Clinic- and Hospital-Based Sentinel Influenza Surveillance, Uganda 2007–2010

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Background. To assess the epidemiology and seasonality of influenza in Uganda, we established a sentinel surveillance system for influenza in 5 hospitals and 5 outpatient clinics in 4 geographically distinct regions, using standard case definitions for influenzalike illness (ILI) and severe acute respiratory illness (SARI).

Methods. Nasopharyngeal and oropharyngeal specimens were collected from April 2007 through September 2010 from patients with ILI and SARI aged ≥2 months, tested for influenza A and B with real-time reverse-transcription polymerase chain reaction, and subtyped for seasonal A/H1, A/H3, A/H5, and 2009 pandemic influenza A (pH1N1).

Results. Among the 2758 patients sampled, 2656 (96%) enrolled with ILI and 101 (4%) with SARI. Specimens from 359 (13.0%) were positive for influenza; 267 (74.4%) were influenza A, and 92 (25.6%) were influenza B. The median age of both patients with ILI and patients with SARI was 4 years (range, 2 months to 67 years); patients aged 5–14 years had the highest influenza-positive percentage (19.6%), and patients aged 0–4 years had the lowest percentage (9.1%). Influenza circulated throughout the year, but the percentage of influenza-positive specimens peaked during June–November, coinciding with the second rainy season.

Conclusions. Continued and increased surveillance is needed to better understand the morbidity and mortality of influenza in Uganda.

Influenza is recognized as an important contributor to respiratory disease and death [1]. In the temperate climate of North America, seasonal annual epidemics are associated with substantial increases in hospitalization and death, especially among older persons [2, 3]. The burden and prevalence of influenza has been well documented in the northern and southern hemispheres [2, 4–6]. Recent publications have described the epidemiology of influenza in certain tropical countries in Africa [7–10]. However, in much of Africa, the epidemiology, seasonality, and disease burden of influenza are poorly understood because influenza surveillance is uncommon [3, 11, 12].

Uganda is a landlocked equatorial East African country of 242,554 km², bordered by Sudan in the north, the Democratic Republic of Congo in the west, Rwanda and Tanzania in the south, and Kenya in the east (Figure 1). In 2006 the population was 28,195,754 persons [13]. The climate is tropical; temperatures range from 15° to 30°C, and precipitation varies from 750 mm in the northeast to 1,500 mm in the high-rainfall areas on the shores of Lake Victoria [13]. Rainy seasons occur during March–May and September–November.

Before 2005, there was little surveillance for influenza in Uganda. In October 2005, in response to the rising global concern regard avian influenza and pandemic influenza, the Ugandan government established a multi-sectorial national task force to develop the National Preparedness and Response Plan for Avian and Human Pandemic Influenza. In 2006, the Uganda Virus Research Institute (UVRI) was recognized by the World Health Organization as a National Influenza Center, and in 2007 the UVRI implemented a national sentinel surveillance system for influenza to assess the epidemiology and seasonality of influenza and detect novel influenza viruses. We describe the epidemiology and seasonality of influenza in Uganda by using surveillance data collected during April 2007 through September 2010.
METHODS

Setting and Surveillance Sites
We selected general hospitals and clinics that provide treatment for all ages of patients to be surveillance sites, in accordance with the Pan American Health Organization and Centers for Disease Control and Prevention (CDC) Generic Protocol for Influenza Surveillance, which recommends choosing diverse sites to ensure national representation [14]. In addition, we selected the majority of our sites in areas of Uganda with high population density and high trade, where influenza transmission during a pandemic would likely be more rapid. In April 2007, we began surveillance in one site, Mengo hospital, located in the capital city of Kampala. We gradually increased the number of sites over the next 2 years to 10 hospitals and clinics. The hospitals offer both outpatient and inpatient care, whereas the clinics offer only outpatient care. As of September 2010, the last month for which we report data, surveillance was being conducted at 5 public hospitals and 5 general outpatient clinics (4 public, 1 private) in 6 districts encompassing 4 geographically distinct regions across Uganda (Figure 1). The population density of the areas

Figure 1. Location of influenza sentinel surveillance sites (black circles) and National Influenza Reference Laboratory (hatched circle) in Uganda.
surrounding the sentinel sites ranges from 91–500 persons/km² for the 5 sentinel sites located in the northwest and western regions of Uganda to 501–4600 persons/km² for the 5 sentinel sites located in central Uganda surrounding the capital city of Kampala (Figure 1).

