Original Article

Infection of Thai Influenza A Viruses Subtype H5N1 Using Tracheal Culture

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Abstract

Avian influenza (AI) is caused by influenza A virus subtype H5N1. The virus causes severe disease and death in several avian and mammalian species. This study investigated the ability of swine and chicken tracheal cultures to support highly pathogenic avian influenza subtype H5N1 (HPAI-H5N1) viral replication. Three Thai HPAI-H5N1 viruses isolated from chicken (A/chicken/Thailand/CU-K2/04), duck (A/duck/Thailand/CU-328/07), and tiger (A/tiger/Thailand /CU-T7/04) were used to infect the swine and chicken tracheal cultures. Our results indicated that the chicken tracheal culture could support virus replication in contrast to the swine tracheal culture, suggesting that pig has low susceptibility to Thai HPAI-H5N1 infection. Immunohistochemical staining and histopathological study showed tracheal epithelial necrosis and exfoliation, confirming HPAI-H5N1 infection. Overall, our results suggest the possibility of using animal tracheal culture as *in vitro* model to study HPAI infection mechanism. The information gained from the study can be used for better understanding of the pathogenesis and prevention and control of HPAI infection in the future.

Keywords: influenza A, Thailand, tracheal culture

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Introduction

Avian Influenza (AI) is an emerging disease in Thailand. The disease is caused by Highly Pathogenic Avian Influenza virus subtype H5N1 (HPAI-H5N1). The HPAI-H5N1 has a profound impact on animal production and public health since it causes disease in poultry, mammals and humans. HPAI-H5N1 causes disease in poultry with clinical signs of respiratory distress, excessive lacrimation, edema of the head, diarrhea, neurological symptoms, decreased egg production and death. In Thailand, as of June 2014, HPAI-H5N1 was reported to infect 25 people with 17 deaths (WHO, 2014) and has been documented in many avian species as well as in several mammal species such as leopards, tigers, cats, dogs and humans (Keawcharoen et al., 2004; Chotpitayasunondh et al., 2005; Thanawongnuwech et al., 2005; Ungchusak et al., 2005; Amonsin et al., 2006; Songserm et al., 2006a; Songserm et al., 2006b).

HPAI-H5N1 infection in other species besides avian species was documented. HPAI-H5N1 was reported to infect tigers and leopards that ingested chicken carcasses (Keawcharoen et al., 2004). Nucleotide sequences of the viruses isolated from tigers and leopards were closely related to those of the viruses isolated from chickens (Amonsin et al., 2006). Moreover, there are evidences supporting that HPAI-H5N1 can infect cats and dogs in Thailand (Songserm et al., 2006a; Songserm et al., 2006b). The nucleotide sequences of the viruses isolated from those infected cats and dogs were also related to the viruses isolated from the avian species in the same periods of HPAI H5N1 outbreak (Amonsin et al., 2007).

Pig is an intermediate host that can be infected with influenza viruses and also play an important role in viral infections and reassortment. Incidences of direct transmission of influenza virus from avian species to pig have been documented including infection of H1N1 and H9N2 avian strains in swine in China (Guan et al., 1996; Peiris et al., 2001) and H4N6 in Canada (Karasin et al., 2000). It has also been known that pig can be infected with both avian and human influenza A virus (IAV) since pig has two types of IAV receptors, α 2,3 and α 2,6-linked Sialic acid on the epithelial lining of upper respiratory tracts (Peiris et al., 2001). Thus, the co-infection situation can increase a chance for genetic reassortment of IAV within pigs. In human, pandemic H1N1 (pH1N1) was first reported in April 2009 and then spread worldwide. Subsequently, pH1N1 was first isolated from Thai swine population in November 2009 (Sreta et al., 2010) and the novel reasserted IAV in pig in Thailand was found in 2010 (Kitikoon et al., 2011).

At present, several models for IAV infection in animals have been used. Both *in vivo* and *in vitro* techniques have been applied to investigate the pathogenesis of interested organisms. The models include animal challenge study (Govorkova et al., 2005; Thiry et al., 2007), cell culture study (Zaffuto et al., 2008) and tracheal culture study (Mostow et al., 1977). The animal challenge study, an *in vivo* technique, could be the best system for study of host response to the infection. While the cell culture or organ culture studies, which are *in vitro* techniques, provide an opportunity to study mechanism of the infection under highly controlled conditions (Zaffuto et al., 2008). Moreover, the use of *in vitro* techniques can reduce the number of laboratory animals used in each study.

