

Seroincidence of Influenza Among HIV-infected and HIV-uninfected Men During the 2009 H1N1 Influenza Pandemic, Bangkok, Thailand

Shikha Garg,¹ Sonja J. Olsen,^{2,3} Stefan Fernandez,⁴ Charung Muangchana,⁵ Kamonthip Rungrojcharoenkit,⁴ Prabda Prapasiri,³ Jacqueline M. Katz,² Marcel E. Curlin,^{1,3} Robert V. Gibbons,⁴ Timothy H. Holtz,^{1,3} Anupong Chitwarakorn,⁶ and Fatimah S. Dawood²

¹Division of HIV/AIDS Prevention and ²Influenza Division, Centers for Disease Control and Prevention, Atlanta, Georgia; ³Thailand MoPH-U.S. CDC Collaboration, Nonthaburi; ⁴Armed Forces Research Institute of Medical Sciences, Bangkok, ⁵National Vaccine Institute, Nonthaburi, and ⁶Department of Disease Control, Ministry of Public Health, Thailand

Among 368 Thai men who have sex with men with paired serum samples collected before and during the 2009 H1N1 influenza pandemic, we determined influenza A (H1N1) pdm09 seroconversion rates (≥ 4 -fold rise in antibody titers by hemagglutination inhibition or microneutralization assays). Overall, 66 of 232 (28%) participants seroconverted after the first year of A(H1N1)pdm09 activity, and 83 of 234 (35%) participants seroconverted after the second year. Influenza A(H1N1)pdm09 seroconversion did not differ between human immunodeficiency virus (HIV)-infected (55 of 2157 [35%]) and HIV-uninfected (71 of 2211 [34%]) participants ($P = .78$). Influenza A(H1N1)pdm09 seroconversion occurred in approximately one third of our Thai study population and was similar among HIV-infected and HIV-uninfected participants.

Keywords. A(H1N1)pdm09; HIV; influenza; pandemic; serology; Thailand.

In April 2009, the influenza A (H1N1)pdm09 virus emerged and rapidly gave rise to the first influenza pandemic in 40

years [1]. Serologic surveys have been used to estimate cumulative incidence of infection in populations worldwide. A recent global meta-analysis of A(H1N1)pdm09 serologic surveys estimated an overall incidence of 20% in the first year of virus circulation, with substantial variation across age groups and regions [2]. Because most serologic surveys were based on samples of the general population, data remain limited on the incidence of A(H1N1)pdm09 infection among persons with underlying conditions associated with an increased risk for severe influenza, particularly from developing countries.

Human immunodeficiency virus (HIV) infection increases the risk of severe illness and complications from influenza [3–5], although it is unknown whether HIV infection increases susceptibility to influenza. Data on A(H1N1)pdm09 infection in persons infected with HIV are largely limited to studies from developed countries. Understanding the impact of influenza pandemics and whether susceptibility differs among persons with and without HIV infection could inform global and national prioritization strategies for influenza vaccination, particularly early in an influenza pandemic when global vaccine supply is likely to be limited.

In Thailand, the first laboratory-confirmed cases of A(H1N1)pdm09 infection occurred in the first week of May 2009. Emergence of the virus subsequently resulted in 3 distinct waves of circulation during the first 2 years after being identified in the population (Figure 1) [6]. The A(H1N1)pdm09 monovalent vaccine became available in Thailand in January 2010, and access to the vaccine was limited with only 2 million doses purchased by the government (Thai population, 66 million). Access to seasonal influenza vaccine was also limited in Thailand [7]. We estimated the strain-specific incidence of influenza among a cohort of Thai men who have sex with men (MSM) with and without HIV infection in Bangkok.

METHODS

Setting

Men who have sex with men residing in Bangkok were enrolled into the Bangkok Men's Cohort Study (BMCS) starting in April 2006 as part of an ongoing study to estimate HIV incidence [8]. Serum specimens were collected from BMCS participants at enrollment and every 4 months for those with HIV infection and every 12 months for those without. The BMCS participants consented to storage of specimens for future testing at enrollment. This study was approved by the Institutional Review Board at the Centers for Disease Control and Prevention and the Ministry of Public Health, Thailand.