**Surveillance Methods**
The study population included outpatients with influenza-like illness (ILI) and hospitalized patients with severe acute respiratory illness (SARI). Standardized case definitions were used for ILI and SARI. We defined a case of ILI as an outpatient aged ≥2 months with a measured fever (temperature ≥38°C) and either cough or sore throat that did not require hospital admission. We defined cases of SARI according to one of the following sets of criteria: (1) a child aged 2 months to <5 years requiring hospitalization, with recent onset of cough or difficulty breathing and ≥1 of the following signs or symptoms: inability to drink or breast-feed, lethargy, unconsciousness, vomiting of all feedings, convulsions, nasal flaring, grunting, chest indrawing, stridor, or tachypnea (case definition adapted from Integrated Management of Childhood Illness criteria for severe pneumonia [15]); or (2) a patient aged ≥5 years requiring hospitalization, with a measured fever (temperature ≥38°C) and cough, shortness of breath, or difficulty breathing.

In the outpatient departments, nurses and clinical officers identified patients with ILI by surveying patients who presented with a measured fever (temperature ≥38°C) and respiratory symptoms. In the inpatient departments, nurses and clinical officers identified patients with SARI by reviewing the charts of all patients admitted with a clinical diagnosis of acute pneumonia.

Each weekday during the surveillance period, nurses and clinical officers interviewed and sampled the first patients with ILI who presented to the clinic (maximum, 10 patients), and all patients with SARI. Using a standardized case investigation form, clinical officers interviewed patients and abstracted clinical data from medical records to obtain demographic and clinical data. Clinical officers also collected nasopharyngeal and oropharyngeal swab specimens from patients aged >1 year; oropharyngeal swab specimens alone were collected from children aged <1 year. Immediately after specimen collection, swab specimens were placed in a single vial with 1 mL of viral transport media containing Dulbecco’s modified Eagle medium, 2.5% bovine serum albumin fraction V, 1% glutamine, 2% HEPES, 1% penicillin-streptomycin and fungi zone (250 µg/mL). The specimens were immediately frozen in dry shippers containing liquid nitrogen on site, and every 2 weeks the dry shippers were transported to the National Influenza Reference Laboratory in Entebbe in liquid nitrogen.

**Laboratory Testing**
Nasopharyngeal and oropharyngeal swab specimens were tested at the UVRI for influenza viruses A and B by using the CDC’s real-time reverse-transcription polymerase chain reaction (rRT-PCR) protocols and primers. Positive influenza A specimens were further subtyped for seasonal A/H1, A/H3, A/H5, and 2009 pandemic influenza A (H1N1) (pH1N1). All positive controls and PCR primer pairs for ribonucleoprotein controls, and influenza typing and subtyping were provided by the CDC (Atlanta, Georgia).

We inoculated specimens positive for influenza A virus and negative for H5 by rRT-PCR, into Madin-Darby canine kidney cells for virus isolation. A detailed protocol describing our viral culture methodology is available on request. Selected specimens were sent to CDC Atlanta for external verification, identification of nonsubtypable isolates, and further virological analysis.

**Statistical Analysis**
Each submitted specimen and case investigation form was assigned a unique identification number and entered into an EpiInfo (CDC, Atlanta) database. Case classification of ILI and SARI was verified by blind reclassification of cases by using clinical information derived from the standardized case investigation form. Demographic and clinical characteristics of ILI and SARI cases were calculated as proportions and compared by using Fisher’s exact or Pearson’s χ² test; differences were considered statistically significant at P < .05, and all reported P values are 2 sided. The seasonality of type and subtype circulation was assessed and presented graphically by using absolute counts and percentage positivity per month throughout the study period. Statistical analysis of data was conducted by using EpiInfo v3.5.1 and Microsoft Excel 2007 software (Microsoft).

**Ethical Considerations**
Verbal consent for influenza screening was obtained from all identified patients aged ≥18 years. A proxy verbal consent was obtained from parents or legal guardians for patients <18 years. This surveillance was determined to be nonresearch by the ethical review committees of the Ugandan Ministry of Health, the UVRI, and the US CDC.

