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An influenza A (H3N2) virus outbreak in the Kingdom of Cambodia during the COVID-19 pandemic of 2020

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ABSTRACT

Background: Global influenza virus circulation decreased during the COVID-19 pandemic, possibly due to widespread community mitigation measures. Cambodia eased some COVID-19 mitigation measures in June and July 2020. On 20 August a cluster of respiratory illnesses occurred among residents of a pagoda, including people who tested positive for influenza A but none who were positive for SARS-CoV-2.
Methods: A response team was deployed on 25 August 2020. People with influenza-like illness (ILI) were asked questions regarding demographics, illness, personal prevention measures, and residential arrangements. Respiratory swabs were tested for influenza and SARS-Cov-2 by real-time reverse transcription PCR, and viruses were sequenced. Sentinel surveillance data were analyzed to assess recent trends in influenza circulation in the community.
Results: Influenza A (H3N2) viruses were identified during sentinel surveillance in Cambodia in July 2020 prior to the reported pagoda outbreak. Among the 362 pagoda residents, 73 (20.2%) ILI cases were identified and 40 were tested, where 33/40 (82.5%) confirmed positive for influenza A (H3N2). All 40 were negative for SARS-CoV-2. Among the 73 residents with ILI, none were vaccinated against influenza, 47 (64%) clustered in 3/8 sleeping quarters, 20 (27%) reported often wearing a mask, 27 (36%) reported often washing hands, and 11 (15%) reported practicing social distancing. All viruses clustered within clade 3c2. A1 close to strains circulating in Australia in 2020.
Conclusions: Circulation of influenza viruses began in the community following the relaxation of national COVID-19 mitigation measures, and prior to the outbreak in a pagoda with limited social distancing. Continued surveillance and influenza vaccination are required to limit the impact of influenza globally.

Introduction

Human seasonal influenza is a vaccine-preventable disease with a global burden of 300,000–650,000 deaths annually (Iuliano et al., 2018). However, during the global coronavirus disease 2019 (COVID-19) pandemic in 2020, there was also a worldwide reduction in influenza virus circulation (WHO, 2020e). It has been hypothesized that this reduction may be attributable to increased social distancing and other community mitigation interventions employed to control COVID-19 worldwide (Olsen et al., 2020).

As of 2 August 2020, the Kingdom of Cambodia (Cambodia) reported 240 confirmed cases of COVID-19 after testing 52,711 individuals. Most cases were reported during March, June, and July in 2020 (WHO, 2020b). Similar to many other countries in Southeast Asia, Cambodia implemented community mitigation measures, including school/night venue closures in mid-March 2020, and the cancellation of mass gatherings and restrictions on in-country travel in April (WHO, 2020a). The Cambodian Ministry of Health (MoH) also recommended that people stay at home if possible and related prevention messages were communicated to the public via posters, radio, and television. Easing of restrictions on mass gatherings began in June, with other restrictions easing in July, and limited school re-openings began in August 2020.

In Cambodia, influenza virus detection typically increases in May to July (Horm et al., 2014; Horwood et al., 2019), but few

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influenza viruses were detected in this period during 2020 (WHO, 2020d), which coincided with the implementation of COVID-19 mitigation measures. In late July, following the easing of some community mitigation measures, an increase in influenza A (H3N2) detection was measured using these same systems. In addition to increased detection by influenza-like illness (ILI) surveillance systems, several outbreaks of influenza A (H3N2) were detected through event-based surveillance in Cambodia (WHO, 2020d). In this context, we report the results of an investigation of an outbreak of influenza A (H3N2) virus in a pagoda with resident monks in Cambodia during August 2020. We also discuss the possible implications of this outbreak for future influenza virus circulation in the context of the COVID-19 pandemic.