In this study, tracheal cultures from chickens and pigs were used to study the ability of different HPAI-H5N1 strains to infect and replicate in two different tracheal culture assays. Results showed the ability of HPAI-H5N1 infection and replication especially in avian and mammal tracheal culture models. These findings will provide useful information to understand the infection of Thai HPAI-H5N1 viruses isolated from different animal species.

Materials and Methods

HPAI-H5N1 viruses: Thai HPAI-H5N1 viruses used in this study were selected (n=3) with the following criteria. First, HPAI-H5N1 viruses recovered from chicken (n=1), duck (n=1) and tiger (n=1) were selected. Second, availability of the viruses in culture collections of the Veterinary Diagnostic Laboratory, Faculty of Veterinary Science, Chulalongkorn University was required. Third, only viruses with whole genome sequences were selected.

The HPAI-H5N1viruses were compared in the level of nucleotide and amino acid sequences by computer programs (Bioedit and MegAlign software; DNASTAR, Madison, WI) to identify polymorphisms in antigenic and virulent determinants on their 8 genes. Each virus was propagated in chicken embryonated eggs (Senne, 1998) in order to yield the stock virus at the optimized titer for infection (10^5 ELD_{50}). Virus titers were determined by 50% egg lethal dose (ELD_{50}) in chicken embryonated eggs (Villegas, 1998) and calculated for the virus titers by the Reed and Muench method (Reed and Muench, 1938). A list of the HPAI-H5N1viruses used in the study is shown in Table 1.

Table 1 HPAI-H5N1 viruses examined in this study

ID	Host	Location	Year	GenBank accession number
A/chicken/Thailand/CU-K2/04 (CU-K2)	Chicken	Nakhon Pathom	2004	AY551934 AY550147 AY590578-81 AY590567-68
A/duck/Thailand/CU-328/04 (CU-328)	Duck	Bangkok	2007	EU616835-42
A/tiger/Thailand/CU-T7/04 (CU-T7)	Tiger	Chonburi	2004	AY866475-76 AY972551-54 AY907671 AY907674

Infection of HPAI-H5N1 viruses in chicken and swine tracheal cultures

Tracheal culture preparation: Tracheal cultures in this study were obtained from 4-week-old healthy pigs from a farm that proved to be free of Porcine Reproductive and Respiratory Syndrome (PRRS), Aujeszky's disease (AD) and Swine fever (SF), and from 6-week-old healthy broilers from a farm with high bio-security system. In total, 12 animals for each species (4 animals per each viral isolate) were included in this study. All animals were tested as sero-negative for HPAI-H5N1 virus antibodies. This project was reviewed and approved under the Chula's IACUC approval number 0381042.

To prepare a tracheal-ring culture, a trachea was rinsed 3 times with phosphate buffer saline (PBS) containing penicillin 400,000 IU/L and streptomycin 0.4 g/L. A tracheal ring was cut approximately 0.5 cm thick using a sterile technique. Then, the tracheal ring was placed into a 24-well plate with a culture medium (RPMI Medium 1640 GIBCO®) to which 5% fetal bovine serum, penicillin 400,000 IU/L and streptomycin 0.4 g/L were added, and incubated at 37°C with 5% CO_2. The tracheal cultures were prepared 30-60 min before HPAI-H5N1 virus infection was performed.

Infection of HPAI-H5N1 viruses: In this study, all activities involving HPAI-H5N1 infections were performed in Bio-safety level 3 laboratory (Veterinary Diagnostic Laboratory, Faculty of Veterinary Science, Chulalongkorn University). For each HPAI-H5N1 virus, tracheal-ring cultures from 2 pigs were infected and performed in duplicate. Chicken tracheal-ring cultures were performed similarly to the swine tracheal-ring cultures.

Tracheal culture infection was performed as previously described (Thompson et al., 2006). To perform HPAI-H5N1 viral infection in tracheal cultures, the culture medium from tracheal-ring cultures was removed from each well. Then, 1 ml of HPAI-H5N1 virus with the titer for infection 10^5 ELD_{50} was inoculated into the chicken and swine tracheal-ring cultures. The virus inoculums were completely removed after incubation at 37°C with 5% CO_2 for 1 h. Then, the tracheal-ring cultures were washed twice with a culture medium without fetal bovine serum. The culture medium from the second wash was harvested for virus quantitation at the beginning of viral replication (at time point 0; T0). A fresh culture medium was added to the tracheal-ring cultures prior to incubation at 37°C with 5% CO_2. Negative control cultures were included using the culture medium without virus inoculation.

