Impact of Influenza A(H1N1)pdm09 Virus on Circulation Dynamics of Seasonal Influenza Strains in Kenya

Janet Majanja, Rose N. Njoroge, Rachel Achilla, Eyako K. Wurapa, Meshack Wadegu, Silvanos Mukunzi, Josephat Mwangi, James Njiri, George Gachara, and Wallace Bulimo*

U.S. Army Medical Research Unit, Kenya, Nairobi, Kenya; Department of Medical Laboratory Sciences, Kenyatta University, Nairobi, Kenya

Abstract. We describe virus variations from patients with influenza-like illness before and after the appearance of influenza A(H1N1)pdm09 in Kenya during January 2008–July 2011. A total of 11,592 nasopharyngeal swabs were collected from consenting patients. Seasonal influenza B, A/H1N1, A/H3N2, A/H5N1, and influenza A(H1N1)pdm09 viruses were detected by real-time reverse transcription–polymerase chain reaction. Of patients enrolled, 2073 (17.9%) had influenza. A total of 1,524 (73.4%) of 2,073 samples were positive for influenza A virus and 549 (26.6%) were positive for influenza B virus. Influenza B virus predominated in 2008 and seasonal A(H1N1) virus predominated in the first half of 2009. Influenza A(H1N1)pdm09 virus predominated in the second half of 2009. Influenza A/H3N2 virus predominated in 2010, and co-circulation of influenza A(H1N1)pdm09 virus and influenza B virus predominated the first half of 2011. The reduction and displacement of seasonal A(H1N1) virus was the most obvious effect of the arrival of influenza A(H1N1)pdm09 virus. The decision of the World Health Organization to replace seasonal A(H1N1) virus with the pandemic virus strain for the southern hemisphere vaccine was appropriate for Kenya.

INTRODUCTION

Influenza pandemics are typically caused by the introduction of a virus with a new hemagglutinin surface protein (HA) and/or neuraminidase (NA) surface protein(s) to the human population. Historically, influenza pandemics have been associated with displacement and replacement of previously circulating influenza A subtypes.1,2 The 1918 H1N1 variants circulated for 39 years before being replaced by an H2N2 subtype in 1957. This new subtype circulated for 11 years before being replaced in 1968 by a new H3N2 subtype.3 It is also theorized that the emerging H1N1 viruses probably replaced pre-circulating H3-like viruses in 1918.4 The exception to this trend was observed in 1977 when an H1N1 virus from the 1950s reappeared and remained in parallel with the circulating H3N2 seasonal virus until 2009.9,11 It has been postulated that viral factors in new strains that enable their rapid transmission between humans and the immune status of the current population drive the extinction of old strains, their sudden replacement by new strains, or co-existence of the two strains.5 It is important to understand whether emerging strains replace or co-exist with previously existing strains because this will directly influence the selection of viruses to include in influenza vaccines.

In April 2009, a novel H1N1 virus emerged in Mexico and proceeded to spread worldwide.4,6 Because its detection in the Northern Hemisphere coincided with decreasing seasonal influenza activity, the impact on the circulation of seasonal influenza viruses could not be fully assessed.7 In contrast, the first wave of the influenza A(H1N1)pdm09 virus (hereafter referred to as pH1N1) in the Southern Hemisphere coincided with the onset of the winter influenza and respiratory virus season. Thus, data obtained from the 2009 Southern Hemisphere winter provide an opportunity to examine the circulation dynamics of pandemic and seasonal viruses during the early pandemic period.7 Information from the Southern Hemisphere during the first wave of the 2009 pandemic indicated that the pandemic strain displaced the circulating seasonal A/H1N1 viruses (hereafter referred to as sH1N1) and to a smaller extent the H3N27 and eventually completely replaced sH1N1.5,9

