

Avian Influenza (AI) Task Force at Chulalongkorn University

Alongkorn Amonsin

Avian influenza (AI) is caused by influenza type A virus. The virus can be classified into several subtypes (H1-16 and N1-9) based on Hemagglutinin (HA) and Neuraminidase (NA) antigens. In Thailand, the Highly Pathogenic Avian Influenza (HPAI) H5N1 outbreak in avian species was first reported in early 2004. The disease has significant impact on animal production and public health, since it causes severe disease in avian and mammal species as well as human. Since 2004, 7 waves of HPAI outbreaks were reported in the country.

During the early 2004 AI outbreak, the AI task force at Chulalongkorn University was established for emergency response of the outbreaks as well as for scientific researches of AI in Thailand. In the past 5 years, the AI task force has collaborated and worked with Thai authorities for AI outbreaks investigation as well as disease prevention and control. In addition, the task force has conducted research projects related to the AI virus and disease. The information gained from our research provides basic information of Thai AI virus and disease situation of the country. Moreover the information from the studies has also been used for planning of disease prevention and control strategies. AI research projects at Chulalongkorn University can be grouped into 4 categories of which some parts have already been published in many peer-review journals with high impact factors. The 4 categories include; 1. Epidemiology and surveillance of AI in Thailand. 2. Characterization of Thai AI viruses. 3. Development of AI diagnosis 4. AI database in Thailand

1. Epidemiology and surveillance of AI in Thailand

The CU-AI task force has conducted the studies related to the epidemiology of Avian Influenza and disease surveillance. The task force worked closely with

the Department of Livestock Development of Thailand (DLD), Faculty of Medicine, and College of Public Health, Chulalongkorn University as well as the faculty of Veterinary Sciences, Kasetsart University. The studies were focused on investigations of AI outbreaks in several avian species and mammals. The reports of AI infection in mammal species in Thailand including tigers, dog and cat are the first reports of AI infection in other mammals apart from humans. Publications and reports related to the epidemiology and surveillance of AI in Thailand are:

1. Research reports "Molecular epidemiology of Avian influenza (H5N1) in Thailand" by Amonsin et al., 2004. The project was supported by National Research Council of Thailand (NRCT), fiscal year 2004. This project was received the outstanding project award from Ratchadaphisek Somphot Endowment Fund, fiscal year 2005.
2. Highly pathogenic avian influenza H5N1, Thailand, 2004 (Tiensin et al., 2005)
3. Avian influenza H5N1 in tigers and leopards (Keawcharoen et al., 2004)
4. Probable tiger-to-tiger transmission of avian influenza H5N1 (Thanawongnuwech et al., 2005).
5. Avian influenza H5N1 in naturally infected domestic cat (Songserm et al., 2006^a).
6. Fatal avian influenza A H5N1 in a dog (Songserm et al., 2006^b).
7. Surveillance activities and molecular analysis of H5N1 highly pathogenic avian influenza viruses from Thailand, 2004-2005 (Buranathai et al., 2007).
8. Geographic and temporal distribution of highly pathogenic avian influenza A virus (H5N1) in Thailand, 2004-2005: an overview (Tiensin et al., 2007).
9. New strain of influenza A virus (H5N1), Thailand (Chutinimitkul et al., 2007^a).

Chulalongkorn University Emerging and Re-emerging Infectious Diseases in Animals Center (CUEIDA)*, Faculty of Veterinary Sciences, Chulalongkorn University, Henri-Dunant Rd., Pathumwan, Bangkok, 10330, Thailand

*The establishment of the Chulalongkorn University Emerging and Re-emerging Infectious Diseases in Animals Center (CUEIDA) was supported by Chulalongkorn University (100 years Chulalongkorn University Fund)

10. Influenza virus (H5N1) in live bird markets and food markets, Thailand (Amonsin et al., 2008).

2. Characterization of Thai AI viruses

The CU-AI task force team has an expertise on AI genome sequencing, molecular characterization of the viruses and genetic monitoring of the viruses isolated from avian and mammal species in Thailand. The information related to molecular characteristics of AI viruses is crucial for determining of the prevention and control strategies in the country. The information of Thai AI virus characterization are published in:

1. The genome sequence analysis of H5N1 avian influenza A virus isolated from the outbreak among poultry populations in Thailand (Viseshakul et al., 2004)

2. Characterization of the hemagglutinin and neuraminidase genes of recent influenza virus isolates from different avian species in Thailand (Keawcharoen et al., 2005).

