EPIDEMIOLOGICAL CHARACTERISTICS, CLINICAL PRESENTATION AND DIAGNOSIS AT POINT-OF-CARE DURING THE FIRST WAVE OF THE H1N1 INFLUENZA PANDEMIC IN CAMBODIA

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Abstract. We conducted clinic-based surveillance for influenza virus among cases with acute febrile illness at 9 medical clinics in south-central Cambodia during 2006-2009. Patients greater than or equal to 24 months old presenting with acute fever (>38ºC) were enrolled. In late July 2009, the study identified its first case of pandemic H1N1 (pH1N1) influenza virus infection. The prevalence of pH1N1 infections increased rapidly during August and September and by October, pH1N1 infections had peaked replacing H3N2 as the dominant subtype. The incidence of pH1N1 subsequently decreased, with only one case identified in late December. From late July through December 2009, 42.4% of all influenza cases were caused by pH1N1. Except for headache, less frequently reported among pH1N1-infected patients, patients infected with the pH1N1 reported symptoms (*eg*, cough, diarrhea, vomiting and nausea) similar to seasonal H3N2 and B virus infections. Among children 6 to 12 years old, there was a higher number of hospitalizations campared to other age groups. Identification of influenza virus types A and B using the QuickVue© rapid diagnostic test was found to be equally sensitive for pH1N1 (50.4%), H3N2 (51.7%) and influenza B (53.9%) viruses, although the sensitivity was low among all subtypes. The pH1N1 virus rapidly became the dominant virus subtype in 2009 in Cambodia, but no symptoms consistently distinguished the pandemic strain from other influenza virus subtypes. The QuickVue© test was as sensitive for detecting pH1N1 viral as well as other circulating seasonal influenza viruses.

Keywords: epidemiology, clinical, influenza, pandemic, mortality, Cambodia

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INTRODUCTION

Pandemic H1N1 (pH1N1) is an influenza A virus with genetic characteristics of North American and European swine, avian, and human influenza viruses (Dawood *et al*, 2009). In April 2009, human pH1N1 infections were first identified in southern California, the United States. Those patients were quickly linked to influenza cases that had emerged in Mexico.

Subsequently, this virus spread rapidly from North America to other countries, including Asian nations. For Southeast Asia, the first official report of pH1N1 infections to the World Health Organization (WHO) was in Thailand (WHO, 2009a). Other countries in the region quickly reported cases, including Malaysia (WHO, 2009b), Vietnam (WHO, 2009c), Lao PDR (WHO, 2009d) and Cambodia (WHO, 2009e). By 11 June 2009, the virus was identified across the globe and a pandemic was declared by the World Health Organization.

We have conducted passive surveillance for the etiology of acute febrile illnesses in south-central Cambodia since December 2006. Continuous, daily enrollment of acute febrile patients at out patient clinics provides data for tracking the seasonality and clinical impact of influenza virus infections, as well as providing a population to test new methods of diagnosis, including point-of-care tests.

In this study, we examined several aspects of the pH1N1 infection, particularly those issues of interest to clinicians and public health personnel (Kamigaki and Oshitani, 2010). First, we examined the epidemiological characteristics of this infection, in particular, which ages and in which months are patients likely to be infected. Second, the authors observed the clinical presentation of pH1N1 and noted whether the presentation of this virus differed clinically relative to other seasonal influenza infections, as has been reported elsewhere (Dawood *et al*, 2009; WHO, 2009f; Bautista *et al*, 2010). This data will provide a clinical baseline for evaluating future changes in the severity of pH1N1 as it has been suggested that subsequent waves of pandemic influenza may differ clinically from the initial wave (Morens *et al*, 2010; Potter, 2001). Finally, for clinicians using point-of-care tests designed for detection of H3N2, H1N1, and B viruses, we evaluated whether one of these tests, QuickVue©, was as sensitive and specific for pH1N1 as for detection of H3N2 and B influenza virus subtypes.

