGPSI	TAGVPESMREEYRQEQ
A/duck/Indonesia/MS/2004	MSLLTEVET	PTRNEWECH	RCSDSSDPI	LVVAASIIO	ILHLILWILD	RLFFKCIYRF	LKYGLKRGPSI	TAGVPESMREEYRQEQ
A/Indonesia/CDC596/2006	MSLLTEVET	PTRNEWECH	RCSDSSDPI	LVVAANIIO	ILHLILWILD	RLFFKFIYRF	LKYGLKRGPSI	TAGVPESMREEYRQEQ
A/Indonesia/CDC599/2006	MSLLTEVET	PTRNEWECH	RCSDSSDPI	LVVAANIIG	ILHLILWILD	RLFFKFIYRF	LKYGLKRGPSI	TAGVPESMREEYRQEQ
A/Indonesia/CDC887/2006	MSLLTEVET	PTRNEWECH	KCIDSSDPI	LAVAASIIC	ILHLILWILD	RLFFKFIYRF	LKYDLKRGPSI	TAGVPESMREEYRQEQ
A/Indonesia/CDC938/2006	MSLLTEVET	PTRNEWECH	KCIDSSDPI	LAVAASIIC	ILHLILWIID	RLFFKFIYRF	LKYDLKRGPSI	TAGVPESMREEYRQEQ
A/turkey/Turkey/1/2005	XXXLTEVET	PTRNEWECH	RCSDSSDPI	LVVAASIIC	ILHLILWILD	RLFFKCIYRF	LKYGLKRGPSI	TEGVPESMREEYRQEQ
A/whooper swan/Mongolia/3/2005	MSLLTEVET	PTRNEWECH	RCSDSSDPI	LVVAASIIO	ILHLILWILD	RLFFKCIYRF	LKYGLKRGPSI	TEGVPESMREEYRQEQ
A/goose/Guangdong/1/1996	MSLLTEVET	PTKNEWECH	KCSDSSDPI	LVVAASIIO	ILHLILWILD	RLFFKCIYRF	LKYGLKRGPSI	TEGVPESMREEYRQEQ
A/bar-headed goose/Qinghai/5/2005	MSLLTEVET	PTRNEWECH	RCSDSSDPI	LVVAASIIO	ILHLILWILD	RLFFKCIYRF	LKYGLKRGPSI	TEGVPESMREEYRQEQ
A/duck/Fujian/668/2006	MSLLTEVET	PTRNEWECH	RCSDSSDPI	LVFAASIIG	ILHLILWILD	RLFFKCIYRF	LKYGLKRGPSI	TEGVPESMREEYRQEQ
A/duck/Guangxi/150/2006	MSLLTEVET	PTRNEWECE	RCSDSSDPI	VVAASIIC	ILHLILWILD	RLFFKCIYRF	LKYGLKRGPST	TEGVPESMREEYRQEQ

- Amantadine resistant amino acid
- T Human/Avian-like amino acid

**Figure 19**: Comparison of deduced amino acid of M2 protein at position 26, 27, 30, 31, 64, and 66 involving amantadine resistance (grey triangles) and at position 16, 28, and 55 representing avian-like and human-like characteristics (grey arrows).

### ศูนย์วิทยทรัพยากร จุฬาลงกรณ์มหาวิทยาลัย

### Polymerase gene (PB2, PB1, PA)

Figure 20, 21, and 22 show genetic relatedness of PB2, PB1, and PA gene of avian influenza H5N1 viruses isolated in this study and comparing to other viruses from Thailand and other countries. Genetic relatedness of PB2, PB1, and PA gene were consistented with genetic relatedness of other genes and are clustered in the same group of Vietnam-Thailand lineage and separated from China and Indonesia lineages. Table 14 and Figure 23, 24, and 25 show genetic analysis of three polymerase proteins of avian influenza virus.

In this study, genetic analysis of PB2 protein at positions 627 and 355 of CU-347 isolate were E627 and R355 indicating the characteristic of non-virulence in mammal. Some polymorphisms at amino acid residues 199, 661, 667 and 702 of CU-347 contains avian like amino acids; A119, A661, V667 and K702 respectively. Based on the analysis of PB1 protein at position 198 of the CU-347 isolate is K198 representing non-virulence in mammal. Sequence analysis of PA gene at position 409 of the CU-347 isolate has characterization of avian-like amino acids (S409).

### ศูนย์วิทยทรัพยากร จุฬาลงกรณ์มหาวิทยาลัย

### PB2 phylogenetic analysis



**Figure 20**: Phylogenetic analysis of PB2 gene of H5N1 viruses isolated in this study compared to other H5N1 viruses from Thailand and other countries. The analysis was based on nucleotides 1-2277 of the PB2 gene.



### PB1 phylogenetic analysis



**Figure 21**: Phylogenetic analysis of PB1 gene of H5N1 viruses isolated in this study compared to other H5N1 viruses from Thailand and other countries. The analysis was based on nucleotides 1-2271 of the PB1 gene.



### PA phylogenetic analysis



**Figure 22**: Phylogenetic analysis of PA gene of H5N1 viruses isolated in this study compared to other H5N1 viruses from Thailand and other countries. The analysis was based on nucleotides 1-2151 of the PA gene.



	20	1973	PB2	a,b			PB1 <sup>c</sup>	PA <sup>d</sup>
Virus		H/A li	ike <sup>a</sup>	-	Virule	nce <sup>b</sup>	Virulence	H/A like
	199	661	667	702	627	355	198	409
A/chicken/Thailand/CU-347/2008	А	А	V	K	E	R	К	S
A/duck/Thailand/CU-34 <mark>5/2007</mark>	-*	-*	_*	_*	_*	-*	_*	S
A/chicken/Thailand/CU-346/2007	-*	_*	-*	_*	_*	-*	_*	-*
A/Thailand/16/2004	А	А	V	K	К	R	К	S
A/chicken/Thailand/CU-K2/2004	А	А	V	К	E	R	К	S
A/Thailand/676/2005	А	А	V	К	К	R	К	S
A/chicken/Thailand/CK-162/2005	A	А	V	К	Е	R	К	S
A/chicken/Thailand/NP-172/2006	А	А	1	К	E	R	К	S
A/chicken/Thailand/PC-168/2006	А	А	V	К	Е	R	К	S
A/chicken/Thailand/PC-170/2006	А	А	V	K	Е	R	К	S
A/duck/Thailand/CU- <mark>328</mark> /2007	А	А	V	К	Е	R	К	S
A/duck/Thailand/KU-56/2007	А	А	I.	К	Е	R	К	S
A/chicken/Thailand/ST-351/2008	А	А	V	К	E	R	К	S
A/chicken/NIAH11484 <mark>3/</mark> 2008	А	А	V	К	Е	R	К	S
A/Viet Nam/1194/2004	А	А	V	К	К	R	К	S
A/Viet Nam/1203/2004	А	А	V	К	к	R	К	S
A/duck/Vietnam/1228/2005	А	А	V	К	Е	R	К	S
A/duck/Vietnam/5/2007	А	А	V	К	Е	R	К	S
A/chicken/Indonesia/4/2004	А	А	V	К	Е	R	К	S
A/duck/Indonesia/MS/2004	А	А	V	К	Е	R	к	S
A/Indonesia/CDC596/2006	А	Т	V	К	E	R	к	S
A/Indonesia/CDC599/2006	А	Т	V	K	Е	R	к	S
A/Indonesia/CDC887/2006	А	А	V	К	Е	R	К	Ν
A/Indonesia/CDC938/2006	А	А	V	К	Е	R	К	S
A/turkey/Turkey/1/2005	А	А	V	К	К	R	К	S
A/whooper swan/Mongolia/3/2005	А	А	V	К	К	R	К	S
A/goose/Guangdong/1/1996	А	А	V	к	Е	К	К	S
A/bar-headed goose/Qinghai/5/2005	А	A	V	к	К	R	К	S
A/duck/Guangxi/150/2006	А	А	V	к	Е	R	К	S
A/duck/Fujian/668/2006	А	А	V	К	Е	R	К	S

 Table 14: Genetic analysis of deduced amino acid of PB2, PB1, and PA genes at key

 determinant residues relating to human or avian-like and virulence characteristics.

<sup>a</sup> Amino acid at position 199, 611, 667, 702: A (Alanine), V (Valine), K (Lysine)

<sup>b</sup> Amino acid at position 627, 355: E (Glutamic acid), K (Lysine), R (Arginine)

<sup>c</sup> Amino acid at position 198: K (Lysine)

<sup>d</sup> Amino acid at position 409: S (Serine)

### PB2 analysis

Consensus	LIGNLQILKIRV	HEGYEEFTMV	LPFAAAPPEQS	RMQFSSLTVNV	RGSGMRILVRGNSI	PVFNYNKATKR	LTVLGKD2
28 Sequences	350	360	620 6	30 64	0 650	660	670
CU347PB2	LIGNLOILKIRVE	HEGYEEFTMV	LPFAAAPPEOS	RMOFSSLTVNV	RGSGMRILVRGNSI	PVFNYNKATKR	LTVLGKD
A/Thailand/16/2004	LIGNLQILKIRVE	HEGYEEFTMV	LPFAAAPPKQS	RMQFSSLTVNV	RGSGMRILVRGNSI	PVFNYNKATKR	LTVLGKDA
A/chicken/Thailand/CU-K2/2004	LIGNLQILKIRVE	HEGYEEFTMV	LPFAAAPPEQS	RMQFSSLTVNV	RGSGMRILVRGNSI	PVFNYNKATKR	LTVLGKDA
A/Thailand/676/2005	LIGNLQILKIRVE	HEGYEEFTMV	LPFAAAPPKON	IRMQFSSLTVNV	RGSGMRILVRGNS	PVFNYNKATKR	LTVLGKDA
A/chicken/Thailand/CK-162/2005	LIGNLQILKIRVE	HEGYEEFTMV	LPFAAAPPEQN	RMQFSSLTVNV	RGSGMRILVRGNSI	PVFNYNKATKR	LTVLGKDA
A/chicken/Thailand/NP-172/2006	LIGNLQILKIRVE	HEGYEEFTMV	LPFAAAPPEQS	RMQFSSLTVNV	RGSGMRILVRGNS	PVFNYNKATKR	LTILGKDA
A/chicken/Thailand/PC-168/2006	LIGNLQILKIRVE	HEGYEEFTMV	LPFAAAPPEQS	SRMQFSSLTVNV	RGSGMRILIRGNS	PVFNYNKATKR	LTVLGKD
A/chicken/Thailand/PC-170/2006	LIGNLQILKIRVE	HEGYEEFTMV	LPFAAAPPEQN	IRMQFSSLTVNV	RGSGMRILVRGNS	PVFNYNKATKR	LTVLGKD
A/duck/Thailand/CU-328/2007	LIGNLQILKIRVE	HEGYEEFTMV	LPFAAAPPEQN	IRMQFSSLTVNV	RGSGMRILVRGNSI	PVFNYNKATKR	LTVLGKDA
A/duck/Thailand/KU-56/2007	LIGNLQILKIRVE	HEGYEEFTMV	LPFAAAPPEQS	RMQFSSLTVNV	RGSGMRILVRGNS	PVFNYNKATKR	LTILGKDA
A/chicken/Thailand/ST-351/2008	LIGNLQILKIRVE	HEGYEEFTMV	LPFAAAPPEQS	RMQFSSLTVNV	RGSGMRILVRGNS	PVFNYNKATKR	LTVLGKDA
A/chicken/Sukhothai/NIAH114843/2008	LIGNLQILKIRVE	HEGYEEFTMV	LPFAAAPPEQS	KMQFSSLTVNV	RGSGMRILVRGNSI	PVFNYNKATKR	LTVLGKDA
A/Viet Nam/1194/2004	LIGNLQILKIRVE	HEGYEEFTMV	LPFAAAPPKQS	RMQFSSLTVNV	RGSGMRILVRGNSI	PVFNYNKATKR	LTVLGKDA
A/Viet Nam/1203/2004	LIGNLQILKIRVE	HEGYEEFTMV	LPFAAAPPKQS	RMQFSSLTVNV	RGSGMRILVRGNS	PVFNYNKATKR	LTVLGKDA
A/duck/Vietnam/1228/2005	LIGNLQILKIRVY	EGYEEFTMV	LPFAAAPPEQS	RMQFSSLTVNV	RGSGMRILVRGNS	PVFNYNKATKR	LTVLGKDA
A/duck/Vietnam/5/2007	LIGNLQILKIRVY	EGYEEFTMV	LPFAAAPPEQS	RMQFSSLTVNV	RGSGMRILVRGNS	PVFNYNKATKR	LTVLGKDA
A/chicken/Indonesia/4/2004	LIGNLQILKIRVE	HEGYEEFTMV	LPFAAAPPEQS	RMQFSSLTVNV	RGSGMRILVRGNS	PVFNYNKATKR	LTVLGKDA
A/duck/Indonesia/MS/2004	LIGNLQILKIRVE	HEGYEEFTMV	LPFAAAPPEQS	RMQFSSLTVNV	RGSGMRILVRGNSI	PVFNYNKATKR	LTVLGKDA
A/Indonesia/CDC596/2006	LIGNLQILKIRVE	HEGYEEFTMV	LPFAAAPPEQS	RMQFSSLTVNV	RGSGMRILVRGNS	PVFNYNRTTKR	LTVLGKDA
A/Indonesia/CDC599/2006	LIGNLQILKIRVE	HEGYEEFTMV	LPFAAAPPEQS	RMQFSSLTVNV	RGSGMRILVRGNSI	PVFNYNRTTKR	LTVLGKD
A/Indonesia/CDC887/2006	LIGNLQILKIRVE	HEGYEEFTMV	LPFAAAPPEQS	RMQFSSLTVNV	RGSGMRILVRGNS	PVFNYNKATKR	LTVLGKDA
A/Indonesia/CDC938/2006	LIGNLQILKIRVE	HEGYEEFTMV	LPFAAAPPEQS	RMQFSSLTVNV	RGSGMRILVRGNS	PVFNYNKATKR	LTVLGKDA
A/turkey/Turkey/1/2005	LIGNLQILKIRVE	HEGYEEFTMV	LPFAAAPPKQS	RMQFSSLTVNV	RGSGMRILIRGNS	PVFNYNKATKR	LTVLGKDA
A/whooper swan/Mongolia/3/2005	LIGNLQILKIRVE	HKGYEEFTMV	LPFAAAPPKQS	RMQFSSLTVNV	RGSGMRILIRGNS	PVFNYNKATKR	LTVLGKD
A/goose/Guangdong/1/1996	LIGNLQILKIKVE	HEGYEEFTMV	LPFAAAPPEPS	RMQFSSLTVNV	RGSGMRILVRGNS	PVFNYNKATKR	LTVLGKD
A/bar-headed goose/Qinghai/5/2005	LIGNLQILKIRVE	HEGYEEFTMV	LPFAAAPPKQS	RMQFSSLTVNV	RGSGMRILIRGNS	PVFNYNKATKR	LTVLGKD
A/duck/Guangxi/150/2006	LIGNLQILKIRVE	HEGYEEFTMV	LPFAAAPPEQS	RMQFSSLTVNV	RGSGMRILVRGNS	PVFNYNKATKR	LTVLGKDA
A/duck/Fujian/668/2006	LIGNLQILKIRVE	HEGYEEFTMV	LPFAAAPPEQS	SRMQFSSLTVNV	RGSGMRILVRGNS	PVFNYNKATKR	LTVLGKD
			-			T	T
						_	_
IIXI Consensus		KKEEL	DCKIAPLMVAY	FLILGKEDKR	YGPALSINELSNLA		
28 Sequences		190	200	700	710	•	
CU347PB2		KKEEL	ODCKTAPLMVAY	FLILGKEDKR	YGPALSINELSNLA		
A/Thailand/16/2004	1 N 1975	KKEEL	ODCKTAPLMVAY	FLILGKEDKR	YGPALSINELSNLA		
A/chicken/Thailand	/CU-K2/2004	KKEEL	ODCKTAPLMVAY	FLILGKEDKR	YGPALSTNELSNLA		
A/Thailand/676/200	5	KKEEL	ODCKTAPLMVAY	FLILGKEDKR	YGPALSTNELSNLA		
A/chicken/Thailan	I/CK-162/2005	KKEEL	ODCKTAPLMVAY	FLILGKEDKR	YGPALSINELSNLA		
A/chicken/Thailan	/NP-172/2006	KKEEL	ODCKTAPLMVAY	FLILGKEDKR	YGPALSINELSNLA		
A/chicken/Thailand	PC-168/2006	KKEEL	ODCKTAPLMVAY	FLILGKEDKR	YGPALSINELSNLA		
A/chicken/Thailand	PC-170/2006	KKEEL	ODCKTAPLMVAY	FLILGKEDKR	YGPALSINELSNLA		
A/duck/Thailand/CL	1-328/2007	KKEEL	ODCKTAPLMVAY	FLILGKEDKR	YGPALSTNELSNLV		
A/duck/Thailand/KU	1-56/2007	KKEEL	ODCKIAPLMVAY	FLILGKEDKR	YGPALSINELSNLA		
A/chicken/Thailand	I/ST-351/2008	KKEEL	ODCKIAPLMVAY	FLILGKEDKR	YGPALSINELSNLA		
A/chicken/Sukhotha	i/NIAH114843/	2008 KKEEL	ODCKIAPLMVAY	FLILGOEDKR	YGPALSINELSNLA		
A/Viet Nam/1194/20	004	KKEEL	ODCKIAPLMVAY	FLILGKEDKR	YGPALSINELSNLA		
A/Viet Nam/1203/20	004	KKEEL	ODCKIAPLMVAY	FLILGKEDKR	YGPALSINELSNLA		
A/duck/Vietnam/122	28/2005	KKEEL	ODCKIAPLMVAY	FLILGKEDKR	YGPALSINELSNLA		
A/duck/Vietnam/5/2	2007	KKEEL	DCKIAPLMVAY	FLILGKEDKR	YGPALSINELSNLA		