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Correspondence: Fatimah S. Dawood, MD, Centers for Disease Control and Prevention, 1600 Clifton Road, Mailstop A32, Atlanta, GA 30333 (hgj0@cdc.gov).

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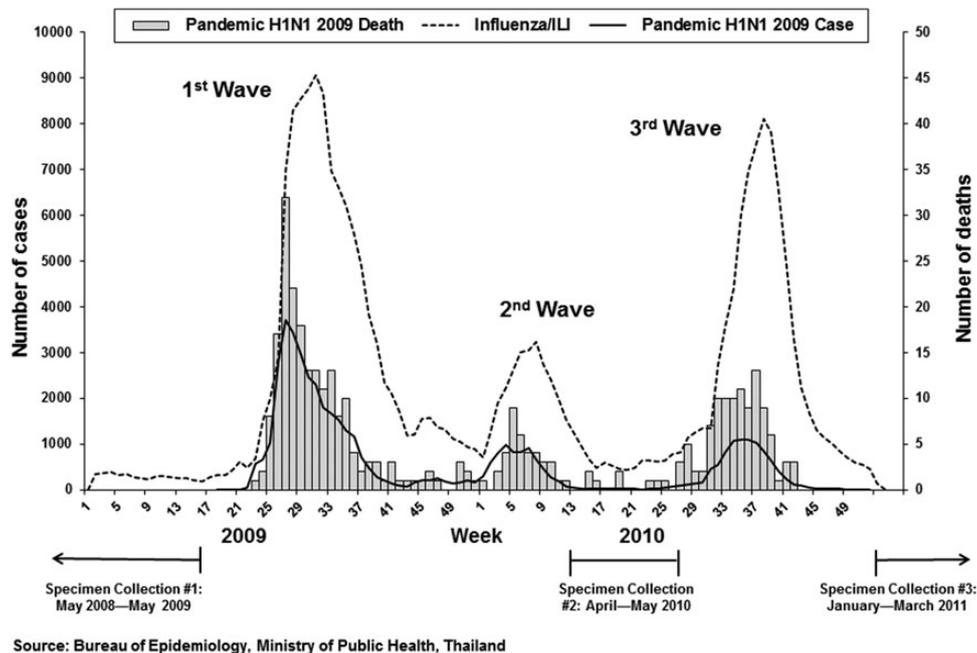


Figure 1. The 3 waves of the 2009 H1N1 influenza pandemic in Thailand based on national Thai surveillance of influenza-like illness, laboratory-confirmed cases of A(H1N1)pdm09 and A(H1N1)pdm09-associated deaths (reproduced with permission from *Siriraj Med J.* 2011;64).

Specimen Selection

Stored serum specimens were selected from BMCS participants who had blood drawn at least once during May 2008–May 2009 and at least once during April–July 2010 or January–March 2011. These time points were chosen to obtain baseline serum specimens before A(H1N1)pdm09 circulation in Thailand and follow-up serum specimens either after the first 2 waves or third wave of A(H1N1)pdm09 circulation (Figure 1) [6]. For participants who had serum specimens available at all 3 time points, paired specimens for after the first waves and after the third wave of the pandemic were treated independently.

Laboratory Testing

Serum was tested by hemagglutination inhibition (HI) assays using guinea pig erythrocytes for antibody response to A/California/08/2009, A/Brisbane/59/2007, A/Perth/16/2009, and B/Brisbane/60/2008 and by microneutralization (MN) assays against A/California/08/2009. Specimens were tested at the Armed Forces Research Institute of Medical Science in Bangkok, Thailand using the standard World Health Organization protocol [9, 10]. For A(H1N1)pdm09, seroconversion was defined as ≥ 4 -fold rise in HI or MN antibody titers and a minimum titer on the second sample of 40. For all other virus types and subtypes, seroconversion was defined as ≥ 4 -fold rise in HI antibody titers only and a minimum HI titer on the second sample of 40. Because several studies have suggested that a minimal titer of 20 may be optimal for assessing seroconversion to novel viruses, we also assessed A(H1N1)pdm09 seroconversion

based on a minimal titer on the second sample of 20 [11, 12]. If a baseline titer was < 10 (lower limit of detection), the titer was set equal to 5 for analytic purposes. Human immunodeficiency virus testing methods for the BMCS have been previously described [8]. Although access to influenza vaccine is limited in Thailand with $< 5\%$ coverage in high-risk groups in 2010 and 2011, it is not known whether BMCS participants received influenza vaccine before baseline serum collection.