MATERIALS AND METHODS

The study methods were outlined in detail elsewhere (Blair *et al*, 2009). In brief, patients enrolled sought care for acute fever at one of nine outpatient clinics. All clinics were located in non-urban areas in south-central Cambodia, about 50 km outside of Phnom Penh, the nation's capitol. Each patient was clinically evaluated by a physician or medical assistant and the patient's presenting symptoms recorded. A patient was enrolled if they had a tympanic membrane temperature >38ºC for at least 24 hours but not longer than 10 days, were greater than 23 months old and informed consent was given by the patient and/or a guardian. At enrollment, a questionnaire and medical examination were carried out, including obtaining a throat and nasal swab. All participants were enrolled as volunteers in accordance with protocols approved by the Institutional Review Board of the US NAMRU2 and the National Ethics Committee for Human Research of the Royal Kingdom of Cambodia's Ministry of Health.

Specimen collection

To obtain the nasal specimen, a dry polyester swab was inserted into the nostril, parallel to the palate, withdrawn and placed in 2 to 3 ml of viral transport media (VTM). For throat swabs, tonsils and posterior pharynx were swabbed and then the swab was placed in VTM. All specimens were received within 24 to 72 hours at our laboratory in Phnom Penh.

Influenza virus detection

Ribonucleic acid was extracted from nasal and throat swabs by QIAamp viral RNA mini kits (Qiagen, Hilden, Germany) according to the manufacturer's instructions and stored at -70ºC. Influenza virus genome was detected using a real-time reverse transcriptase polymerase chain reaction assay (real-time RT-PCR) developed by the US Centers for Disease Control and Prevention to detect influenza A and B viruses and subtypes H1 and H3. In July 2009, a real-time RT-PCR was introduced for the detection of pandemic H1N1 influenza virus that was developed by the US Centers for Disease Control and Prevention and recommended by the WHO (2009g). A Roto-Gene 6000 real-time thermocycler (Corbett Life Science, Sydney, Australia) was used to detect influenza A (M-gene) and B (NS-gene) and subtypes H1 and H3. Pandemic H1N1 influenza was detected (MP-gene) and subtyped (HA-gene) using an Applied Biosystems 7500 real time thermocycler (Life Technologies, Grand Island, NY). While we did not target the neuraminidase gene, we presumed, based on currently circulating subtypes (WHO, 2010), H1 detection indicates H1N1 and H3 detection indicates H3N2.

Point-of-care testing

During the study, physicians were provided a point-of-care test (QuickVue©, Quidel Corporation, San Diego, CA) for the identification of influenza virus infection. Medical personnel were instructed to test patients who presented with respiratory symptoms or an influenza-like illness. Not all patients were evaluated with the point-of-care test. The QuickVue© test identifies influenza type A and B antigens. For each patient, laboratory staff inserted a sterile polyester tipped swab into the left nostril and gently rotated upwards until resistance was met at the level of the turbinate. The swab was then rotated gently against the nasal wall. The swab was then removed and placed in a small clear tube with test reagent solution; the virus particles, if any, were disrupted, exposing internal viral nucleoproteins. An influenza specific test strip was placed in the tube with the reagent solution. If influenza antigens were present, a pink to red test line along with a blue control line appeared indicating a positive result. A pink line above the control line was classified as type A virus and a pink line below the blue line was classified as type B virus. If influenza antigens were absent or at low levels, only the blue control line was present. To ensure proper implementation of the test, clinic laboratory staff initially took digital, color photographs with the patient's identification number printed on the picture. These were re-read by a laboratory technician at NAMRU2. After the clinic laboratory staff showed competency in performing and reading the test, proper use of the test was confirmed by NAMRU2 staff through regular visits and competency assessments.

Definitions

Influenza infection was defined as an enrolled patient, positive by real-time RT-PCR for the presence of influenza virus. Influenza-like illness (ILI) was defined by fever (>38ºC) with two or more of the following symptoms: cough, sore throat, headache, myalgia, or rhinorrhea (Navarro-Mari, 2005). A current smoker was defined as an adult who reported smoking. A current student was anyone 6 to 18 years old who was enrolled in school at the time he or she sought care. Education was defined as a person who did or did not complete six years of classes (*ie*, primary school). Travel outside Cambodia was defined as leaving Cambodia

for another country in the previous two months.

