Virological Surveillance of Influenza-Like Illness Among Children in Ghana, 2008–2010

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Background. The global annual attack rate for influenza is estimated to be 10%–20% in children, although limited information exists for Africa. In 2007, Ghana initiated influenza surveillance by routine monitoring of acute respiratory illness to obtain data on circulating strains. We describe influenza surveillance in children <11 years old who had influenza-like illness (ILI) from January 2008 to December 2010.

Methods. Oropharyngeal swabs from pediatric outpatients with ILI attending any of 22 health facilities across the country were submitted. We tested swabs for influenza virus using molecular assays, virus isolation, and hemagglutination assays.

Results. Of the 2810 swabs, 636 (23%) were positive for influenza virus. The percentage of positives by gender was similar. The proportion of ILI cases positive for influenza increased with age from 11% (31/275) in infants (aged 0–1 years) to 31% (377/1219) among children aged 5–10 years (P < .001). The majority of cases were influenza A (90%), of which 60% were influenza A(H1N1)pdm09. In all 3 years, influenza activity appeared slightly higher during May through July.

Conclusions. During the 3 years of influenza surveillance in Ghana, children aged <11 years bore a high burden of influenza-associated ILI.

Influenza is associated with highly contagious respiratory infections throughout the world [1, 2]. Influenza spreads during seasonal epidemics, resulting in the deaths of 250 000–500 000 people every year [3], and influenza pandemics can cause millions of deaths [3]. Influenza viruses attack all age groups, although the young and old are the most severely affected by seasonal strains [4, 5]. An estimated 13.8–16 million cases of influenza-associated respiratory illness occur annually among people <20 years of age [6]. The global annual attack rate for influenza is estimated to be 10%–20% in children, though the burden in developing countries is not well known [7]. For every 100 children, up to 6–15 outpatient visits and 3–9 courses of antibiotics per year may be due to influenza-like illness (ILI) [8].

Because influenza in children can produce different symptoms than in adults, and many other etiologies of respiratory tract infection (RTI) exist, pediatric influenza infections are often missed. In many health centers, laboratory diagnostics for viral infections do not exist. In Ghana, young children suffer from a high burden of RTI, which is the second most common outpatient diagnosis after malaria [9].

In order to obtain current information about the national activity of influenza, to monitor circulating influenza strains, and to better understand the epidemiology of ILI, the Ghana Health Service and the National Influenza Center (NIC) initiated national ILI surveillance for all ages in 2007, in collaboration with the World Health Organization (WHO), the US Centers for Disease Control and Prevention (CDC), and the US Naval Medical Research Unit No. 3. We describe here the results from the first 3
complete years of influenza surveillance in Ghana, from January 2008 to December 2010.

MATERIALS AND METHODS

Setting
Sentinel sites for routine national influenza virus surveillance in Ghana were set up as part of the Integrated Disease Surveillance and Response (IDSR) system of the Ghana Health Service, which is the health delivery wing of the Ministry of Health. The Noguchi Memorial Institute for Medical Research (NMIMR) was designated as the NIC by the Ghana Health Service in 2007 and formally recognized by the WHO in 2010. Its role is to monitor the circulation of influenza viruses in Ghana and provide information to the WHO Global Influenza Surveillance and Response System (GISRS). In September 2007, 9 sentinel sites were established in 3 of the country’s 10 regions. The number of sites increased to 16, covering 4 additional regions in 2009, and to 22 sites, covering all regions (Figure 2) by December 2010. Tropical climatic conditions prevail in Ghana; warm and comparatively dry along the southeast coast, hot and humid in the southwest, hot and dry in the north. Surveillance sites include outpatient health centers and outpatient departments at hospitals.

Subjects
At all sites, we targeted patients aged 0–10 years who presented with ILI. Children within this age range were targeted because of the paucity of information concerning ILI in this age group in sub-Saharan Africa and reports from elsewhere in the world showing that influenza disease burden is significant in children. We used the WHO case definition for ILI: sudden onset of fever >38°C with at least 1 respiratory symptom, such as cough or sore throat, in the absence of another known cause of symptoms [10]. Oropharyngeal swabs (Ropewalk; and Becton, Dickinson, and Company) were taken from all ILI patients by trained healthcare workers following informed consent from the parent or guardian. Ethical approval for the surveillance of influenza virus in acute respiratory illness in Ghana was obtained from the Institutional Review Board of NMIMR. Demographic and clinical details of patients were recorded on a case investigation form designed for ILI surveillance that accompanied the swab.