Virulence determinant amino acid

A/duck/Vietnam/5/2007 A/chicken/Indonesia/4/2004

A/duck/Indonesia/MS/2004

A/Indonesia/CDC596/2006

A/Indonesia/CDC599/2006

A/Indonesia/CDC887/2006

A/Indonesia/CDC938/2006

A/turkey/Turkey/1/2005 A/whooper swan/Mongolia/3/2005

A/goose/Guangdong/1/1996

A/duck/Guangxi/150/2006

A/duck/Fujian/668/2006

A/bar-headed goose/Qinghai/5/2005

THuman/Avian-like amino acid

Figure 23: Comparison of deduded amino acid of PB2 protein at position 355 and 627 which involving with virulence determinant amino acids of virus (triangles) and at position 199, 661, 667, and 702 which represent of avian-like and human-like characteristics (arrows).

FLILGKEDKRYGPALSINELSNLA

FLILGKEDKRYGPALSINELSNLA

FLILGKEDKRYGPALSINELSNLA

FLILGKEDKRYGPALSINELSNLA

FLILGKEDKRYGPALSINELSNLA

FLILGKEDKRYGPALSINELSNLA

FLILGKEDKRYGPALSINELSNLA

FLILGREDKRYGPALSINELSNLA

FLILGKEDKRYGPALSINELSNLA

FLILGKEDKRYGPALSINELSNLA

KKEELQDCKIAPLMVAY FLILGKEDKRYGPALSINELSNLA

KREELQDCKIAPLMVAY FLILGKEDKRYGPALSINELSNLA

KKEELQDCKIAPLMVAY

KKEELQDCKIAPLMVAY

**KKEELQDCKIAPLMVAY** 

KKEELODCKIAPLMVAY

KKEELQDCKIAPLMVAY

KKEELQDCKIAPLMVAY

KKEELQDCKIAPLMVAY

KKEELQDCKIAPLMVAY

KKEELODCKIAPLMVAY

### PB1 analysis

Consensus	MEITTHE	QRKRRVR	JNM1KKMV1Q	RITCKKKÖRI	LNKKSYLIR
28 Sequences	180	190	200	210	220
CU347PB1	MEITTHE	QRKRRVR	DNMTKKMVTQ	RTIGKKKQRI	LNKKSYLIR
A/Thailand/16/2004	MEITTHE	QRKRRVR	DNMTKKMVTQ	RTIGKKKQRI	LNKKSYLIR
A/chicken/Thailand/CU-K2/2004	MEITTHE	QRKRRVR	DNMTKKMVTQ	RTIGKKKQRI	LNKKSYLIR
A/Thailand/676/2005	MEITTHE	QRKRRVR	DNMTKKMVTQ	RTIGKKKQRI	LNKKSYLIR
A/chicken/Thailand/CK-162/2005	MEITTHE	QRKRRVR	DNMTKKMVTQ	RTIGKKKQKI	LNKKSYLIR
A/chicken/Thailand/NP-172/2006	MEITTHE	QRKRRVR	DNMTKKMVTQ	RTIGKKKQRI	LNKRSYLIR
A/chicken/Thailand/PC-168/2006	MEITTHE	QRKRRVR	DNMTKKMVTQ	RTIGKKKQRI	LNKKSYLIR
A/chicken/Thailand/PC-170/2006	MDITTHE	QRKRRVR	DNMTKKMVTQ	RTIGKKKQKI	LNKKSYLIR
A/duck/Thailand/CU-328/2007	MEMTTHE	QRKRRVR	DNMTKKMVTQ	RTIGKKKQKI	LNKKSYLIR
A/duck/Thailand/KU-56/2007	MEITTHE	QRKRRVR	DNMTKKMVTQ	RTIGKKKQRI	LNKRSYLIR
A/chicken/Thailand/ST-351/2008	MEITTHE	QRKRRVR	DNMTKKMVTQ	RTIGKKKQRI	LNKKSYLIR
A/chicken/Sukhothai/NIAH114843/2008	MDITTHE	QRKRRVR	DNMTKKMVTQ	RTIGKKKQKI	LNKKSYLIR
A/Viet Nam/1194/2004	MEITTHE	QRKRRVR	DNMTKKMVTQ	RTIGKKKQRI	LNKKSYLIR
A/Viet Nam/1203/2004	MEITTHE	QRKRRVR	DNMTKKMVTQ	RTIGKKKQRI	LNKKSYLIR
A/duck/Vietnam/1228/2005	MEITTHE	QRKRRVR	DNMTKKMVTQ	RTIGKKKQRI	LNKKSYLIR
A/duck/Vietnam/5/2007	MEITTHE	QRKRRVR	DNMTKKMVTQ	RTIGKKKQRI	LNKKSYLIR
A/chicken/Indonesia/4/2004	MEITTHE	QRKKRVR	DNMTKKMVTQ	RTIGKKKQRI	LNKRSYLIR
A/duck/Indonesia/MS/2004	MEITTHE	QRKRRVR	DNMTKKMVTQ	RTIGKKKQRI	LNKRSYLIR
A/Indonesia/CDC596/2006	MEITTHE	QRKRRVR	DNMTKKMVTQ	RTIGKKKQRI	LNKRSYLIR
A/Indonesia/CDC599/2006	MEITTHE	QRKRRVR	DNMTKKMVTQ	RTIGKKKQRI	LNKRSYLIR
A/Indonesia/CDC887/2006	MEITTHE	QRKRRVR	DNMTKKMVTQ	RTIGKKKQRI	LNKRSYLIR
A/Indonesia/CDC938/2006	MEITTHE	QRKRRVR	DNMTKKMVTQ	RTIGKKKQRI	LNKRSYLIR
A/turkey/Turkey/1/2005	MEITTHE	QRKRRVR	DNMTKKMVTQ	RTIGKKKQRI	LNKKSYLIR
A/whooper swan/Mongolia/3/2005	MEITTHE	QRKRRVR	DNMTKKMVTQ	RTIGKKKQRI	LNKKSYLIR
A/goose/Guangdong/1/1996	MEIITHE	QRKRRVR	DNMTKKMVTQ	RTIGKKKQRI	LNKRSYLIR
A/bar-headed goose/Qinghai/5/2005	MEITTHE	QRKRRVR	DNMTKKMVTQ	RTIGKKKQRI	LNKKSYLIR
A/duck/Fujian/668/2006	MEITTHE	QRKRRVR	DNMTKKMVTQ	RTIGKKKQRI	LNKRSYLIR
A/duck/Guangxi/150/2006	MEITTHE	QRKRRVR	DNMTKKMVTO	RTIGKKKQRI	LNKRSYLIR



Virulence determinant amino acid

Figure 24: Comparison of deduced amino acid of PB1 protein at position 198 which involving with virulence of virus (triangle).

## ศูนย์วิทยทรัพยากร จุฬาลงกรณ์มหาวิทยาลัย

PA analysis

Consensus	CKDVSDLKQID	ODFLESKOP4	SWIYSEINKA	CEPID29MIE	TUEIGED
29 Sequences	390	400	410	420	430
CU345PA	CKDVSDLRQYD	SDEPESRSLA	SWIQSEFNKA	CELTDSIWIE	LDEIGED
CU347PA	CKDVSDLRQYD	SDEPESRSLA	SWIQSEFNKA	CELTDSIWIE	LDEIGED
A/Thailand/16/2004	CKDVSDLRQYD	SDEPESRSLA	SWIQSEFNKA	CELTDSSWIE	LDEIGED
A/chicken/Thailand/CU-K2/2004	CKDVSDLRQYD	SDEPESRSLA	SWIQSEFNKA	CELTDSSWIE	LDEIGED
A/Thailand/676/2005	CKDVSDLRQYD	SDEPESRSLA	SWIQSEFNKA	CELTDSSWIE	LDEIGED
A/chicken/Thailand/CK-162/2005	CKDVSDLRQYD	SDEPESRSLA	SWIQSEFNKA	CELTDSSWIE	LDEIGED
A/chicken/Thailand/NP-172/2006	CKDVGDLKQYD	SDEPEPRSLS	SWIQSEFNKA	CELTDSSWIE	LDEIGED
A/chicken/Thailand/PC-168/2006	CKDVSDLRQYD	SDEPESRSLI	SWIQSEFNKA	CELTDSSWIE	LDEIGED
A/chicken/Thailand/PC-170/2006	CKDVGDLRQYD	SDEPESRSLA	SWIQSEFNKA	CELTDSSWIE	LDEIGED
A/duck/Thailand/CU-328/2007	CKDVSDLRQYD	SDEPESRSLA	SWIQSEFNKA	CELTDSSWIE	LDEIGED
A/duck/Thailand/KU-56/2007	CKDVSDLKQYD	SDEPEPRSLS	SWIQSEFNKA	CELTDSSWIE	LDEIGED
A/chicken/Thailand/ST-351/2008	CKDVSDLRQYD	SDEPESRSLA	SWIQSEFNKA	CELTDSSWIE	LDEIGED
A/chicken/Sukhothai/NIAH114843/2008	CKDVSDLRQYD	SDEPESRSLA	SWIQSEFNKA	CELTDSIWIE	LDEIGED
A/Viet Nam/1194/2004	CKDVSDLRQYD	SDEPESRSLA	SWIQSEFNKA	CELTDSSWIE	LDEIGED
A/Viet Nam/1203/2004	CKDVSDLRQYD	SDEPESRSLA	SWIQSEFNKA	CELTDSIWIE	LDEIGED
A/duck/Vietnam/1228/2005	CKDVSDLRQYD	SDEPESRSLA	SWIQSEFNKA	CELTDSSWIE	LDEIGED
A/duck/Vietnam/5/2007	CKDISDLRQYD	SDEPESRSLA	SWIQSEFNKA	CELTDSSWIE	LDEIGED
A/chicken/Indonesia/4/2004	CKDVSDLRQYD	SDEPESRSLA	SWIQSEFNKA	CELTDSSWIE	LDEIGED
A/duck/Indonesia/MS/2004	CKDVSDLRQYD	SDEPESRSLA	SWIQSEFNKA	CELTDSSWIE	LDEIGED
A/Indonesia/CDC596/2006	CKDISDLRQYD	SDEPESRSLA	SWIQSEFNKA	CELTDSSWIE	LDEIGED
A/Indonesia/CDC599/2006	CKDISDLRQYD	SDEPESRSLA	SWIQSEFNKA	CELTDSSWIE	LDEIGED
A/Indonesia/CDC887/2006	CKDVSDLAQYN	SDEPESRSLA	SWIQNEFNKA	CELTDSSWIE	LDEIGED
A/Indonesia/CDC938/2006	CKDVSDLAQYN	SDEPESRSLA	SWIQSEFNKA	CELTDSSWIE	LDEIGED
A/turkey/Turkey/1/2005	CKDVSDLKQYD	SDEPESRSLA	SWIQSEFNKA	CELTDSSWIE	LDEIGED
A/whooper swan/Mongolia/3/2005	CKDVSDLRQYD	SDEPESRSLA	SWIQSEFNKA	CELTDSSWIE	LDEIGED
A/goose/Guangdong/1/1996	CKDVSDLRQYD	SDEPKPRSLA	SWIQSEFNKA	CELTDSSWIE	LDEIGED
A/bar-headed goose/Qinghai/5/2005	CKDVSDLRQYD	SDEPESRSLA	SWIQSEFNKA	CELTDSSWIE	LDEIGED
A/duck/Guangxi/150/2006	CKDVSDLKQYD	SDEPEPRSLS	SWIQSEFNKA	CELTDSSWVE	LDEIGED
A/duck/Fujian/668/2006	CKDVSDLRQYD	SDEPEPKSLS	SWIQSEFNKA	CELTDSSWIE	LDEIGED

Γŀ

Human/Avian<mark>-like</mark> amino acid

**Figure 25**: Comparison of decuded amino acid of PA protein at position 409 which involving with characterization of avian-like and human-like characteristics (arrow).

### ศูนย์วิทยทรัพยากร จุฬาลงกรณ์มหาวิทยาลัย

### Nonstructural protein gene

Genetic relatedness of Nonstructural protein gene of avian influenza H5N1 viruses is shown in Figure 26. Avian influenza H5N1 viruses (CU-345 and CU347) are clustered in the same group as Vietnam-Thailand lineage. Mutation analysis of Nonstructural protein gene of avian influenza viruses at virulence determinant are demonstrated in Table 15 and Figure 27. In this study, avian influenza H5N1 viruses (CU-345 and CU347) carried 5 amino acid deletions at position 80-84 and have C-terminal ESEV that are virulence features. However, position 92 poseses Aspartic acid (D) which indicating non-virulence in mammals (Seo et al., 2004).

### NS phylogenetic analysis



**Figure 26:** Phylogenetic analysis of NS gene of H5N1 viruses isolated in this study comparing to other H5N1 viruses from Thailand and other countries. The analysis was based on nucleotides 1-690 of the NS gene.

Table 15: Genetic analysis of deduced amino acid of NS1 gene at key determinant residues related to virulence characteristics.

