

Short Communication

Study on molecular characterization of avian influenza H5N1 viruses isolated from Sukhothai and Uthai Thani provinces of Thailand in 2008

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In 2004, outbreak of highly pathogenic avian influenza (HPAI) subtype H5N1 virus was firstly reported both in poultry and humans in Thailand which caused dramatic economic losses to poultry industry and continued to pose a serious threat to public health (Tiensin *et al.*, 2005). Previously, 6 major waves of HPAI H5N1 virus outbreaks have occurred in poultry (Nuansrichay *et al.*, 2008). The first wave was reported in January-May 2004, the second in July 2004-April 2005, the third in July-November 2005, the fourth in January-November 2006, the fifth in January-June 2007 and the sixth in January-April 2008. Recently, the seventh wave occurred in Sukhothai and Uthai Thani provinces in early November 2008. In this literature, we sequenced all 8 gene segments of 2 HPAI H5N1 viruses isolated from the outbreaks in Sukhothai and Uthai Thani provinces in the lower northern part of Thailand and submitted to Genbank as follows: A/chicken/Sukhothai/NIAH114843/2008 (H5N1) (FJ455874, and FJ750820-FJ750826) from Sukhothai province and A/chicken/Uthai Thani/NIAH115067/2008 (H5N1) (FJ743696, and FJ750813-750819) from Uthai Thani province.

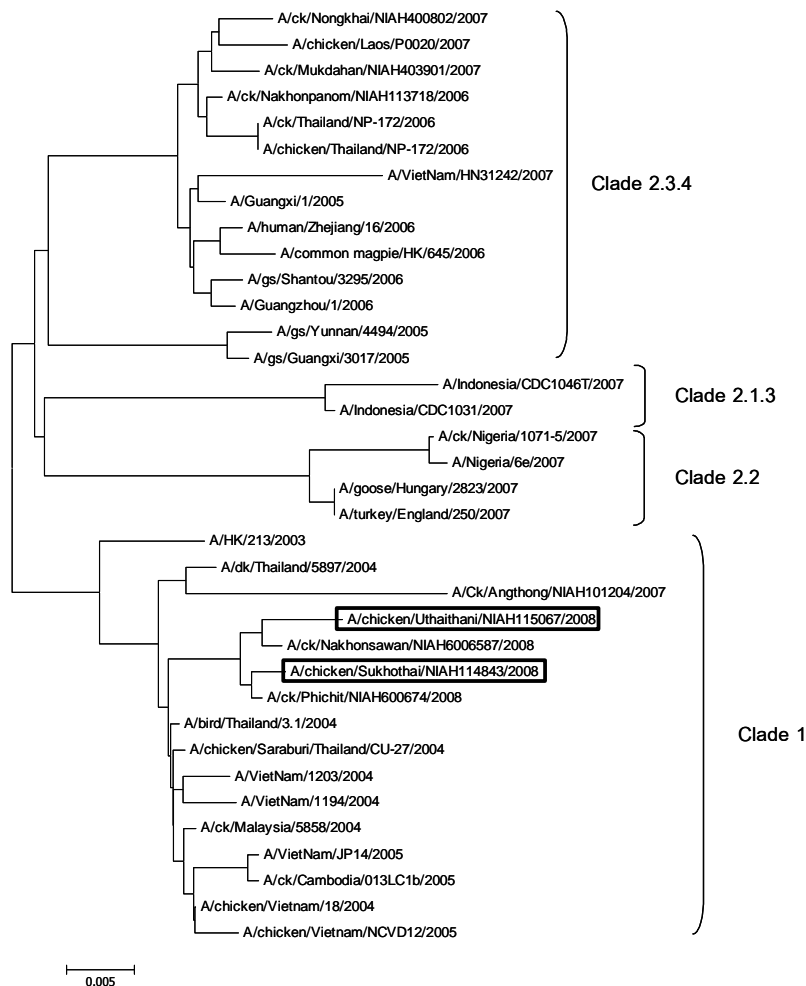
On 27 October 2008, the suspected HPAI H5N1 case was initially found in native chickens at Thung Saliang sub-district, Thung Saliang district, Sukhothai province. Two weeks later, H5N1 outbreak occurred in native chickens at Thung Pho sub-district, Nongchang district, Uthai Thani province. Preliminary investigations in both provinces revealed that the affected birds exhibited the signs of H5N1 infection, such as oedema of face, cyanosis of comb and wattle, and sudden death. The cumulative mortalities of birds in Sukhothai and Uthai Thani

provinces were 5 out of 17 and 5 out of 7, respectively. The visceral organs of native chickens from Sukhothai province and a carcass of native chicken from Uthai Thani province were submitted to National Institute of Animal Health, Department of Livestock Development for confirmation of H5N1. Each sample was propagated in 9-day-old embryonated eggs and the allantoic fluid was collected and tested for hemagglutination (HA) and hemagglutination inhibition (HI) tests according to Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE, 2004). Thereafter, the infected allantoic fluid from different isolates was harvested and then RNAs were extracted by QIAamp viral RNA extraction kit (QIAGEN, Hilden, Germany) according to manufacturer's recommendation. Viral RNA was amplified by reverse transcription-polymerase chain reaction (RT-PCR) and DNA sequencing analyses were performed as described by Nuansrichay *et al.* (2008).