Methods

On 18 August 2020, a cluster of respiratory illnesses among residents of a Buddhist pagoda was reported to the Cambodian MoH. This outbreak was originally investigated as a possible severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) cluster on 20 August 2020, but 34 monks with respiratory symptoms tested negative for SARS-CoV-2, which is the virus that causes COVID-19, and 28 (82%) tested positive for influenza A viruses. On 25 August 2020, a response team comprising staff from the MoH, US Centers for Disease Control and Prevention, and the World Health Organization (WHO) were deployed to investigate this cluster further. The team met with local MOH officials, civil authorities, and pagoda leaders to identify individuals who lived in the pagoda complex during 18–26 August 2020 and became ill. The individuals identified as ill were interviewed to determine clinical symptoms compatible with ILI defined as sudden onset of fever (temperature >38 °C) and a cough and/or sore throat with onset on or after 15 August. All individuals with ILI completed a standard questionnaire, which included questions regarding age, sex, clinical signs and symptoms experienced since onset of illness, attendance at religious and school events in the past 14 days, social distancing practices in the past 14 days, frequency of hand washing and face mask use in the past 14 days on a Likert scale (i.e., never, sometimes, often, and always), influenza vaccination history, and eating, sleeping, and bathing arrangements. Nasopharyngeal and oropharyngeal swabs were collected and combined in viral transport medium, and tested at the National Institute of Public Health for influenza and SARS-CoV-2 viruses. Further diagnostics, including viral isolation from three specimens and rapid Sanger sequencing of the hemagglutinin and neuraminidase genes were performed by the National Influenza Center at Institute Pasteur Cambodia (Horwood et al., 2019). Presence of an influenza virus infection was identified based on the detection of influenza viral RNA by real-time reverse transcription PCR, with a cycle threshold value of 35 or lower, in the respiratory specimen from a resident of the pagoda. The outbreak investigation team also conducted an environmental assessment to evaluate sleeping, eating, praying, and bathing areas in order to observe and assess social distancing measures, mask use, and general hygiene.

In order to document any changes in national influenza virus circulation during the period of the COVID-19 pandemic in Cambodia, we obtained virological surveillance data on influenza virus circulation from the National ILI Sentinel Surveillance System (Sreng et al., 2010) for 2017–2020. We calculated the percentage of ILI specimens that tested positive for influenza during May to July (the period when influenza virus detection typically increases) relative to the same months in 2017–2019. ILI cases from the outbreak cluster were not represented in these sentinel surveillance data.

Results

During the period from May to July, the percentages of ILI specimens that tested positive for influenza according to the national sentinel surveillance system were 11.0% (34/309) in 2017, 25.5% (82/321) in 2018, 37.9% (113/298) in 2019, and 0.7% (2/270) in 2020. In 2020, influenza A (H3N2) virus was first detected in July 2020, immediately prior to the reported pagoda outbreak (Figure 1).

From 15 to 26 August 2020, 362 pagoda residents slept each night at the pagoda. These residents ate and slept on the premises but this was not a “closed” environment. Residents left the pagoda to buy groceries and attend local attractions, including a field trip to the zoo. They also received visitors and interacted with the community as part of daily alms collection and for performing religious rites.

