Epidemiological and Serological Surveillance of Human Pandemic Influenza A Virus Infections during 2009–2010 in Thailand

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SUMMARY: Since April 2009, the outbreak of human pandemic influenza A (H1N1) virus (pH1N1) infection has spread from North America to other parts of the world, and currently, pH1N1 is the predominant circulating strain of influenza viruses. Our objectives were to perform a serological survey of medical personnel at the Chumphae Hospital in Thailand and to investigate the prevalence of pH1N1 in randomly selected patients diagnosed with respiratory tract disease. Prevalence of pH1N1 in the patients was determined by performing real-time reverse transcription-polymerase chain reaction. The study was carried out between July 2009 and November 2010. Seroprevalence of hemagglutination inhibition (HI) titers among medical personnel was established in three cross-sectional studies at the end of each wave of the pandemic by performing HI assay to detect antibodies against pH1N1. Infection by the pH1N1 peaked between July and October 2009; the second wave was from January to March 2010 and the third wave from June to November 2010. The HI titers after the first, second, and third waves were 48.2%, 22.4%, and 25.7%, respectively. After the second and third waves, 52.1% and 45.3% of the medical personnel who had received pH1N1 vaccination had HI titers ≥ 40. These findings show that seasonal influenza strain in Chumphae and the predominant influenza strain from each wave was pH1N1. HI assay results also represent the severity of the attack rate in each wave.

INTRODUCTION

Since April 2009, the outbreak of human pandemic influenza A (H1N1) 2009 virus (pH1N1) infection has spread from North America to other parts of the world, and this H1N1 strain has replaced the currently circulating seasonal influenza virus. On September 10, 2010, the World Health Organization announced the beginning of the post-pandemic period of pH1N1 infection (1).

In Thailand, the first confirmed case of pH1N1 infection was reported on May 12, 2009 by the Bureau of Emerging Infectious Diseases, Department of Disease Control, Ministry of Public Health (2). In the outbreak, pH1N1 cases were reported predominantly from schools and crowded communities; the high infection rate among children and young adults may be the result of group activities in schools (3).

Symptoms of pH1N1 infection are usually mild and similar to those of common cold, which presents with fever, headache, sore throat, and nasal congestion. However, patients with pH1N1 infection having underlying complications or chronic diseases are at an increased risk of hospitalization. The new strain of H1N1 has resulted from triple reassortment of human, avian, and swine influenza viruses, and thus, is antigenically distinct from the currently circulating strains of seasonal influenza H1N1 virus (4,5).

Hemagglutinin (HA) and neuraminidase (NA) are important glycoproteins on the surface of influenza viruses, and these act as surface antigens that can induce an immune response. Hence, administration of antibodies against these surface antigens can protect the host from infection; however, if the existing antibodies are directed against a different antigenic type or subtype of the influenza virus, they might not protect the host from the antigenic variant of the virus. The pH1N1 spread worldwide because previously developed antibodies to the antigenic site of this particular virus were not present in most of the people (6,7).

Protective antibody titers against an infection would develop either in response to annual vaccination or because of natural infection with the virus. Vaccination against circulating strains, which usually change every year because of antigenic drift in the virus, would optimize protection against the viral infection.

In this study, we conducted a cross-sectional serological survey of medical personnel at the Chumphae Hospital, Khon Kaen province, Thailand, by performing the hemagglutination inhibition (HI) assay. These patients represented the high-risk group. We also investigated the prevalence of pH1N1 infection by performing real-time reverse transcription-polymerase chain reaction (real-time RT-PCR) on nasal or throat swab samples obtained from randomly selected patients diagnosed with respiratory tract disease.
MATERIALS AND METHODS

Specimen collection: Chumphae district of Khon Kaen province in northeast Thailand was chosen as the site for epidemiological studies and surveillance on pH1N1 as it represents a suburban area of Thailand. The research protocol was approved by the Ethics Committee of the Faculty of Medicine, Chulalongkorn University. Permission for pH1N1 surveillance was granted by the director of the Chumphae Hospital. The participants were informed about the objective of this study, and their written consents were obtained. All the specimens and sera were sent to the Center of Excellence in Clinical Virology, Faculty of Medicine, Chulalongkorn University within 48 h of collection and were stored at −20°C until analysis.