Sample Size Calculation

The BMCS contains ~ 500 participants infected with HIV and 1000 HIV-uninfected (negative for HIV at the time of the first sample collection) participants. To obtain representative estimates of influenza seroincidence among HIV-infected and HIV-uninfected participants separately in the BMCS, we needed paired serum samples from a minimum of 141 HIV-infected persons and 164 HIV-uninfected persons after the first 2 waves and after the third wave of the pandemic. These calculations were based on an assumption of a type I error of 5%, an expected cumulative A(H1N1)pdm09 influenza incidence of 15%, and 5% precision.

Analysis

We calculated the proportion of HIV-infected and HIV-uninfected participants who seroconverted after the first 2 waves or after the third wave of the pandemic; participants with specimens collected at all 3 time points were included in both analyses. To increase our sample size and allow for comparisons

between HIV-infected and HIV-uninfected participants, we used χ^2 tests to compare the proportion of participants who seroconverted at any time point for each of the circulating influenza types or subtypes. Participants with specimens collected at all 3 time points were counted only once. Tests were 2-tailed, and a *P* value of .05 was considered significant. Analyses were conducted using SAS, version 9.2 (SAS Institute, Cary, North Carolina).

RESULTS

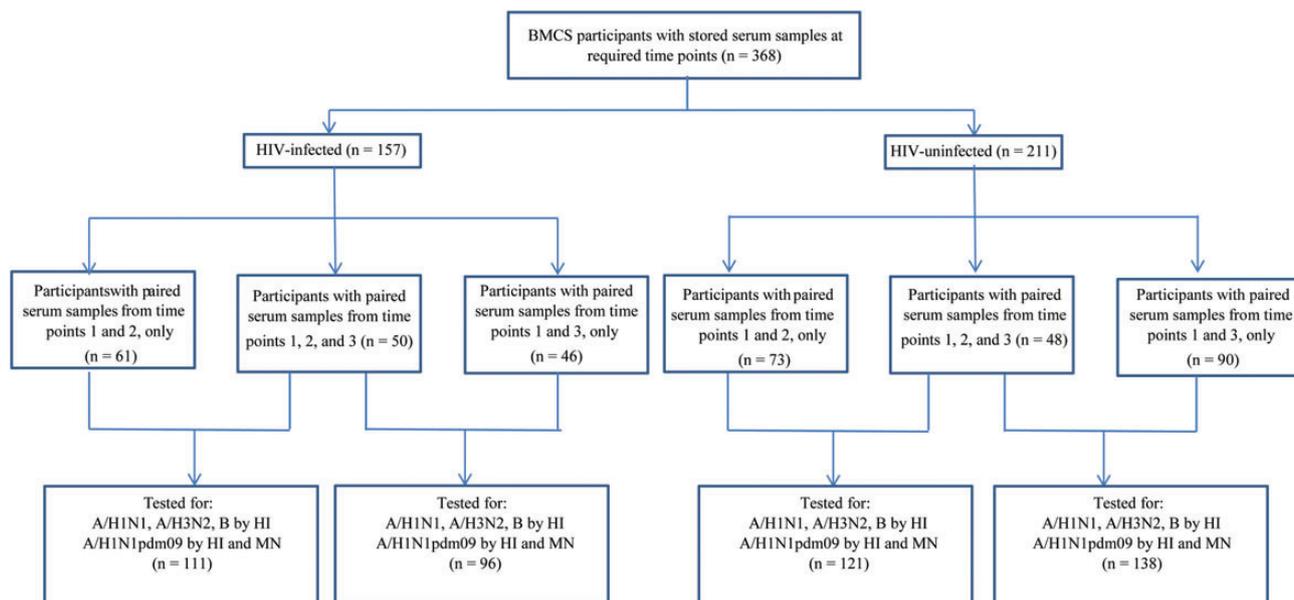
Paired serum samples were available for 368 participants (157 HIV-infected and 211 HIV-uninfected). The BMCS members included in our study did not differ from those not included in our study based on age. Among the 157 HIV-infected men who had samples available from before the pandemic, 61 had paired samples available after the first 2 waves, 46 after the third wave of the pandemic, and 50 after both the first 2 waves and the third wave of the pandemic. Among the 211 HIV-uninfected men who had samples from before the pandemic, 73 had paired samples available after the first 2 waves, 90 after the third wave, and 48 after both the first 2 waves and the third wave of the pandemic (Figure 2).