Kenya lies in both the Northern and Southern Hemispheres, and the World Health Organization (WHO) places it in the Eastern Africa influenza transmission zone along with 18 other geographically related countries with similar influenza transmission patterns.10 However, the Kenya Ministry of Public Health and Sanitation recommends the use of Southern Hemisphere vaccine formulation for its residents because the influenza season in Kenya coincides with the winter in the Southern Hemisphere.11 A partnership between the U.S. Army Medical Research Unit–Kenya (USAMRU-K), the Kenya Medical Research Institute (KEMRI), the Kenya Ministry of Health, and the Centers for Disease Control and Prevention–Kenya (CDC-Kenya) undertakes influenza surveillance activities in Kenya, under the WHO global influenza surveillance program. USAMRU-K’s sentinel surveillance network consists of eight sentinel surveillance sites located in district hospitals throughout the country. The current study sought to use this surveillance data to determine the circulation dynamics of the pandemic and seasonal influenza strains in Kenya, a country in the East Africa Transmission Zone. These data also enabled us to determine the usefulness of the Southern Hemisphere vaccine for Kenya.

MATERIALS AND METHODS

Ethical considerations. Eligible participants voluntarily enrolled in accordance with an Institutional Review Board protocol approved by the KEMRI and the Walter Reed Army Institute of Research review boards. Written consent was obtained from patients ≥ 18 years of age. For patients < 18 years of age, written consent was obtained from a parent or legal guardian. In addition, written consent was obtained from patients 7–17 years of age. For all persons, informed consent was verbally explained and questions were solicited.

Collection of clinical samples. The active surveillance field sites were composed of the following eight hospitals chosen...
geographically and depending on population demographics; Mbagathi, New Nyanza, Port Reitz, Alupe, Malindi, Isiolo, Kericho, and Kisii (Figure 1). Patients > 2 months of age who came to hospital outpatient clinics with fever (temperature $\geq 38^\circ C$) and a cough or sore throat were recruited by dedicated project clinical officers. Persons with exudative pharyngitis or tonsillitis or symptom onset $> 72$ hours were excluded from enrollment.

None of the patients sampled had previously been vaccinated against influenza. Patients’ age, sex, occupation, residence, work place, history of influenza-like illness within the household, travel history, and animal contact were ascertained and a clinical examination was completed.

Duplicate nasopharyngeal samples were collected by using flocked dacron swabs and placed in cryovials containing 1 mL of virus transport medium, kept at 4°C and stored in a liquid nitrogen dry shipper within 8 hours of collection. All samples were transported weekly from the surveillance sites to the National Influenza Center laboratory within KEMRI (Nairobi, Kenya) and were maintained in a cold chain throughout transport.

**Polymerase chain reaction.** Specimens were tested for influenza A and B viruses. Nucleic acid was extracted by using the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany), according to the manufacturer’s instructions. In brief, a starting volume of 100 $\mu$L of each sample was used for the extraction. The RNA was eluted from columns with RNase-free water containing 0.04% sodium azide and used immediately for real-time reverse transcription–polymerase chain reaction (RT-PCR) or stored at $-80^\circ C$.

Detection of sH1N1, H3N2, influenza B, H5N1 and pH1N1 viruses was carried out by using the Ag Path-ID One Step Real Time Quantitative Reverse Transcription PCR (Real-Time qRT-PCR) Kit (Applied Biosystems, Foster City, CA) with CDC Human Influenza Virus Real-Time RT-PCR Detection and Characterization Panels (CDC, Atlanta, GA).

Primers and probes used in this study were specific for the matrix gene for influenza virus typing and for the hemagglutinin gene for influenza A virus subtyping. Human RNase P gene served as an internal positive control for human nucleic acid in all specimens. Cycle threshold values $< 35.0$ were regarded as positive. This protocol is available under a material transfer agreement from the CDC upon request.

**Virus isolation and influenza B virus characterization.** Influenza virus isolations were performed in Madin-Darby canine kidney cells, followed by hemagglutination inhibition assay using guinea pig erythrocytes and reference antiserum in accordance with CDC protocols. Inoculated cells were
incubated at 33°C in an atmosphere of 5% CO2 and observed daily for 10 days for visual cytopathic effect by using an inverted microscope. When a cytopathic effect was observed, the supernatant fluid was collected and the hemagglutination titer was measured. Hemagglutination inhibition testing to antigenically characterize the viruses was conducted on high-titer samples, and isolates with low hemagglutination titers were passaged once.

