

กรณีศึกษา :

## ความชุกของไวรัส Avian Influenza H5N1 ตัวอย่างจากโพรงจมูกของสุกรใน เขตจังหวัดเชียงใหม่และลำพูนในประเทศไทย

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**บทคัดย่อ** จากตำแหน่งการเกิดอุบัติการณ์การระบาดของโรคไข้หวัดนก H5N1 ในสัตว์ปีกจำนวน 19 จุดในจังหวัดเชียงใหม่และลำพูน เลือกรักษาในฟาร์มสุกรที่อยู่ในรัศมี 10 กิโลเมตรจากตำแหน่งที่มีการระบาดของตัวอย่างจากโพรงจมูกในสุกรเพื่อเพาะแยกเชื้อและจำแนกชนิดไวรัสไข้หวัดนกตามข้อแนะนำของ OIE และใช้แบบสอบถามประเมินระบบความปลอดภัยทางชีวภาพของฟาร์ม ตัวอย่างจากโพรงจมูกของสุกรจำนวน 1,040 ตัวอย่าง จากฟาร์มสุกรจำนวน 50 ฟาร์ม ในจังหวัดเชียงใหม่และลำพูนไม่พบผลบวกสำหรับการเพาะแยกเชื้อไวรัส เกษตรกรผู้เลี้ยงสุกรมีความระมัดระวังการติดต่อของเชื้อไข้หวัดนกทางนกป่าและมีระบบความปลอดภัยทางชีวภาพที่ดีในฟาร์ม เช่น การเผาและทำลายนกที่ตายในฟาร์ม ไม่พบเหตุการณ์ของเชื้อไวรัสไข้หวัดนกติดต่อถึงสุกรในพื้นที่ที่เคยมีการระบาดของเชื้อไข้หวัดนกในสัตว์ปีก ดังนั้นบทบาทของการผสมผสานไวรัสไข้หวัดนกในตัวอย่างสุกรน่าจะจำกัดในประเทศไทย อย่างไรก็ตามการศึกษาดังกล่าวในกลุ่มประชากรสุกรตลอดทั้งปี น่าจะทำให้เกิดความเข้าใจถึงโครงสร้างประชากรของไวรัสไข้หวัดนกมากขึ้น

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**คำสำคัญ :** ไวรัสไข้หวัดนก H5N1, สุกร, ตัวอย่างจากโพรงจมูก, จังหวัดเชียงใหม่, จังหวัดลำพูน

### Introduction

Influenza viruses can infect many species of animals including avian, swine, equine and human. There is a high potential for cross-species transmission of influenza viruses in nature. Pig is an important host in influenza virus ecology since they are susceptible both avian and human influenza A viruses, often being involved in inter-species transmission, facilitated by regular close contact with humans or birds<sup>(1)</sup>. Pigs serve as major reservoirs of H1N1 and H3N2 influenza viruses, and the maintenance of these viruses in pigs and the frequent introduction of viruses from other species may be

important in the generation of 'new' strains of influenza, some of which may have the potential to transmit to other species including humans<sup>(2)</sup>.

It has been shown that humans occasionally contact influenza viruses from pigs. The internal protein genes of human influenza viruses show percent homology with the genes of some swine influenza viruses. It is clear that the swine influenza outbreak in Hong Kong in 1998 was the result of avian influenza virus crossing the species barrier from poultry to pig<sup>(3-4)</sup>. More importantly, swine influenza viruses could infect humans as shown when a soldier died of swine influenza infection in Fort Dix, New Jersey in

1976<sup>(5)</sup>. Pigs are an important vessel for Avian Influenza virus to cross the species barrier since they have cell surface receptors for both human and avian influenza viruses. If avian influenza viruses and human influenza viruses infect the same pigs, they can rearrange their genetic material in pigs. Thus allow a more severe strain of avian influenza virus to infect human easily.

In Thailand, the highly pathogenic avian influenza (H5N1) outbreak in human appeared to emerge in three major episodes. The first outbreak of H5N1 avian influenza was reported in Thailand in early January 2004<sup>(6)</sup>. It lasted until March, leading to 12 human cases and 8 of them died. The second outbreak occurred in July 2004 had run its course until the end of the year leaving in its wake five human cases with four fatalities<sup>6</sup>. In October-December 2005, the third outbreak was reported resulting in five human cases with two fatalities. One of fatal case reported in October 2005, was a 48-year-old chicken slaughterer in Kanchanaburi province, western part of Thailand. Moreover, his son was hospitalized and confirmed to be infected by H5N1. The other one was reported in December 2005, was a 5-year-old boy in the central province of Nakorn Nayok. In addition, two infected individuals were reported as a worker in a local chicken farm in Nontaburi province and an 18-month-old boy in the suburban of Bangkok<sup>(7-9)</sup>.

Many reports indicated that avian influenza virus (H5N1) is the main cause for several outbreaks in human in the world and these outbreaks may be the result of direct avian-to-human transmission. Therefore, it is of public health concern to determine the role of pigs as mixing vessel of avian and human influenza in Thailand. In order to achieve the aforementioned goal, we survey the prevalence of avian influenza virus in pigs in northern Thailand.