Harvest of HPAI-H5N1 viruses: The culture medium was harvested 6, 12, 24, 48 and 72 h after virus inoculation (T6, T12, T24, T48 and T72). All samples of culture medium were stored at -80°C for further analysis. Samples of negative control were harvested and stored similarly. All tracheal rings at each time point were collected and fixed in 10% neutral buffered formalin for histopathological and immunohistochemical studies.

Evaluation of virus replication

Virus replication: A single-step quantitative real-time RT–PCR (rRT-PCR) was performed for evaluation of virus replication. All samples were subjected to RNA isolation using a viral RNA extraction kit (RBC-BIOSCIENCE®). In this study, rRT-PCR was performed by using a probe and primer set specific for M gene of HPAI-H5N1 virus (Payungporn et al., 2006). In addition, GAPDH, a house keeping gene that is present in every cell (Di Trani et al., 2006), was included as an internal control in each rRT-PCR reaction. To quantify the virus, fluorescence of M gene and GAPDH detected from rRT-PCR reactions was reported in terms of threshold cycle (Ct). In each reaction a copy number of M gene and GAPDH was calculated from the Ct compared to the standard. Then, the copy number of viral RNA was normalized with the GAPDH. The quantity of normalized HPAI-H5N1 viruses from rRT-PCR result was then evaluated for viral replication at each time point of infection (T0-T72).

Histopathological changes: In this study, Hematoxilin & Eosin (H&E) staining, microscopic examination and immunohistochemistry were performed. The tracheal rings were processed through an automatic tissue processor and a tissue embedding system before staining. The H&E staining was used for investigation into histopathological changes in tracheal cultures post-infection especially in the epithelial lining, which is the target cells of viral infection. Microscopic findings were examined and compared among the infection groups and the negative control group. Immunohistochemistry using a mouse-derived monoclonal antibody specific for IAV nucleoprotein antigen was performed to detect influenza A antigen to confirm IAV infection. For interpretation, a positive result of immunohistochemical staining for IAV infected cells demonstrated dark-brown staining in the nucleus of the cell.

Statistical analysis: Differences in replications among the three HPAI-H5N1 viruses on each tracheal culture assay were analyzed by using Oneway ANOVA (SPSS version 17, IBM, USA).

Results

HPAI-H5N1 viruses isolated from chicken, duck and tiger in Thailand: Thai HPAI -H5N1 viruses recovered from chicken (A/chicken/Thailand/CU-K2/04; CU-K2), duck (A/duck/Thailand/CU-328/07; CU-328) and tiger (A/tiger/Thailand/CU-T7/04; CU-T7) were included in this study. Description of the selected HPAI-H5N1 viruses including ID, host, location, year of isolation and GenBank accession number is shown in Table 1. Differences in nucleotide and amino acid sequences among CU-K2, CU-328 and CU-T7 were 1) HA gene at antigenic site E (amino acid position 86), 2) PB1 gene at virulent determinant (amino acid position 317), 3) PB2 gene at virulent determinant (amino acid position 627) and amino acid related to host specificity (amino acid position 119). For example, amino acid position PB2-627 in CU-T7 contained K (Lysine), while in CU-K2 and CU-328 contained E (Glutamic acid). More details about the comparison of nucleotide and

amino acid sequences of the viruses are shown in Table 2. The viral titers of the stock viruses recovered from chicken (CU-K2), duck (CU-328) and tiger (CU-T7) was 10^8, $10^7$and $10^{9.5}$ ELD_{50}, respectively. The stock viruses were freshly diluted with the culture medium (RPMI) in order to yield the titer 10^5 ELD_{50} for HPAI-H5N1 virus infection.