RESULTS

A total of 11,592 respiratory specimens were collected during January 2008–July 2011 from the eight sentinel surveillance sites of USAMRU-K. Of these samples, 2,073 (17.9%) were PCR-positive for influenza viruses. Influenza A viruses were detected in 1,524 (73.4%) of the samples, and influenza B viruses were detected in the remaining 549 (26.5%) samples. Of the influenza A virus–positive samples, 345 (22.6%) were pH1N1, 387 (25.4%) were H3N2, 143 (9.4%) were sH1N1, and 649 (42.6%) were unsubtyped. Of the influenza B virus–positive samples, we were able to characterize 291 (52%) samples. The 2008 viruses belonged to the B/Yamagata lineage and 2009–2011 viruses belonged to the B/Victoria lineage. These results are summarized in Table 1. Influenza activity was detected throughout the surveillance period. In 2008, activity peaked in August at 36%. In 2009, a peak of 52% was detected in October (Figure 2). In 2010, activity peaked in July at 19%. In 2011, a peak of 27% was detected in March (Figure 2).

In 2009 with the appearance of the pandemic, in addition to the July–August peak, there was an additional peak in October coinciding with the peak of the new strain. In 2008–2009, influenza activity displayed distinct seasonality with two clear peaks and lows. However, this trend was disrupted after the appearance and establishment of the pandemic strain from January 2010 (Figure 2). Thus in 2010, influenza activity did not decrease below 6% during any month compared with lows of 1.6% in 2008 and 0.4% in 2009 (Figure 2). Furthermore, in 2010 influenza activity also did not increase above 20% compared with peaks of 36%, 52%, and 27% in 2008, 2009, and 2011, respectively (Figure 2). In 2011, influenza activity peaked in July (Figure 2).

Different influenza viruses also showed different circulation, co-circulation, and dominance patterns in the 3.5-year study period (Figure 2). Pre-pandemic (during February–July 2008), influenza B viruses predominated throughout the country and H3N2 and pH1N1 were second and third in order of prevalence (Figure 2). During July–December 2008, the circulating strains in order of predominance were H3N2, influenza B, and sH1N1. In early 2009 up to July, influenza A viruses, especially sH1N1

<table>
<thead>
<tr>
<th>Year</th>
<th>No. samples positive for influenza viruses</th>
<th>pH1N1</th>
<th>H3N2</th>
<th>Seasonal H1N1</th>
<th>Influenza A (unsubtyped)</th>
<th>B/Victoria-like</th>
<th>B/Yamagata-like</th>
<th>B (unsubtyped)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008</td>
<td>577</td>
<td>0 (0)</td>
<td>108 (18.7)</td>
<td>37 (6.4)</td>
<td>249 (43.2)</td>
<td>2 (0.3)</td>
<td>107 (18.5)</td>
<td>74 (12.8)</td>
</tr>
<tr>
<td>2009</td>
<td>893</td>
<td>185 (20.7)</td>
<td>92 (10.3)</td>
<td>106 (11.9)</td>
<td>328 (36.7)</td>
<td>59 (6.6)</td>
<td>2 (0.2)</td>
<td>121 (13.5)</td>
</tr>
<tr>
<td>2010</td>
<td>351</td>
<td>50 (14.2)</td>
<td>178 (50.7)</td>
<td>0 (0)</td>
<td>71 (20.2)</td>
<td>16 (4.6)</td>
<td>0 (0)</td>
<td>36 (10.3)</td>
</tr>
<tr>
<td>2011</td>
<td>252</td>
<td>110 (43.6)</td>
<td>9 (3.6)</td>
<td>0 (0)</td>
<td>1 (0.4)</td>
<td>105 (41.7)</td>
<td>0 (0)</td>
<td>27 (10.7)</td>
</tr>
<tr>
<td>Total</td>
<td>2,073</td>
<td>345 (16.6)</td>
<td>387 (18.7)</td>
<td>143 (6.9)</td>
<td>649 (31.3)</td>
<td>182 (8.8)</td>
<td>109 (5.3)</td>
<td>258 (12.4)</td>
</tr>
</tbody>
</table>

Figure 2. Total influenza-like illness (ILI) samples collected in Kenya and distribution of influenza-positive samples by subtype as a proportion of total samples collected per month in Kenya during 2008–2011.
were predominant (Figure 2). During the pandemic (August–December 2009), four influenza viruses (sH1N1, pH1N1, H3N2, and influenza B) co-circulated with influenza B and the pandemic strain predominating (Figure 2).