	NS1 gene <sup>®</sup>							
Virus	5 amino acid deletion <sup>b</sup>	Virul	lence determinant					
	80-84 <sup>b</sup>	92 <sup>°</sup>	Carboxyl-terminal <sup>d</sup>					
A/chicken/Thailand/CU-347/2008	5 aa deletion	D	ESEV					
A/duck/Thailand/CU-345/2007	5 aa deletion	D	ESEV					
A/chicken/Thailand/CU-346/2007	_*	_*	_*					
VThailand/16/20 <mark>04</mark>	5 aa deletion	D	ESEV					
Vchicken/Thailand/CU-K2/2004	5 aa deletion	D	ESEV					
VThailand/676/2005	5 aa deletion	D	ESEV					
Vchicken/Thailand/CK-162/2005	5 aa deletion	D	ESEV					
Vchicken/Thailand/NP-172/2006	5 aa deletion	D	ESEV					
Vchicken/Thailand/PC-168/2006	5 aa deletion	D	ESEV					
Vchicken/Thailand/PC-170/2006	5 aa deletion	D	ESEV					
Vduck/Thailand/C <mark>U-3</mark> 28/2007	5 aa deletion	D	ESEV					
Vduck/Thailand <mark>/KU-</mark> 56/2007	5 aa deletion	D	ESEV					
Vchicken/Thailand/ST-351/2008	5 aa deletion	D	ESEV					
Vchicken/NIAH114843/2008	5 aa deletion	D	ESEV					
Viet Nam/1194/2004	5 aa deletion	D	ESEV					
Viet Nam/1203/2004	5 aa deletion	D	ESEV					
Vduck/Vietnam/1228/2005	5 aa deletion	D	ESEV					
/duck/Vietnam/5/2007	5 aa deletion	D	ESEV					
/chicken/Indonesia/4/2004	5 aa deletion	D	ESEV					
/duck/Indonesia/MS/2004	5 aa deletion	D	ESEI					
/Indonesia/CDC596/2006	5 aa deletion	D	ESEV					
VIndonesia/CDC599/2006	5 aa deletion	D	ESEV					
VIndonesia/CDC887/2006	5 aa deletion	D	ESEV					
VIndonesia/CDC938/2006	5 aa deletion	D	ESEV					
Vturkey/Turkey/1/2005	5 aa deletion	D	ESKV					
/whooper swan/Mongolia/3/2005	5 aa deletion	D	ESKV					
Vgoose/Guangdong/1/1996	no deletion	D	ESEV					
√bar-headed	5 aa deletion	D	ESEV					
oose/Qinghai/5/2005								
/duck/Guangxi/150/2006	5 aa deletion	D	ESEV					
Vduck/Fujian/668/2006	5 aa deletion	D	ESEV					

<sup>b</sup> amino acid at position 80-84 of NS1 gene have 5 amino acid deletion

<sup>c</sup> amino acid at position 92 of NS1: D (Aspartic acid)

<sup>d</sup> amino acid at carboxy terminal of NS1: ESEV (Glutamic acid, Serine, Glutamic acid, and Valine)

### NS1 analysis

🖂 Consensus	ESDKALK		PASRYLTDMT	NOKRKMART	IESEV-
29 Sequences		io i	90	220	230
CU345NS	ESDKALK		PASRYLTDMT	NQKRKMART	IESEV.
CU347NS	ESDKALK		PASRYLTDMT	NQKRKMART	IESEV.
A/Thailand/16/2004	ESDKALK		PASRYLTDMT	NQKRKMART	IESEV.
A/chicken/Thailand/CU-K2/2004	ESDKALK		PASRYLTDMT	NQKRKMART	TESEV.
A/Thailand/676/2005	ESDKALK		PASRYLTDMT	NQKRKMART	IESEV.
A/chicken/Thailand/CK-162/2005	ESDKALK		PASRYLTDMT	NQKRKMART	IESEV.
A/chicken/Thailand/NP-172/2006	ESDEALK		PISRYLIDMI	NQKRKMART	TESEV.
A/chicken/Thailand/PC-168/2006	ESDKALK		PISRYLIDMI	NQKRKMART	IESEV.
A/chicken/Thailand/PC-170/2006	ESDKALK		PASRYLTDMT	NQKRKVART	IESEV.
A/duck/Thailand/CU-328/2007	ESDKALK		PASRYLTDMT	NQKRKMART	IESEV.
A/duck/Thailand/KU-56/2007	ESDEALK		PISRYLIDMI	NQKRKMART	VESEV.
A/chicken/Thailand/ST-351/2008	ESDKALK		PASRYLTDMT	NQKRKMART	TESEV.
A/chicken/Sukhothai/NIAH114843/2008	ESDKALK		PASRYLTDMT	NQKRKVART	IESEV.
A/Viet Nam/1194/2004	ESDKALK		PASRYLTDMT	NQKRKMART	IESEV.
A/Viet Nam/1203/2004	ESDKALK		PASRYLTDMT	NQKR.MART	TESEV.
A/duck/Vietnam/1228/2005	ESDKALK		PVSRYLTDMT	NQNRKMART	IESEV.
A/duck/Vietnam/5/2007	ESDKALK		PVSRYLTDMT	NQNRKMART	IESEV.
A/chicken/Indonesia/4/2004	ESDEALK	XXXXX	PASRYLTDMT	NQKRKMART	IESEV.
A/duck/Indonesia/MS/2004	ESDEALK		PISRYLIDMI	NQKRKMART	IESEI.
A/Indonesia/CDC596/2006	EFDEALKE		PASRYLTDMT	NQKRKMART	IESEV.
A/Indonesia/CDC599/2006	EFDEALKE		PASRYLTDMT	NQKRKMART	IESEV.
A/Indonesia/CDC887/2006	ESDEALKE		PASRYLTDMT	NQKRKMART	IESEV.
A/Indonesia/CDC938/2006	ESDEALKE		PASRYLTDMS	NQKRKMART	TESEV.
A/turkey/Turkey/1/2005	ESDEALK		PASRYLTDMT	DQKRKMART	IESKV.
A/whooper swan/Mongolia/3/2005	ESDEALK		PASRYLTDMT	DQKRKMART	IESKV.
A/goose/Guangdong/1/1996	ETNENLKI	AIASS	PAPRYITDMS	KQKRYMAKR	VESEV.
A/duck/Fujian/668/2006	ESDEALK		PASRYLTDMT	NQKRKMART	IESEV.
A/duck/Guangxi/150/2006	ESDEALK		PISRYLIDMI	NQKRKMART	IESEV.
A/bar-headed goose/Qinghai/5/2005	ESDEALK		PASRYLTDMT	DQKRKMART	IESKV.

**Figure 27**: Comparison of deduced amino acid of NS1 protein at position 80-84 which involving virulence determinant amino acid of virus (indicated in box), 92 (triangle) and C-terminal (dashed).


## Nucleoprotein gene

Genetic relatedness of Nucleoprotein gene of avian influenza H5N1 viruses is shown in Figure 28. Avian influenza H5N1 viruses (CU-345, CU 346, and CU347) are clustered in group related to the outbreaks in Thailand and separated from China and Indonesia lineages. Genetic analysis of Nucleoprotein gene of avian influenza viruses at key determinant residues such as of avian-like and human-like characteristics is shown in Table 16 and Figure 29. In this study, avian influenza H5N1 viruses (CU-345, CU 346, and CU347) carried Leucine (L) indicating avian-like characteristics.



**Figure 28**: Phylogenetic analysis of NP gene of H5N1 viruses isolated in this study compared to other H5N1 viruses from Thailand and other countries. The analysis was based on nucleotides 1-1481 of the NP gene.

60

**Table 16:** Genetic analysis of deduced amino acid of NP gene at key determinantresidues relating to human or avian-like and virulence characteristics.

Virus	Human/Avian like characteristics
	136 <sup>ª</sup>
A/chicken/Thailand/CU-347/2008	
A/duck/Thailand/CU-345/2007	
A/chicken/Thailand/CU-346/2007	L
A/Thailand/16/2004	L
A/chicken/Thailand/CU-K2/2004	L
A/Thailand/676/2005	L
A/chicken/Thailand/CK-162/2005	L
A/chicken/Thailand/NP-172/2006	L
A/chicken/Thailand/PC-168/2006	L
A/chicken/Thailand/PC-170/2006	
A/duck/Thailand/CU-328/2007	L
A/duck/Thailand/KU-56/2007	L
A/chicken/Thailand/ST-351/2008	L
A/chicken/NIAH114843/2008	L
A/Viet Nam/1194/2004	<u>с</u>
A/Viet Nam/1203/2004	L
A/duck/Vietnam/1228/2005	L
A/duck/Vietnam/5/2007	1 and 1
A/chicken/Indonesia/4/2004	
A/duck/Indonesia/MS/2004	
A/Indonesia/CDC596/2006	
A/Indonesia/CDC599/2006	I
A/Indonesia/CDC887/2006	11
A/Indonesia/CDC938/2006	
A/turkey/Turkey/1/2005	0./
A/whooper swan/Mongolia/3/2005	~ 011 01 n n r
A/goose/Guangdong/1/1996	М
A/bar-headed goose/Qinghai/5/2005	on D III o
A/duck/Guangxi/150/2006	L
A/duck/Fujian/668/2006	

<sup>a</sup> Amino acid at position 136 of Nucleoprotein gene: M (Methionine), L (Leucine)

NP analysis

🔀 Consensus	EIRRIWRQAN	NGEDATAGLT	HLMIWHSNLN	DATYORTRALV	RIGMDE
30 Sequences	120	130	140	150	160
CU345NP	EIRRIWRQAN	NGEDATAGLT	HLMIWHSNLN	DATYQRTRALV	RIGMDE
CU346NP	EIRRIWRQAN	NGEDATAGLT	HLMIWHSNLN	DATYQRTRALV	RIGMDE
CU347NP	EIRRIWRQAN	NGEDATAGLT	HLMIWHSNLN	DATYQRTRALV	RIGMDE
A/Thailand/16/2004	EIRRIWRQAN	NGEDATAGLT	HLMIWHSNLN	DATYQRTRALV	RIGMDE
A/chicken/Thailand/CU-K2/2004	EIRRIWRQAN	NGEDATAGLT	HLMIWHSNLN	DATYORTRALV	RIGMDE
A/Thailand/676/2005	EIRRIWRQAN	NGEDATAGLT	HLMIWHSNLN	DATYQRTRALV	RIGMDE
A/chicken/Thailand/CK-162/2005	EIRRIWRQAN	NGEDATAGLT	HLMIWHSNLN	DATYQRTRALV	RIGMDE
A/chicken/Thailand/NP-172/2006	EIRRIWRQAN	NGEDATAGLT	HLMIWHSNLN	DATYQRTRALV	RIGMDE
A/chicken/Thailand/PC-168/2006	EIRRIWRQAN	NGEDATAGLT	HLMIWHSNLN	DATYQRTRALV	RIGMDE
A/chicken/Thailand/PC-170/2006	EIRRIWRQAN	NGEDATAGLT	HLMIWHSNLN	DATYQRTRALV	RIGMDE
A/duck/Thailand/CU-328/2007	EIRRIWRQAN	NGEDATAGLT	HLMIWHSNLN	DATYQRTRALV	RIGMDE
A/duck/Thailand/KU-56/2007	EIRRIWRQAN	NGEDATAGLA	HLMIWHSNLN	DATYQRTRALV	RIGMDE
A/chicken/Thailand/ST-351/2008	EIRRIWRQAN	NGEDATAGLT	HLMIWHSNLN	DATYQRTRALV	RIGMDE
A/chicken/Sukhothai/NIAH114843/2008	EIRRIWRQAN	NGEDATAGLT	HLMIWHSNLN	DATYQRTRALV	RTGMDE
A/Viet Nam/1194/2004	EIRRIWRQAN	NGEDATAGLT	HLMIWHSNLN	DATYQRTRALV	RTGMDE
A/Viet Nam/1203/2004	EIRRIWRQAN	NGEDATAGLT	HLMIWHSNLN	DATYQRTRALV	RTGMDE
A/duck/Vietnam/1228/2005	EIRRIWRQAN	NGEDATAGLT	HLMIWHSNLN	DATYQRTRALV	RTGMDE
A/duck/Vietnam/5/2007	EIRRIWRQAN	NGEDATAGLT	HLMIWHSNLN	DATYQRTRALV	RTGMDE
A/chicken/Indonesia/4/2004	EIRRIWRQAN	NGEDATAGLT	HLMIWHSNLN	DATYQRTRALV	RTGMDE
A/duck/Indonesia/MS/2004	EIRRIWRQAN	NGEDATAGLT	HLMIWHSNLN	DATYQRTRALV	RTGMDE
A/Indonesia/CDC596/2006	EIRRIWRQAN	NGEDATAGLT	HLMIWHSNLN	DATYQRTRALV	RTGMDE
A/Indonesia/CDC599/2006	EIRRIWRQAN	NGEDATAGLT	HLMIWHSNLN	DATYQRTRALV	RTGMDE
A/Indonesia/CDC887/2006	EIRRIWRQAN	NGEDATAGLT	HLMIWHSNLN	DATYQRTRALV	RTGMDE
A/Indonesia/CDC938/2006	EIRRIWRQAN	NGEDATAGLT	HLMIWHSNLN	DATYQRTRALV	RTGMDE
A/turkey/Turkey/1/2005	EIRRIWRQAN	NGEDATAGLT	HLMIWHSNLN	DATYQRTRALV	RTGMDE
A/whooper swan/Mongolia/3/2005	EIRRIWRQAN	NGEDATAGLT	HLMIWHSNLN	DATYQRTRALV	RTGMDE
A/goose/Guangdong/1/1996	EIRRIWRQAN	NGEDATAGLT	HMMIWHSNLN	DATYQRTRALV	RTGMDE
A/bar-headed goose/Qinghai/5/2005	EIRRIWRQAN	NGEDATAGLT	HLMIWHSNLN	DATYQRTRALV	RTGMDE
A/duck/Fujian/668/2006	EIRRIWRQAN	NGEDATAGLT	HLMIWHSNLN	DATYQRTRALV	RIGMDE
A/duck/Guangxi/150/2006	EIRRIWRQAN	NGEDATAGLT	HLMIWHSNLN	DATYORTRALV	RTGMDE

Figure 29: Comparison of deduced amino acid of NP protein at position 136 which represents avian-like and human-like characteristics (indicated by arrows).



## CHAPTER V

## DISCUSSION

In Thailand, avian influenza H5N1 virus is a new emerging virus, which was first reported at the beginning of year 2004. To date, avian influenza virus has spread periodically with at least 7 waves of Al outbreaks reported in the country. The outbreaks of avian influenza in Thailand are mostly caused by avian influenza H5N1 virus classified as Genotype Z or clade 1 (Li et al., 2004; Viseshakul et al., 2004). Genetic characteristics of avian influenza H5N1 viruses from Thailand were found to be similar to those in Vietnam; therefore, the viruses have been classified as Thailand-Vietnam lineage or genotype Z (Amonsin et al., 2006; Li et al., 2004; Viseshakul et al., 2004; Webster and Govorkova, 2006). WHO/OIE/FAO and team (2008) have summarized avian influenza H5N1 virus outbreaks in many countries around the world. They reported that avian influenza H5N1 virus can be divided into several groups or clades by using the comparison of genetic sequence of HA gene. For example, avian influenza H5N1 virus from Thailand can be classified into in clade 1 that is the virus spreading in Thailand, Vietnam, and Malaysia (Boltz et al., 2006; Li et al., 2004). The outbreaks of avian influenza H5N1 in Thailand were previously caused by clade 1 viruses. Currently, there are at least 2 clades (i.e. clade 1 and 2.3.4) or 2 genotypes (i.e. genotype Z and V) of avian influenza H5N1 viruses found to emerge and cause the outbreaks in the country. Chutinimitkul found influenza H5N1 and team (2007)that avian virus "A/chicken/Thailand/NP-172/2006" that is a virus isolated from chickens at Nakhon Phanom and has the genetic characteristics of genotype V. The virus, NP-172 (genotype V) is different from the genotype Z that causes most AI outbreaks in Thailand. It is noted that the genotype V virus has PA gene that exhibited low nucleotide similarity from the genotype Z (Chutinimitkul et al., 2007).