Sample Collection and Processing
Oropharyngeal swabs were placed in a vial (Sarstedt) containing 1 mL of virus transport medium (VTM) and transported within 72 hours in a cold box with ice packs at 4°C to the laboratory at the NIC. Upon arrival in the laboratory, each vial with the swab was vortexed for 30 seconds; an aliquot of 200 µL was then transferred to a new vial for molecular and virus isolation attempts. The original swab and residual VTM were cryopreserved at −70°C. All specimens were processed by real-time reverse-transcription polymerase chain reaction (rRT-PCR) within 72 hours of collection.

Genomic Detection of Influenza Viruses
Viral RNA was extracted from 140 µL of the sample using the QIAamp viral RNA kit (Qiagen) according to the manufacturer’s instructions. The RNA was eluted from the spin columns with 60 µL of RNase/DNase-free elution buffer provided with the kit. The RNA was then subjected to an rRT-PCR assay for human influenza virus detection and characterization from the CDC. The rRT-PCR Flu assay is a nucleic acid amplification assay that detects seasonal influenza A and B viruses and further characterizes influenza A subtypes A/H1, A/H3, and A/H5. The panel includes a set of oligonucleotide primers and dual-labeled hydrolysis (TaqMan) probes for qualitative detection and characterization of human influenza viruses. The SuperScript III PlatinumOne-Step quantitative RT-PCR kit (Invitrogen) or AgPath-ID One-Step RT-PCR Kit (Ambion) were used with a 25-µL reaction mixture under the following conditions: 0.5 U of kit-supplied enzyme mixture, 40 µM of each primer, and 10 µM of each TaqMan probe. The reverse-transcription step for all primer sets was fixed for 30 minutes at 50°C and 2 minutes at 95°C. A 2-step PCR cycling protocol of 45 cycles of 95°C for 15 seconds and 55°C for 30 seconds was performed. All temperature transition rates were set at a maximum of 20. Fluorescence data were acquired at the end of each annealing step. The rRT-PCR assays were performed on real-time PCR instruments (Applied Biosystems) with SDS software version 1.4 (Applied Biosystems). Results of the rRT-PCR assays were determined by the analyses of cycle threshold values generated by SDS auto-analysis on samples against reference positive and negative controls obtained from the CDC (rRT-PCR Flu Panel 2008/2009).

Virus Isolation From Cultures and Typing
Virus isolation from cell cultures was attempted for all samples that were initially positive by rRT-PCR. Madin-Darby canine kidney and sialytransferase cell lines propagated at 37°C in Eagle’s minimum essential medium supplemented with fetal calf serum (10% [vol/vol]) were inoculated using a sample from the vortexed VTM. Cell cultures were then maintained in serum-free medium in the presence of 0.25 µg/mL of beef pancreas trypsin (Sigma-Aldrich) and incubated for 4 days at 37°C. Supernatant medium was tested using a hemagglutination (HA) assay [11] to determine growth of influenza virus. Positives cultures were then subjected to HA and hemagglutination inhibition (HAI) assays for influenza based on CDC protocols [11]. Identification of influenza A virus subtypes was performed using reference sera and antigens described in the WHO manual on influenza diagnosis and surveillance [10]. A subsample of influenza A(H1N1)pdm09
virus isolates was sent to the WHO Influenza Collaborating Center, National Institute for Medical Research, London, UK, for phylogenetic analyses and antiviral susceptibility testing by sialidase inhibition assays.

**Data Analysis**

Demographic and clinical data on patients, along with results from the rRT-PCR and antigenic typing based on HAI, were entered in an Excel spreadsheet (Microsoft Office 2003). No specific identifying information on patients was entered. Basic analyses were performed using Microsoft Excel and Statistical Package for the Social Sciences (SPSS 2007), using the \( \chi^2 \) test for difference in proportions between categories.

