Influenza Viral Infection in 2005-2006 in Samitivej Hospital†

Sawang Saenghirunvattana MD*, Piboon Laohathai MD*,
Pranee Thawatsupha BSc*, Rungrueng Kitplati MD, FETP**,
Naruemol Masakul BSc, MBA*, Wimonthip Jaturaphunsathaporn BA*

† *Supported by Prasert Prasartthongosot Foundation*
** Samitivej Sukumvit Hospital, ** Ministry of Public Health*

Objective: *Set an alarm system for early detection of respiratory viral infection.*
Material and Method: *The authors prospectively investigated avian flu and SARS between March 2005 and April 2006. Specimens from a nasopharyngeal swab or bronchial washing were analyzed for influenza A, B, parainfluenza, adenovirus, respiratory syncytial virus and avian flu and SARS by using technique of PCR and immunofluorescence by the Department of Medical Sciences.*
Results: *Eighty-nine patients who were enrolled in the present study. Peak of the incidence was 43% on April 2005 and there was no incidence of the influenza from December 2005 till April 2006. This may be due to the mass campaign of influenza vaccination or seasonal variation.*
Conclusion: *Vaccination remains the primary strategy for the prevention of influenza, and the broadened recommendations should lead to protection of a larger portion of the population.*

Keywords: *Influenza vaccine, Broncho-alveolar lavage*

J Med Assoc Thai 2007; 90 (3): 448-51
Full text. e-Journal: *http://www.medassocthai.org/journal*

Pandemic infection of respiratory viruses such as SARS, influenza and avian influenza causes serious illness, morbidity, mortality, and economic damage.

The impact of influenza infections is a global concern each year when the disease develops in approximately 20% of the world's population. In the United States, influenza infections occur in epidemics each winter, generally between late December and early March. Recent events, including human cases of avian influenza, have heightened awareness of the threat of a pandemic and have spurred efforts to develop plans for its control.

Although vaccination is the primary strategy for the prevention of influenza, there are a number of likely scenarios where vaccination is inadequate and effective antiviral agents would be of the utmost importance. During any influenza season, antigenic drift in the virus may occur after formulation of the year's vaccine has taken place. This is reducing the vaccine's protection, thus outbreaks could occur more easily among high-risk populations. In the course of a pandemic, vaccine supplies would be inadequate. Vaccine production by current methods cannot be carried out with the speed required to halt the progress of a new strain of influenza virus. Therefore, it is likely that a vaccine would not be available for the first wave of the spreading virus.

Pandemics result from the emergence of an influenza virus strain that a large numbers of the population have not been exposed, a virus that would be transmitted readily from person to person and a virus that would cause human disease. Highly pathogenic avian influenza A (H5N1) has now fulfilled two of these three criteria. H5N1 viruses are expanding their mammalian host range. There are sporadic human infections that have high fatality rates. This is continuing to occur in Vietnam, Thailand, and Cambodia. These events increase the alarming likelihood that there will be an opportunity for further adaptation of H5N1 to a human host[1-6].

Correspondence to : Saenghirunvattana S, Samitivej Sukumvit Hospital, Bangkok 10110, Thailand.

The expanding geographic distribution of avian influenza A (H5N1) infections, with recent outbreaks in Kazakstan, Mongolia, and Russia, indicates that more human populations are at risk[1,2].

The authors' hospital is a private hospital. Each day there are approximately 30% of the 1,000 out patients that are foreigners including Japanese, Europeans, and Americans. These foreigners travel around the world on business trips often, risking spreading a disease more rapidly.

In 2005 and 2006, the authors planned to set an alarm system, for early detection of the infections, to help control diseases. Influenza, avian flu, and SARS must be detected early to be contained. Therefore, the authors included foreigners, who travel and may carry the viruses into the country, in an alarm system. The authors also planned for a vaccine production by using the data obtained in 2005-2006.