At the time the first serum sample was drawn, the median age of HIV-infected men was 28 years (range: 19–49 years), and the median age of HIV-uninfected men was 28 years (range: 19–55 years). Among the 157 HIV-infected men, 8 (5%) had CD4 cell

counts <200 cells/mm³, 32 (21%) had CD4 cell counts of 200–350 cells/mm³, and 116 (74%) had CD4 cell counts >350 cells/mm³. No individuals had a CD4 cell count <50 cells/mm³. The median plasma HIV viral load was 28 900 copies/mL (range: 0–1 480 000 copies/mL). Human immunodeficiency virus viral load was <47 copies/mL (lower limit of detection) in 4 (3%) men infected with HIV. Only 19 (12%) men were on antiretroviral therapy. Among the 211 men who were HIV-uninfected at the first serum sample collection, 60 (28%) seroconverted to HIV-infected during the study.

Four influenza virus types and subtypes circulated during the study period: A(H1N1), A(H1N1)pdm09, A(H3N2), and B viruses. Among HIV-infected men, seroconversion by HI against any influenza virus type or subtype was found in 14 of 111 (13%) after the first 2 waves and 12 of 96 (12%) after the third wave of the pandemic. Among HIV-uninfected men, seroconversion by HI against any influenza virus was found in 12 of 121 (10%) after the first 2 waves and 16 of 138 (12%) after the third wave of the pandemic (Table 1).

Among HIV-infected men, cumulative A(H1N1)pdm09 seroincidence was 30 of 111 (27%) after the first 2 waves and 35 of 96 (36%) after the third wave of the pandemic based on HI or MN. Among HIV-uninfected men, cumulative A(H1N1)pdm09 seroincidence was 36 of 121 (30%) after the first 2 waves and 48 of 138 (35%) after the third wave of the pandemic based on HI or MN. Cumulative A(H1N1)pdm09 seroincidence at any time point was similar among HIV-infected and HIV-uninfected



Time point 1: May 2008–May 2009; time point 2: April–July 2010; time point 3: January–March 2011
 HI: hemagglutination inhibition
 MN: microneutralization

Figure 2. Influenza serology testing among BMCS participants with stored serum samples available before and after the first 2 waves and third wave of the 2009 H1N1 influenza pandemic. Abbreviations: BMCS, Bangkok Men’s Cohort Study; HIV, human immunodeficiency virus.

Table 1. Influenza Seroincidence by Hemagglutination Inhibition and Microneutralization Assay After the First Two Waves,^a After the Third Wave,^b and After the First Two Waves or Third Wave of the 2009 H1N1 Influenza Pandemic Among HIV-Infected and HIV-Uninfected Men Who Have Sex With Men: Bangkok, Thailand, 2009–2011

