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,5 Prabda Prapasiri,5 Jacqueline M. Katz,1 Marcel E. Curlin,1,3 Robert V. Gibbons,4 Timothy H. Holtz,1,3 Anupong Chitwarakorn,6 and Fatimah S. Dawood4

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.
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 $\chi^2$ 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/mm$^3$, 32 (21%) had CD4 cell counts of 200–350 cells/mm$^3$, and 116 (74%) had CD4 cell counts $>350$ cells/mm$^3$. No individuals had a CD4 cell count $<50$ cells/mm$^3$. 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

---

**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.
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 $\geq 40$. Based on MN testing, 91 (25%) men had baseline A(H1N1)pdm09 antibody titers $\geq 40$.

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/mm$^3$. 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 $\geq 40$ 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-infected 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.

Acknowledgments

We thank Marc-Alain Widdowson for assistance with study design and planning; Wanee Chonwattana for laboratory assistance; Wichuda Sukwicha and Philip Mock for data management assistance; and Dr. Iamsirithaworn Sopon for sharing national Thai surveillance data.

Disclaimer. The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention or the Department of Defense.

Financial support. This work was supported by the US Centers for Disease Control and Prevention.

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

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

References

1. New influenza A (H1N1) virus: global epidemiological situation, June 2009. Wkly Epidemiol Rec 2009; 84:249–57.
2. Van Kerkhove MD, Hirve S, Koukounari A, et al. Estimating age-specific cumulative incidence for the 2009 influenza pandemic: a meta-analysis of A(H1N1)pdm09 serological studies from 19 countries. Influenza Other Respir Viruses 2013; 7:872–86.
3. Cohen C, Moyes J, Tempia S, et al. Severe influenza-associated respiratory infection in high HIV prevalence setting, South Africa, 2009–2011. Emerg Infect Dis 2013; 19:1766–74.
4. Ormsby CE, de la Rosa-Zamboni D, Vazquez-Perez J, et al. Severe 2009 pandemic influenza A (H1N1) infection and increased mortality in patients with late and advanced HIV disease. AIDS 2011; 25:435–9.
5. Sheth AN, Althoff KN, Brooks JT. Influenza susceptibility, severity, and shedding in HIV-infected adults: a review of the literature. Clin Infect Dis 2011; 52:219–27.
6. Iamsirithaworn S, Akarasewi P, Yingyong T, et al. Three Waves of the 2009 H1N1 Influenza Pandemic in Thailand. Siriraj Med J 2011; 63:64–7.
7. Gupta V, Dawood FS, Muangchan C, et al. Influenza vaccination guidelines and vaccine sales in southeast Asia: 2008–2011. PloS One 2012; 7:e52842.
8. van Griensven F, Thienkrua W, McNicholl J, et al. Evidence of an explosive epidemic of HIV infection in a cohort of men who have sex with men in Thailand. AIDS 2013; 27:825–32.

9. World Health Organization. The 2011–2012 WHO Influenza Reagent Kit for Identification of Influenza Isolates. Available at: http://www.influenzareagentresource.org/Portals/6/PDFS/WHO%20Insert%202011-2012.pdf. Accessed 10 December 2013.

10. World Health Organization. WHO Global Influenza Surveillance Network Manual for the laboratory diagnosis and virological surveillance of influenza. Available at: http://whqlibdoc.who.int/publications/2011/9789241548090_eng.pdf. Accessed 31 March 2014.

11. Papenburg J, Baz M, Hamelin ME, et al. Evaluation of serological diagnostic methods for the 2009 pandemic influenza A (H1N1) virus. Clin Vaccine Immunol 2011; 18:520–2.

12. Veguilla V, Hancock K, Schiffer J, et al. Sensitivity and specificity of serologic assays for detection of human infection with 2009 pandemic H1N1 virus in U.S. populations. J Clin Microbiol 2011; 49:2210–5.

13. Lerdsamran H, Pittayawongnon G, Pooruk P, et al. Serological response to the 2009 pandemic influenza A (H1N1) virus for disease diagnosis and estimating the infection rate in Thai population. PLoS One 2011; 6:e16164.

14. Althoff KN, Eichelberger M, Gange SJ, et al. Seroincidence of 2009 H1N1 infection in HIV-infected and HIV-uninfected women prior to vaccine availability. AIDS 2011; 25:1229–32.

15. Hung CC, Zhao TL, Sun HY, et al. Seroepidemiology of novel influenza A (H1N1) infection among HIV-infected patients in the era of highly active antiretroviral therapy. J Microbiol Immunol Infect 2013; 46:151–7.

16. Kok J, Tudo K, Blyth CC, et al. Pandemic (H1N1) 2009 influenza virus seroconversion rates in HIV-infected individuals. J Acquir Immune Defic Syndr 2011; 56:91–4.

17. Cagigi A, Nilsson A, Pensieroso S, et al. Dysfunctional B-cell responses during HIV-1 infection: implication for influenza vaccination and highly active antiretroviral therapy. Lancet Infect Dis 2010; 10: 499–503.

18. Centers for Disease Control and Prevention. Prevention and control of seasonal influenza with vaccines. Recommendations of the Advisory Committee on Immunization Practices—United States, 2013–2014. MMWR Recomm Rep 2013; 62:1–43.

19. Hancock K, Veguilla V, Lu X, et al. Cross-reactive antibody responses to the 2009 pandemic H1N1 influenza virus. N Engl J Med 2009; 361:1945–52.