**RESULTS**

**Characteristics of the Study Population**

A total of 2810 children <11 years old with ILI were evaluated from January 2008 to December 2010. Oropharyngeal swabs were obtained from 227 children in 2008, 882 in 2009, and 1696 in 2010 (Table 1). Of all the children evaluated, 1485 (53%) were male. Children in all age groups presented with a cough. Swabs were taken between 1 and 15 days after the reported onset of ILI symptoms (mean, 3.6 days); 90% of all swabs were obtained within 3 days of symptom onset (Supplementary Figure 1).

**Influenza Virus Detection by rRT-PCR Assays**

Overall, 636 (23%) of the 2810 respiratory samples were positive for either influenza A or B by rRT-PCR. Of the samples tested, 11% were positive for influenza in 2008, compared with 24% in 2009 and 23% in 2010. The proportion of samples testing positive increased with age of the child, from 11% (31/275) in infants <1 year old to 31% (377/1219) among those 5–10 years of age (\( P < .001 \)). The rate of influenza virus detection peaked at three days after onset of ILI symptoms (Supplementary Figure 1). Throat swabs taken within the first 3 days of onset of ILI were more likely to be influenza positive (31%) compared with specimens collected 7–15 days after symptom onset (6%) (\( P < .02 \)). Of the 397 influenza-positive specimens identified in 2010 (Table 1), 342 (86%) tested positive for influenza A(H1N1)pdm09.

In all 3 years, influenza activity appeared to be slightly higher during the period May to July, which corresponds to the rainy season in Ghana (Figure 2). The greatest number of ILI cases occurred in March and April of 2010 (625 and 385, respectively); during these 2 months approximately 30% of ILI cases were positive for influenza. Among all positive cases, 90% were influenza A (Supplementary Table 1), and the rest were influenza B. More than half (57%) of the influenza A cases were influenza A(H1N1)pdm09; 26% were influenza A (H3N2).

**Virus Isolation and Characterization**

In all 3 years, we isolated and typed 29/63 (46%) influenza B specimens, compared with 152/573 (27%) influenza A specimens (Table 1). Antigenic and phylogenetic analyses of the pandemic influenza strains were performed on 13 specimens. These analyses revealed that the influenza A(H1N1)pdm09 strains were antigenically similar to the virus used for the initial pandemic influenza vaccine—A/California/7/2009. All 13 virus isolates screened on sialidase inhibition assays were susceptible to oseltamivir and zanamivir (sialidase inhibitors).

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**Table 1. Characteristics of Children Aged <11 Years With Influenza-Like Illness, January 2008–December 2010, Ghana**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Characteristic</th>
<th>Cases of ILI, No. (%); N = 2810</th>
<th>Influenza Positive/Isolate, No. (%); n = 636</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008</td>
<td></td>
<td>232 (8.3)</td>
<td>24 (3.8)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>2009</td>
<td></td>
<td>882 (31.4)</td>
<td>215 (33.8)</td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td></td>
<td>1696 (60.4)</td>
<td>397 (62.4)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>1485 (52.8)</td>
<td>340 (53.5)</td>
<td>.73</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>1325 (47.2)</td>
<td>296 (46.5)</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>&lt; 1</td>
<td>275 (9.8)</td>
<td>31 (4.9)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td></td>
<td>1–4</td>
<td>1316 (46.8)</td>
<td>228 (35.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5–10</td>
<td>1219 (43.4)</td>
<td>377 (59.3)</td>
<td></td>
</tr>
<tr>
<td>Symptoms</td>
<td>Reported fever (( \geq )38°C)</td>
<td>2545 (90.6)</td>
<td>507 (79.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cough</td>
<td>2675 (95.2)</td>
<td>590 (92.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sore throat</td>
<td>1247 (44.4)</td>
<td>233 (36.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Runny nose</td>
<td>1313 (46.7)</td>
<td>256 (40.3)</td>
<td></td>
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<tr>
<td></td>
<td>Myalgia</td>
<td>293 (10.4)</td>
<td>63 (9.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Headache</td>
<td>806 (28.7)</td>
<td>237 (37.3)</td>
<td></td>
</tr>
</tbody>
</table>
We established a successful influenza virus surveillance system in Ghana that has the ability to perform rapid virus detection by rRT-PCR assays, to isolate viruses through cell culture, and to perform HAI assays to monitor circulating influenza virus strains. Although the surveillance system was initially limited in 2007, surveillance sites are now present in all 10 regions of the country, and regularly submit samples from ILI cases to the NIC for influenza virus testing.