Influenza Virus Type/ Subtype	Influenza Seroincidence Overall (<i>n</i> = 368); no. (%)			Influenza Seroincidence Among HIV-Infected MSM (<i>n</i> = 157); no. (%)			Influenza Seroincidence Among HIV-uninfected MSM (<i>n</i> = 211); no. (%)			<i>P</i> Value ^c
	1st 2 Waves (<i>n</i> = 232)	3rd Wave (<i>n</i> = 234)	1st 2 Waves or 3rd Wave (<i>n</i> = 368)	1st 2 Waves (<i>n</i> = 111)	3rd Wave (<i>n</i> = 96)	1st 2 Waves or 3rd Wave (<i>n</i> = 157)	1st 2 Waves (<i>n</i> = 121)	3rd Wave (<i>n</i> = 138)	1st 2 Waves or 3rd Wave (<i>n</i> = 211)	
Seroincidence by HI										
A (H1N1)pdm09	10 (4)	12 (5)	18 (5)	6 (5)	6 (6)	11 (7)	4 (3)	6 (4)	7 (3)	0.10
A (H1N1)	8 (3)	9 (4)	15 (4)	4 (4)	3 (3)	6 (4)	4 (3)	6 (4)	9 (4)	0.83
A (H3N2)	4 (2)	8 (3)	12 (3)	2 (2)	3 (3)	5 (3)	2 (2)	5 (4)	7 (3)	0.94
B	7 (3)	7 (3)	13 (4)	4 (4)	2 (2)	5 (3)	3 (2)	5 (4)	8 (4)	0.76
Any influenza virus type	26 (11)	28 (12)	47 (13)	14 (13)	12 (12)	23 (15)	12 (10)	16 (12)	24 (11)	0.35
Seroincidence by MN										
A (H1N1)pdm09	66 (28)	83 (35)	126 (34)	30 (27)	35 (36)	55 (35)	36 (30)	48 (35)	71 (33)	0.78
Seroincidence by HI or MN										
A (H1N1)pdm09	66 (28)	83 (35)	126 (34)	30 (27)	35 (36)	55 (35)	36 (30)	48 (35)	71 (34)	0.78

Abbreviations: HI, hemagglutination inhibition; HIV, human immunodeficiency virus; MN, microneutralization assay; MSM, men who have sex with men.

^a Time period after the second wave of the pandemic: April 1, 2010–July 31, 2010.

^b Time period after the third wave of the pandemic: January 1, 2011–March 31, 2011.

^c Based on comparison of influenza seroincidence after first 2 waves or third wave of pandemic combined among HIV-infected and HIV-uninfected MSM.

MSM based on HI alone, MN alone, or HI or MN (Table 1). When the minimal titer required for seroconversion on the second sample was lowered to 20, overall seroconversion against A (H1N1)pdm09 by HI increased from 10 (4%) to 31 (13%) after the first 2 waves and 12 (5%) to 46 (20%) after the third wave. Based on HI testing, 2 (0.5%) men had baseline A(H1N1)pdm09 antibody titers ≥ 40 . Based on MN testing, 91 (25%) men had baseline A(H1N1)pdm09 antibody titers ≥ 40 .

In a subanalysis of 61 men who were classified as HIV-uninfected but who seroconverted to HIV-infected during the study, the cumulative A(H1N1)pdm09 seroincidence was 12 of 38 (32%) after the first 2 waves and 18 of 55 (33%) after the third wave of the pandemic based on HI or MN.

DISCUSSION

Among Thai MSM participating in the BMCS, approximately one third seroconverted to A(H1N1)pdm09 during the first 3 waves of the A(H1N1)pdm09 pandemic, roughly corresponding to the first 2 years of A(H1N1)pdm09 circulation in Thailand. HIV infection did not increase the risk of seroconversion in our study population, in which the majority of men had CD4 cell counts >200 cells/mm³. The cumulative seroincidence of seasonal influenza viruses was similar to A(H1N1)pdm09 during

the first 3 waves of the pandemic, highlighting the added burden of seasonal influenza in Thailand during the pandemic.

Our findings show a higher seroincidence of A(H1N1)pdm09 based on seroconversion by HI or MN than pooled results from a meta-analysis of 12 studies with paired sera that estimated a cumulative A(H1N1)pdm09 seroincidence after the first year of virus circulation of 20% (range: 13%–26%) among persons aged 20–44 years [2]. However, comparisons between the seroincidence rates in our study and those of other studies should be made with caution because most published studies on A(H1N1)pdm09 seroincidence were based largely on studies using HI alone [2]. In comparison to our findings, another Thai study that estimated A(H1N1)pdm09 infection rates in various populations after the first wave of the pandemic using an HI titer ≥ 40 found infection rates of 3% in adults in the general population, consistent with our finding of an A(H1N1)pdm09 seroincidence of 4% after the first wave of the pandemic based on HI alone [13]. Although using a minimal HI titer of 40 as a marker of immunity is standard for serologic studies, several studies have found that in polymerase chain reaction-confirmed influenza A(H1N1)pdm09 infections, the optimal HI titer cut-off value was 20 for identifying persons with prior infection [11, 12]; this lower cutoff increased the positivity of our HI assay 3- to 4-fold and may more accurately reflect true seroconversion rates by HI.