### Materials and methods

Location of sampling of pigs: From the site of 19 incidences of avian influenza outbreak in poultry in 2005 shown in Chiang Mai and

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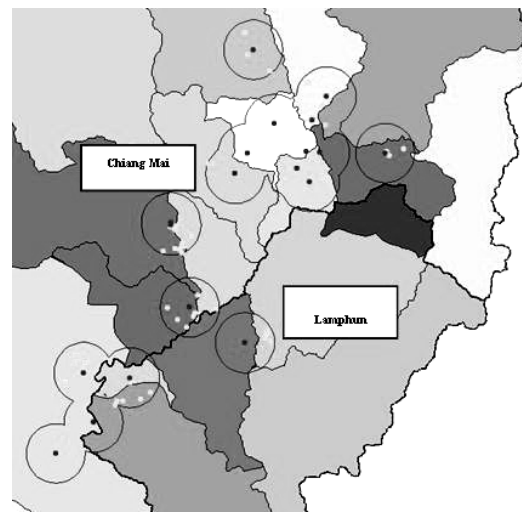


Fig. 1. Map of northern part of Thailand. The site of 19 incidences of avian influenza outbreak in poultry in 2005 shown in Chiang Mai-Lamphun province. \*The black point positions are the incident of avian influenza virus in poultry and the white point position are choosing the pig farm.

Sampling of pigs: Nasal swab were collected randomly from pigs in selected farms at Chiang Mai and Lamphun provinces in Thailand on a monthly basis between January-July 2006. A total of 1,014 nasal swab samples from 50 farms in Chiang Mai and Lamphun provinces were collected for the isolation and identification of avian influenza virus according to OIE guideline. Questionnaires were used to assess biosecurity system of the farms

Viral isolation: Nasal swab samples were inoculated onto Marline Derby Canine Kidney cells (MDCK cells) culture tubes in Eagles Minimum Essential Medium without fetal calf serum but containing trypsin-versin (2 µg/ml). Virus isolates were passaged and identified using haemagglutination inhibition (HAI) test and neuraminidase inhibition (NAI) test using a panel of reference sera. Re-isolation was attempted from the original specimen.

Viral RNA extraction and multiplex RT-PCR assays: These were carried out as described previously<sup>(10-12)</sup>

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## Results

From 1,014 nasal swab samples from 50 farms in Chiang Mai and Lamphun provinces, none were positive for virus isolation. The farmers were aware of avian influenza transmission via free living birds and had biosecurity system in place such as bury or burn dead bird found on the farms. 95% of farmer has the best biosecurity system such as the using disinfection, the controlling visitor come to the farm, and the eradicated for illness pigs (Table1.). Approximated 60% of pig farms were standard farm which were accredited by Department of Livestock Development of Thailand.

## Discussion and conclusion

Pig in particular serves as mixing vessels to generate reassortants which are potential candidates for new pandemic strains, since they are susceptible to both avian and human influenza viruses<sup>(13-14)</sup>. It has been proposed that pigs, by being permissive to both avian and human influenza viruses, act as an intermediate

host allowing reassortment and adaptation of avian viruses to human. The pig has been the leading contender for the role of intermediate host for influenza A viruses. Pigs are the only mammalian species, which are domesticated, reared in abundance and are susceptible to, and allow productive replication of avian influenza viruses. This susceptibility is due to the presence of both  $\alpha$ 2,3- and  $\alpha$ 2,6-galactose sialic acid linkages in cells lining the pig trachea which can result in modification of the receptor binding specificities of avian influenza viruses from  $\alpha$ 2,3 to  $\alpha$ 2,6 linkage<sup>(14)</sup>. Recently, H9N2 viruses have apparently been introduced to pigs in South-East Asia, possibly from poultry, although the potential of these viruses to spread and persist in pigs remain unknown. Transmission of influenza A viruses from pig to humans occurs on a regular basis, however, subsequent transmission of these viruses within the human population is very rare.

Following interspecies transmission and/or genetic reassortment, an influenza virus may undergo many pig-to-pig transmissions because of the continual availability of susceptible pigs. The mechanisms whereby an avian virus is able to establish a new lineage in pigs remain unclear, although following the introduction of an avian virus into pigs in Europe in 1979, the virus was relatively unstable for approximately 10 years<sup>(15)</sup>, but the mutation rate this virus did not subsequently increase<sup>(16)</sup>. It would appear that the adaptive processes can take many years as occurred, following transmission of both avian H1N1 and human H3N2 viruses to pig.

In the present study, there was no evidence of avian influenza virus infecting pigs in the same area where there were avian influenza outbreaks in poultry. The role of pigs as mixing vessel in Thailand may be limited. However, a longitudinal study of pig population throughout the year may provide a clearer understanding of the influenza virus population structure.

## Acknowledgement

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**Table 1;** Summary data of biosecurity system in farm.

Data	Percent
Standard farm	64%(32/50)
Type of housing	
- Open housing	64%(32/50)
- Close housing	36%(18/50)
Using disinfection	
- before go through the farm	92%(46/50)
- dip pond in front of housing	82%(41/50)
- used disinfectant into the farm	100%(50/50)
The eradicated for illness pigs	96%(48/50)
Bury and burn dead bird immediately	98%(49/50)

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