Viral replication in tracheal cultures: In this study, the replications of each HPAI-H5N1 viruses in swine and chicken tracheal cultures were evaluated. The copy numbers of the viruses were calculated from the quantity of M gene and normalized by GAPDH. The normalized copy numbers of HPAI-H5N1 viruses (CU-K2, CU-328, CU-T7) at 0, 6, 12, 24, 48 and 72 h post-infection from the swine and chicken tracheal cultures are shown in Table 3. Figure 1 shows the growth curve of average normalized copy number (mean ± SD) of HPAI-H5N1 viruses (CU-K2, CU-328, and CU-T7) from both swine and chicken tracheal cultures. In the swine tracheal cultures, there was no significant difference in the normalized copy numbers of viral RNA during 0-72 h (T0-T72) post-infection. In the chicken tracheal cultures, for CU-328, no significant difference in the normalized copy numbers of viral RNA during T0-T72 post-infection was found. For CU-K2, there were significant differences in the normalized copy numbers of viral RNA from T0 to T6 and from T12 to T48 ($p < 0.05$). For CU-T7, there were significant differences in the normalized copy numbers of viral RNA during T6 and T12 and during T12 and T24 ($p < 0.05$) (Table 3 and Fig 1).

Table 2 Genetic comparison of nucleotide and amino acid sequences of HPAI-H5N1 viruses (CU-K2, CU-328 and CU-T7)

Virus	Isolate Description	Host	HA gene			PB1 gene	PB2 gene	
			Connecting peptide	Antigenic site E		Virulence determinant	Virulence determinant	Virulence determinant
			323-329	83	86	317	627	119
CU-K2	A/Chicken/Thailand /CU-K2/04	Chicken	RERRRKK	A	V	Y	E	A
CU-328	A/Duck/Thailand/CU-328/07	Duck	RERRRKK	A	A	M	E	F
CU-T7	A/Tiger/Thailand/CU-T7/04	Tiger	RERRRKK	A	V	M	K	F

Table 3 Normalized copy number (median ± SD) of HPAI-H5N1 viruses (CU-K2, CU-328, CU-T7) at 0, 6, 12, 24, 48 and 72 h post-infection from swine and chicken tracheal cultures

Time (hour)	Normalized copy number (median ± SD) (log)					
	Swine tracheal culture			Chicken tracheal culture		
	CU-K2	CU-328	CU-T7	CU-K2	CU-328	CU-T7
0	2.30 ± 0.20	2.96 ± 0.11	0.00 ± 0.08	3.09 ± 0.13[a]	3.98 ± 0.42	1.38 ± 0.42
6	2.56 ± 1.22	3.09 ± 0.32	1.21 ± 1.63	3.74 ± 0.62[aa]	4.16 ± 0.74	1.49 ± 0.48[c]
12	2.25 ± 1.09	2.80 ± 0.51	0.50 ± 1.01	3.41 ± 0.79[b]	4.67 ± 0.48	2.93 ± 0.48[cc,d]
24	2.52 ± 0.98	2.92 ± 0.97	0.59 ± 0.97	3.96 ± 0.72	4.42 ± 0.22	4.90 ± 1.52[dd]
48	2.46 ± 1.29	3.06 ± 0.72	0.32 ± 1.87	5.00 ± 0.82[bb]	4.54 ± 0.42	4.70 ± 0.55
72	2.33 ± 0.87	2.94 ± 0.99	0.92 ± 1.16	4.85 ± 1.78	4.29 ± 0.26	4.85 ± 0.56

Statistical significance ($p < 0.05$) of normalized copy number of CU-K2 from T0 (a) to T6 (aa) and from T12 (b) to T48 (bb)
Statistical significance ($p < 0.05$) of normalized copy number of CU-T7 from T6 (c) to T12 (cc) and from T12 (d) to T24 (dd)