Material and Method

Specimens from a nasopharyngeal swab or bronchial washing was dropped in the special media provided by the Department of Medical Sciences and transported at 4°C to the laboratory for detection of influenza A, B, parainfluenza, adenovirus, respiratory syncytial virus, avian flu, and SARS (if requested) by using the technique of PCR and immunofluorescence.

Cell culture isolation

Swabs were placed in viral transport media (VTM) and immediately chilled and transported to the Thai National Influenza Center (NIC). Upon receipt, the specimens were centrifuged at 3,000 rpm at 4°C for 30 minutes. Supernatant was then inoculated into MDCK (Madin Darby Canine Kidney) cells. The cell culture was observed daily for cytopathic effect (CPE), characterized by a rounding of cells starting in small clusters and spreading to effect the entire cell sheet[7].

Immunofluorescence assay (IFA)

The infected cells were smeared onto a multiwell slide and fixed chilled acetone for IFA assay. For influenza typing, two pools of WHO specific monoclonal antibodies against influenza type A and B anti-mouse IgG FITG conjugate were stained on the slides. Then the slides were examined under fluorescence microscope at 100-200X. The infected cells exhibited the green apple of fluorenscein isothicyanate (FITC). Influenza A positive cell cultures were further identified H1, H3 or H5 using WHO monoclonal antibodies against these subtypes by IFA[8].

RT-PCR method

RNA was extracted from all specimens using the Nucleospin RNA virus kit (Machercy Nagel Gmbh & KG, Germany). CDNA was synthesized and subjected for amplification using Qiagen one-step RT-PCR kit. Each two primers sets used to amplify genes specific for influenza A, B subtype H1, H3, H5 neuraminidase, and B-actin. The sizes of amplicons were 244, 820, 724, 1010, 154/159/219, 615 and 629 bp respectively. The positive was demonstrated by the size of the amplicons[9,10]. The protocol was approved by the hospital's ethic committee.

Results

Eighty-nine patients were enrolled between March 2005 and April 2006. This included 85 adult patients that ranged in age from 18 to 92 years old (average age 42.7 years) and four children that ranged in age from 5 to 12 years old. Among the adult, there were 62 males (73%) and 13 females (27%). History of smoking was quite high (85%). Forty-one Japanese (48%) participated in this study.

Only influenza A (11%) and B (4%) were detected in the present study.

Table 1 demonstrates the high incidence of the influenza infection. The influenza infection peaked to 33% in September 2005. After November, the authors

Table 1. Demonstrates the incidence of influenza A, B from March 2005 to April 2006

Month	H_3N_2	B	Positive	Total	%Yield
2005					
March	-	2	4	14	30
April	2	-	3	7	29
May	3	-	2	8	25
June	2	-	1	6	17
July	1	-	0	4	0
Aug	-	1	1	7	14
Sep	-	-	1	3	33
Oct	1	-	0	2	0
Nov	-	1	2	9	22
Dec	1	-	0	7	0
2006					
Jan	-	-	0	4	0
Feb	-	-	0	9	0
March	-	-	0	8	0
April	-	-	0	1	0
Total	10	4	14	89	16

could not detect either influenza A or B till the end of the study in April 2006.

There were no cases of parainfluenza virus, adenovirus, suspected, and respiratory syncytial virus. Furthermore, there were no cases of avian flu and SARS. Interestingly, other than nasopharyngeal swab, 60% of the adult patient's specimens came from bronchial washings. This had not been done elsewhere.

Discussion

The reason that there was no incidence of influenza infection between December 2005 and April 2006, in the presented population, may be due to the authors' heavy campaign of influenza vaccination in early 2005.

Two elderly patients with COPD (chronic obstructive pulmonary disease) developed acute respiratory distress syndrome, were intubated in the intensive care unit, and survived. This is due to early administration of oseltamivir. Both of the patients' bronchial washing specimens revealed positive for influenza A.

Three patients who got influenza A infection in April 2005 were members of the same family.