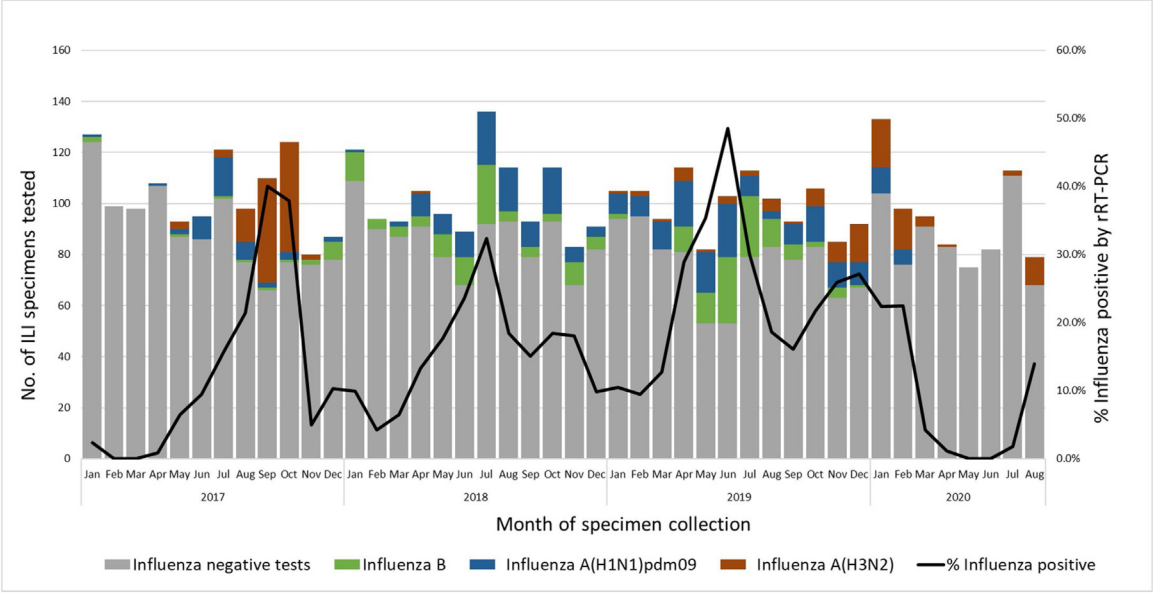

Figure 1. Influenza-like illness sentinel specimens tested and influenza virus detection in the Kingdom of Cambodia during 2017–2020.

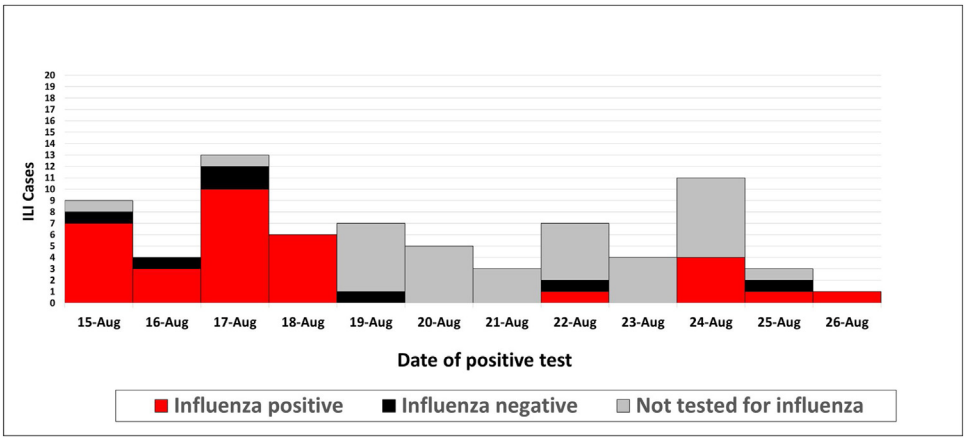

Figure 2. Influenza virus laboratory results among residents with influenza-like illness in a pagoda in Takeo Province, Cambodia, during 15–26 August 2020 (N = 73).

All individuals were assessed for ILI. Among the 362 residents of the pagoda, 73 (20.2%) met the case definition for ILI. Dates of illness onset ranged from 15 to 26 August (Figure 2). Among these 73 people with ILI, specimens were collected and tested for influenza and SARS-CoV-2 from 40 (including the 34 cases from the initial investigation). Thirty-three people (82.5%) tested positive for influenza A (H3N2) virus and all 40 tested negative for SARS-CoV-2.