Statistical analysis

As it has been suggested that the clinical characteristic of influenza viruses differs by age of patient (Cox and Subbarao, 1999). The results for each virus subtype (*ie*, pH1N1, H3N2, and B) were stratified by age as follows: 2 to 5 years (preschool), 6 to 12 years (schoolchildren), 13 to 18 years (adolescents), and 19 years and older (adults). A chi-square test was employed to test for statistical differences among proportions within each age group for each clinical feature and a chi-square test of trend was used to examine changes in clinical characteristics by age (Rosner, 2005). The point-of-care test identifies influenza type, not subtype. In this study, we determined if there was a statistically significant difference in the proportion of influenza A viruses identified by rapid test for patients infected with pH1N1 *vs* patients infected with H3N2. We also compared detection of influenza pH1N1 with detection of B viruses. The sensitivity and specificity of the QuickVue© test results were given with 95% confidence intervals based on normal approximation to binomial distribution (Rosner, 2005). Statistical significance was set at $p<0.05$.

RESULTS

Patients

From 1 January through 31 December 2009, 5,857 patients were enrolled. Of these patients, 53.4% were male and 18.6, 23.6, 11.6 and 46.2% were aged 2 to 5, 6 to 12, 13 to 18, and ≥19 years, respectively. Influenza-like illness was identified among 67.0% of these patients.

Of the total enrolled patients, 5,846 (99.8%) were tested for influenza viruses; 0.2% of samples collected were found to be unsuitable for testing due to poor specimen quality. Of the enrolled patients, 818 (14.0%) were PCR positive. Of the influenza viruses, 293 (35.8%), 282 (34.4%) and 243 (29.7%) were subtypes H3N2, pH1N1, and B, respectively. Influenza positive patients were more likely to be male, similar to influenza negative patients (57.1% and 52.8%, $p=0.22$) (Table 1). Influenza positive cases were younger than influenza negative cases ($p<0.001$); 77.1% of influenza cases ≤18 years old compared to 50.0% of influenza negative cases. Patients infected with influenza viruses were more likely to present with an influenza-like illness (91.0% *vs* 63.1%, $p<0.0001$).

Epidemic profile

The first case of pH1N1 infection among enrolled patients occurred 31 July 2009 and the last case occured 11 December 2009 (Fig 1). During this period, which roughly corresponds with the monsoon season, 661 of 3,211 enrolled patients (20.6%) were infected with influenza viruses. During the pH1N1 outbreak, 282 (42.4%), 195 (29.3%), and 188 (28.3%) were positive for subtypes pH1N1, H3N2, and B, respectively. Although seasonal H1N1 was detected in 2008 (Blair *et al*, 2009), no seasonal H1N1 cases were detected during 2009. Among enrolled patients, the prevalence of pH1N1 patients increased rapidly in August and September, and by October 2009, pH1N1 had peaked replacing H3N2 as the dominant subtype. Beginning in November, cases decreased monthly until only three influenza infections -one of each subtype- were detected in late December.

Epidemiological features

The proportion of patients infected with each subtype did not statistically differ by gender or education level (Table 2). Children and adolescents attending school

Table 1
Sex, age, and presence of influenza-like illness among patients infected and not infected with influenza viruses, Cambodia, 2009.

Characteristics	Influenza positive (*n*=818) % (*n*)	Influenza negative (*n*=5,028) % (*n*)	*p*-value
Sex			0.022
Female	42.9 (351)	47.2 (2,372)	
Male	57.1 (467)	52.8 (2,653)	
Age (years)			<0.0001
2 to 5	19.2 (157)	18.5 (931)	
6 to 12	38.4 (314)	21.1 (1,063)	
13 to 18	19.6 (160)	10.3 (518)	
≥19	22.9 (187)	50.0 (2,515)	
Influenza-like Illness			<0.0001
Present	91.0 (744)	63.1 (3,174)	
Absent	9.0 (74)	36.9 (1,854)	

Missing data from each covariate accounts for percentage differences; data may not add up to 100% because of rounding.