**RESULTS**
From April 2007 through September 2010, specimens and complete case investigation forms were collected from 3874 patients. Of those, 1116 (29%) did not meet the ILI or SARI case definition and were excluded from this analysis. Among the 2758 specimens included in our study, 2656 (96%) were from patients with ILI, and 102 (4%) were from patients with SARI (Table 1).

Among the patients with SARI or ILI, 1529 (55.4%) were female. The median age of both patient groups was 4 years (standard error, 0.25 years; range: 2 months to 67 years). The majority of patients in the sentinel surveillance system were
from 2 hospitals: Entebbe hospital (1266 patients [45.9% of total]), a district hospital in Southern Uganda, and Arua Hospital (469 patients [17.0% of total]), a district hospital in northern Uganda (Table 1). The number of patients enrolled per day varied by sentinel site and season; the maximum allowed number of patients enrolled per day (n = 10) was reached by sentinel sites only 1.1% of the time (64 of 5885 days).

Of the 2758 specimens, a total of 359 (13.0%) were positive for influenza; 353 of 2656 specimens (13%) from patients with ILI and 6 of 101 (6%) from patients with SARI were positive for influenza (Table 1). During 2008–2009, the only full calendar years of surveillance, 279 of 1620 total specimens (17.2%) were positive for influenza, including 276 of 1606 (17.2%) from patients with ILI and 3 of 14 (21.4%) from patients with SARI. Of the 359 influenza-positive specimens overall, 267 (74.4%) were influenza A and 92 (25.6%) were influenza B (Supplementary Table 1). Among the 267 influenza A viruses, 152 (42.3%) were A/H3, 73 (20%) were A/H1, 21 (7%) were pH1N1, and 21 (4%) were unsubtypable (Supplementary Table 1). During 2007, 2008, and 2010, influenza A/H3 was the predominant virus identified. However, in 2009, influenza B predominated; during that year 48 of 131 specimens (36.6%) were influenza B, and 39 of 131 (29.8%) were A/H1.

Among the 2465 enrolled patients with ILI with valid onset dates, 13.1% (296 of 2264 patients) with illness onset ≤3 days before specimen collection tested positive for influenza, compared with 18.7% (26 of 139) with illness onset 4–7 days before specimen collection (P = .06) and 9.7% (6 of 62) with illness onset >7 days before specimen collection (P = .43). Among the 98 patients with SARI with onset dates, 6.9% (6 of 87 patients) with illness onset ≤3 days before specimen collection tested positive for influenza, compared with 0.0% (0 of 6) with illness onset 4–7 days before specimen collection.

### Table 1. Demographic Characteristics and Case Classification of Patients Enrolled in the Influenza Sentinel Surveillance System in Uganda by Year, 2007–2010

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>Total Tested</th>
<th>Influenza Positive</th>
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<tr>
<td>Total cases</td>
<td>112</td>
<td>710</td>
<td>910</td>
<td>1026</td>
<td>2758</td>
<td>359 (13)</td>
</tr>
<tr>
<td>ILI</td>
<td>112</td>
<td>708</td>
<td>898</td>
<td>938</td>
<td>2656</td>
<td>353 (13)</td>
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<tr>
<td>SARI (adult)</td>
<td>0</td>
<td>2</td>
<td>10</td>
<td>27</td>
<td>39</td>
<td>6 (15)</td>
</tr>
<tr>
<td>SARI (child)</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>61</td>
<td>63</td>
<td>2 (0)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>69</td>
<td>398</td>
<td>507</td>
<td>555</td>
<td>1529</td>
<td>181 (12)</td>
</tr>
<tr>
<td>Female</td>
<td>43</td>
<td>310</td>
<td>401</td>
<td>469</td>
<td>1223</td>
<td>178 (15)</td>
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<tr>
<td>Unknown</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Age range, y</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>0–4</td>
<td>71</td>
<td>374</td>
<td>477</td>
<td>565</td>
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<td>5–14</td>
<td>12</td>
<td>133</td>
<td>144</td>
<td>220</td>
<td>509</td>
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<td>15–64</td>
<td>29</td>
<td>203</td>
<td>289</td>
<td>238</td>
<td>759</td>
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<tr>
<td>≥65</td>
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<td>0</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>1 (33)</td>
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<tr>
<td>Sentinel site</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Entebbe</td>
<td>42</td>
<td>363</td>
<td>403</td>
<td>458</td>
<td>1266</td>
<td>161 (13)</td>
</tr>
<tr>
<td>Arua</td>
<td>140</td>
<td>249</td>
<td>80</td>
<td>469</td>
<td>1731</td>
<td>36 (8)</td>
</tr>
<tr>
<td>Fort Portal</td>
<td>403</td>
<td>51</td>
<td>2</td>
<td>0</td>
<td>4</td>
<td>10 (0)</td>
</tr>
<tr>
<td>Kisenyi</td>
<td>14</td>
<td>62</td>
<td>82</td>
<td>158</td>
<td>32 (20)</td>
<td></td>
</tr>
<tr>
<td>Koboko</td>
<td>30</td>
<td>71</td>
<td>71</td>
<td>172</td>
<td>10 (6)</td>
<td></td>
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<tr>
<td>Kiswa</td>
<td>14</td>
<td>62</td>
<td>82</td>
<td>158</td>
<td>32 (20)</td>
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<tr>
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<td>61</td>
<td>46</td>
<td>263</td>
<td>51 (19)</td>
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<tr>
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<td>62</td>
<td>82</td>
<td>158</td>
<td>32 (20)</td>
<td></td>
</tr>
<tr>
<td>Mengo</td>
<td>70</td>
<td>7</td>
<td>27</td>
<td>34</td>
<td>2 (6)</td>
<td></td>
</tr>
<tr>
<td>The Surgery</td>
<td>57</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>5 (7)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ILI, influenzalike illness; NA, not applicable (sentinel site not enrolled during this period); SARI, severe acute respiratory illness.