The first laboratory-confirmed case of pH1N1 influenza in Kenya was identified on June 29, 2009. However, we did not detect the pandemic strain from our sentinel sites until August 2009 at Mbagathi, Kisii, and Malindi. As the pandemic strain became more established, pH1N1 virus was then detected at Port Reitz District Hospital in Mombasa in September 2009. In October 2009, the pandemic strain was detected in Isiolo, Alupe, and Kericho. After the appearance of the pandemic strain in August 2009, it replaced influenza B virus and became dominant in October 2009 (Figure 2). In 2010, a decrease in the circulation of pH1N1 virus was observed during April–September. The H3N2 virus became the predominant circulating strain in the same period (Figure 2). The pH1N1 virus resurfaced in September 2010, peaked in December, and remained in circulation until July 2011. The sH1N1 virus was not detected after September 2010 in our sentinel network (Figure 2). In 2011, the predominant co-circulating strains were pH1N1 and influenza B viruses (Figure 2). The 2010 pH1N1 virus peak was not as pronounced as when the virus first came into the population in 2009. A peak in sH3N2, pH1N1 and influenza B viruses was seen starting in July 2011 (Figure 2).

Overall, in 2008, influenza B virus composed 31.7% of the circulating influenza strains and influenza A virus composed 68.3%; H3N2 and sH1N1 viruses composed 18.7% and 6.4%, respectively (Table 1). In 2009, influenza A virus was the predominant type, composing 79.6% of the viruses in circulation. Among the influenza A type viruses, the pandemic strain, sH1N1, and H3N2 composed 20.7%, 11.9%, and 10.3%, respectively, of the viruses detected (Table 1). In 2010, influenza A was also the predominant virus type, composing 85% of the viruses in circulation (Table 1). The H3N2 virus was the most prevalent of the circulating influenza A viruses (50.7%). The pH1N1 virus composed 14.2% of the circulating influenza A viruses, but sH1N1 virus was not detected during this period (Table 1). In 2011, influenza A and B co-circulated with equal intensity with each composing 47.6% and 52.4% of the circulating viruses, respectively. The pH1N1 virus was the predominant influenza A subtype, composing 43.6% of the total subtyped influenza A viruses (Table 1). The H3N2 viruses composed a meager 3.6% and no sH1N1 virus was detected (Table 1).

**DISCUSSION**

This report summarizes the results of diagnostic testing for influenza and the impact of the pH1N1 virus on circulating seasonal influenza viruses in Kenya. The displacement and replacement of sH1N1 virus was the most notable outcome of the introduction of the pandemic strain, and the virus co-circulated preferentially with influenza B viruses. The pandemic strain was detected in our sentinel network a month after the first laboratory confirmed case of pH1N1 influenza in Kenya. However, after its appearance in the sentinel network, pH1N1 virus exhibited rapid transmission and displaced seasonal influenza viruses to become the dominant strain for at least three months. Thereafter, the activity of this virus decreased dramatically and disappeared for at least six months before reemerging. There was also an overall change in the trend of influenza activity after the appearance of the pH1N1 virus with seasonality becoming less marked.

Displacement of all previously circulating non-pH1N1 viruses by the incoming pH1N1 virus is a trend observed in the Northern and Southern Hemispheres. The pandemic strain later completely replaced sH1N1 virus in both hemispheres. This displacement and subsequent replacement of previously circulating seasonal influenza viruses by an incoming pandemic strain has been described after previous pandemics. Viral immune escape and systematic interference are among mechanisms hypothesized for the displacement/replacement phenomenon. Evolution of the influenza HA gene can create amino acid changes on the surface, which disrupt the binding of HA neutralizing antibodies (antigenic drift). The arising escape mutants are more fit and displace preceding strains as the dominant strains. A hypothetical advantage of the new strain that could lead to the disappearance of older strains is that older strains might have reached their biological limit; it could no longer produce a viable variant that differs antigenically from previously occurring variants. Systematic interference between competing strains occurs when the infection with an influenza strain generates partial immunity against closely related subtypes. This partial immunity suppresses a second infection by the same strain and related subtypes but not infection from a different influenza type. Maintenance of the older subtype will be handicapped by the decreased susceptible hosts and the consequent reduction of total shedding of the older virus.