Nasopharyngeal/throat swab sample collection: Nasopharyngeal or throat swab samples were collected on a weekly basis between July 2009 and November 2010. Samples were collected from approximately 20 patients (15 outpatients and 5 inpatients), who presented with symptoms of acute respiratory tract infection such as fever, sore throat, cough, rhinorrhea, and nasal congestion. The samples were then transported to the Center of Excellence in Clinical Virology in a viral transport medium (placed on ice) containing antibiotics (penicillin G, 2 × 10⁶ U/L and streptomycin, 200 mg/L) within 48 h of collection.

The samples were screened for pH1N1, human seasonal influenza A (H1N1 and H3N2) viruses, and influenza B virus by performing real-time RT-PCR analysis of the samples.

Study populations for HI antibody testing: (i) Medical personnel: Cross-sectional serum samples were obtained from medical personnel of the Chumphae Hospital, including cleaners, electricians, and people who volunteered after every wave of the pandemic influenza. After the first wave in the second week of December 2009, 255 samples were obtained. After the second wave in June 2010, 397 serum samples were collected, and 366 serum samples were collected after the third wave of the pandemic in December 2010. The participants were volunteers. In addition to the blood samples collected, pH1N1 vaccination history, health status, and informed consents were also obtained from the participants.

Laboratory methods: (i) Detection of influenza viruses by real-time RT-PCR: RNA was extracted from 200 μL of the nasopharyngeal swab using the Viral Nucleic Acid Extraction Kit (HiYield™; RBC Bioscience Corp., Taipei, Taiwan) according to the manufacturer’s instructions. The influenza virus was detected using the extracted RNA as a template. Primers, specific TaqMan probes (BioDesign Co., Thailand), and thermal profiles for the reaction were the same used in previous studies (3,8,9). Real-time RT-PCR was performed using the SuperScript III Platinum One-Step Quantitative RT-PCR system (Invitrogen, Foster City, Calif., USA) in Rotor-Gene 3000 (Corbett Research, New South Wales, Australia).

(ii) Influenza virus propagation: The virus used in this study was A/Thailand/CU-H88/09 (accession numbers, HM446345 and HM446344). The virus was propagated in the allantoic cavity of 10-day-old embryonated chicken eggs placed in an egg incubator at 36.5°C for 48 h. Allantoic fluid was then harvested, and the virus titers were determined by the HI assay.

(iii) HI assay: The serum samples were treated with receptor destroying enzyme (RDE) obtained from the bacterium Vibrio cholerae Ogawa type 558 (Denka Seiken, Tokyo, Japan) according to the manufacturer’s instructions. The sera were diluted tenfold with phosphate buffered saline (PBS). RDE-treated sera were twofold serially diluted in a V-shaped 96-well plate (Greiner Bio-One GmbH, Kremsmuenster, Austria) and incubated with 25 μL of 8 HA units of virus for 30 min; this was followed by the addition of 50 μL of 0.5% turkey erythrocytes to the sera and incubation at room temperature for 30 min (10). Samples with HI titers greater than or equal to 1:40 were considered seropositive.

Statistical analysis: We calculated the geometric mean titer (GMT) for the titer values obtained from the HI test for medical personnel who enrolled in HI test only; HI titers below 40 were excluded from the calculation. Analysis was performed using SPSS software, version 17 for Windows. P-value < 0.05 was considered statistically significant.

RESULTS

Detection of influenza viruses using real-time RT-PCR: In total 1,355 patients with age ranging from 25 days to 86 years were screened for influenza virus infections. The percentage of influenza cases diagnosed by performing real-time RT-PCR is shown in Fig. 1. During the study, there were three waves of influenza activity dominated by pH1N1. The activity of pH1N1 peaked for the first time between July and October 2009 (11), for the second time between January and March 2010, and for the third time between June and September 2010. By October 2010, influenza activity had started to decrease.

HI assay: Table 1 shows the characteristics of the medical personnel observed after every wave of the pandemic influenza. The percentage of antibody-positive medical personnel is shown in Table 2.

HI antibodies against pH1N1 after the first wave: The HI assay was performed on samples collected from 255 medical personnel; 123 samples (48.2%) showed positive HI titers (HI titers ≥ 40) against pH1N1. Mean age was 34.0 years (range, 19–56 years). GMT after the first wave was 62.8 (95% CI, 49.2–76.3). Results of comparison between the risk groups have been reported elsewhere (11).