In this study, we have collected the samples from 42 districts of 13 provinces in the border of Thailand, Myanmar and Laos. These locations were selected based on the criteria as follows: 1) an area that has a joint border of Thailand with Myanmar and Laos 2) an area that has an international crossing point, a temporary border crossing, a temporarily permitted between Thailand and neighboring countries, which are essential for disease surveillance and poultry movement) and 3) an area with the outbreaks of avian influenza previously reported in the last 4-5 years. Provinces that are located along the borders of Thailand and neighboring countries, Myanmar and Laos with the report of avian influenza outbreaks are Nakorn Phanom, Nong Khai and Chiang Mai, etc. (Amonsin et al., 2006; Chutinimitkul et al., 2007). In this study, 2,175 samples were collected from several poultry species from 42 districts of 13 provinces between September 2007 and June 2008. Sixty-eight samples (3.13% (68 / 2,175)) were tested positive for the HA. Out of 68 HA positive samples, 15 samples were tested positive as influenza A H5N1 virus using multiplex RT-PCR, realtime RT-PCR and PCR-ELISA assays. We have selected only 3 avian influenza H5N1 viruses which are the representatives from each province of Loey, Chiang Rai, and Prachuap Khiri Khan for whole gene sequencing of the virus.

In the present study, we used 3 PCR-based assays to identify influenza A H5N1 virus. The samples that yield positive results by using multiplex RT-PCR samples were further confirmed by realtime RT-PCR using M, HA, and NA gene specific primers (Payungporn et al., 2006; Payungporn et al., 2004; Suwannakarn et al., 2008) and PCR-ELISA using H5N1 specific probes (Chaharaein et al., 2009). Multiplex RT-PCR can be identified 15 samples as influenza A virus. Multiplex RT-PCR can also further identify 15 influenza A into H5 (7 samples) and N1 (12 samples).When the realtime RT-PCR and PCR-ELISA were used to all isolates were confirmed as influenza A subtype H5N1. Test results showed that multiplex PCR methods may not be sensitive enough to find subtype viruses. Therefore, test methods together with realtime RT-PCR and PCR-ELISA could be identifying as H5N1.

In this study, only 3 avian influenza H5N1 viruses (CU-345, CU-346 and CU-347) were selected for whole gene sequencing. The genetic information from whole genome sequences were used for phylogenetic analysis and genotype analysis of the viruses. We were able to elucidate whole genome sequences (8 genes) of only one virus (CU-

347). Due to the limitation of RNA quality, only 4 genes of the other two viruses (CU-345 and CU-346) were sequenced. Reasons for the unsuccessful whole gene sequencing may be spend many times to handle or using RNA in any PCR method that RNA could be degrade or the low virus titers may be affect the amount of extracted RNA which was not good enough for whole genome sequencing of the viruses.

Genetic relatedness of avian influenza H5N1 viruses isolated in this study was evaluated by phylogenetic analysis. Our analysis indicated that avian influenza H5N1 viruses (CU-345, CU 346 and CU-347) in this study are arranged in the same group of Vietnam-Thailand lineage, which was related to most of the AI outbreaks in Thailand, Vietnam, and Malaysia. The phylogenetic analysis of 8 genes of the viruses showed that the genetic relatedness of 3 viruses in each gene were generally consistent. The result of this study indicated that avian influenza H5N1 viruses in Thailand derived from the ancestor "Goose/Guangdong/96-lineage" (Chen et al., 2006). In clade classification system, the CU-345, CU-346 and CU-347 avian influenza H5N1 viruses belonged to clade 1, which was considered as an important clade and caused the outbreaks of avian influenza in Thailand and Vietnam as well as Cambodia, Laos and Malaysia.

We determined the mutations in key determinant residues of 8 genes of the viruses and compared with the avian influenza H5N1 virus circulating in Thailand and other countries. The analysis of HA cleavage site at the position 323-329 in HA gene showed the characteristics of multiple basic amino acids insertion. This characteristic designated the virus as highly pathogenic avian influenza (HPAI) (Claas et al., 1998). The avian influenza H5N1 viruses in this study had the characteristics of HA cleavage site type "RERRRKK". The cleavage site can be found in other avian influenza H5N1 viruses in Thailand that have multiple insertion of basic amino acids at different types such as "RERKRKK", "REKRRKK" and "RERRRKK" (Amonsin et al., 2006). In this study, avian influenza H5N1 viruses contained avian specific-receptor binding properties at position 222-224 (Q222 and G224). The presence of Q222 and G224 can affect the ability of the virus to bind the host receptor ( $\alpha$ -2,3 linkage), typical for avian but not the human virus (Connor et al., 1994). The mutation of amino acids at these positions may

affect the virulence of infection particularly in mammals (Matrosovich et al., 1999; Shinya et al., 2006; Webster et al., 1997). Amino acids related to the receptor binding pockets were also analyzed. These amino acids had highly positive selection pressure (Smith et al., 2006). We found no mutations of amino acids at position 129 and 175 (L129 and L175), that was related to the virulence of avian influenza H5N1 virus infection. The analysis of glycosylation sites of avian influenza viruses showed that the viruses contain all 7 glycosylation sites related to the virulence of avian influenza H5N1 virus (Matrosovich et al., 1999; Shortridge et al., 1998). In addition, the comparative analysis of amino acids of 5 locations of the HA1 protein including antigenic site A-E at amino acids positions 83, 86, 138, 140, and 141 were analyzed due to their highly positive selection pressure (Smith et al., 2006). The analysis showed that avian influenza H5N1 virus had amino acid mutations at position 86, 138, 140, and 141. These mutations was previously shown to result in the changes of antigenic epitopes, which reflect the adaptation of the virus to escape host immunity (de Jong and Hien, 2006).

The previous studies demonstrated the presence of 20 amino acid deletion at NA stalk region of NA gene in avian influenza H5N1 viruses from Thailand, Vietnam, Indonesia and many countries in Asia and Europe during the year 2003-2007 (Amonsin et al., 2006; Chen et al., 2006; Li et al., 2004; Salzberg et al., 2007; Viseshakul et al., 2004). This 20 amino acid deletion is missing in avian influenza H5N1 virus isolated from goose "Goose/Guangdong/1/196". The change of amino acids at NA stalk region was the result of the adaptation or evolution occuring from the infection in wild aquatic birds to domestic poultry (Matrosovich et al., 1999). In this study, 3 viruses have 20 amino acid deletion at position 49-68 of NA stalk region, which were consistent with avian influenza H5N1 viruses circulating in Thailand. Amino acids associated with Oseltamivir resistance were previously identified at positions 119 (E to V), 293 (R to K), and 295 (N to S) (Kiso et al., 2004) and at position 275 (H to Y) (Gubareva et al., 2000). However, such amino acids were not found in this study.

The particular amino acid positions or concensus in M gene were found to be specific to avian virus (avian-like amino acids) or mammal virus (human-like amino acids) (Matrosovich et al., 1999), for example, amino acids in M2 protein at position 16 (E /G) (avian/human-like), position 28 (I/V) and position 55 (L/F). The results of this study found that amino acids at position 16 and 55 were E and L which were avian-like amino acids. Amino acid at position 28 was V, the human-like amino acid. In addition, amino acid associated with amatadine resistance I 26 and 31 N were identified. In general, the amantadine resistances were related to the mutations of amino acids at position 26, 27, 30, 31, 64, 66 of M2 protein (Cheung et al., 2006). These mutations are involved in the structure of protein that may affect the ability of antiviral drugs (Cheung et al., 2006; Pinto et al., 1992).

Amino acids of the PB2 gene at position 199, 661, 667 and 702 were Alanine (A), Alanine (A), Valine (V) and Lysine (K) respectively. These amino acid represented the characteristics of the virus in avian (avian-like amino acids), while the characteristics of virus in mammal (human-like amino acids) had the amino acid as Serine (S), Threonine,(T) Glutamic acid (E), and Arginine (R) (Puthavathana et al., 2005). The analysis of amino acids in polymerase gene (PB2) can indicate virulence characteristics of viruses in mammals (Shinya et al., 2004) such as in the PB2 gene at positions 627 and 355. However, in this study the virus (CU-347) contained E627 and R355 which were the characteristics of non-virulence in mammals. The amino acid of the PB1 protein at position 198 was Lysine (K) indicating non-virulence characteristics. Moreover, the amino acid of PA protein at position 409 was Serine (S), which is the characteristic of the avian-like amino acids. Therefore, genetic analysis of the polymerase genes of avian influenza H5N1 viruses isolated in this study indicated the characteristics of viruses both in avian and mammal. However, single position of amino acids was not the only a marker representing the virulence of avian influenza.

Previous study revealed that some amino acid deletion in NS gene may increase the virulence of avian influenza H5N1 virus (Lipatov et al., 2005). The current study revealed that avian influenza H5N1 virus had 5 amino acid deletions at positions 80-84. This characteristic was previously found in avian influenza H5N1 viruses in Thailand, Vietnam, Indonesia, China, Europe and Africa. However, no deletion was found in avian influenza H5N1 virus from China "Goose/Guangdong/1/96" (Duan et al., 2008). The mutation of amino acids at position 92 from aspartic acid (D) to glutamic acid (E) may affect the virulence of the virus, especially in mammal strains (Seo et al., 2004). Avian influenza H5N1 virus in this study possed D92 indicating non-virulent characteristics in mammal. This amino acid of the NS1 gene is one of several markers that reflect the virulence of virus. Another virulence determinant in the NS1 protein was carboxyl-terminal. This virulence determinant was related to the capture of host proteins in PDZ domain (Obenauer et al., 2006). Avian influenza H5N1 virus mostly contained c-terminal motif ESEV, whereas non virulent avian influenza H5N1 virus may has c-terminal motif as RSKV (Obenauer et al., 2006). In this study, avian influenza H5N1 virus contained c-terminal motif of the NS1 protein commonly found in virulent avian influenza H5N1 virus that was the position of L136. In contrast, the "Goose/Guangdong/1/96" virus had Methionine (M), human-like amino acid (Reid et al., 2004).

Genotype analysis of avian influenza H5N1 virus in this study was done in a H5N1 virus (CU-347) that had whole genome sequenced. It can be concluded that avian influenza H5N1 virus isolated from provinces located at joint border of Myanmar, Laos, Thailand was avian influenza H5N1 viruses, genotype Z. Currently, the classification system named "clade" of avian influenza H5N1 virus is developed. Clade nomenclature system is based on principles of the HA gene evolutions. The analysis of HA gene of three H5N1 viruses (CU-345, CU-346 and CU-347) indicated that the viruses belonged to clade 1 responsible for the outbreaks of avian influenza in Thailand and Vietnam including Cambodia, Laos and Malaysia.

## Conclusion and suggestion

The present study collected 2,715 samples from the several poultry species in 42 districts of 13 provinces that joint border of Thailand, Myanmar and Laos between September 2007 and June 2008. Sixty eight samples were tested positive for the HA test (3.13% (68 / 2,175)). Out of 68 samples, 15 samples were identified as avian influenza H5N1 by multiplex RT-PCR, realtime RT-PCR and PCR-ELISA methods. Three avian influenza H5N1 viruses from Loey (CU-345), Chiang Rai (CU-346), and Prachuap Khiri Khan (CU-347) were selected for whole gene sequencing and genotype classification. Total numbers of sequence of avian influenza H5N1 viruses available in this study were 16 sequences.

Genetic relatedness and genotype analysis of avian influenza H5N1 viruses revealed that avian influenza H5N1 viruses isolated from border of Thailand, Laos and Myanmar were classified as genotype Z or clade1. These viruses were closely related and arranged in the same group with the viruses from avian and human in Vietnam-Thailand lineage, which is responsible for most AI outbreaks in Thailand, Vietnam, Cambodia, and Malaysia. Genetic analysis of these viruses demonstrated no mutations at key determinant residues such as HA cleavage site, receptor binding site in HA protein, NA stalk region, antiviral drug resistant residues in NA protein, antiviral drug resistant residues in M2 protein, and virulence determinants in PB2, PB1, and NS protein. The H5N1 viruses had characteristics of both avian-like and Human-like viruses.

According to the results of present study, monitoring of influenza A H5N1 virus from avian species in border area between Thailand and neighboring countries should be following up continuously. The genetic characterizations of the virus to examine mutations or genetic changes of avian influenza H5N1 virus in Thailand are required.

Data obtained could be beneficially used as follows:

- 1. To know evidence of avian influenza H5N1 viruses in the border areas between Thailand and neighboring countries (Laos and Myanmar).
- 2. To be published genetic data in GenBank database.
- 3. To explain spreading and mutations of avian influenza H5N1 virus.
- 4. To be used as a resource of an origin or a primary cause of outbreak of avian influenza A viruses.
- 5. To be used as a fundamental data to select and develop a candidate influenza (H5N1) vaccine strain.

From the results, the suggestion for further studies could be as follows:

- 1. Information of this outbreak will be used to plan control and prevention strategies of AI. Therefore, intensive active surveillances are recommended.
- 2. Genetic of avian influenza H5N1 virus could be mutating easily, genetic characterizations of the virus to examine are required.
- 3. Avian influenza surveillance should require cooperation from various parties and have adequate equipment. Therefore, coordination with local agencies before the surveillance will be work easier.
- Researcher should be vaccinated against influenza before they will collect the sample. In field, researcher should wear protective equipment such as mask, glove, and Tyvec suite etc.

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## จุฬาลงกรณ์มหาวิทยาลัย

APPENDICES

## APPENDIX A

Standard amino acid at	obreviations
Amino Acid	Letter
Alanine	Ala, A
Arginine	Arg, R
Asparagine	Asn, N
Aspartic acid	Asp, D
Cysteine	Cys, C
Glutamic acid	Glu, E
Glutamine	GIn, Q
Glycine	Gly, G
Histidine	His, H
Isoleucine	lle, I
Leucine	Leu, L
Lysine	Lys, K
Methionine	Met, M
Phenylalanine	Phe, F
Proline	Pro, P
Serine	Ser, S
Threonine	Thr, T
Tryptophan	Trp, W
Tyrosine	Tyr, Y
Valine	Val, V

## APPENDIX B

## Reagents and preparations

1.