The authors also compared the incidence of isolation in Samitivej Hospital (Table 2) from 1996 to 2000. During that period, other than influenza A and B, various types of viruses were also identified such as adenovirus, respiratory syncytial viruses, and para-influenza. The authors were not able to identify even a single isolate at this time.

Table 2. Demonstrates the viral causes of acute pharyngitis, rhinitis in 719 patients from April 18, 1996 to July 12, 2000

	Positive virus N (%)
Total case	296 cases (41.17%)
Influenza A	159 (53.71)
Influenza B	87 (29.39)
Adenovirus	25 (8.44)
RSV	23 (7.77)
Para-influenza type III	11 (3.71)
RSV Sub gr. B	7 (2.36)
Para-influenza type I	5 (1.69)
Total viruses	317*

* There were two viruses in one patient = 21 cases
The age range 4 days - 60 years

The recent data reinforced the authors' belief that the important viral isolates are still influenza A and B, and this will offer updated information for local governments to develop their own vaccines.

Conclusion

Fortunately, the two patients who developed acute respiratory distress syndrome, survived. One important part could be the early administration of antiviral drugs. Therefore, antiviral agents form an important part of a rational approach to epidemic influenza and are critical to planning for a pandemic.

The authors could not detect influenza infection between December 2005 and April 2006. This may be due to the mass campaign of vaccination. Vaccination remains the primary strategy for the prevention of influenza, and the broadened recommendations should lead to protection of a larger portion of the population. However, although the neuraminidase inhibitors clearly cannot substitute for vaccination, they can be valuable in developing information for the government to launch its own vaccines.

Acknowledgement

The authors wish to thank Mrs. Sandra Miller for her help in preparing this manuscript.

References

1. Chen H, Smith GJ, Zhang SY, Qin K, Wang J, Li KS, et al. Avian flu: H5N1 virus outbreak in migratory waterfowl. Nature 2005; 436: 191-2.
2. Liu J, Xiao H, Lei F, Zhu Q, Qin K, Zhang XW, et al. Highly pathogenic H5N1 influenza virus infection in migratory birds. Science 2005; 309: 1206.
3. Ungchusak K, Auewarakul P, Dowell SF, Kitphati R, Auwanit W, Puthavathana P, et al. Probable person-to-person transmission of avian influenza A (H5N1). N Engl J Med 2005; 352: 333-40.
4. Stohr K. Avian influenza and pandemics - research needs and opportunities. N Engl J Med 2005; 352: 405-7.
5. World Health Organization. Cumulative number of confirmed human cases of avian Influenza A/ (H5N1) reported to WHO, 2005. [Accessed 2005 Sep 2). Available at: http://www.who.int/csr/disease/avian_influenza/country/case_table_2005_ 08/en/index.html)
6. Monto AS. The threat of an avian influenza pandemic. N Engl J Med 2005; 352: 323-5.

การติดเชื้อไข้หวัดใหญ่ ระหว่างปี พ.ศ. 2548 - พ.ศ. 2549 ในผู้ป่วยโรงพยาบาลสมิติเวช

สว่าง แสงหิรัญวัฒนา, พิบูลย์ เลาหทัย, ปราณี ธวัชสุภา, รุ่งเรือง กิจผาติ, นฤมล มาสกุล, วิมลทิพย์ จตุรพันธุ์สถาพร

ระหว่างปี พ.ศ. 2548 - พ.ศ. 2549 ได้ทำการตรวจผู้ป่วยติดเชื้อทางเดินหายใจ โดยส่งเสมหะและน้ำล้างปอดตรวจหาไวรัส ไข้หวัดใหญ่ A, B พบอุบัติการณ์สูงสุด 43% ในเดือนเมษายน พ.ศ.2548 และไม่พบอีกเลย ตั้งแต่ ธันวาคม พ.ศ. 2548 จนถึง เมษายน พ.ศ. 2549 น่าจะเป็นผลจากการรณรงค์ฉีดวัคซีนไข้หวัดใหญ่หรือ ลดลงตามฤดูกาล