Results from the complete genome analysis showed that the 2 isolates from Sukhothai and Uthai Thani provinces closely resembled H5N1 strains occurred in Thailand during 2004-2005 with minor changes. In addition, the whole genome phylogenetic analyses also showed that these viruses belonged to genotype Z.

The phylogenetic tree of the HA gene (Figure 1) indicated that Sukhothai and Uthai Thani DNA sequences were similar and belonged to the cluster of Thai and Vietnam isolates during 2004 and 2005 which belonged to Clade 1. Both isolates had the multiple basic amino acids (PQRERRRKKR/GLF) at the connecting peptide between HA1 and HA2 genes which indicated that these viruses were highly virulent strains (OIE, 2004). In the neuraminidase (NA) gene, these 2 viruses had 20 amino acids deletion in the stalk region (amino acid positions from 49 to 68) and amino acid residues (H) at position 274. This finding suggested that the 2 isolates might be susceptible to treatment with oseltamivir (Smith *et al.*, 2006). The amino acid residue at position 92 (D) in NS gene was observed from the 2 isolates suggesting that these viruses were sensitive to treatment with interferon and tumor necrosis factor- α (Krug, 2006). The viruses contained amino acid residue (E) at position 627 in the PB2 gene, suggesting that these viruses had low efficiency for replication capability in mammalian hosts (Shinya *et al.*, 2004). The M2 gene of the viruses had 2 amino acid changes (from L to I at amino acid position 26 and S to N at amino acid position 31) which implied resistance for amantadine and rimantadine (Scholtissek *et al.*, 1998).

Figure I. Hemagglutinin gene of H5N1 viruses isolated in Sukhothai and Uthai Thani provinces in 2008 compared with other H5N1 sequences in GenBank. The phylogenetic tree was generated by using MEGA 4 program (neighbor-joining analysis method and with Tamura -Nei model). Analysis was based on nucleotides 49 to 1570 (nucleotides 1521 base pairs).



In conclusion, the genetic sequences of HPAI H5N1 viruses in Sukhothai and Uthai Thani provinces are similar to HPAI H5N1 virus isolated in 2004 and 2005 with minor changes but their pathogenicity remain unchanged. Since the first outbreak of HPAI H5N1 in 2004, the Thai government has implemented strict control measures without vaccination. Therefore, this result may indicate that HPAI subtype H5N1 virus in Thailand does not share genetic materials with HPAI subtype H5N1 strain from neighboring countries since 2006. However, concerning the surveillance program, control measures and public awareness should be emphasized to prevent the reintroduction and spread of HPAI subtype H5N1 in the future.

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Abstract

Study on the molecular characterization of all 8 gene segments of 2 highly pathogenic avian influenza (HPAI) viruses subtype H5N1 isolated from the outbreaks in Sukhothai and Uthai Thani provinces, Thailand, in 2008 by DNA sequencing and phylogenetic analysis showed that these viruses belong to Clade 1, similar to HPAI H5N1 outbreak in poultry during 2004-2005. In addition, the viruses were resistant to amantadine and rimantadine, but they were sensitive to oseltamivir carboxylate. The reassortment with HPAI subtype H5N1 strain from neighboring countries was not found.

Keywords : highly pathogenic avian influenza virus subtype H5N1, phylogenetic analysis, sequence analysis

การศึกษาคุณสมบัติทางด้านโมเลกุลของเชื้อไวรัสไข้หวัดนก H5N1 ที่ระบาดในจังหวัดสุโขทัยและอุทัยธานีของ ประเทศไทย ในปี พ.ศ. 2551

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บทคัดย่อ

การศึกษาคุณสมบัติทางด้านโมเลกุลของเชื้อไวรัสไข้หวัดนก สายพันธุ์ H5N1 จำนวน 2 สายพันธุ์ ที่ระบาดในจังหวัดสุโขทัยและอุทัยธานีของประเทศไทย ในปี พ.ศ. 2551 โดยการเรียงลำดับเบสของรหัสพันธุกรรม (DNA sequencing) และ การวิเคราะห์ทาง Phylogenetic ของจีโนม ทั้ง 8 ชิ้น พบว่า เชื้อไวรัสไข้หวัดนกทั้ง 2 สายพันธุ์ อยู่ใน Clade 1 ที่มีความคล้ายคลึงกับกลุ่มเชื้อไวรัสไข้หวัดนก สายพันธุ์ H5N1 ที่เคยมีการระบาดในสัตว์ปีก ระหว่างปี พ.ศ. 2547-2548 นอกจากนี้เชื้อไวรัสไข้หวัดนกทั้ง 2 สายพันธุ์ ตี้อต่อยา amantadine และ rimantadine แต่ไม่พบการตี้อยา oseltamivir carboxylate และไม่พบการผสมกลายพันธุ์ (reassortment) กับไวรัสไข้หวัดนก สายพันธุ์ H5N1 ใหม่จากประเทศเพื่อนบ้าน

คำสำคัญ : เชื้อไวรัสไข้หวัดนก H5N1, phylogenetic analysis, sequence analysis