Among the 73 residents with ILI, 62 were monks, four were cooks, and seven were in training to be monks. Except for the four cooks (aged 38–54 years), all were aged 22 years or younger. The median age was 13 years (range 7–54 years) and 69 (94%) were male. None had ever been vaccinated against influenza. However, 64% (47/73) of the residents with ILI (and 76%, 25/33 of those testing positive for influenza) were clustered in three of the eight buildings with sleeping areas. In addition, 55/73 (75%) of the residents with ILI reported attending religious events in the pagoda that included outside visitors, and 20/73 (27%) left the pagoda to collect alms in the 14 days before they were ill. In the 14 days before illness onset, 20 (27%) of the residents with ILI reported wearing a face mask “often,” 27/73 (36%) reported washing their hands “often,” 11 (15%) reported practicing social distancing behaviors during prayers, study, or meals, and 48/73 (65%) reported attending school on the pagoda grounds. Most residents (68/73, 93%) indicated that they shared bathrooms and sleeping quarters, and 32/73 (46%) reported close contact with any person with acute respiratory illness during 14 days before illness onset. When interviewed by the response team, the head of the pagoda

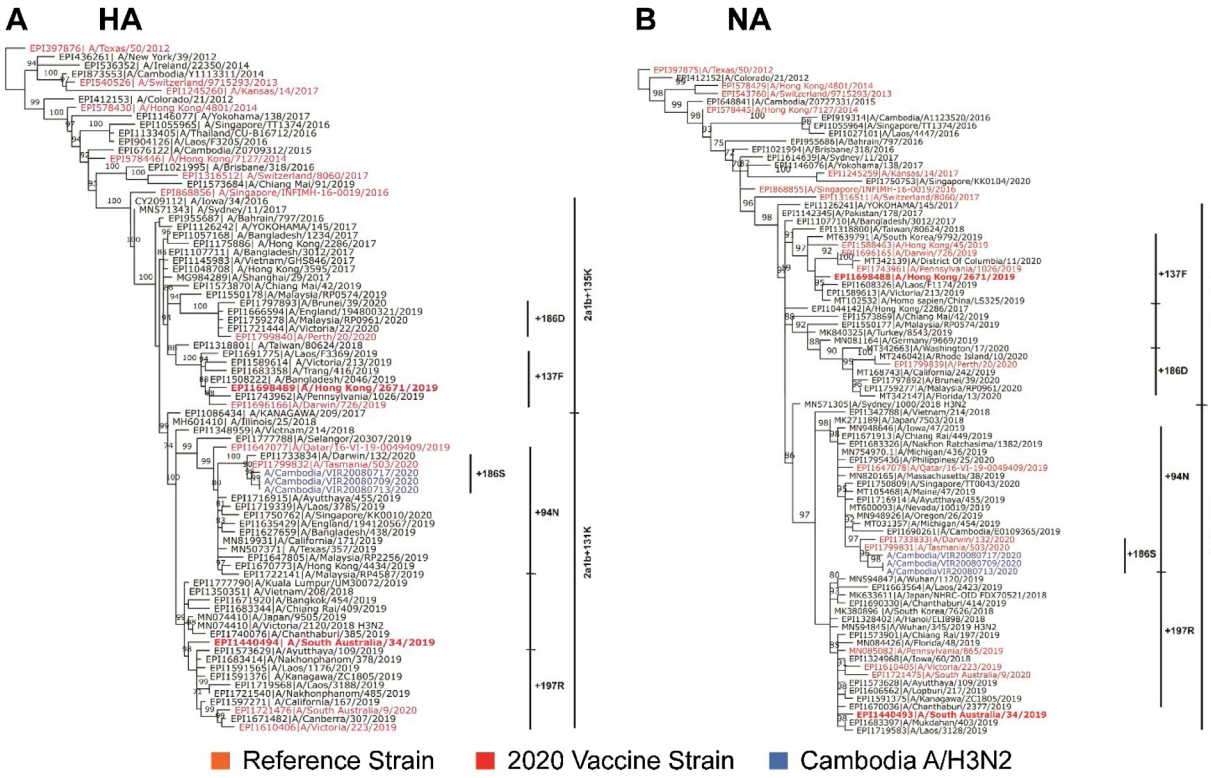

Figure 3. Phylogenetic analysis of (A) hemagglutinin (HA) and (B) neuraminidase (NA) gene sequences from influenza A (H3N2) viruses from the outbreak in Cambodia during August 2020. The maximum likelihood phylogeny was inferred using a general time reversible nucleotide substitution model with a gamma distribution for the among-site rate variation (GTR + Γ) in RAxML v8 and visualized using Figtree v1.4 (http://tree.bio.ed.ac.uk/software/figtree/). Branch support values were generated using 1000 bootstrap replicates. Reference and vaccine strains were obtained from GISAID (www.gisaid.org).

also indicated that there were no changes or relaxations in these practices by the residents over time. The environmental assessment found close quarter living arrangements, with sleeping and eating areas forcing frequent and prolonged encounters within a 1-m distance, and shared shower and toilet facilities. The team also observed inconsistent and infrequent face mask use, and frequent mixing with the local population both inside and outside the pagoda.

Seven residents (six monks and one trainee) with laboratory-confirmed influenza had an onset of illness date of 15 August, so a single index case for this outbreak was not apparent. However, among these seven residents, four reported contact with other persons with respiratory illness, all attended school, three attended the outside market, and two attended daily alms collections. In addition, five reported never wearing masks, four reported never washing hands, and none reported practicing social distancing.