Phosphate Buff	fer Saline (PBS)		
Sodium ch	nloride (NaCl)	8	g
Potassium	n chloride (KCI)	0.2	g
Potassium	di-hydrogen phosphate (KH <sub>2</sub> PO <sub>4</sub> )	0.2	g
Di-sodium	hydrogen phosphate (Na2HPO4)	1.15	g

Gentle stir on stirrer for 30 min and adjust pH to 7.2 and sterilize immediately by autoclave

## Reagents for agarose gel electrophoresis

1.	. 10 mg/ml Ethidium bromide		
	Ethidium bromide	1	g
	Distilled water	1,000	ml

Stir few hours for dye has dissolved, wrap container in aluminum foil and transfer to a dark bottle and store at 4°C

2. 2% Agarose gel			
Agarose (ultrapure)		0.3	g
1X TBE		20.0	ml
10 mg/ml Ethidium bro	mide	1.0	μΙ

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## APPENDIX C

## Nucleotide sequences of A/duck/Loei/Thailand/CU-345/07

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CU-345-PA: Polymerase acidic gene (PA)
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LOCUS DEFINITIO ACCESSION VERSION KEYWORDS	CU-345-PA 516 bp DNA linear 23-DEC-2008 DN Influenza A virus strain A/duck/Loei/Thailand/CU-345/07(H5N1) N
SOURCE	Influenza A virus
ORGANISM	Influenza A virus Unclassified.
REFERENCI	E = 1 (bases 1 to 516)
AUTHORS	Lapkuntod, J., Chuatakul, C., Tantilertcharoen, R.,
	Poovorawan, Y. and Amonsin, A.
TITLE	Monitoring of influenza A virus from avian species in border
	areas of Thailand
JOURNAL	Unpublished
REFERENCI	E = 2 (bases 1 to 516)
AUTHORS	Lapkuntod, J., Chuatakul, C., Tantilertcharoen, R.,
	Poovorawan, Y. and Amonsin, A.
TITLE	Direct Submission
JOURNAL	Submitted (23-DEC-2008) Chulalongkorn University, Department of
Veterina	ry Public Health, Rama 4 Rd. Pathumwan, Bangkok 10330, Thailand
FEATURES	Location/Qualifiers
source	1516
	/organi <mark>s</mark> m="Influenza A virus"
	/mol_type="genomic_DNA"
	/strain="A/duc <mark>k/Loei/Thailand/CU-</mark> 345/07(H5N1)"
	/serotype="H5N1"
	/segment="PA"
	/country="Thailand"
BASE COUL	NT 177 a 101 c 129 g 109 t
ORIGIN	
1 a	acgccctctc agactacctg atgggcctcc ttgctctcag cggtcgaagt ttttgctgat
61 (	ggatgccctt aaattaagca tcgaagaccc gagtcatgag ggggaggggga taccactata
121 (	cgatgcaatc aaatgcatga agacattttt cggatggaaa gagcccaaca tcgtgaaacc
181 a	acatgaaaag ggtgttaact ccaattacct cctggcttgg aagcaggtgc tggcagaact
241 0	ccaagatatt gaaaatgagg agaaaatccc aaaaacaaag aacatgaaaa aaacaagcca
301 (	yttgaagtgg acactcggtg agaacatggc accagagaaa gtagactttg aggactgcaa
361 a	agatgttagc gacctaagac agtatgacag tgatgaacca gagtctagat cactagcaag
421 0	ctggattcag agtgaattca acaaggcatg tgaattgaca gattcgattt ggattgaact
481 -	tgatgaaata ggagaagacg tagctccaat tgagca//

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CU-345-HA: Hemagglutinin gene (HA)
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LOCUS	CU-345-HA 455 bp DNA linear 23-DEC-2008
DEFINITION	Influenza A virus strain
A/duck/Loei	/Thailand/CU345/07(H5N1)
ACCESSION	
VERSION	
SOURCE	· Influenza A wirus
ORGANISM	Influenza A virus
ORGANISH	Unclassified
REFERENCE	1  (bases 1 to 455)
AUTHORS	Lapkuntod, J., Chuatakul, C., Tantilertcharoen, R.,
	Poovorawan, Y. and Amonsin, A.
TITLE	Monitoring of influenza A virus from avian species in
	border areas of Thailand
JOURNAL	Unpublished
REFERENCE	2 (bases 1 to 455)
AUTHORS	Lapkuntod, J., Chuatakul, C., Tantilertcharoen, R.,
	Poovorawan,Y. and Amonsin,A.
TITLE	Direct Submission
JOURNAL	Submitted (23-DEC-2008) Chulalongkorn University,
	Department of Veterinary Public Health, Rama 4 Rd.
	Pathumwan, Bangkok 10330, Thailand
FEATURES	Location/Qualifiers
Source	1455
	/organism="influenza A virus"
	/mol_type="genomic_DNA"
	/scrature="H5N1"
	/segment="HA"
	/country="Thailand"
BASE COUNT	164 a 81 c 113 q 97 t
ORIGIN	
1 tggaaatt	tc attgctccag aatatgcata caaaattgtc aagaaagggg actcaacaat 63
tatgaaaagt	gaattggaat atggtaactg caacaccaag tgtcaaactc caatgggggc
121 gataaact	ct agtatgccat tccacaatat acaccetete accategggg aatgeeecaa
181 atatgtga	aa tcaaatagat tagtccttgc gactgggctc agaaatagcc ctcaaagaga
241 gagaagaa	ga aaa <mark>aagagag gattatttgg agctata</mark> gca ggttttatag agggaggatg
301 gcagggaa	tg gtagatggtt ggtatgggta ccaccatagc aatgagcagg ggagtgggta
361 cgctgcag	ac aaagaatcca ctcaaaaggc aatagatgga gtcaccaata aggtcaactc
421 gataattg	ac aaaatgaaca ctcagtttga ggccg//

L	OCUS	CU-345-NP 5	54 bp DNA l	inear 23-DE	C-2008	
DI	EFINITION	Influenza A	virus stra:	in		
		A/duck/Loei,	/Thailand/C	J345/07 (H5N	1)	
A	CCESSION					
V	ERSION					
K	EYWORDS					
S	OURCE	Influenza A	virus			
0	RGANISM	Influenza A	virus			
		Unclassifie	d.			
R	EFERENCE	1 (bases 1	to 554)			
A	UTHORS	Lapkuntod, J	., Chuataku	l,C., Tanti	lertcharoen	,R.,
Ρ	oovorawan,Y	. and Amons.	in,A.			
Т	ITLE	Monitoring	of influenz	a A virus f	rom avian s	pecies in
		order areas	of Thailar	id		F
J	OURNAL	Unpublished				
R	EFERENCE	2 (bases 1	to 554)			
A	UTHORS	Lapkuntod, J	., Chuataku	l.C., Tanti	lertcharoen	,R.,
		Poovorawan,	Y. and Amon	sin.A.		, .,
Т	ITLE	Direct Subm	ission	,		
- .T	OURNAL	Submitted (	23 - DEC - 2008	) Chulalong	korn Univer	sitv.
Ũ	ooraan	Department	of Veterina	ry Public F	lealth. Rama	4 Rd.
		Pathumwan.	Bangkok 103	30. Thailand	iouroni, maine	1 1000
म	EATURES	Location/Ou	alifiers			
s	ource	1554				
0	04200	/organism="	Influenza A	virus"		
		/mol type="	genomic DNA	"		
		/strain="A/	duck/Loei/T	hailand/CII-	345/07 (H5N1	) "
		/serotype="	H5N1"	inallania, oo	0 10, 0, (110111	,
		/segment="N	p"			
		/country="T	hailand"			
В	ASE COUNT	175 a	108 c	163 a 10	8 +	
0	RIGIN	1/0 4	100 0	100 9 10	0 0	
1	atagogtete	aaggcaccaa	acgatettat	gaacagatgg	aaactootoo	ggaacgccag
61	aatactacta	agatcaggg	atctattaa	agaatgatta	ataacattaa	gagattetac
121	atacagatgt	gcacagaact	caaactcagt	gactatgaag	gaaggetaat	ccagaacago
181	ataacaataq	agagaatggt	actetetaca	tttgatgaaa	gaaggaagaa	atacctoraa
241	gaacacccca	atacaaaaa	qqacccqaaq	aagactggaag	gtccaattta	tcagaggaa
301	gaccocceed	gegeggggaaa	actaattctd	tacqacaaaq	aggagatcag	aagaatttaa
361	catcaaacaa	acaatugaga	ggacgcaact	actantetta	cccacctgat	gaggaccegg
421	tccaatctaa	atgatgccac	atatcagaga	acqaqaqctc	tcatacatac	tagaatagaa
481	ccaaggatgt	actetetat	acaaqqqtca	acteteceta	ggagatctog	aactaccaat
541	acaacaataa	aggg//	ycaayyycca	uccolocita	ggagaceegg	ugetyeeygt
711	yeayeayeaa	uggg//				

Ct	J-345-NA: 1	Neuraminidase gene (NA)
L	DCUS	CU-345-NA 1352 bp DNA linear 23-DEC-2008
DI	EFINITION	Influenza A virus strain A/duck/Loei/Thailand/CU- 345/07(H5N1)
A	CCESSION	
VI	TRETON	
NI S(	TIRCE	· Influenza A wirus
OI	RGANISM	Influenza A virus
D		Unclassified.
RI	SFERENCE	I (bases I to 1352) Leolunted I. Chustelul C. Mentileutsheveen D
A	JTHORS	Lapkuntod, J., Chuatakul, C., Tantilertcharoen, R.,
m.		Monitoring of influence A wirwe from awien encoded in
1.	1116	border areas of Thailand
J	JURNAL	Unpublished
RI	EFERENCE	2 (bases 1 to 1352)
AU	JTHORS	Lapkuntod, J., Chuatakul, C., Tantilertcharoen, R.,
		Poovorawan, Y. and Amonsin, A.
T	ITLE	Direct Submission
J	JURNAL	Submitted (23-DEC-2008) Chulalongkorn University,
		Department of veterinary Public Health, Rama 4 Rd.
רי		Location/Qualifiers
E 1 97		11352
		/organism="Influenza A virus"
		/mol type="genomic DNA"
		/strain="A/duck/Loei/Thailand/CU-345/07(H5N1)"
		/serotype="H5N1"
		/segment="NA"
		/country="Thailand"
BZ	ASE COUNT RIGIN	400 a 245 c 343 g 364 t
1	l atgaatcca	aa <mark>ataagaagat aataaccatc ggat</mark> caatct gtatggtaac tggaatggct
61	agcttaato	gt tacaa <mark>attgg gaacttgatc tcaa</mark> tatggg tcagtcattc aattcacaca
121	gggaatcaa	ac acaaagctga accaatcagc aatactaatt ttcttactga gaaagctgtg
181	gcttcagta	aa aattagcggg caattcatct ctttgcccca ttaatggatg ggctgtatac
241	agtaaggad	ca acagtataag gatcggttcc aaggggggatg tgtttgttat aagagagcca
301		
201 421	tatectate	ag atgaaggetee etceccatat aacteaagget ttgagtetat taettgatea
481	gcaagtact	tt gccatgatgg caccagttgg ttgacaattg gaatttotgg cocagacagt
541	gagactato	gg ctgtattgaa atacaatggc ataataacag acactatcaa gagttggagg
601	aataacata	ac tgagaactca agagtetgaa tgtgcatgtg taaatqqctc ttqctttact
661	. gtaatgact	tg acggaccaag taatggtcag gcatcacata agatcttcaa aatggaaaaa
721	gggaaagto	gg ttaaatcagt cgaattggat gctcctaatt atcactatga ggaatgctcc
781	tgttatcct	tg atgccggcga aatcacatgt gtgtgcaggg ataattggca tggctcaaat
841	cggccatgo	gg tatctttcaa tcaaaatttg gagtatcaaa taggatatat atgcagtgga
901	gttttcgga	ag acaatccacg ccccaatgat ggaacaggta gttgtggtcc ggtgtcctct
961	aacggggca	at atggggtaaa agggttttca tttaaatacg gcaatggtgt ctggatcggg
1021	agaacaaaa	aa gcactaattc caggagcggc tttgaaatga tttgggatcc aaatgggtgg
1081	actgaaaco	gg acagtagctt ttcagtgaaa caagatatcg tagcaataac tgattggtca
1141	ggatatago	cg ggagttttgt ccagcatcca gaactgacag gactagattg cataagacct
1201		gg tigagtigat cagagggcgg cccaaagaga gcacaattig gactagiggg
126J	agcagcata	al cullingtgg tgtaaatagt gacactgtgg gttggtottg gocagaoggt
1321	gelgagtte	ye calleaceal lgaeaagtag ll//

CU-345-NS:	Nonstructural gene (NS)	
LOCUS DEFINITION	CU-345-NS 824 bp DNA linear 23-DEC-2008 Influenza A virus strain A/duck/Loei/Thailand/CU- 345/07(H5N1)	
ACCESSION		
VERSION		
KEYWORDS		
SOURCE	· Influenza Autrus	
OPCANTEM	Influenza A virus	
ORGANISM		
REFERENCE	1 (Dases 1 to 824)	
AUTHORS	Lapkuntod, J., Chuatakul, C., Tantilertcharoen, R.,	
	Poovorawan, Y. and Amonsin, A.	
TITLE	Monitoring of influenza A virus from avian species in	
	border areas of Thailand	
JOURNAL	Unpublished	
REFERENCE	2 (bases 1 to 824)	
AUTHORS	Lapkuntod, J., Chuatakul, C., Tantilertcharoen, R.,	
	Poovorawan,Y. and Amonsin,A.	
TITLE	Direct Submission	
JOURNAL	Submitted (23-DEC-2008) Chulalongkorn University,	
	Department of Veterinary Public Health, Rama 4 Rd.	
	Pathumwan, Bangkok 10330, Thailand	
FEATURES	Location/Qualifiers	
SOURCE	1 824	
DOULCC	/organism="Influenza A virus"	
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	/serature="""	
	/second = "NS"	
	/segment NS	
DACE COUNT	262 - 169 - 200 - 104 +	
BASE COUNT	262 a 166 C 200 g 194 L	
ORIGIN 1 stanstta		
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61 cgatttgc	ag accaagaact gggtgatgee ceatteettg accggetteg eegagateag	
121 aagteeta	a gaggaagagg caacactett ggtetggaea tegaaacage taetegegea	
181 ggaaagca	ja tagtggagcg gattetggag gaggagtetg ataaggeaet taaaatgeeg	
241 gcttcacg	et acctaactga catgactete gaagaaatgt caagggaetg gtteatgete	
301 atgcccaa	yc agaaagtggc aggttccctt tgcatcaaaa tggaccaggc aataatggat	
361 aaagtcato	ca tattgaaagc aaacttcagt gtgatttttg accggttgga aaccctaata	
421 ctacttaga	ag ctttcacaga agaaggagca atcgtgggag aaatctcacc attaccttct	
481 cttccagga	ac atactggtga ggatgtcaaa aatgcaattg gcgtcctcat cggaggactt	
541 gaatggaat	tg ataacacagt tcgagtcact gaaactatac agagattcgc ttggagaagc	
601 agtgatga	gg atgggagact tccactccct ccaaatcaga aacggaaaat ggcgagaaca	
661 attgagtca	ag aagtttgaag aaataaggtg gctgattgaa gaagtaagac ataqattgaa	
721 aattacaga	aa aacagcttcg aacagataac gtttatgcaa gccttacaac tactgcttga	
781 agtggagga	aa gagataagag cettetegtt teagettatt taat//	
5-50-50		