Data on A(H1N1)pdm09 seroincidence among individuals infected with HIV are limited. We found no differences in A(H1N1)pdm09 seroincidence among HIV-infected and HIV-uninfected MSM. Likewise, a US study comparing HIV-infected women with CD4 cell counts ≥ 350 cells/mm³ and HIV-uninfected women found no differences in A(H1N1)pdm09 seroincidence [14]. A Taiwanese study comparing HIV-infected and HIV-uninfected men also found no difference in A(H1N1)pdm09 seroconversion based on HI [15]. In Australia, A(H1N1)pdm09 seroprevalence among HIV-infected persons based on single serum samples was similar to national seroprevalence estimates, and results were not different based on CD4 cell count or HIV viral load [16]. Although our study and prior studies suggest that HIV infection does not confer an increased susceptibility to influenza virus infection, HIV-infected persons in these studies were not severely immunocompromised, making it difficult to draw conclusions about risk among HIV-infected persons with low CD4 cell counts. It is possible that individuals infected with HIV were unable to mount detectable antibody responses after influenza infection due to B-cell dysfunction [17], thus accounting for the lack of differences in susceptibility to influenza infection seen among HIV-infected and HIV-uninfected individuals. However, the fact that seroincidence was similar among HIV-infected and HIV-uninfected individuals rather than higher among HIV-uninfected individuals would suggest that HIV-infected individuals do mount a detectable antibody response to influenza infection. Prior studies have demonstrated that HIV infection confers an increased risk for severe influenza infection, supporting the importance of targeted influenza vaccination among persons infected with HIV [18].

Several points should be considered when interpreting our findings. First, one quarter of men had elevated titers to A(H1N1)pdm09 at baseline based on MN, whereas only 0.5% of men had elevated baseline titers based on HI. The MN findings may suggest that Thai adults had relatively high levels of preexisting cross-reactive antibody to A(H1N1)pdm09 from prior infection with other influenza viruses [19]. However, detection of cross-reactive antibodies to previously circulating influenza strains by MN is unlikely to have impacted our results because we defined A(H1N1)pdm09 infection as evidence of a 4-fold rise in A(H1N1)pdm09 antibody titers using paired sera rather than as a single titer of ≥ 40 , as some prior studies have done based on HI testing results. Second, although A(H1N1)pdm09 seroincidence was similar among HIV-infected and HIV-uninfected MSM, our study may not have had adequate power to detect differences in seroincidence by HIV infection status. Third, A(H1N1)pdm09 seroincidence varies by age group, and our data reflect only seroincidence among younger adults in Thailand. Strengths of our study include testing of paired sera to determine seroincidence rather than just seroprevalence of antibodies to A(H1N1)pdm09 and availability of sera

from several time points allowing estimation of seroincidence after both the first and second years of A(H1N1)pdm09 circulation in Thailand.

CONCLUSIONS

As seen in other populations in many countries, a substantial proportion of Thai MSM in Bangkok seroconverted to A(H1N1)pdm09 during the 2009 pandemic in the absence of A(H1N1)pdm09 vaccine and in the setting of limited availability of seasonal influenza vaccine. Although HIV infection did not appear to increase the probability of A(H1N1)pdm09 seroconversion, the majority of HIV-infected MSM in this study were young and had relatively preserved immune function. It remains unclear whether severely immunocompromised persons infected with HIV are more susceptible to A(H1N1)pdm09 influenza virus and to seasonal influenza viruses in general. Given that persons infected with HIV are at risk for more complicated illness once infected with influenza, these individuals should continue to be prioritized for influenza vaccination during seasonal influenza epidemics and pandemics.

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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