3. Genetic characterization of H5N1 influenza A viruses isolated from zoo tigers in Thailand (Amonsin et al., 2006^b)

4. Genetic characterization of influenza A viruses (H5N1) isolated from 3rd wave of Thailand AI outbreaks (Amonsin et al., 2006^a)

5. Genetic analysis of Influenza A virus (H5N1) derived from domestic cat and dog in Thailand {Amonsin, 2007 #2}

6. Genetic characterization of H1N1, H1N2 and H3N2 swine influenza virus in Thailand (Chutinimitkul et al., 2008).

3. Development of AI diagnosis

The CU-AI task force team has developed the molecular assays for AI identification. The developed assays have provided rapid virus identification and differentiation in animals and humans. In addition, the developed assay have been applied and used in many outbreak investigations. The development of assays for AI identification and differentiation can be found in the publications.

1. Single-step multiplex reverse transcription-polymerase chain reaction (RT-PCR) for influenza A virus subtype H5N1 detection (Payungporn et al., 2004).

2. Single step multiplex real-time RT-PCR for H5N1 influenza A virus detection (Payungporn et al., 2006^a).

3. Discrimination between highly pathogenic and low pathogenic H5 avian influenza A viruses (Payungporn et al., 2006^b).

4. Assay to detect H5N1 oseltamivir resistance (Suwannakarn et al., 2006).

5. H5N1 Oseltamivir-resistance detection by real-time PCR using two high sensitivity labeled TaqMan probes (Chutinimitkul et al., 2007^b).

6. Typing (A/B) and subtyping (H1/H3/H5) of influenza A viruses by multiplex real-time RT-PCR assays (Suwannakarn et al., 2008)

7. Detection of influenza virus types A and B and type A subtypes (H1, H3, and H5) by multiplex polymerase chain reaction (Boonsuk et al., 2008).

4. AI database in Thailand

CU-AI task force has developed the AI database in Thailand containing information of AI viruses isolated in Thailand. The established database is available through the web page (<http://www.thai-ai-database.org>) (Figure 1). This database contains the information about the description of each virus isolate, the nucleotides and amino acid sequences of the viruses, the genetic monitoring of the viruses in Thailand. The database also contains search engine function that provides the user to find the information of any Thai AI viruses for their nucleotide sequences or genetic monitoring results. This Thai-AI database is very useful for both scientific communities and general public.

Current and future activities

In 2009, Chulalongkorn University has established “Emerging and re-emerging Animal Diseases, research unit”. The goal of this research unit is to conduct and support the researches and developments that related to emerging and re-emerging animal diseases in Thailand. Our future activities will including 1). Develop BSL3 facilities for researches and studies on emerging and re-emerging animal diseases 2). Acquire or provide funding and facilities to projects on emerging and re-emerging animal diseases especially Avian Influenza to provide new knowledge and field applications 3). Monitor and investigate emerging and re-emerging animal diseases in Thailand and establish the databases on disease information, epidemiology, pathology, virology, and nucleotide sequences of those emerging and re-emerging pathogens 4). Establish laboratory networks both in national and international levels for the prevention and control of emerging and re-emerging animal diseases.



Acknowledgement

We gratefully thank the Department of Livestock Development of Thailand for their collaborations and supports. The research projects awarded to CU-AI task force were funded by Chulalongkorn University (100 years Chulalongkorn University Fund and Ratchadaphisek Somphot Endowment Fund), National Research Council of Thailand (NRCT), Thailand Research Fund (TRF), Minnesota Center of Excellence for Influenza Research and Surveillance (MCEIRS) supported by National Institutes of Health/National Institute of Allergy and Infectious diseases (NIH/NIAID) and International Cooperative Zoonotic Influenza Research Center (ICZIRC) supported by Center for Disease Control and Prevention (CDC).

References

Amonsin, A., Choatrakol, C., Lapkuntod, J., Tantilertcharoen, R., Thanawongnuwech, R., Suradhat, S., Suwannakarn, K., Theamboonlers, A. and Poovorawan, Y. 2008. Influenza virus (H5N1) in live

bird markets and food markets, Thailand. *Emerg. Infect. Dis.* 14: 1739-1742.