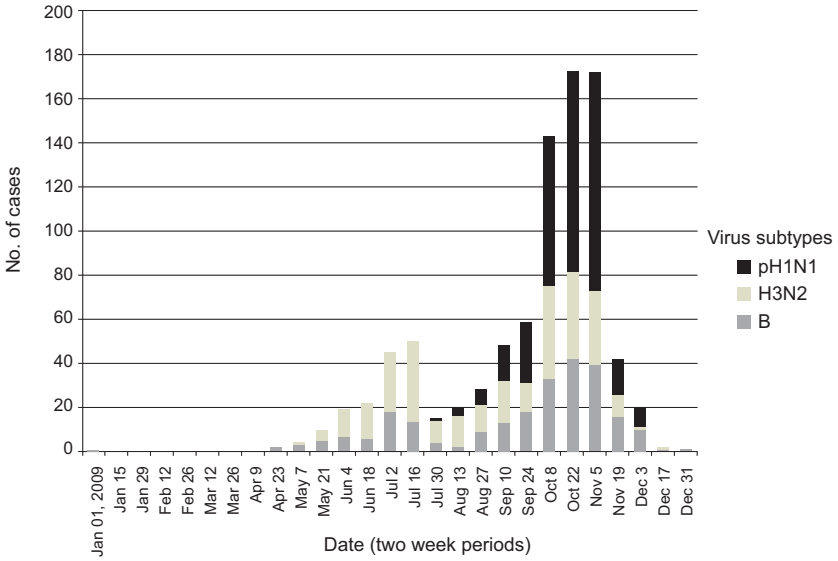

Fig 1–Influenza positive cases by virus subtype, south-central Cambodia, 2009.

were as likely to be infected with any one of the three circulating strains as those not attending school. Only one patient with H3N2 and none infected with pH1N1 or B viruses reported recent travel outside of Cambodia. Among adults, smoking was rare among patients and was not statistically associated with infection of any virus subtype. Persons infected with pH1N1 H3N2 or B were less likely to report they knew someone else who had the same illness than persons infected with H3N2 and

Table 2
Epidemiological and clinical features of pandemic H1N1 virus infections compared to H3N2 and B influenza viruses, 30 July to 31 December 2009, Cambodia.

	Preschool children 2 to 5 years			School children 6 to 12 years			Adolescents 13 to 18 years			Adults 19 years and older		
	pH1N1	H3N2	B	pH1N1	H3N2	B	pH1N1	H3N2	B	pH1N1	H3N2	B
Epidemiological and clinical features	(*n*=32)	(*n*=49)	(*n*=43)	(*n*=96)	(*n*=71)	(*n*=77)	(*n*=63)	(*n*=34)	(*n*=38)	(*n*=90)	(*n*=39)	(*n*=29)
Female	53.1	40.8	48.8	40.6	39.4	45.5	41.3	35.3	34.2	46.7	43.6	27.6
Completed primary education (Yes)	NA	NA	NA	NA	NA	NA	38.1	26.5	28.9	43.3	33.3	27.6
Currently student (Yes)	NA	NA	NA	93.8	94.4	87.0	84.1	85.3	89.5	NA	NA	NA
Traveled outside Cambodia	0	0	0	0	0	0	0	0	0	0.0	2.6	0.0
Knew someone with same illness	3.1	20.4	30.2*	7.3	22.5	20.8*	9.5	11.8	15.8	5.6	7.7	17.2
Currently smoking	NA	NA	NA	NA	NA	NA	NA	NA	NA	3.3	0.0	3.4
Influenza-like illness	96.9	95.9	97.7	95.8	93.0	94.8	96.8	97.1	94.7	86.7	92.3	89.7
Mean temperature (°C)	39.2	39.1	39.3	39.1	39.0	39.0	39.1	39.1	39.1	39.0	39.0	39.0
Days since illness began, median	3	3	3	3	3	3	3	3	3	3	3	4
Sore throat	50.0	65.3	60.5	69.8	70.4	72.7	77.8	79.4	86.8	76.7	79.5	75.9
Cough	87.5	93.9	93.0	93.8	85.9	90.9	88.9	85.3	84.2	83.3	84.6	82.8
Headache	40.6	55.1	69.8*	67.7	77.5	77.9	71.4	88.2	92.1*	83.3	87.2	96.6
Nausea and/or vomiting	3.1	20.4	20.9	16.7	16.9	16.9	7.9	17.6	15.8	26.7	20.5	13.8
Diarrhea	9.4	2.0	4.7	1.0	1.4	1.3	1.6	2.9	0.0	2.2	2.6	0.0
Shortness of breath	6.3	2.0	7.0	2.1	2.8	0.0	1.6	2.9	0.0	2.2	12.8	6.9*
Muscle aches and/or joint pain	0.0	6.1	14.0	13.5	11.3	14.3	22.2	44.1	21.1*	53.3	66.7	62.1
Patients admitted or referred	6.2	4.1	11.6	7.3	1.4	1.3*	0	2.9	0	8.9	7.7	10.3