MOH staff recommended that additional physical distancing, hand washing, mask use, and visitor restrictions be applied within the pagoda, including specific physical distancing practices for classrooms, dining rooms, and prayer halls. The investigation team also recommended moving the local live bird market from inside the pagoda to a location outside the main gate to reduce exposure to non-residents. Longer term recommendations included adding more living space with upgraded airflow, installing individual shower facilities, and further improvements in environmental and water sanitation procedures on site.

Sequencing of three of the influenza A(H3) viruses detected in this outbreak indicated that the viruses belonged to clade 3c.2A1b + 131 K + 94 N (Figure 3), which is the same clade as that determined for the influenza viruses detected in mainland Australia and Tasmania earlier in 2020. The viruses detected in Cambodia also contained a G186S mutation that is common in this clade. One strain comprising A/Cambodia/VIR20080713/2020 also had a Y233H mutation in the hemagglutinin gene. All three viruses were 97–99.5% similar to the prototype Northern and Southern Hemisphere vaccine strains for 2020 at both the nucleotide and amino acid levels. No antiviral resistance mutations that might affect neuraminidase inhibitors were detected in the neuraminidase genes.

Following the investigation of this outbreak, several additional outbreaks of influenza A (H3N2) were reported in other closed and semi-closed settings in Cambodia from August to September 2020, thereby indicating that influenza circulation was widespread throughout the country (Figure 4).

Discussion

In Cambodia, ILI sentinel surveillance indicated an unusually low level of influenza circulation during 2020 (WHO, 2020d) despite testing similar numbers of specimens as previous years. This reduction coincided with measures implemented to mitigate COVID-19 transmission in Cambodia. However, influenza detection increased following the easing of COVID-19 mitigation measures in June and July, possibly indicating a delayed start to the influenza season. Cambodia has reported imported cases of COVID-19 and most recently on 2 October 2020, but no community transmission has been reported. In addition, no cases were reported by the sentinel ILI and influenza virological surveillance systems despite ongoing testing of these specimens for SARS-CoV-2 since 1 December 2019. Face mask usage, hand washing, physical distancing measures, and vaccine uptake were reported to be

Figure 4. Influenza A (H3N2) virus outbreaks in Cambodia from August to September during 2020. The initial outbreak investigated in Takeo Province, Cambodia (inset box), is indicated by a red star. Subsequent outbreaks detected in pagodas and prisons are indicated by orange circles.

uniformly low in this pagoda with an outbreak of influenza, and the cases clustered in specific housing locations. There were no reported changes in these practices by residents over time, thereby suggesting that influenza virus circulating in the surrounding community following the easing of general mitigation measures may have entered this relatively closed and susceptible pagoda population. Indeed, following this outbreak, influenza A (H3) virus infections were detected in five additional pagoda and prison populations in Cambodia by September 2020, and more are being investigated as broader COVID-19 preventive measures continue to ease further.