Amonsin, A., Chutinimitkul, S., Pariyothorn, N., Songserm, T., Damrongwattanapokin, S., Puranaveja, S., Jam-On, R., Sae-Heng, N., Payungporn, S., Theamboonlers, A., Chaisingh, A., Tantilertcharoen, R., Suradhat, S., Thanawongnuwech, R. and Poovorawan, Y. 2006^a. Genetic characterization of influenza A viruses (H5N1) isolated from 3rd wave of Thailand AI outbreaks. *Virus Res.* 122: 194-199.

Amonsin, A., Payungporn, S., Theamboonlers, A., Thanawongnuwech, R., Suradhat, S., Pariyothorn, N., Tantilertcharoen, R., Damrongwattanapokin, S., Buranathai, C., Chaisingh, A., Songserm, T. and Poovorawan, Y. 2006^b. Genetic characterization of H5N1 influenza A viruses isolated from zoo tigers in Thailand. *Virology* 344: 480-491.

Boonsuk, P., Payungporn, S., Chieochansin, T., Samransamruajkit, R., Amonsin, A., Songserm, T., Chaisingh, A., Chamnanpood, P., Chutinimitkul, S., Theamboonlers, A. and Poovorawan, Y. 2008. Detection of influenza virus types A and B and type A subtypes (H1, H3, and H5) by multiplex polymerase chain reaction. *Tohoku J. Exp. Med.* 215: 247-255.

Buranathai, C., Amonsin, A., Chaisingh, A., Theamboonlers, A., Pariyothorn, N. and Poovorawan, Y. 2007. Surveillance activities and molecular analysis of H5N1 highly pathogenic avian influenza viruses from Thailand, 2004-2005. *Avian Dis.* 51: 194-200.

Chutinimitkul, S., Songserm, T., Amonsin, A., Payungporn, S., Suwannakarn, K., Damrongwattanapokin, S., Chaisingh, A., Nuansrichay, B., Chieochansin, T., Theamboonlers, A. and Poovorawan, Y. 2007^a. New strain of influenza A virus (H5N1), Thailand. *Emerg. Infect. Dis.* 13: 506-507.

Chutinimitkul, S., Suwannakarn, K., Chieochansin, T., Mai le, Q., Damrongwattanapokin, S., Chaisingh, A., Amonsin, A., Landt, O., Songserm, T., Theamboonlers, A. and Poovorawan, Y. 2007^b. H5N1 Oseltamivir-resistance detection by real-time PCR using two high sensitivity labeled TaqMan probes. *J. Virol. Methods* 139: 44-49.

Chutinimitkul, S., Thippamom, N., Damrongwattanapokin, S., Payungporn, S., Thanawongnuwech, R., Amonsin, A., Boonsuk, P., Sreta, D., Bunpong, N., Tantilertcharoen, R., Chamnanpood, P., Parchariyanon, S., Theamboonlers, A. and Poovorawan, Y. 2008. Genetic characterization of H1N1, H1N2 and