All values are percents except where stated; NA, not applicable; * $p<0.05$

Table 3
Sensitivity and specificity of Quickvue© influenza A+B test to detect influenza pandemic H1N1 (pH1N1), H3N2, and B viruses identified by polymerase chain reaction, 31 July to 31 December 2010, Cambodia.

Rapid test	Influenza A subtypes		B	Negative	Total
	pH1N1	H3N2			
Influenza A	133 (50.4)[a]	93 (51.7)[a]	7	8	241
Influenza B	1	2	89 (53.9)[a]	9	101
A and B	0	0	4	0	4
Negative	130	85	65	1,977 (99.1)[b]	2,257
Total	264	180	165	1,994	2,603

[a] Number positive (sensitivity); [b] Number negative (specificity)

B; the difference was significant ($p<0.05$) among preschool and schoolchildren.

Clinical features of patients infected with pH1N1, H3N2, and B subtypes

When stratified by age, no significant differences in mean temperature, median time from illness onset to treatment, percent with sore throat, percent with cough, percent with nausea and/or vomiting, percent with diarrhea, percent reporting muscle aches or joint pains was noted. For all subtypes there was a significant association between influenza infection and muscle and/or joint pains in each age group ($p<0.0001$, trend test). In all age groups, persons infected with pH1N1 were less likely to report headache; this was significant among preschool children ($p<0.05$) and adolescents ($p<0.05$). The proportion of pH1N1 patients reporting headache increased with age ($p<0.0001$, trend test). Adults infected with pH1N1 were less likely to report shortness of breath ($p<0.05$) but not the other age groups. Adolescents were less likely to report muscle or joint pains, although there was only a significant difference between persons infected with H3N2 and pH1N1. Children aged 6 to 12 years infected with pH1N1 were more likely to be hospitalized or referred for hospitalization than other age groups.

Evaluation of point-of-care test

From 31 July to 11 December 2009, we evaluated 3,211 patients for influenza with PCR. Of these, 2,604 (81.1%) were also tested using the QuickVue© influenza A+B test (Table 3). Physicians were instructed to use the point-of-care test for patients with an ILI; 81.4% of those tested had an ILI. Persons tested with the rapid test tended to be younger (21.1 *vs* 25.4 years, $p<0.0001$) and less likely to be male (50.7 *vs* 67.4%, $p<0.0001$). Among evaluated patients, the prevalences of pH1N1, H3N2, and B were 10.1, 6.9, and 6.3% (Table 2). The remaining cases were negative (76.6%). The sensitivities of the QuickVue© to identify pH1N1 was 50.4% (95% CI 44.4-56.4), H3N2 was 51.7% (95% CI 44.4-58.9), and B viruses was 53.9% (95% CI 46.3-61.4). The specificity was 99.1% (95% CI: 98.6-99.5). There were no significant differences in sensitivity of QuickVue© by subtypes pH1N1, H3N2, and B viruses ($p=0.92$).

DISCUSSION

In this study, we examined the epidemiological characteristics of pH1N1, the clinical characteristics of pH1N1 infections compared with seasonal influenza infection, and tested the sensitivity and specificity of a commercially available point-of-care test frequently used by clinicians. This information will be useful for health and medical personnel.