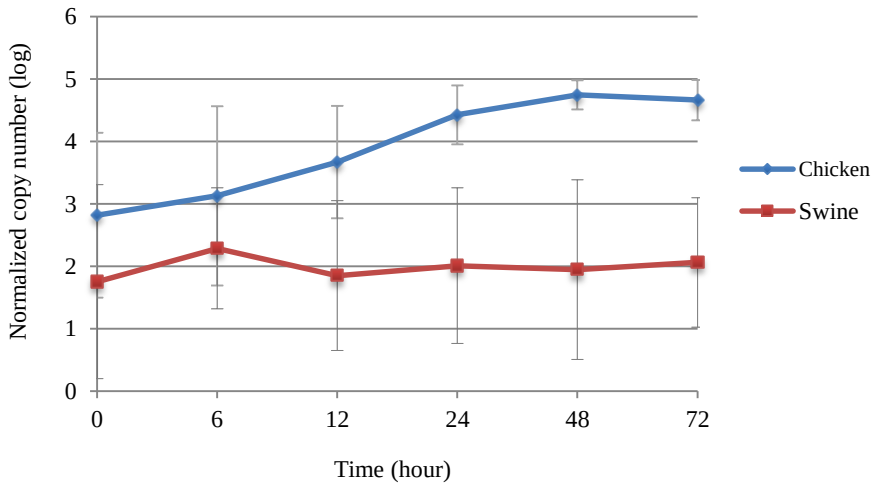

Figure 1 Average normalized copy number (± SD) of HPAI-H5N1 viruses (CU-K2, CU-328, and CU-T7) at 0, 6, 12, 24, 48 and 72 h post-infection from swine and chicken tracheal cultures

Histopathological changes in HAPI-H5N1 infected tracheal cultures: The histopathological changes in swine and chicken tracheal cultures were examined from T0 to T72 in the HPAI-H5N1 infected and non-infected groups (Fig 2). Sloughing of tracheal epithelium was found 24 h and 12 h after performing the tracheal cultures in non-infected swine and chicken tracheal cultures, respectively. Then, most of the tracheal epithelia were extremely exfoliated at 48 and 72 h.

In the HPAI-H5N1 infected swine tracheal cultures, partial necrosis of tracheal epithelium was found at 12 h post-infection. Then, tracheal epithelial exfoliation and cytoplasmic droplet at the surface of tracheal epithelium were found at 24 h post-infection. At 48 h, extreme tracheal epithelial exfoliation (more than 80%) and necrosis of tracheal epithelium were found. Subsequently, most epithelial cells were exfoliated at 72 h post-infection. Interestingly, the tracheal epithelial exfoliation was found in the tracheal cultures infected with CU-328 12 h earlier than those infected with the other HPAIs.

In the HPAI-H5N1 infected chicken tracheal cultures, cloudy swelling degeneration and/or hydropic degeneration of tracheal epithelium were found at 12 h post-infection. Subsequently, extreme tracheal epithelial exfoliation (more than 90%) and necrosis of tracheal epithelium were found at 24 h post-infection. Most epithelia of the chicken tracheal cultures were exfoliated at 48 h post-infection. Interestingly, the tracheal epithelial exfoliation was found in the tracheal cultures infected with CU-328 as early as 12 h before those infected with the other HPAIs.

Immunohistochemical changes in infected tracheal cultures: Histopathological sections of kidney from the chicken infected with HPAI (H5N1) were used as positive controls and the non-infected tracheal culture was used as negative control in the immunohistochemical staining (Fig 2). In most tracheal-ring sections, the immunohistochemical staining showed positive with dark-brown stain in nucleus of the cell. However, it should be noted that the immunohistochemistry of tracheal rings with exfoliated tracheal epithelium could not be evaluated.