## Nucleotide sequences of A/chicken/Chiang Rai/Thailand/CU-346/07

## CU-346-HA: Hemagglutinin gene (HA)

DEFINITIO	ON Influenza A virus strain A/chicken/Chiang
	Rai/Thailand/CU-346/07(H5N1).
ACCESSION	Ν
VERSION	
KEYWORDS	
SOURCE	Influenza A virus
ORGANIS	SM Influenza A virus
	Unclassified.
REFERENCE	E 1 (bases 1 to 754)
AUTHORS	S Lapkuntod, J., Chuatakul, C., Tantilertcharoen, R.,
	Poovorawan, Y. and Amonsin, A.
TITLE	Monitoring of influenza A virus from avian species in
	border areas of Thailand
JOURNAI	L Unpublished
REFERENCE	E 2 (bases 1 to 754)
AUTHORS	S Lapkuntod,J., Chuatakul,C., Tantilertcharoen,R.,
	Poovorawan, Y. and Amonsin, A.
TITLE	Direct Submission
JOURNAI	L Sub <mark>mitte</mark> d (23-DEC-2008) Chulalongkorn University,
	Department of Veterinary Public Health, Rama 4 Rd.
	Pathumwan, Bangkok 10330,Thailand
FEATURES	Location/Qualifiers
source	1754
	/strain="A/chicken/Chiang Rai/Thailand/CU-346/0/(H5N1)" /serotype="H5N1" /segment="HA" /country="Thailand"
BASE COUN	NT 276 a 138 c 175 g 165 t
ORIGIN	
1 agaaat	tgtgg tatggettgt caaaaagaac agtacatace caacaattaa gaggageta
61 aataat	tacca accaagaaga tettttggta etgtggggga tteaceatee taatgatge
121 gcagac	gcaga caaagctcta tcaaaaccca accacctata tttctgttgg gacatcaac
181 ctaaac	caga gattggtacc aagaatagct actagatcca aagtaaacgg gcaaagtgg
241 aggato	ggagt tettttggac aattttaaaa eegaatgatg caateaaett tgagagtaa
301 ggaaat	ttca ttgctccaga atatgcatac aaaattgtca agaaagggga ctcaacaat
361 atgaaa	aagtg aattggaata tggtaactgc aacaccaagt gtcaaactcc aatggggggc
421 ataaac	tota gtatgocatt ccacaatata caccototca ccatogggga atgococaa
481 tatgto	jaaat caaatagatt agteettgeg actgggetea gaaatageee teaaagagag
541 agaaga	aagaa aaaagagagg attatttgga gctatagcag gttttataga gggaggatg
601 caggga	aatgg tagatggttg gtatgggtac caccatagca atgagcaggg gagtgggta
661 gctgca	agaca aagaatccac tcaaaaggca atagatggag tcaccaataa ggtcaactc
721 ataatt	:gaca aaatgaacac tcagtttgag gccg//

## CU-346-NP: Nucleoprotein gene (NP)

L	OCUS	CU-346-NP	554 bp	DNA l	inear	23-DEC-2008
D	EFINITION	Influenza A	virus stra	in A/chick	en/Chiang	
		Rai/Thailand	d/CU-346/07	(H5N1).		
A	CCESSION					
V	ERSION					
K	EYWORDS	0				
S	OURCE	Influenza A	virus			
	ORGANISM	Influenza A	virus			
		Unclassified	d.			
R	EFERENCE	1 (bases 1	to 554)			
	AUTHORS 📃	Lapkuntod, J	., Chuataku	l <mark>,C.,</mark> Tant	ilertcharoen	,R.,
P	oovorawan <mark>,</mark>	and .				
		Amonsin, A.				
	TITLE	Monitoring of	of influenz	a A virus	from avian s	pecies in
		border area	s of Thaila	nd		
	JOURNAL	Unpublished				
R	eferenc <mark>e</mark>	2 (bases 1	to 554)			
	AUTHORS	Lapkuntod,J	., Chuataku	l,C., Tant	ilertcharoen	,R.,
		Poovorawan,	Y. and Amon	sin,A.		
	TITLE	Direct Subma	ission			
	JOURNAL	Submitted (2	23-DEC-2008	) Chulalon	gkorn Univer	sity,
		Department (	of Veterina	ry Public	Health, Rama	a 4 Rd.
		Pathumwan, 1	Bangkok 103	30, Thaila	nd	
F	EATURES	Location/Qua	alifiers			
S	ource	1554				
		/organism="	Influenza A	virus"		
		/mol_type="c	genomic DNA	"		
		/strain="A/o	chicken/Chi	ang Rai/Th	ailand/CU-34	6/07(H5N1)"
		/serotype="l	H5N1"			
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В	ASE COUNT	175 a	108 c	163 g 1	08 t	
0	RIGIN					
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61	aatgctactg	agatcagggc	atctgttgga	agaatggtta	a gtggcattgg	gaggttctac
121	atacagatgt	gcacagaact	caaactcagt	gactatgaag	ggaggctgat	ccagaacagc
181	ataacaatag	agagaatggt	actctctgca	tttgatgaaa	gaaggaacag	atacctggaa
241	gaacacccca	gtgcgggaaa	ggacccgaag	aagactggag	gtccaattta	tcggaggaga
301	gacgggaaat	gggtgagaga	actaattctg	tacgacaaag	aggagatcag	gaggatttgg
361	cgtcaagcga	acaatggaga	ggacgcaact	gctggtctta	cccacctgat	gatatggcat
421	tccaatctaa	atgatgccac	atatcagaga	acgagagete	tcgtgcgtac	tggaatggac
481	ccaaggatgt	gctctctgat	gcaagggtca	actctcccta	ggagatctgg	agctgccggt
541	gcagcagtaa	aggg//				

จุฬาลงกรณ์มหาวิทยาลัย

### CU-346-NA: Neuraminidase gene (NA)

```
CU-346-NA 1380 bp
                                                           23-DEC-2008
  LOCUS
                                     DNA
                                              linear
  DEFINITION Influenza A virus strain A/chicken/Chiang
              Rai/Thailand/CU-346/07(H5N1).
  ACCESSIONVERSION
  KEYWORDS
              .
  SOURCE
              Influenza A virus
    ORGANISM Influenza A virus
              Unclassified.
  REFERENCE
             1 (bases 1 to 1380)
    AUTHORS Lapkuntod, J., Chuatakul, C., Tantilertcharoen, R.,
             Poovorawan, Y. and Amonsin, A.
              Monitoring of influenza A virus from avian species in
    TTTLE
              border areas of Thailand
    JOURNAL
              Unpublished
  REFERENCE 2 (bases 1 to 1380)
    AUTHORS
             Lapkuntod, J., Chuatakul, C., Tantilertcharoen, R.,
             Poovorawan, Y. and Amonsin, A.
    TITLE
             Direct Submission
    JOURNAL Submitted (23-DEC-2008) Chulalongkorn University,
             Department of Veterinary Public Health, Rama 4 Rd.
              Pathumwan, Bangkok 10330, Thailand
  FEATURES
              Location/Qualifiers
              1..1380
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              /mol type="genomic DNA"
              /strain="A/chicken/Chiang Rai/Thailand/CU-346/07(H5N1)"
              /serotype="H5N1"
              /segment="NA"
              /country="Thailand"
  BASE COUNT
                                              378 t
                  410 a 249 c
                                    343 g
  ORIGIN
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  61 agettaatgt tacaaattgg gaacttgate teaatatggg teagteatte aatteacaea
 121 ggaaatcaac acaaagctga accaatcagc aatactaatt ttcttattga gaaagctgtg
 181 gcttcagtaa aattagcagg caattcatct ctttgcccca ttaatggatg ggctgtatac
 241 agtaaggaca acagtataag gatcggttcc aaggggggatg tgtttgttat aagagagcca
 301 ttcatctcat gctcccactt ggaatgcaga actttctttt tgactcaggg agccttgctg
 361 aatgacaagc actccaatgg gactgtcaaa gacagaagcc ctcacagaac attaatgagt
 421 tgtcctgtgg gtgaggctcc ctccccatat aactcaaggt ttgagtctgt tgcttggtca
 481 gcaagtgctt gccatgatgg caccagttgg ttgacaattg gaatttctgg cccagacagt
 541 ggggctgtgg ctgtattgaa atacaatggc ataataacag acactatcaa gagttggagg
 601 aataacatac tgagaactca agagtctgaa tgtgcatgtg taaatggctc ttgctttact
 661 gtaatgactg acggaccaag taatggtcag gcatcacata agatcttcaa aatggaaaaa
 721 gggaaagtgg ttaaatcagt cgaattggat gctcctaatt atcactatga ggaatgctcc
 781 tgttatcctg atgccggcga aatcacatgt gtgtgcaggg ataattggca tggctcaaat
 841 cggccatggg tatctttcaa tcaaaatttg gagtatcaaa taggatatat atgcagtgga
 901 gttttcggag acaatccacg ccccaatgat ggaacaggta gttgtggtcc ggtgtcctct
961 aacqqqqcat atqqqqtaaa aqqqttttca tttaaatacq qcaatqqtqt ctqqatcqqq
1021 agaacaaaaa gcactaattc caggagcggc tttgaaatga tttgggatcc aaatgggtgg
1081 actgaaacgg acagtagctt ttcagtgaaa caagatatcg tagcaataac tgattggtca
1141 ggatatagcg ggagttttgt ccagcatcca gaactgacag gactagattg cataagacct
1201 tgtttctggg ttgagttgat cagagggcgg cccaaagaga gcacaatttg gactagtggg
1261 agcagcatat ctttttgtgg tgtaaatagt gacactgtgg gttggtcttg gccagacggt
1321 gctgagttgc cattcaccat tgacaagtag tttgttcaaa aaactccttt gtttctacta
```

CU-3	47-pb2:	Polymerase 1	oasic 2 gene	(PB2)			
LOCU	S	CU-347-PB2	2281 bp	DNA li	near	17-DEC-2008	
DEFI	NITION	Influenza A Khan/Thaila	virus strain nd/CU <mark>-</mark> 347/08	A/chicken/: (H5N1).	Prachuap Kh	iri	
ACCE	SSION						
VERS	ION						
KEYW	ORDS						
SOUR	CE	Influe <mark>nza</mark> A	virus				
OR	GANISM	Influenza A Unclassifie	virus				
REFE	RENCE	1 (bases 1	to 2281)				
AIJ	THORS	Lapkuntod, J	. Chuatakul.	C., Tantile	rt.charoen.R		
		Poovorawan,	. and Amonsin	n,A.		- /	
TI	TLE	Monitoring	of influenza	A virus from	n avian spe	cies in border	2
		areas of Tha	iland		and the second sec		
JO	URNAL	Unpublished					
REFE	RENCE	2 (bases 1	to 2281)				
AU	THORS	Lapkuntod, J	. Chuatakul.	C., Tantile	rt.charoen,R		
		Poovorawan.	and Amonsi	n.A.		- /	
TI	TLE	Direct Subm	ssion	,			
JO	URNAL	Submitted (	7-DEC-2008)	Chulalongko:	rn Universi	tv, Bangkok,	
		1Department	of Veterinar	v Public He	alth, Rama	4 Rd.	
		Pathumwan, H	angkok 10330	, Thailand			
FEAT	URES	Lo	ation/Oualif	iers			
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		/mol type="	renomic DNA"				
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61	acaaaaaa	cca ctgtggac	ca tatggccata	atcaagaaat	acacatcagg	aagacaagag	
121	aagaacco	ctg ctctcaga	at gaaatggatg	atggcaatga	aatatccaat	cacagcggac	
181	aagagaat	taa tagagatga	at tcctgaaagg	aatgaacaag	ggcagacgct	ctggagcaag	
241	acaaatga	atg ctggatcg	ja cagggtgatg	gtgtctcccc	tagctgtaac	ttggtggaat	
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361	aaggttga	aaa ggttaaaa	a tggaaccttc	ggtcccgttc	atttccgaaa	ccaagttaaa	
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481	gatgtcat	ca tggaggtc	gt tttcccaaat	gaagtgggag	ctagaatatt	gacatcagag	
541	tcgcaatt	tga caataacga	a agagaagaaa	gaagagctcc	aagattgtaa	gattgctccc	
601	ttaatggt	tg catacatg	t ggaaagggaa	ctggtccgca	aaaccagatt	cctaccggta	
661	gcaggcgg	gaa caagcagto	gt gtacattgag	gtattgcatt	tgactcaagg	gacctgctgg	
721	gaacagat	gt acactcca	yg cggagaagtg	agaaatgacg	atgttgacca	gagtttgatc	
781	atcgctgd	cca gaaacatto	gt taggagagca	acggtatcag	cggatccact	ggcatcactg	
841	ctggagat	gt gtcacage	ac acaaattggt	gggataagga	tggtggacat	ccttaggcaa	
901	aatccaad	ctg aggaacaa	gc tgtggatata	tgcaaagcag	caatgggtct	gaggatcagt	
961	tcttcctt	ta gctttgga	gg cttcactttc	aaaagaacaa	gtggatcatc	cgtcaagaag	

## Nucleotide sequences of A/chicken/Prachuap Khiri Khan/Thailand/CU-347/08

1021	gaagaggaag	tgcttacagg	caacctccaa	acattgaaaa	taagagtaca	tgagggatat
1081	gaggaattca	caatggttgg	gcggagggca	acagctatcc	tgaggaaagc	aactagaagg
1141	ctgattcagt	tgatagtaag	tggaagagac	gaacaatcaa	tcgctgaggc	aatcattgta
1201	gcaatggtgt	tctcacagga	ggattgcatg	ataaaggcag	tccgaggcga	tctgaatttc
1261	gtaaacagag	caaaccaaag	attaaacccc	atgcatcaac	tcctgagaca	ttttcaaaag
1321	gatgcaaaag	tgctatttca	gaattgggga	attgaaccca	ttgataatgt	catggggatg
1381	atcggaatat	tacctgacat	gactcccagc	acagagatgt	cactgagagg	agtaagagtt
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1501	ttcttaaggg	ttcgagatca	gcgggggaac	gtactcttat	ctcccgaaga	ggtcagcgaa
1561	acccagggaa	cagagaaatt	gacaataaca	tattcatcat	caatgatgtg	ggaaatcaac
1621	ggtcctgagt	cagtgcttgt	taacacttat	cagtggatca	tcagaaactg	ggagactgtg
1681	aagattcaat	ggtctcaaga	ccccacgatg	ctgtacaata	agatggagtt	tgaaccgttc
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1981	gcaaccaaaa	ggcttaccgt	tcttggaaag	gacgcaggtg	cattaacaga	ggatccagat
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2101	gacaaaaggt	atggaccagc	attgagcatc	aatgaactga	gcaatcttgc	gaagggggag
2161	aaagcta <mark>at</mark> g	tgctgatagg	gcaaggagac	gtggtgttgg	taatgaaacg	aaaacgggac
2221	tctagcatac	ttactgacag	ccagacagcg	accaaaagaa	ttcggatggc	catcaattag
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CU-347-PE	31: Poly	ymerase bas:	ic 1 gene (1	PB1)		
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	/mol	l_type="gene	omic DNA"			
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61 acat	tccctt	atactogaga	ccctccatac	agccatggaa	cagggacagg	atacaccato
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181 acto	qaqcac	cccaactcaa	cccgattgat	qqaccactqc	ctgaggataa	tgagcccagt
- 241 gggt	atgcac	aaacagattg	tgtattggaa	gcaatggctt	tccttgaaga	atcccaccca
301 ggga	atctttg	aaaactcgtg	tctagaaaca	atggaaattg	ttcaacaaac	aagagtggat
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541 ataa	acaacac	atttccagag	aaagagaagg	gtgagggaca	acatgaccaa	aaaaatggtc
601 acad	caaagaa	caatagggaa	gaaaaaacaa	aggctgaaca	aaaagagcta	cctgataaga
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961 atga	ataacgt	acatcacaag	gaaccagcca	gaatggtttc	ggaatgtctt	aagcattgct
1021 ccta	ataatgt	tctcaaacaa	gatggcgaga	ctaggaaaag	gatacatgtt	cgaaagtaag
1081 agca	atgaagt	tacgaacaca	aataccagca	gaaatgcttg	caaacattga	tcttaaatac
1141 ttca	atgaat	taacgaaaaa	gaaaattgag	aaaataaggc	ctctattaat	agatggtaca