We identified our first case of pH1N1 infection in late July 2009, approximately three months after initial reports in North America demonstrating the rapid worldwide spread of this respiratory virus. By October 2009, our data showed pH1N1 had replaced H3N2 subtype as the dominant strain in Cambodia. A lack of population-wide immunity to pH1N1 combined with acquired immunity to H3N2 and B subtypes from previous infections in older patients likely explains the rapid increase in pH1N1 cases. No patients reported traveling outside Cambodia prior to infection possibly implying person-to-person transmission occured among Cambodians residing in south-central Cambodia. Our data suggest children enrolled in school were as likely to be infected with pH1N1 as other subtypes, which could suggest pH1N1 was not more common in that environment. Children have a school holiday in August and September and returned to school in October, which limits their risk for exposure at school. Other variables were not associated with different rates of infection, including gender, education, and smoking in adults, although few adults reported smoking.

We noted that patients identified with pH1N1 virus infection were less likely than patients with H3N2 or B virus infections to report knowing a person with a similar illness. Since influenza patients are unaware of the infecting virus subtype, this may suggest pH1N1 patients were infected by patients who were asymptomatic or pre-symptomatic, although it has been proposed these symptom-free patients may be of marginal importance to the spread of influenza virus infections (Lau *et al*, 2010). While beyond the scope of this study, it may be appropriate to examine whether influenza infections lose virulence as they are transmitted from the index case to secondary cases within households explaining why early infections sought care but not later infections with pH1N1. These preliminary observations should be investigated with household transmission studies, with the understanding that the provision of antiviral medications to symptomatic contacts would likely limit the ability to assess differences in clinical presentation.

There are reports that pH1N1 influenza virus infections are milder or produce symptoms that are distinguishable from seasonal influenza (Cao *et al*, 2009; Dawood *et al*, 2009; Tinoco *et al*, 2009). Recognizing that our results are from patients with acute fever, a subset that does not include afebrile, pH1N1 infected patients (Gerrard, 2009), our data suggest among preschoolers, schoolchildren, adolescents, and adults that cough, sore throat, nausea and/or vomiting at evaluation does not differ consistently by virus subtype. It has also been stated vomiting and diarrhea were more common in pH1N1 infected patients (Dawood *et al*, 2009), but our data do not support that finding. Adult patients were less likely to report shortness of breath suggesting less serious illness, but this finding was not consistent among other age groups. Schoolchildren were more likely to be hospitalized or referred for hospitalization; this was not observed in other age groups and this age group

did not have any significant differences in presentation (*eg*, cough, fever, shortness of breath) that could explain the increased referrals to tertiary care. This age group should be monitored during the next influenza seasons to determine if there is a consistently increased likelihood of hospitalization. pH1N1-infected patients were more likely to report headache than patients infected with H3N2 and B viruses; this difference was significant among preschool children and adolescents. While the causes of headache are multiple, this finding may imply less sinus inflammation among those with pH1N1 infections than other viral subtypes.

To the best of our knowledge, no previous studies from developing countries have compared the clinical characteristics of pH1N1 patients to patients infected with other seasonal influenza viruses. An influenza study from Wisconsin, USA, comparing pH1N1, seasonal H1N1, and H3N2 infections found the clinical features of pH1N1 were similar to other Influenza A viruses (Belongia *et al,* 2010).

Like other rapid influenza diagnostic tests, the commercially available QuickVue© rapid diagnostic test for influenza antigens does not distinguish among subtypes of influenza A viruses. It has been suggested the sensitivity of QuickVue© and other rapid diagnostic tests varies by subtype (CDC, 2009). A study conducted in the United States using influenza positive specimens from nasopharyngeal or oropharyngeal swabs and provided by state health laboratories found QuickVue© was more sensitive for H3N2 than pH1N1 (CDC, 2009). Test results likely vary depending on the patient's viral titer, time of specimen collection, patient's age, and conditions of transportation and storage prior to testing (CDC, 2009). In this study, we transported the specimens within 24 hours and stored them at -70°C until RNA extraction and PCR testing. The median time from symptoms to seeking treatment was three days. When compared to real-time RT-PCR test results, the sensitivity of detecting influenza A viruses pH1N1 and H3N2 appeared equal and as effective as detecting B viruses.

In conclusion, by the end of 2009, pH1N1 had replaced H3N2 and B viruses as the dominant subtype in Cambodia. Clinically, symptoms of pH1N1 were similar to other circulating influenza subtypes. A rapid diagnostic test had low sensitivity and high specificity but was as accurate for detecting pH1N1 as it was for H3N2 and B viruses.

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