HI antibodies against pH1N1 after the second wave: Of the 397 study participants, 89 (22.4%) showed positive HI titers. Their mean age was 47.6 years (range, 14–87 years). GMT of medical personnel after the second wave was 117.2 (95% CI, 69.4–164.9). Of the 83 samples obtained from the participants who had been tested after the first wave for the presence of antibody against pH1N1, 26 (31.3%) showed HI titers against pH1N1 (GMT = 56.6). However, the results of the assay conducted in June 2010 for HI titers against pH1N1 showed that only 6 participants of this cohort had antibody titers against pH1N1 (GMT = 89.8).

Monovalent pH1N1 vaccination (calculation of GMT 3 months after vaccination): Seventy-one participants
Table 1. Demographic data of medical personnel

<table>
<thead>
<tr>
<th>Age Group</th>
<th>First wave</th>
<th>Second wave</th>
<th>Third wave</th>
</tr>
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<tbody>
<tr>
<td>15–30</td>
<td>115</td>
<td>68</td>
<td>29</td>
</tr>
<tr>
<td>31–40</td>
<td>76</td>
<td>66</td>
<td>33</td>
</tr>
<tr>
<td>&gt;40</td>
<td>64</td>
<td>263</td>
<td>304</td>
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<table>
<thead>
<tr>
<th>Sex, no. (%)</th>
<th>First wave</th>
<th>Second wave</th>
<th>Third wave</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>59 (23.1)</td>
<td>90 (22.7)</td>
<td>76 (20.8)</td>
</tr>
<tr>
<td>Female</td>
<td>196 (76.9)</td>
<td>307 (77.3)</td>
<td>290 (79.2)</td>
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</table>

<table>
<thead>
<tr>
<th>pH1N1 monovalent vaccination, no. (%)</th>
<th>First wave</th>
<th>Second wave</th>
<th>Third wave</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>71 (17.9)</td>
<td>75 (20.5)</td>
<td>75 (20.5)</td>
</tr>
<tr>
<td>No</td>
<td>326 (82.1)</td>
<td>291 (79.5)</td>
<td>304</td>
</tr>
</tbody>
</table>

had been vaccinated with pH1N1 monovalent vaccine (Sanofi Pasteur, Lyon, France) between February and March, 2010. Mean age was 51.1 years (range, 21–75 years), and 37 (52.1%) of the 71 participants had a positive titer in the HI test. GMT of the vaccinated participants was 166.1 (95% CI, 68.1–264.1). In the unvaccinated participants (n = 326), 52 had positive protective HI titers (>40), with a GMT of 91.4 (95% CI, 55.4–127.4). GMT of the vaccinated cohort was significantly higher than that of the unvaccinated group (P < 0.05).

**HI antibodies against pH1N1 after the third wave:** In total 94 of the 366 medical personnel showed positive HI titers (25.7%). The GMT for the medical personnel (mean age, 54.5 years; range, 17–86 years) calculated after the third wave was 104.3 (95% CI, 69.1–139.6). GMT of the medical personnel who had received the pH1N1 monovalent vaccine was 94.8 (95% CI, 65.6–124.0). Of the medical personnel who had not been vaccinated (n = 291), 74 had protective HI titers (>40), with a GMT of 97.4 (95% CI, 53.9–140.9). There was a significant difference between the GMT of the vaccinated and unvaccinated groups (P < 0.05).

**DISCUSSION**

In this study, we investigated the prevalence of pH1N1 from July 2009 to November 2010. The pH1N1 strain of influenza virus has become the predominant influenza strain circulating in the Chumphae district and has taken the place of the circulating seasonal influenza strains (influenza A/H1, A/H3, and B) since July 2009. The pH1N1 has shown a tendency to continue its activity and become the standard circulating seasonal strain in the future. In Thailand, influenza activity occurs in a biphasic pattern with sporadic infection throughout the year (12) as seen in other temperate countries of the northern hemisphere. Influenza is more prevalent in the rainy season than in the winter, as has been observed in other tropical countries (13).

Serological tests were performed on medical personnel who represented the high-risk group susceptible to infectious disease. Furthermore, if the medical personnel acquire an infection, it may be transmitted to patients, such as those with underlying chronic medical conditions, pregnant women, and immunosuppressed and immunocompromised patients, who are at a high risk of acquiring infections.