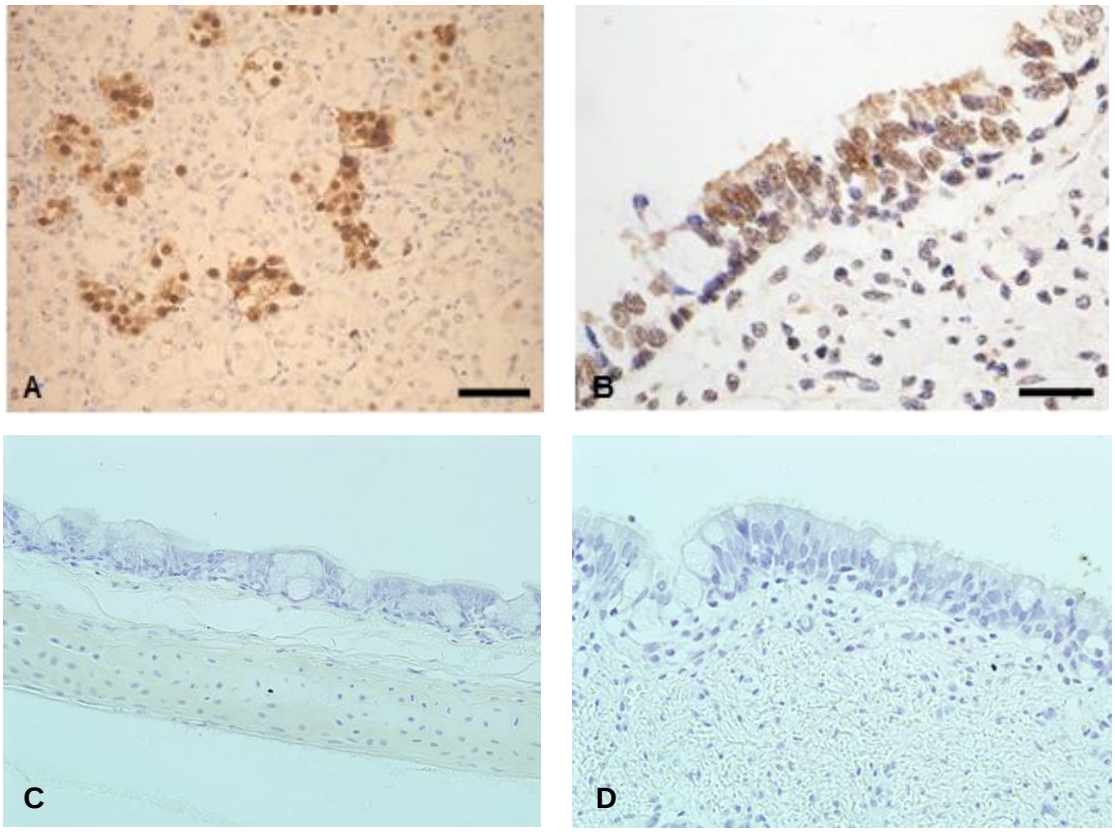

Figure 2 Immunohistochemistry findings. A: Positive control; positive result from HPAI-H5N1 infected chicken kidney tissue, 400X (Bar = 25µm). B: Positive result from HPAI-H5N1 infected swine tracheal culture, 400X (Bar = 25 µm). C: Negative control chicken tracheal tissue, 400X (Bar = 25µm). D: Negative control; swine tracheal tissue, 400X (Bar =25µm)

Discussion

HPAI-H5N1 viruses used in this study were CU-K2 (Viseshakul et al., 2004), CU-328 (Amonsin et al., 2008) and CU-T7 (Amonsin et al., 2006). All viruses were clustered in clade 1 (Vietnam-Thailand lineage), which was responsible for the HPAI-H5N1 outbreaks in Thailand (Li et al., 2004). The comparison of nucleotide sequences among the 3 viruses revealed common characteristics of HPAI-H5N1 including multiple basic amino acids at the HA cleavage site which are composed of "RERRRKK" pattern, 20 amino acids deletion in NA stalk region and 5 amino acids deletion in NS1 gene. Among these 3 viruses, minor differences in antigenic and virulent determinants

were observed especially PB2-627 in CU-T7 containing K (Lysine) suggesting preferential binding to mammalian host.

In this study, the HPAI-H5N1 replication in swine and chicken tracheal cultures was quantified after virus inoculation (T6, T12, T24, T48 and T72). The increase in copy number of viral RNA after virus inoculations proves that swine tracheal culture can be infected with CU-K2, CU-328 and CU-T7. However, there was no significant increase in the normalized copy number. This finding may imply that swine tracheal culture has low susceptibility to the Thai HPAI-H5N1 viruses. This result correlates well with a report studying *in vivo* infection that piglets (2-3 weeks) could support HPAI-H5N1 infection, but the susceptibility was low (Lipatov et al., 2008). Moreover, pigs at 4 weeks old could be infected with HPAI-H5N1 isolated in Vietnam and Thailand 2004, but showed low titer (Choi et al., 2005). The HPAI-H5N1 infection in chicken tracheal cultures revealed that the viruses isolated from chicken (CU-K2) and tiger (CU-T7) had the ability to replicate in chicken tracheal culture. In contrast, the virus isolated from duck (CU-328) could infect, but the viral replication was not significant. Our results implied the differences in susceptibility of swine and chickens tracheal cultures for the infection of Thai HPAI-H5N1 viruses. In addition, the abilities of viral replication in tracheal cultures were different among the HPAI-H5N1 viruses isolated from chicken, duck and tiger.