The global influenza virus circulation is low (WHO, 2020e) but our experience in Cambodia highlights the importance of maintaining sentinel and event-based surveillance systems during the COVID-19 pandemic to detect illnesses caused by other infectious respiratory diseases. Our experience also provides an important reminder that influenza has not disappeared, and other countries should remain vigilant and prepare for the possible late arrival of the influenza season following the easing of community and personal mitigation measures.

Influenza surveillance and timely virus information sharing with the WHO Global Influenza Surveillance and Response System remain critical for informing vaccine strain selection (Russell et al., 2008). Details of isolates and sequences are especially vital in 2020 because global influenza virus contributions to inform vaccine virus selection have decreased greatly. In addition, existing respiratory surveillance systems for influenza can and should be leveraged to support national SARS-CoV-2 surveillance (WHO, 2020c).

Several countries observed decreases in influenza circulation following the implementation of non-pharmaceutical interventions (NPIs) to prevent the spread of COVID-19, thereby demonstrating that interventions other than vaccines can play important roles in reducing influenza circulation (Cowling et al., 2020; Lee et al., 2020; Soo et al., 2020). During the next few months, increases in influenza circulation may occur in locations where COVID-19 prevention measures have eased or lapsed, and there could be an opportunity for authorities to target NPIs to reduce the impacts of both influenza and SARS-CoV-2 in high-risk communities, such as semi-closed environments like pagodas. Influenza vaccination remains the most important prevention tool for protecting the population from influenza infection (Grohskopf et al., 2020). In addition, given that influenza virus infections and SARS-CoV-2 may often be clinically indistinguishable, influenza vaccination is particularly important during this COVID-19 pandemic because it may reduce the burden of ILI illnesses on medical care providers (Maltezou et al., 2020).

The data obtained from the outbreak investigation in Cambodia suggest a temporal relationship between the circulation of influenza and the national relaxation of NPIs, but these observations remain an ecological comparison. The head of the pagoda reported that the behaviors of residents had not changed over time, but we lacked individual-level data from before the outbreak. In addition, we were only able to interview ill people, so we could not perform risk factor analysis.

Further studies are needed to identify both the personal protective measures and community-level measures with the greatest impact on influenza virus circulation during the COVID-19 pandemic. These studies combined with assessments of community-specific attitudes toward implementation, as well as economic and social evaluations of their impacts, may help to identify feasible NPI options for future influenza seasons. Following the 2009 H1N1 pandemic, evidence increased regarding the effectiveness and feasibility of NPIs, but many studies were observational (Bin-Reza et al., 2012; Liang et al., 2020; Qualls et al., 2017).

In conclusion, the current outbreak of A (H3N2) in Cambodia suggests that additional outbreaks of influenza could occur globally as personal and community restrictions associated with COVID-19 mitigation are relaxed. In Cambodia, it is crucial to ensure that these outbreaks are prevented and contained as the country continues to prevent the introduction and potential widespread transmission of COVID-19. Continued vigilant surveillance, global virus sharing, and timely influenza vaccination will also help to understand and mitigate the impact of influenza as global circulation inevitably increases.

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Conflict of interests

All authors have signed statements indicating that there are no conflicts of interest regarding the contents of this manuscript.

Ethical approval

This investigation was undertaken as part of a public health outbreak response and it was considered a non-research investigation by the Cambodian MOH. This activity was also reviewed and determined to be a non-research activity by the Human Subjects Contacts at the US Centers for Disease Control and Prevention. This activity was also undertaken in a manner consistent with applicable federal law and US Centers for Disease Control and Prevention policy.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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