Although pH1N1 virus replaced sH1N1 virus, it preferentially co-circulated with influenza B viruses compared with H3N2 virus. We attribute this preference to lower competitive interference between influenza A and B virus types compared with two subtypes from the same influenza type. We postulate that for strains of the same subtype to co-circulate, they need to be variable enough in a way that minimizes cross-immunity, but also that this partial immunity, which is a function of time, should have decayed enough by the time the second strain is emerging. Co-circulation patterns of the different influenza A virus subtypes are important because they dictate the possibility of co-infection and reassortment events.

In this report, we also showed that pH1N1 and H3N2 viruses co-circulated during August–December 2009, September 2010, and March 2011, and both viruses started showing increased activity again in June 2011. These three periods offered opportunities for H3N2 and pH1N1 virus co-infections and reassortment. Our sequence data reported elsewhere confirms this phenomenon and showed that 4 of the 32 H3N2 viruses isolated in 2010 had acquired the M gene segment of the pH1N1 virus by reassortment.

Before the pandemic in 2009, influenza B and sH1N1 viruses were the predominant circulating strains. However, after the pandemic, pH1N1 virus quickly became the dominant strain. Our data supports the higher transmissibility of pH1N1 virus in comparison with other non-pH1N1 viruses, as reported elsewhere. This phenomenon can be partially explained by a population highly susceptible to pH1N1 virus than to non-pH1N1 viruses to which the population might have acquired partial immunity in previous years by prior natural exposure to antigenically related strains. A virus with a novel subtype spreads without restraint; newly arising pandemic strains thus typically infect a higher than normal fraction of the human population.
The speed of the spread seemed to co-relate with the proximity of the sentinel site to the introduction foci. Thus, our sentinel site to detect the pandemic strain was Mbagathi in Nairobi, which was one of the foci, followed by New Nyanza Hospital, which is located in Kisumu in Western Kenya, which was another foci. In addition, our sentinel sites were located at district hospitals, which are third from the top of the pyramidal public health delivery system in Kenya. Because of the heightened state of alert regarding the pandemic, the initial pH1N1 cases were likely referred to top tier facilities (referral or provincial hospitals). This finding is demonstrated by earlier detection of the pandemic strain by CDC-Kenya whose sentinels are based exclusively at provincial and referral hospitals. CDC-Kenya detected the first pandemic case on June 29, 2009, at Kenyatta National Referral Hospital in Nairobi.

The pH1N1 virus decreased from dominance after three months and was undetected for at least six months before reappearing. We hypothesize that the strain generated enough herd immunity through immunization by exposure to suppress its transmission. However, this immunity wanes with time, enabling the virus to reemerge. It is also possible for viruses to reemerge if they have mutated enough to escape immunity generated against the parent strain. However, we ruled out this possibility because the emerging pH1N1 viruses have been shown to be antigenically homogeneous and closely related to the vaccine virus A/California/7/2009.20

Worldwide influenza virologic surveillance data is posted on a WHO database known as FluNet.21 We used this data to compare influenza patterns in Kenya with those in other countries in the same East African transmission zone. Unfortunately, most (11 of 19) of these countries had no data available. Data from another five of these countries could not be used because of either extremely low sample numbers or unavailability of pre-pandemic data. This finding underscores the relevance of influenza data for Kenya because it is the only country in this transmission zone with extensive and comprehensive influenza surveillance. However, a comparison was made with Uganda and Madagascar, the only other countries in that transmission zone whose data preceded the pandemic. In 2008, Kenya and Uganda reported presence of influenza B virus during their surveillance. However, this virus co-circulated with H3N2 and sH1N1 viruses in Kenya but only with sH1N1 virus in Uganda. In the same year, Madagascar reported no influenza B virus; sH1N1 virus was the predominant strain, which later co-circulated with H3N2 virus. In 2009 before the pandemic, influenza resulted from co-circulation of sH1N1 and H3N2 viruses in Kenya and Madagascar. However, unlike Madagascar, Kenya also experienced low levels of influenza B virus. In the same period, Uganda had co-circulation of sH1N1 and influenza B viruses.