The microscopic examination indicated the different time points of tracheal epithelial exfoliation between the infected swine and chicken tracheal cultures. In the chicken tracheal cultures, the tracheal epithelial exfoliation and necrosis were found earlier than those in swine. Increase in the severity of lesions was signified in the infected groups when compared with the normal group. This finding could be influenced by HPAI-H5N1 virus inoculation and correlated with the positive immunohistochemical results. However, this study provides results from *in vitro* infection that might be insufficient in details about host responses to the HPAI-H5N1 infection. For example, an *in vivo* study showed degeneration and necrosis of bronchial epithelium, leukocytes infiltration and apoptosis in pigs inoculated intranasally with HPAI-H5N1 viruses (Lipatov et al., 2008). Moreover, the immunohistochemical staining confirmed HPAI-H5N1 infection in most tracheal epithelia. Unfortunately, the interpretation at 48 and 72 h post-infection was limited due to the extreme exfoliation and necrosis of the tracheal epithelium.

In conclusion, the HPAI-H5N1 viruses isolated from chicken, duck and tiger in Thailand could infect the swine and chicken tracheal cultures. The abilities of viral replication in the chicken tracheal cultures were different among the viruses, whereas the swine tracheal cultures had low susceptibility to the Thai HPAI-H5N1 viruses. Our results suggest the possibility of using animal tracheal culture as *in vitro* model to study HPAI-H5N1 infection mechanism. This information will be useful for the prevention and control of HPAI-H5N1 infection in the future.

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บทคัดย่อ

การติดเชื้อไวรัสไข้หวัดนก (H5N1) ที่แยกได้ในประเทศไทยในเนื้อเยื่อหลอดลมเพาะเลี้ยง

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โรคไข้หวัดนก (Avian influenza) เกิดจากเชื้อไวรัส Influenza type A สายพันธุ์ H5N1 มีรายงานการระบาดของโรคไข้หวัดนกในสัตว์ปีกและสัตว์เลี้ยงลูกด้วยนมหลายชนิด การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อทดสอบความสามารถในการเพิ่มจำนวนของเชื้อไข้หวัดนกชนิดก่อโรครุนแรง (highly pathogenic avian influenza) สายพันธุ์ H5N1 ในหลอดลมเพาะเลี้ยงของไก่และสุกร โดยคัดเลือกเชื้อไข้หวัดนกจำนวน 3 ตัวอย่างที่แยกได้ในประเทศไทยจากไก่ (A/chicken/Thailand/CU-K2/04) เป็ด (A/duck/Thailand/CU-328/07) และเสือ (A/tiger/Thailand /CU-T7/04) มาทดสอบการติดเชื้อในหลอดลมเพาะเลี้ยงของไก่และสุกร การศึกษาพบว่าเชื้อไวรัสทั้ง 3 ตัวอย่างสามารถเพิ่มจำนวนได้ในหลอดลมเพาะเลี้ยงของไก่ ส่วนในหลอดลมเพาะเลี้ยงของสุกรพบว่าเชื้อไวรัสทั้ง 3 ตัวอย่างเพิ่มจำนวนเพียงเล็กน้อยและไม่มีนัยสำคัญทางสถิติ ซึ่งสอดคล้องกับงานวิจัยที่ผ่านมาที่พบว่าสุกรมีความไวรับต่ำต่อเชื้อไข้หวัดนก การศึกษาทางจุลพยาธิวิทยาการติดเชื้อไวรัสในหลอดลมเพาะเลี้ยงพบว่าในกลุ่มติดเชื้อไข้หวัดนกมีการตายและลอกหลุดของเซลล์เยื่อบุหลอดลมรุนแรงกว่าในกลุ่มควบคุม และสามารถพิสูจน์ยืนยันการติดเชื้อไข้หวัดนกในเซลล์เยื่อบุหลอดลมได้ด้วยเทคนิคอิมมูโนฮิสโตเคมี การศึกษาในครั้งนี้ได้ให้ข้อมูลความสามารถในการเพิ่มจำนวนของเชื้อไข้หวัดนกที่แยกได้จากไก่ เป็ด และเสือ ในประเทศไทยต่อชนิดของการเพาะเลี้ยงเนื้อเยื่อจากอวัยวะของสัตว์ทดลอง (ไก่และสุกร) ซึ่งเป็นข้อมูลที่จะทำให้เกิดความเข้าใจการก่อโรคของเชื้อไข้หวัดนกได้ดียิ่งขึ้น รวมทั้งข้อมูลเหล่านี้จะเป็นข้อมูลสนับสนุนการป้องกันและควบคุมการติดเชื้อไข้หวัดนกในอนาคต

คำสำคัญ: ไข้หวัดใหญ่ชนิดเอ ประเทศไทย หลอดลมเพาะเลี้ยง

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