The baseline serum samples collected from individuals in Chum Saeng district, Nakorn Sawan Province in 2008 had shown very low positive HI titers against pH1N1 (11). Furthermore, tests showed no cross-reactivity between the seasonal influenza virus and the H1N1 virus in the HI assay, even though pH1N1 are antigenically distinct from the current seasonal strains and vaccination against the seasonal strain cannot provide protection against pH1N1 (14). In the previous study, HI assay performed after the first wave in the general population that had a lower risk of infection than the medical personnel did, showed that 36% of the study participants exhibited HI titers against pH1N1 with high seroprevalence in the age group of 11–20 years (11).

According to a previous study (11), 48.2% (123/255)
of the study participants developed HI antibody titers after the first wave, while 22.41% (89/397) of the participants developed the same against pH1N1 after the second wave that occurred 6 months later. After the third wave, 25.7% of the participants showed HI antibody titers. The HI titers after the first wave were high, and this can be explained by the fact that pH1N1 had spread globally in 2009. Although serum samples were collected 4 months after the first wave had peaked, at that point in time, the majority of the population had not developed antibodies against the virus. After the second and third waves, the HI titers were lower than that observed after the first wave, possibly because a proportion of the population had been infected during the first wave and had developed immunity against pH1N1.

Mean age of the participants in the first wave was lower (34.0 years) than that of the participants in the second and third waves (47.6 years and 54.5 years, respectively). The number of participants in the age group of 15–20 years in the first wave was higher than that in the second and third waves, and the number of participants in the age group above 40 years was lower in the first wave than in the second and third waves. This might also explain the higher HI titers after the first wave, as the pH1N1 infection rates were higher among the young adults as shown in Table 2. Seroprotective HI titers observed in the age group of 15–30 years were higher than those observed in other age groups during all the three waves.

This phenomenon can also be explained by the persistence of antibodies against HA, whose titers can decrease to two- and tenfold relative to the peak titers observed after the first 6 months (7). Hence, antibodies produced during the first wave did not persist long enough to be detectable during the second wave, and consequently, the overall rate of seropositive samples was low. On the other hand, the percentage of HI titers correlated with the pH1N1 peak during the second and third waves because HI titers observed at the peak of the second and third waves were lower than those in the first wave, indicating low infection rate. This may be another reason why HI titers observed after the first wave were higher than those observed after the two subsequent waves.

GMT calculated after the first wave (62.8) was lower than that calculated after the second (117.2) and third waves (104.3), indicating that although the number of persons who developed immune response during the second wave was low, GMT of the HI titers was higher after the second wave than after the first wave. This finding may be attributed to a booster effect in the individuals, since serum samples were collected after the peak of the second and third waves.

In the group that had the second blood sample drawn from the same participants (83/397), 26 were seropositive as per the previous HI assay; however, only 6 (21.4%) participants displayed HI titers after the second wave. This phenomenon also showed that the levels of the protective antibody had declined over time. The incubation period of the influenza virus during an infection is rather short (2–5 days); however, the body’s im-
mune response, which includes the release of antibodies, is initiated a few weeks after the onset of infection, and lasts for short durations. To optimize protection against influenza infections, protective antibodies against circulating influenza strains are required. Hence, annual influenza vaccination is required to assure that a rapid and effective immune response can be initiated in the hosts.

Of all the participants who had received 1 dose of pH1N1 monovalent vaccine after the second wave, 52.1% showed seroprotective antibody levels (HI titers of at least 40). This showed that annual influenza vaccination can induce an immune response against the virus. However, in the third wave, only 45.3% of the participants showed seroprotective titers, since the influenza vaccination period was from February–March 2010. This showed a decline in the seroprotective levels of the antibodies after 6 months; this finding was in contrast to that of a US trial (15) in which more than 93% of the adults below the age of 65 years showed seroprotective HI levels after 1 dose of the monovalent pH1N1 vaccine.

HI assay is used to screen patients for seroprotective antibody levels and to estimate the infection rate in the population by measuring the antibodies against HA, which is the major target for most of the neutralizing antibodies. HI assaying is widely used for influenza epidemiological surveillance and vaccine studies because it specifically detects HA. In contrast, microneutralization assays are more sensitive, but expert laboratory staff are required to ascertain health and safety (16).

In conclusion, to determine the seroprevalence in a defined population, a serological surveillance study was conducted to estimate changes in the seroprotective antibody titers due to the viral infection occurring in pandemic waves. Continuous epidemiological surveillance of pH1N1 prevalence in the population will help understand the pattern of transmission of influenza and the pH1N1 infection and will assist in designing suitable methods of intervention before the next pH1N1 wave.

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Conflict of interest None to declare.

REFERENCES