* Surveillance performed from April through December.

* Surveillance performed from January through September.

* Children <2 mo of age were excluded.
(P > .99) and 0.0% (0 of 5) with illness onset >7 days before specimen collection (P > .99).

Patients aged 5–14 years had the highest percentage of influenza-positive specimens (19.6%; 100 of 509 specimens); the lowest percentage (9.1%; 136 of 1487 specimens) was among patients aged 0–4 years (Table 1). Patients aged 5–14 years were more likely to test positive for influenza than patients aged 0–4 years (odds ratio, 2.43; 95% confidence interval, 1.82–3.25). A higher percentage of female patients tested positive for influenza virus, compared with male patients (14.6% vs 11.8%; P = .04).

Sentinel site hospitals account for 8.5% of public hospitals (5 of 59) and 4.4% of all hospitals (5 of 113) in Uganda. Sentinel site clinics account for <1% of all public health clinics (5 of 2242) and <1% of all health clinics (5 of 3125) in Uganda. Among the 10 sentinel sites, the percentage of influenza-positive specimens ranged from 0.0% (0 of 10 specimens) at the outpatient clinic in Fort Portal to 77.2% (44 of 57 specimens) at The Surgery, a private 24-hour emergency care clinic in Kampala (Table 1). The samples from the latter site were collected during a 10-month period in 2009, and the majority of the influenza-positive specimens (34 [77.3%]) were seasonal A/H1.

The proportion of samples that were positive for influenza throughout the study period and the total number of influenza-positive samples were higher during May–November, compared with other months. The mean monthly percentage of influenza-positive specimens was 13% (standard error, 14%). The percentage of influenza-positive specimens was highest during October 2009, when 47% of all specimens were positive (Figure 2). The first case of pH1N1 at a sentinel site in Uganda was identified on 30 June 2009; the largest number of pH1N1 cases occurred during November 2009, when pH1N1 accounted for 66.7% (4 of 6) of all influenza cases. From December 2009 through April 2010, pH1N1 continued to cocirculate. However, pH1N1 was displaced as the dominant strain in May 2010 by Influenza A/H3 (Figure 2).

**DISCUSSION**

We report results of the first study of the epidemiology and seasonality of influenza in Uganda. Surveillance throughout 4 years revealed that influenza occurred year-round in Uganda, with seasonal peaks, and among infected persons of all age groups. Increased influenza activity occurred during May–November, and influenza activity was most pronounced during October–November. Although no corresponding climatology data were analyzed for this study, temperature varies little throughout the year in Uganda, with the coldest average temperatures occurring during June–September. Rainy seasons typically occur during 2 periods in Uganda: late March–June and September–November, when the more intense rains occur [13]. Therefore, the periods of increased influenza activity in Uganda overlapped with periods of colder temperatures and increased rainfall. Although studies have reported viral diseases, including influenza, to be associated with cool and dry seasons in West Africa, our findings in Uganda are consistent with the influenza seasonality that has been described in regard to the tropical countries of Brazil, Singapore, and Thailand, where increased influenza activity has been associated with periods of increased rainfall [5, 6, 8, 16–18].