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1261	gtttcaatcc	tgaatcttgg	acagaaaagg	tacaccaaaa	ccacatattg	gtgggacgga
1321	ctccaatgct	ctgatgattt	cgctctcatc	gtaaatgcac	cgaatcatga	gggaatacaa
1381	gcaggagtgg	ataggtttta	taggacttgt	aaactagttg	gaatcaatat	gagcaagaag
1441	aagtcttaca	taaatcggac	agggacattt	gaattcacga	gctttttcta	ccgctatgga
1501	tttgtagcca	atttcagtat	ggagctgccc	agttttggag	tgtctggaat	taatgaatcg
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1621	ccagcaacag	ctcagatggc	tcttcagtta	ttcatcaagg	actacagata	cacataccga
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1741	gagcaaaccc	gttcaaaggc	aggactgttg	gtttcagatg	gaggaccaaa	tctatacaat
1801	atccgaaatc	tccatattcc	tgaagtctgc	ttaaaatggg	aattgatgga	tgaagattac
1861	cagggcagac	tgtgtaatcc	tctgaatcca	tt <mark>cgtcagcc</mark>	ataaggaaat	tgaatctgtc
1921	aacaatgctg	tagtaatgcc	agctcatggc	cc <mark>ggccaaga</mark>	gtatggaata	tgatgccgtt
1981	gcaactacac	attcatggat	tcctaaaagg	aaccgttcca	ttctcaatac	gagtcaaagg
2041	ggaattcttg	aggatgaaca	gatgtaccag	aagtgctgca	atttattcga	gaaattcttc
2101	cccagcagtt	catatcggag	gccagttgga	atttccagca	tggtggaggc	catggtgtct
2161	agggcccg <mark>aa</mark>	ttgacgcacg	aattgacttc	gagtctggaa	ggattaagaa	agaagagttt
2221	gctgagatca	tgaagatctg	ttccaccatt	gaagaactca	gacggcaaaa	atagtgaatt
2281	tagcttgtcc	ttc//				

CU-347-PA:	Polymerase	acidic	gene	(PA)

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ACCESSION VERSION KETWORDS SOURCE Influenza A virus ORGANISM Influenza A virus Unclassified. REFERENCE 1 (cases l to 2220) AUTHORS Lapkuncod, J., Chutakul, C., Tantilertcharoen, R., Foovorawan, Y. and Amonsin, A. TITLE Monitoring of influenza A virus from avian species in border areas of Thailand JOURNAL Uppblished REFERENCE 2 (bases l to 2220) AUTHORS Lapkuncod, J., Chutakul, C., Tantilertcharoen, R., Foovorawan, Y. and Amonsin, A. TITLE Jirect Submission JOURNAL Submitted (17-DSC-2008) Chulalongkorn University, Bangkok, Ibepartment of Veterinary Public Health, Rama 4 Rd. Pethuwan, Bangkok 10330, Thailand FEATURES Location/Qualifiers source 1.,2220 /organism="Influenza A virus" /mol_type="genomic DNA" /strain="%/chicken/Frachuag Khiri Khan/Thailand/CU-347/08(H5N1)" /ssegmet="PA" /country="Thailand" CDS 12220 /ordon_start=1 EASE COUNT 730 a 438 c 533 g 519 t ORIGIN 1 atgragagat tiggragas teogaateg caacqaaca agtitgeteg atatagcaca 181 ataatgag aatetggaga teogaateg tigaatagta accqaacgat 181 ataatgag aatetggaga teogaateg tigaatagta accqaacgat catagagat 181 ataatgag aatetggaga teogaateg atacagcaca agtitgeteg atatagcaca 181 ataatgag aatetggaga teogaateg atacagcaca agtitgeteg atatagcaca 181 ataatgagaa attggaga teogaateg atacagaata agaaccgata agtagcaatag 181 gagaagcac atattcacat attctcatta accgattga atatatgaa 181 gagaagcac dattgagag teogaateg atacagaata 181 gagaagcac dattgagag teogaateg atacagaata agaaccgata agtagcaaca 181 gagaagcac atttcacat attctcatta accgatgat catagagag 181 gagaagcac atttcacat attctcatta accgatgat catagagag 181 gagaagcac atttcacat attcacat attcacata accgategat 181 gagaagcac dattgagag atteggaga atteggaga atteggagag atteggagag atteggagag 181 gagaagcac tattgagag atteggaga atteggagag atteggagagag atteggagag atteggagagag atteggagag atteggagag atteggagag atteggagag	DEFINITION	Khan/Thailand/CU-347/08(H5N1).	
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<pre>UnclassIfied. DEFFERENCE 1 (bases 1 to 2220) AUTHORS Lapkuntod, J., Chuatakul, C., Tantilertcharoen, R., Poovorawan, Y. and Amonsin, A. TITLE Monitoring of influenza A virus from avian species in border areas of Thailand JOURNAL Unpublished REFERENCE 2 (bases 1 to 2220) AUTHORS Lapkuntod, J., Chuatakul, C., Tantilertcharoen, R., Poovorawan, Y. and Amonsin, A. TITLE Direct Submission JOURNAL Submitted (17-DEC-2008) Chulalongkorn University, Bangkok, 1Department of Veterinary Public Health, Rama 4 Rd. Pathumwan, Bangkok 10330, Thailand FEATURES Location/Qualifiers source 1.,2220 /organism="Influenza A virus" /mol_type="genomic DNA" /strain="A/chicken/Prachuap Khiri Khar/Thailand/CO-347/08(HSN1)" /serotype="HSN1" /country="Thailand" CDS 12220 /codon_start=1 EASE COUNT 730 a 438 c 533 g 519 t CMTGIM 1 atggaagact ttgtgogaca atgetteaat ccaatgattg tegagettge gaaaaggca 61 atgaaggatt ttgtgogaca atgetteaat ccaatgattg tegagettge dataggcacc 181 atattgtag aatetggaga tegaatgea ttattaaaca acegattga aatattgaa 241 ggaaggace gaaggatgge etggaatgtg ttattaacaa caegattga ataattgaa 241 ggaaggace gaaggatgge etggacagte ttattaaaca acegattga ataattgaa 241 ggaaggaca datteccea gagtetga tattaacaa cegagatga 61 ataattgtag aatetggaga tecgaatga tattaacaa acegattga ataattgaa 241 ggaaggaca gaaggatge etgaattgt tgatacaaa gaacegaat caetaggagt 341 gaadgaca dattaccaat attectda tatetggaa aagacegaat caetaggagt 341 gaadgaca dattaccaat attectda tatetggaa acegaate caetaggagt 341 gaadgaca dattaccaat attectgaa atagecaace agaecaace agaecaace 342 gaadggcaa gtaggget tatggdate tttegtaat cegaagaecaac 343 gaadgacaa datteceaat attectgaa acegaate caetaggag 344 gaatggcaa gtaggget tegaattga caetagaecaat cegaagaecaac 344 gaadgaca datteceaat attectgaace atagecaace tateggaag 344 gaatggcaa tategaaga caetagtaa acetagaag acegaate tagagaeca 344 gaaagaaca attegaagaa caetagtaa atagaecaate tagaggagaa 344 gaaaggaca tategaagaa caetagtaa tagaagaaa teceaaaa agaecgaate tagaggaga 344 gaaaggaca tatagaaga caetagtaa atagaecaat tteceaata</pre>	ORGANISM	Influenza A virus	
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<pre>Povorawan,Y. and Amonsin,A. TITLE Direct Submission JOURNAL Submitted (17-DEC-2008) Chulalongkorn University, Bangkok, IDepartment of Veterinary Public Health, Rama 4 Rd. Pathumwan,Bangkok 10330, Thailand FEATURES Location/Qualifiers source 12220 /organism="Influenza A virus" /mol_type="genomic DNA" /strain="A/chicken/Prachuap Khiri Khan/Thailand/CU-347/08 (H5N1)" /serotype="H5N1" /segment="PA" /codon_start=1 EASE COUNT 730 a 438 c 533 g 519 t ORIGIN 1 atggaagaat ttggggaaa atggttcaat ccaatgattg tcgacttcg ggaaaggca 61 atgaaagaat atggggaag tcggaatga tggaaagaac agttggtcg aatatggaa 21 actggaag tctgttcat gtattcggt ttattaaaac acgattga aatattgaa 21 gagaggacc gaaggatg ctggaatga tattaaaaca agtagga aatagtaca 131 atattgtag aattccca agattgt ggaaaga accgaagaac caaggagt 301 gagaaacca atttccc aggttga ttattaaaca acgattga caagaatc 41 gaaggaac attccca agattgt cattcgaa atgataga atagaaga 241 ggaaggaca tattcaat attccatt atattaaaca acgattga aataattgaa 241 ggaaggaca atttccca agattgta gaatagaa acgaagaa 241 ggaaggaca atttccca agattgta gaatagaa acgaagaa 241 ggaaggaca atttccca agattgaac tattaaaaca ggtgtaatca 361 atgaagaa attggaag acggacaa attaaacca ggtagaatca 361 atgaagaac atttccca agattga tataaacaa ggtagaac caagaggt 361 gaatggcaa gaaggdtt tattaaaca atgagga aatggcaa caagagaa 361 atgaagaa attgaaa aattgaa cacggacaa attaaacca ggtgtaca caagagag 361 atgaagaa atttgaaa cactggaac ataacatca tatctgaga atgagaca acgaagaga 361 atgaagaa attgaaa cactggaac ataacatca tatcgaagag agacgaac caaagagag 371 tgaatggca gaaggttc tcaaatta agaaggag atggagaa acgaagaga 371 tgaatggca gaaggtcc atattcaa aatttgaa cactgaaga acgaagagaa tgagagaa tgagagaa 371 tgaatggca gaacgattc caaagta acgaagaga accgaaggaa agacgaac agacgacga 371 tgaatggca gaaccata acaatga atgagagaa tccgaagaa accgaagaga agacgaac agaagacc 371 tgaatggca gaaccata acaatga agagagaa atccaaaga agagagaa accgaagaa tgagagaa 372 tgaatggaa actgaaga accaaatga aagagagaa acccaaaaa agaacatga 373 tgaatgcca tatacaatga aaccaatga aagagagaa acccaaaaa agaacaaga 374 tgaaaaaaaga gcaggtg</pre>	AUTHORS	Lapkuntod.J., Chuatakul.C., Tantilertcharoen.R.,	
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1141 tttgaggagt ggaaagtgt tagggagagta agggaggagata agggaggaga agggaggagatgt	1001 gtgctg	ay aactocaaya tattyaaaat yayyagaaaa toocaaaaac aaagaacatg	
TINT TO DADUACT OCAAADALOL TADCOACCIA ADACADIATO PREDIDEIDE PREMERENT	1141 +++ aaa	Laa yuuayuuyaa yuyyacacuc yyuyayaaca tggcaccaga gaaagtagac	

1201	agatcactag	caagctggat	tcagagtgaa	ttcaacaagg	catgtgaatt	gacagattcg
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1381	aagggagtgt	acataaacac	agccctgttg	aatgcatcct	gtgcagccat	ggatgacttt
1441	caactaattc	caatgataag	caaatgcaga	accaaagaag	gaagacggaa	aactaatctg
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1681	atgttcctgt	atgtaagaac	caatggaacc	tccaagatca	aaatgaaatg	gggcatggaa
1741	atgaggcgat	gccttcttca	atcccttcaa	caaattgaaa	gcatgattga	agccgagtct
1801	tctgtcaaag	agaaggacat	gaccaaagaa	ttctttgaaa	acaaatcaga	aacatggccg
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2101	ttgcttaatg	cgtcttggtt	caactccttc	ctcgcacatg	cactgaaata	gttgtggcaa
2161	tgctactatt	tgctatccat	actgtccaaa	aaagtacctt	gtttctacta	atacgagacg//

## CU-347-HA: Hemagglutinin gene (HA)

LOCUS		CU-34	47-HA	1716	bp	DNA	linear		17-DH	EC-2008	
DEFINIT	ION	Influ	lenza A	viru	is strai	in A/	/chicken/Pra	achuap	Khiri	L	
		Khan,	/Thailar	nd/CU	J-347/08	3 (H51	N1).				
ACCESSI	ON										
VERSION											
KEYWORD	S										
SOURCE		Influ	lenza A	viru	IS						
ORGAN	ISM	Influ	lenza A	viru	IS						
		Uncla	assified	1.							
REFEREN	CE	1 (k	bases 1	to 1	716)						
AUTHO	RS	Lapkı	intod,J.	, Ch	nuatakul	L,C.,	Tantilert	charoen	.,R.,		
		Poovc	rawan,Y	. an	d Amons	in,A					
TITLE		Monit	coring o	of ir	fluenza	аАт	virus from a	avian s	pecie	es in bord	er
		areas	of Tha	ilan	d						
JOURN	AL	Unpuk	olished								
REFEREN	CE	2 (1	bases 1	to 1	.716)						
AUTHO	RS	Lapkı	intod <mark>,</mark> J.	, Ch	nuatakul	l,C.,	, Tantilerto	charoer	,R.,		
		Poove	rawan,Y	. an	d Amons	in,A					
TITLE		Direc	ct Submi	ssic	n						
JOURN	AL	Subm:	itted (1	.7-DE	C-2008)	) Chu	ulalongkorn	Univer	sity,	Bangkok,	
		1Depa	artment	of V	Veterina	ary H	Public Heal	th, Ram	na 4 H	Rd.	
		Pathu	umwan, B	ang k	ok 1033	о, т	hailand				
FEATURES	S	Locat	cion/Qua	lifi	ers						
source		11	716								
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				011			410				
BASE CO	UN'I'		996 a	311	. C 3	397 9	g 412 t				
ORIGIN											_
1 a'	tggag	Jagaa	tagtgct	LCC	tttgca	aata	gtcagtcttg	ttaaaa	igtga	tcagatttg	C L
01 d	LLGGL	Lacc	algcaad	ICaa	CLCgaca	agag	caggilgaca	Cddldd	llgga	aaagaacgt	L
101 a	cigii ataaa	acac	algecea	laga		ggaa	aagacacaca	acggga	aget	clgcgalcl	d
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361 +-	tagee	aaty	+222002	,+++	taaaaa	yyat aatt	cagateatec	acyaay	atto	ttaatacet	a +
421 c	ataaa	ayaa	cattage	raat	ragetea		tatacataca	agggaaa	arto	ctccttttt	C
481 a	naaat	ataa	tatooct	tat	caaaaaa	raac	agtacatacc	caacaa	itaaa	gaggageta	c
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601 au	cagao	rcaga	caaaact	cta	tcaaaa	rcca	accacctata	tttccc	ittaa	racatcaac	9 a
661 c	taaac	rcaga	gattgat	acc	aagaata	arct	actagatoca	aagtaa	acaa	gacaactaa	a
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1081 c	aqqqa	ataa	tagatac	, jj	gtatga	gtac	caccatagca	atgagg	aqqq	qaqtqqqta	c
1141 a	ctaca	iqaca	aagaato	cac	tcaaaad	qca	atagatggag	tcacca	ataa	ggtcaactc	q
2		-	_		-	-					-

1201 atcattgaca aaatgaacac tcagtttgag gccgttggaa gggaatttaa caacttagaa 1261 aggagaatag agaatttaa caagaagatg gaagacgggt tcctagatgt ctggacttat 1321 aatgctgaac ttctggttct catggaaaat gagagaactc tagacttca tgactcaaat 1381 gtcaagaacc tttacgacaa ggtccgacta cagcttaggg ataatgcaaa ggagctgggt 1441 aacggttgtt tcgagttcta tcataaatgt gataatgaat gtatggaaag tgtaagaaac 1501 ggaacgtatg actacccgca gtattcagaa gaagcaagac taaaaagaga ggaaataagt 1561 ggagtaaaat tggaatcaat aggaatttac caaatactgt caatttattc tacagtggcg 1621 agttccctag cactggcaat catggtagct ggtctatcct tatggatgt ctccaatggg 1681 tctttacaat gcagaatttg catttaaatc aggagt//