We found that the proportion of influenza-positive ILI cases was highest in school-aged children compared to infants and preschool-aged children. Because these children are likely to have more close contacts with peers, and therefore have more opportunities to become infected, these findings are consistent with a transmissible virus like influenza, and support observations from previous studies that school-aged children experience high attack rates [12, 13]. School-aged children also play an important role in community-wide transmission [13, 14].

In our surveillance system, sample collection and laboratory testing for influenza surveillance were prompt and efficient; 90% of swabs taken from children were collected within 3 days of onset of symptoms, and all swabs were transferred to the laboratory and processed within the requisite 72 hours.

We saw a large increase in the number of influenza cases among Ghanaian children in 2009–2010, largely due to outbreaks of influenza A(H1N1)pdm09; the pandemic strain accounted for 86% of all type A cases identified in 2010 alone. The number of cases may have further increased due to more children being brought to health facilities for evaluation following press coverage of the first identified infection and heightened public awareness of pandemic influenza [15].

We focused here on influenza surveillance among children as they bear the greatest burden of respiratory infection, and their disease course is often more severe than that in young infants.
adults [16]. Over the 3 years of surveillance, the vast majority of children were infected with influenza A. The predominance of A(H3) and influenza A(H1N1)pdm09 influenza found in the study represents the prevalence of these subtypes among circulating influenza viruses in Ghana at the time. Individuals with influenza strains associated with less severe symptoms, such as seasonal A(H1) and B, may exhibit different health-seeking behaviors [17, 18].

Throughout the 3 years, influenza activity appeared to be slightly higher during May through July, which corresponds to the rainy season in Ghana. The data can therefore be interpreted as showing little seasonality for influenza virus activity. If there was some minor seasonality, it was overwhelmed by pandemic influenza from 2009 through to 2010 (Figure 1). A similar pattern was shown in a study in tropical northeastern Brazil, where increased influenza activity occurred during rainy periods. [19]. The high relative indoor humidity during the rainy season in the tropical and subtropical regions and during the winter months in the temperate zones may prolong the survival of influenza virus in aerosols, contributing to the seasonal spread [20].

The occurrence of ILI in children varied from one year to another during the period of our study. However, we consistently recorded a greater proportion of collected swabs for influenza virus during the peak of ILI activity (Table 1). Influenza accounted for a much greater proportion of positive swabs during the peak weeks of ILI.

Our findings are subject to the following limitations. The respiratory specimens collected initially were not representative of the country, because virological surveillance started in only 3 regions of the country before it was gradually extended to encompass all 10 regions. Moreover, we could not determine the clinic-based burden of ILI because total numbers of child outpatient visits were not recorded. Our data likely underrepresent the attack rate among infants, since sore throat, a criterion for ILI, is difficult to ascertain in very young children. Our sentinel surveillance system, which is based on only oropharyngeal swabs, might have missed some cases that could have been picked up with nasopharyngeal swabs. The use of both nasopharyngeal and oropharyngeal swabs are reported to maximize sensitivity for a large number of viruses including influenza [21].

Data from this study have partly contributed to the integration of influenza surveillance into the IDSR system of the Ministry of Health, which is implemented by the Disease Surveillance Department of the Ghana Health Service.

The establishment of routine influenza virus surveillance among children in Ghana has made a significant contribution to the WHO GISRS, adding to information on the spread of influenza globally, as well as in West Africa. Such data inform the yearly recommendations for vaccine composition for both the Northern and Southern Hemispheres and can indicate the emergence of novel influenza viruses with pandemic potential [22]. The rational and appropriate use of influenza vaccines can reduce the burden of disease in children [23], and data from this study will help inform public health policy in the development of effective strategies for influenza immunization of young children in Ghana. Further evaluation of other viruses that cause ILI in children can help determine the most effective and appropriate clinical management of children presenting with respiratory infections.

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 copyrighted. 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.

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