- H3N2 swine influenza virus in Thailand. *Arch. Virol.* 153: 1049-1056.
- Keawcharoen, J., Amonsin, A., Oraveerakul, K., Wattanodorn, S., Papravit, T., Karnda, S., Lekakul, K., Pattanarangsang, R., Noppornpanth, S., Fouchier, R.A., Osterhaus, A.D., Payungporn, S., Theamboonlers, A. and Poovorawan, Y. 2005. Characterization of the hemagglutinin and neuraminidase genes of recent influenza virus isolates from different avian species in Thailand. *Acta. Virol.* 49: 277-280.
- Keawcharoen, J., Oraveerakul, K., Kuiken, T., Fouchier, R.A., Amonsin, A., Payungporn, S., Noppornpanth, S., Wattanodorn, S., Theamboonlers, A., Tantilertcharoen, R., Pattanarangsang, R., Arya, N., Ratanakorn, P., Osterhaus, D.M. and Poovorawan, Y. 2004. Avian influenza H5N1 in tigers and leopards. *Emerg. Infect. Dis.* 10: 2189-2191.
- Payungporn, S., Chutinimitkul, S., Chaisingh, A., Damrongwattanapokin, S., Buranathai, C., Amonsin, A., Theamboonlers, A. and Poovorawan, Y. 2006^a. Single step multiplex real-time RT-PCR for H5N1 influenza A virus detection. *J. Virol. Methods* 131: 143-147.
- Payungporn, S., Chutinimitkul, S., Chaisingh, A., Damrongwattanapokin, S., Nuansrichay, B., Pinyochon, W., Amonsin, A., Donis, R.O., Theamboonlers, A. and Poovorawan, Y. 2006^b. Discrimination between highly pathogenic and low pathogenic H5 avian influenza A viruses. *Emerg. Infect. Dis.* 12: 700-701.
- Payungporn, S., Phakdeewirot, P., Chutinimitkul, S., Theamboonlers, A., Keawcharoen, J., Oraveerakul, K., Amonsin, A. and Poovorawan, Y. 2004. Single-step multiplex reverse transcription-polymerase chain reaction (RT-PCR) for influenza A virus subtype H5N1 detection. *Viral Immunol.* 17: 588-593.
- Songserm, T., Amonsin, A., Jam-on, R., Sae-Heng, N., Meemak, N., Pariyothorn, N., Payungporn, S., Theamboonlers, A. and Poovorawan, Y. 2006^a. Avian influenza H5N1 in naturally infected domestic cat. *Emerg. Infect. Dis.* 12: 681-683.
- Songserm, T., Amonsin, A., Jam-on, R., Sae-Heng, N., Pariyothorn, N., Payungporn, S., Theamboonlers, A., Chutinimitkul, S., Thanawongnuwech, R. and Poovorawan, Y. 2006^b. Fatal avian influenza A H5N1 in a dog. *Emerg. Infect. Dis.* 12: 1744-1747.
- Suwannakarn, K., Chutinimitkul, S., Payungporn, S., Chieochansin, T., Theamboonlers, A., Amonsin, A., Damrongwattanapokin, S., Mai le, Q., Hanh, N.H. and Poovorawan, Y. 2006. Assay to detect H5N1 oseltamivir resistance. *Emerg. Infect. Dis.* 12: 1995-1996.
- Suwannakarn, K., Payungporn, S., Chieochansin, T., Samransamruajkit, R., Amonsin, A., Songserm, T., Chaisingh, A., Chamnanpood, P., Chutinimitkul, S., Theamboonlers, A. and Poovorawan, Y. 2008. Typing (A/B) and subtyping (H1/H3/H5) of influenza A viruses by multiplex real-time RT-PCR assays. *J. Virol. Methods* 152: 25-31.
- Thanawongnuwech, R., Amonsin, A., Tantilertcharoen, R., Damrongwattanapokin, S., Theamboonlers, A., Payungporn, S., Nanthapornphiphat, K., Ratanamungkalanon, S., Tunak, E., Songserm, T., Vivatthanavanich, V., Lekdumrongsak, T., Kedsangsakonwut, S., Tunhikorn, S. and Poovorawan, Y. 2005. Probable tiger-to-tiger transmission of avian influenza H5N1. *Emerg. Infect. Dis.* 11: 699-701.
- Tiensin, T., Chaitaweesub, P., Songserm, T., Chaisingh, A., Hoonsuwan, W., Buranathai, C., Parakamawongsa, T., Premasathira, S., Amonsin, A., Gilbert, M., Nielen, M. and Stegeman, A. 2005. Highly pathogenic avian influenza H5N1, Thailand, 2004. *Emerg. Infect. Dis.* 11: 1664-1672.
- Tiensin, T., Nielen, M., Songserm, T., Kalpravidh, W., Chaitaweesub, P., Amonsin, A., Chotiprasatintara, S., Chaisingh, A., Damrongwattanapokin, S., Wongkasemjit, S., Antarasena, C., Songkitti, V., Chanachai, K., Thanapongtham, W. and Stegeman, J.A. 2007. Geographic and temporal distribution of highly pathogenic avian influenza A virus (H5N1) in Thailand, 2004-2005: an overview. *Avian Dis.* 51: 182-188.
- Viseshakul, N., Thanawongnuwech, R., Amonsin, A., Suradhat, S., Payungporn, S., Keawcharoen, J., Oraveerakul, K., Wongyanin, P., Plitkul, S., Theamboonlers, A. and Poovorawan, Y. 2004. The genome sequence analysis of H5N1 avian influenza A virus isolated from the outbreak among poultry populations in Thailand. *Virology* 328: 169-176.