## CU-347-NP: Nucleoprotein gene (NP)

ACCESSION VERSION KEYWORDS SOURCE Influenza A virus ORGANISM Influenza A virus Unclassified. REFERENCE 1 (bases 1 to 1522) AUTHORS Lapkuntod,J., Chuatakul,C., Tantilertcharoen,R., Poovorawan,Y. and TITLE Monitoring of influenza A virus from avian species in border areas of Thailand JOURNAL Unpublished REFERENCE 2 (bases 1 to 1522) AUTHORS Lapkuntod,J., Chuatakul,C., Tantilertcharoen,R., Poovorawan,Y. and Amonsin,A. TITLE Direct Submission
SOURCEInfluenza A virusORGANISMInfluenza A virusUnclassified.REFERENCE1 (bases 1 to 1522)AUTHORSLapkuntod, J., Chuatakul, C., Tantilertcharoen, R., Poovorawan, Y.and
<pre>REFERENCE 1 (bases 1 to 1522) AUTHORS Lapkuntod,J., Chuatakul,C., Tantilertcharoen,R., Poovorawan,Y. and Amonsin,A. TITLE Monitoring of influenza A virus from avian species in border areas of Thailand JOURNAL Unpublished REFERENCE 2 (bases 1 to 1522) AUTHORS Lapkuntod,J., Chuatakul,C., Tantilertcharoen,R., Poovorawan,Y. and Amonsin,A. TITLE Direct Submission</pre>
AUTHORS Lapkuntod, J., Chuatakul, C., Tantilertcharoen, R., Poovorawan, Y. and Amonsin, A. TITLE Monitoring of influenza A virus from avian species in border areas of Thailand JOURNAL Unpublished REFERENCE 2 (bases 1 to 1522) AUTHORS Lapkuntod, J., Chuatakul, C., Tantilertcharoen, R., Poovorawan, Y. and Amonsin, A. TITLE Direct Submission
and Amonsin,A. TITLE Monitoring of influenza A virus from avian species in border areas of Thailand JOURNAL Unpublished REFERENCE 2 (bases 1 to 1522) AUTHORS Lapkuntod,J., Chuatakul,C., Tantilertcharoen,R., Poovorawan,Y. and Amonsin,A. TITLE Direct Submission
Amonsin, A. TITLE Monitoring of influenza A virus from avian species in border areas of Thailand JOURNAL Unpublished REFERENCE 2 (bases 1 to 1522) AUTHORS Lapkuntod, J., Chuatakul, C., Tantilertcharoen, R., Poovorawan, Y. and Amonsin, A. TITLE Direct Submission
TITLEMonitoring of influenza A virus from avian species in border areas of ThailandJOURNALUnpublishedREFERENCE2 (bases 1 to 1522)AUTHORSLapkuntod, J., Chuatakul, C., Tantilertcharoen, R., Poovorawan, Y. and Amonsin, A.TITLEDirect Submission
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AUTHORS Lapkuntod, J., Chuatakul, C., Tantilertcharoen, R., Poovorawan, Y. and Amonsin, A. TITLE Direct Submission
Poovorawan, Y. and Amonsin, A. TITLE Direct Submission
TITLE Direct Submission
JOURNAL Submitted (17-DEC-2008) Chulalongkorn University, Bangkok,
1Department of Veterinary Public Health, Rama 4 Rd.
Pat <mark>humwan</mark> , B <mark>a</mark> ngkok 10330, Thailand
FEATURES Lo <mark>cation/Qualifiers</mark>
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1141 ctgagaagca gatattgggc tataagaacc agaagcggag gaaacaccaa ccagcagagg 1201 gcatctgcag gacagatcag cgttcagccc actttctcgg tacagagaaa ccttccttc 1261 gaaagagcga ccattatggc agcattaca ggaaatactg agggcagaac gtctgacatg 1321 aggactgaaa tcataagaat gatggaaagt gccagaccag aagatgtgtc attccagggg 1381 cggggagtct tcgagctctc ggacgaaag gcaacgaacc cgatcgtgcc ttcctttgac 1441 atgaataatg aaggatctta tttcttcgga gacaatgcag aggagtatga caattaaaga 1501 aaaatacct tgtttctact at //
## CU-347-NA: Neuraminidase gene (NA)

LOCUS 2008	CU-	347-NA	1	381 bp	DI	NA line	ear 17-DEC-			
DEFINITION	Influenza A virus strain A/chicken/Prachuap Khiri Khan/Thailand/CU-347/08(H5N1).									
ACCESSION VERSION										
KEYWORDS	•									
SOURCE	Inf	luenza A vi:	rus							
ORGANISM	Inf	luenza A vi:	rus							
	Unc	lassified.								
REFERENCE	1	(bases 1 to	1381)							
AUTHORS	Lap	kuntod,J.,	Chuatakul,C	., Tan	tiler	ccharoen,R.	,			
	Poov	vorawan,Y. a	andAmonsin,	A.						
TITLE	Mon	Monitoring of influenza A virus from avian species in border								
	area	areas of Thailand								
JOURNAL	Unpublished									
REFERENCE	2 (bases 1 to 1381)									
AUTHORS	Lapl Poov	Lapkuntod, J., Chuatakul, C., Tantilertcharoen, R., Poovorawan, Y. and Amonsin, A.								
TITLE	Direct Submission									
JOURNAL	Subr	mitted (17-1	DEC-2008) C	hulalo	ngkorı	n Universit	zy, Bangkok,			
	1Dep	1Department of Veterinary Public Health, Rama 4 Rd.								
	Path	umwan, Bang	jkok 10330,	Thaila	and					
FEATURES	Loca	ati <mark>on/Quali</mark> :	fiers							
source	1	1381								
	/or	g <mark>anism=</mark> "Infi	luenza A vi	rus"						
	/mol	l_type="geno	omic DNA"							
	/st:	rain="A/chi	cken/Prachu	ap Khi	ri					
	Khai	n/Thailand/	CU-347/08(H	5N1)"						
	/se:	rotype="H5N	1"							
	/seg	gment="NA"								
000	/ COI	untry="Thai.	land"							
CDS 11381										
	/ 000	don_start=1								
BASE COUNT		409 2 2	51 c 346	C	375 +					
ORIGIN		105 a 2.	51 C 510	y	575 0					
1 atraat	ccaa	ataagaagat	aataaccatc	ggatc	aatct	gtatggtaac	tagaataact			
61 agetta	atat	tacaaattoo	gaacttgatc	tcaat	ataaa	tcagtcatto	: aattcacaca			
121 gggaat	caac	acaaagetga	accaatcage	aatac	taatt	ttcttactga	gaaagetgtg			
181 gcttca	ataa	aattagcggg	caattcatct	cttta	cccca	ttaatggatg	ggctgtatac			
241 agtaag	gaca	acagtataag	gatcggttcc	aaddd	ggatg	tattattat	aagagagcca			
301 ttcatc	tcat	gctcccactt	ggaatgcaga	acttt	ctttt	tgactcaggo	agcettgetg			
361 aatgac	aaqc	actccaatqq	gactgtcaaa	gacag	aaqcc	ctcacagaac	attaatgagt			
421 tgtcct	ataa	gtgaggctcc	ctccccatat	aactc	aaqqt	ttgagtctgt	tgcttggtca			
481 gcaagt	qctt	gccatgatgg	caccagttgg	ttgac	aattq	qaatttctqc	cccagacagt			
541 ggggct	qtqq	ctgtattgaa	atacaatqqc	ataat	aacaq	acactatcaa	gagttggagg			
601 aataac	atac	tgagaactca	agagtetgaa	tgtgc	atgtg	taaatggcto	: ttgctttact			
661 gtaatg	actg	acggaccaag	taatggtcag	gcatc	acata	agatetteaa	aatggaaaaa			
721 gggaaa	gtgg	ttaaatcagt	cgaattggat	gctcc	taatt	atcactatga	ggaatgctcc			
781 tgttat	cctg	atgccggcga	aatcacatgt	gtgtg	caggg	ataattggca	tggctcaaat			
841 cggcca	tggg	tatctttcaa	tcaaaatttg	gagta	tcaaa	taggatatat	atgcagtgga			
901 gttttc	ggag	acaatccacg	ccccaatgat	ggaac	aggta	gttgtggtco	ggtgtcctct			
961 aacggg	gcat	atggggtaaa	agggttttca	tttaa	atacg	gcaatggtgt	ctggatcggg			
1021 agaaca	aaaa	gcactaattc	caggagcggc	tttga	aatga	tttgggatco	aaatgggtgg			
1081 actgaa	acgg	acagtagctt	ttcagtgaaa	caaga	tatcg	tagcaataac	: tgattggtca			

1141 ggatatagcg ggagttttgt ccagcatcca gaactgacag gactagattg cataagacct 1201 tgtttctggg ttgagttgat cagagggcgg cccaaagaga gcacaatttg gactagtggg 1261 agcagcatat ctttttgtgg tgtaaatagt gacactgtgg gttggtcttg gccagacggt 1321 gctgagttgc cattcaccat tgacaagtag tttgttcaaa aaaactcctt gtttctacta 1381 g //

# ศูนย์วิทยทรัพยากร จุฬาลงกรณ์มหาวิทยาลัย

# CU-347-M: Matrix gene (MA)

LOCUS DEFINITION	CU-347-M 984 bp DNA linear 17-DEC-2008 Influenza A virus strain A/chicken/Prachuap Khiri							
	Khan/Thailand/CU-347/08(H5N1).							
KEYWORDS	·							
SOURCE	Influenza A virus							
ORGANISM	Unal agai fi ad							
DEFEDENCE								
AUTHORS	Lankuntod.I. Chuatakul.C. Tantilertcharoen R							
AUTIONS	Poovorawan V and Amonsin A							
TTTLE	Monitoring of influenza A virus from avian species in border							
	areas of Thailand							
JOURNAL	Unpublished							
REFERENCE	2 (bases 1 to 984)							
AUTHORS	Lapkuntod, J., Chuatakul, C., Tantilertcharoen, R.,							
	Poovorawan, Y. and Amonsin, A.							
TITLE	Direct Submission							
JOURNAL	Submitted (17-DEC-2008) Chulalongkorn University, Bangkok,							
	1Department of Veterinary Public Health, Rama 4 Rd.							
	Pathumwan, Bangkok 10330, Thailand							
FEATURES	Location/Qualifiers							
source	1984							
	/or <mark>g</mark> anis <mark>m="In</mark> fluenza A virus"							
	/m <mark>ol_typ</mark> e="genomic DNA"							
	/strai <mark>n</mark> ="A <mark>/chicken/Prachuap</mark> Khiri							
	Khan/Thailand/CU-347/08(H5N1)"							
	/serotyp <mark>e="H5N1"</mark>							
	/segment="M"							
	/country="Thailand"							
CDS	1984							
	/codon_start=1							
BASE COUNT	280 a 208 c 263 g 233 t							
URIGIN								
L algag								
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181 gatti	tatat tracactoar cataccoart gaggaggar tacaggatag acgettate							
241 cagaat	tacco taaatagaaa tagagatoca aataatatag atagagoagt taagotatat							
301 aagaa	rctga aaagagaaat aacatteeat gggggteed accategg acggggege caggeedede							
361 accord	tocac ttoccaotto catogotete atatacaaca goatogoaac gotoactaco							
421 gaagto	ggett ttgggeetagt gtgtgeeact tgtgageaga ttgeagatte acageategg							
481 tctcad	cagac agatggcaac tatcaccaac ccactaatca gacatgagaa cagaatggtg							
541 ctggc	cagca ctacagctaa ggctatggag cagatggcag gatcaagtga gcaggcagcg							
601 gaage	catgg agatcgctaa tcaggctagg cagatggtgc aggcaatgag gacaattggg							
661 actca	teeta aetetagtge tggtetgaga gataatette ttgaaaattt geaggeetae							
721 cagaaa	acgaa tgggagtgca gatgcagcga ttcaagtgat cctattgttg ttgccgcaaa							
781 tatca	ttggg atcttgcact tgatattgtg gattcttgat cgtcttttct tcaaatgcat							
841 ttatco	gtcgc cttaaatacg gtttgaaaag agggcctgct acggcagggg tacctgagtc							
901 tatgad	gggaa gagtaccggc aggaacagca gagtgctgtg gatgttgacg atggtcattt							
961 tgtcaa	acata gaattggagt aaaa//							

## CU-347-NS: Nonstructural gene (NS)

LOCUS DEFINITION	CU-347-NS 853 bp DNA linear 17-DEC-2008 Influenza A virus strain A/chicken/Prachuap Khiri Khan/Thailand/CU-347/08(H5N1).							
ACCESSION VERSION								
KEYWORDS								
SOURCE	Influenza A virus							
ORGANISM	Influenza A virus							
REFERENCE	1  (bases 1 to 853)							
AUTHORS	Lapkuntod, J., Chuatakul, C., Tantilertcharoen, R.,							
110 1110110	Poovorawan, Y. and Amonsin, A.							
TITLE	Monitoring of influenza A virus from avian species in border							
	areas of Thailand							
JOURNAL	Unpublished							
REFERENCE	2 (bases 1 to 853)							
AUTHORS	Lapkuntod,J., Chuatakul,C., Tantilertcharoen,R.,							
	Poovorawan, Y. and Amonsin, A.							
TITLE	Direct Submission							
JOURNAL	AL Submitted (17-DEC-2008) Chulalongkorn University, Bangkok,							
	Department of Veterinary Public Health, Rama 4 Rd.							
FFATIRES	Pathumwan, Bangkok 10330, Thailand							
source								
Douroo	/organism="Influenza A virus"							
	/mol type="genomic DNA"							
	/strain="A/chicken/Prachuap Khiri							
	Khan/Th <mark>ai</mark> land/CU-347/08(H5N1)"							
	/serotype="H5N1"							
	/segment="NS"							
	/country="Thailand"							
CDS	1853							
	/codon_start=1							
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ORIGIN	275 a 175 c 204 g 201 c							
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61 cgattte	gcag accaagaact gggtgatgcc ccatteettg accggetteg cegagateag							
121 aagtcc	ctaa qaqqaaqaqq caacactett qqtetqqaca teqaaacaqe taeteqeqea							
181 ggaaag	caga tagtggagcg gattctggag gaggagtctg ataaggcact taaaatgccg							
241 gcttca	cgct acctaactga catgactctc gaagaaatgt caagggactg gttcatgctc							
301 atgccca	aagc agaaagtggc aggttccctt tgcatcaaaa tggaccaggc aataatggat							
361 aaagtc	atca tattgaaagc aaacttcagt gtgatttttg accggttgga aaccctaata							
421 ctactta	agag ctttcacaga agaaggagca atcgtgggag aaatctcacc attaccttct							
481 cttcca	ggac atactggtga ggatgtcaaa aatgcaattg gcgtcctcat cggaggactt							
541 gaatgg	aatg ataacacagt tcgagtcact gaaactatac agagattcgc ttggagaagc							
661 attace	yayy alyyyagact tocactocot coaaatoaga aacggaaaaat ggogagaaca							
721 asttac	icay aayiiiyaay aaalaayyiy yiiyaliyad yadyidayat alayaliyad							
781 actors	agaa gagataagag oottotogtt toagottatt taatgataaa aaacacootg							
841 ggtttc	tacc taa//							

# BIOGRAPHY

Mr. Jiradej Lapkuntod was born on November 13, 1983 in Bangkok, Thailand. He graduated from the Faculty of Veterinary Science, Chulalongkorn University, Thailand in 2006. After that, he enrolled the Master degree of Science in the Department of Veterinary Public Health, Faculty of Veterinary Science, Chulalongkorn University since academic year 2009.