After introduction of the pandemic strain, influenza activity increased in all three countries. In Kenya, pH1N1 virus co-circulated with sH1N1, H3N2 and influenza B viruses. In Uganda, pH1N1 virus co-circulated mostly with sH1N1 virus and minimally with H3N2 or influenza B viruses. In contrast, pH1N1 virus in Madagascar was the sole circulating influenza virus strain. In early 2010, Kenya had mainly co-circulation of influenza B and H3N2 viruses and minimal levels of sH1N1 and pH1N1 viruses. Uganda reported co-circulation of influenza B and H3N2 viruses and minimal circulation of sH1N1 virus; pH1N1 virus was not detected. In the same period, Madagascar influenza activity was caused mainly by pH1N1 and influenza B or H3N2 viruses. From the middle to the end of 2010, Kenya had co-circulation of pH1N1 and H3N2 viruses and minimal circulation of influenza B virus. In Uganda, pH1N1 virus reappeared and co-circulated with H3N2 and influenza B viruses and minimally co-circulated with sH1N1 virus. In Madagascar, influenza was attributable to H3N2 and influenza B viruses. In 2010, there was an overall decrease in influenza activity and a decrease in pH1N1 virus in all three countries. In the first half of 2011 in all three countries, no sH1N1 virus was detected. In Kenya and Uganda, pH1N1 virus co-circulated with H3N2 and influenza B viruses. In Madagascar, only H3N2 and influenza B viruses were reported. This comparison indicates that even countries within the same transmission zone will have their own unique influenza circulation dynamics. This observation underscores the need for country-specific influenza virologic surveillance.

A possible shortcoming of this study might result from the potential for sampling and selection bias associated with sentinel surveillance. Therefore, these results might not be representative of the entire population in Kenya. Additional bias in a sentinel study might arise because of the health-seeking behavior of persons in Kenya. Because older persons rarely visit hospitals unless they are sick and our study targeted ambulatory patients, we mainly recruited children less than five years of age. This feature limited analysis of age distribution of viral etiologies. Thus, for example, we cannot infer the target groups for vaccinations. The study also did not collect any denominator data, which preclude us from calculating crucial population rates. However, despite these limitations, our study is the first to provide insight of the effect of the pH1N1 influenza virus on circulation dynamics of seasonal influenza viruses in a tropical African country and in the East African Influenza Transmission Zone.

In conclusion, this study provides another example of the influenza displacement/replacement process, as described after previous pandemics. Data obtained during this study also support the recommendation that seasonal influenza vaccines for the Southern Hemisphere during 2010–2012 contain representative pH1N1 and H3N2 viruses (as well as influenza B virus), but not previously circulating sH1N1 virus.20

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Authors’ addresses: Janet Majanja, Rose N. Njoroge, Rachel Achilla, Eyako K. Wurapa, Meshack Wadegu, Silvanos Mukunzi, Josephat Mwangi, James Njiri, and Wallace Bulimo, U.S. Army Medical Research Unit–Kenya, U.S. Embassy, Nairobi, Kenya, E-mails: janet.majanja@usamru-k.org, rose.njoroge@usamru-k.org, rachel.achilla@usamru-k.org, eyako.wurapa@usamru-k.org, meshack.wadegu@usamru-k.org, silvanos.mukunzi@usamru-k.org, josephat.mwangi@usamru-k.org, james.njiri@usamru-k.org, and wallace.bulimo@usamru-k.org. George Gachara, Department of Medical Laboratory Sciences, Kenyatta University, Nairobi, Kenya, E-mail: ggachara@gmail.com.

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