Although all age groups were affected with influenza, we determined that over half of the influenza cases were among children aged <5 years. However, while the majority of cases occurred among children aged <5 years, the proportion of influenza-positive specimens was highest among children aged 5–14 years, consistent with findings in Bangladesh [19]. The relatively low proportion of influenza cases among children aged <5 years with respiratory illness in Uganda might be explained by the fact that other respiratory viruses (eg, respiratory syncytial virus) play a greater role in respiratory illness among this age group [20]. The age range of persons with influenza in our study was 2 months to 67 years, which is comparable to other studies in countries with low socioeconomic status [6, 19].

During the study period, influenza A was the dominant circulating virus. Influenza A/H3 was the dominant influenza A subtype and accounted for 42.3% of positive specimens (n = 152 patients). pH1N1 first appeared in Uganda in June 2009, within 2 months after pH1N1 was first identified in North America, and continued to circulate throughout the country during the remainder of the study period. Influenza B was predominant in the months immediately following (ie, July–October 2009) the first case of pH1N1 in Uganda.

One limitation of our surveillance system was that our sentinel sites are not evenly distributed throughout the country, and the majority of our sites were located in areas of high population density. Consequently, our findings might not be generalizable to the whole country. Moreover, because of the lack of monthly rainfall and temperature data for each sentinel site, we were unable to assess whether seasonality and climate varied by sentinel site. The familiarity of surveillance officers with the ILI and SARI case definitions varied across the sentinel sites. This led to misclassification of patients and subsequent exclusion of approximately one-third of patients because they did not meet the ILI or SARI case definition. During the first 2 years of surveillance, the focus was on patients with ILI, and SARI surveillance was not initiated until late 2009. Consequently, we had few patients with SARI, and only 6 patients with SARI tested positive for influenza. Therefore, we were unable to characterize type and subtype distribution among hospitalized influenza cases, and we could not describe the epidemiology of more severe influenza disease. Because of resource and personnel constraints, surveying all patients seeking care for ILI was impossible, and screening only patients admitted with a clinical diagnosis of pneumonia resulted in...
for patients with SARI probably caused us to miss SARI cases in patients admitted for other causes. We do not know, therefore, how many total ILI and SARI cases presented to our surveillance sites during the surveillance period, and we cannot determine the contribution of ILI cases, SARI cases, or influenza-associated respiratory illness, to total hospital admissions and outpatient visits. Our surveillance system screens only patients aged >2 months for influenza because of the difficulties in specimen collection, resulting in our inability to characterize the epidemiology of influenza from extremely young children, a group that potentially can be protected by maternal vaccination.

Establishment of influenza surveillance in Uganda has afforded multiple benefits. The current surveillance system for influenza and the cadre of healthcare professionals who have been trained to work in it has improved Uganda's ability to detect potential epidemics of influenza and other respiratory diseases. The surveillance system has allowed us to define the seasonality and epidemiology of influenza in Uganda. This information can be used for people who reside in or travel to Uganda and have access to influenza vaccination and to inform future public health policies. For countries designing similar systems, rigorous training of healthcare personnel regarding case definitions can help avoid concerns related to misclassification that we encountered. Our approach to starting with a limited number of sites, however, made identifying problems with our system possible, and we addressed those problems before expanding the system. Surveillance for influenza, like many other kinds of surveillance, requires human and financial resources, which are inherent problems for sustainability. However, even a smaller surveillance system with fewer sites can provide crucial information about influenza activity.

During April 2007 through September 2010, influenza was a constant contributor to outpatient and inpatient respiratory illness in Uganda. Peak influenza virus circulation coincided with the second rainy season, which included the months of October and November. Continued and increased influenza surveillance in Uganda, with a focus on hospitalized patients, is needed to better understand the seasonality, epidemiology and the burden of severe influenza disease in Uganda and provide information on which influenza education campaigns and policy decisions can be based.

**Supplementary Data**

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary
data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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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. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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