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The Timeline of Influenza Virus Shedding in Children and Adults in a Household Transmission Study of Influenza in Managua, Nicaragua

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Abstract

In a household transmission study in Nicaragua, children under 6 years of age had a longer duration of pre-symptomatic influenza virus shedding than adults. The duration of post-symptomatic influenza virus shedding was longest in children 0–5 years of age, followed by children 6–15 years of age and adults.

Keywords

Influenza virus shedding; pre-symptomatic; post-symptomatic; children; adults

BACKGROUND

Basic knowledge of the timeline of virus shedding is crucial for a full understanding of influenza transmission and control. Children are particularly prone to influenza infection and are an important source of influenza transmission. Duration of influenza virus shedding is commonly measured as time to shedding cessation following symptom onset even though pre-symptomatic virus shedding occurs in children^{1–5}. Some studies have found that the duration of viral shedding in children may be longer than in adults, however, this has not been consistently reported across studies^{2, 3, 6, 7}. In this study, we aim to characterize pre- and post-symptomatic viral shedding duration using data from a case-based ascertainment household study in Nicaragua. The age-specific event times of viral shedding onset and

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Conflict of Interest Statement

The authors declare no conflict of interest.

cessation are described and compared. We also explore how sex, antiviral use and influenza types and subtypes may explain heterogeneity in viral shedding.

METHODS

Participants and procedures

The Nicaraguan Pediatric Influenza Cohort Study is an ongoing study based in the Health Center Sócrates Flores Vivas (HCSFV) in Managua, Nicaragua, which is the government health center that serves the catchment area of District II. Patients who attended the HCSFV for medical care were screened for their eligibility to participate in an influenza household transmission study. Index cases were recruited both from the pediatric cohort study⁸ and the national influenza surveillance program. Patients who 1) had a positive QuickVue Influenza A+B rapid test result, 2) experienced symptom onset of an acute respiratory infection (ARI) within the previous 48 hours, and 3) lived with at least one other household member were invited to participate. A nasal and oropharyngeal swab, and daily symptom history were collected from consented patients at the Health Center.

Household members were monitored for influenza infection through 5 home visits conducted every 2–3 days over a period of 10–14 days beginning with the identification of the index influenza case. During each visit, the index cases and their household members provided additional nasal and oropharyngeal swabs. Additional respiratory samples were collected if the participant presented at the health center in the 30 days following enrollment. Signs and symptoms of ARI included fever or feverish, cough, sore throat, runny nose, malaise, muscle or joint pain, difficulty breathing, respiratory sounds, nasal flaring and chest indrawing.

Participants provided written informed consent and parental permission was obtained from parents or legal guardians of children. In addition, verbal assent was obtained from children aged 6 years and older. The study was conducted in accordance with the Helsinki Declaration and was approved by Institutional Review Boards at the following institutions: 1) University of Michigan, 2) Centro Nacional de Diagnóstico y Referencia, Ministry of Health, Nicaragua, and 3) University of California, Berkeley.

Laboratory methods

Combined nasal and oropharyngeal swabs were collected from all participants and placed in a single tube of viral transport media. Specimens were maintained at 4°C and sent within 48 hours to the National Virology Laboratory at the Nicaraguan Ministry of Health on the day of collection. The samples were tested in the National Virology Laboratory by real-time reverse-transcription polymerase chain reaction (rRT-PCR) using standard protocols validated by the Centers for Disease Control and Prevention⁹.

Statistical analyses

The primary outcome measure for viral shedding was rRT-PCR positivity. Survival analyses estimated 1) time from symptom onset to viral shedding onset, 2) time from symptom onset to viral shedding cessation, and 3) viral shedding duration, i.e. the duration between viral

shedding onset and cessation. The analyses allowed event times of viral shedding onset and cessation to be censored, and took into account within-household clustering¹⁰. Viral shedding onset of index cases was left censored whereas left, right or interval censoring may occur for shedding in household contacts. All statistical analyses were conducted in R version 3.1.3. Technical details of the statistical methods are presented in the supplementary materials.

RESULTS

A total of 168 index cases and 668 household contacts were recruited during 2012–2014, when influenza A(H1N1)pdm09, A/H3N2/Perth/16/2009-like, A/Texas/50/2012-like, and B (Yamagata and Victoria-lineages) circulated. Among those recruited, 144 index cases and 112 household contacts had rRT-PCR confirmation of influenza infection. Eight vaccinated rRT-PCR positive participants were excluded because this study is underpowered to estimate the effect of vaccination. These analyses focus on describing the timeline of viral shedding in relation to symptom onset date. Twenty-eight rRT-PCR positive household contacts who did not show any signs or symptoms of acute respiratory infection (ARI) were excluded from the main analyses. The percentage of rRT-PCR confirmed household contacts 0–5 years of age who showed signs or symptoms of ARI was 32.1%, compared with 21.4% and 20.5% among contacts 6–15 years of age and adult contacts respectively. The mean body temperature on the date of symptom onset (if any) for rRT-PCR confirmed cases among household contacts were 38.0°C for children 0–5 years of age, 37.9°C for 6–15 years of age and 38.0°C for adults. The main analyses included 220 rRT-PCR positive individuals who showed ARI symptoms; among them, 61% received Oseltamivir.

rRT-PCR positivity was observed as early as 5 days before symptom onset, and at the latest 21 days after symptom onset. Therefore, event times were corrected by +5 days to allow for pre-symptomatic shedding in our analyses.

Time from illness onset to onset of viral shedding

Univariate analyses estimated that 69% of children 0 to 5 years of age, 67% of children 6 to 15 years of age and 45% of adults (16 years of age or above) showed pre-symptomatic viral shedding. The median time from symptom onset to viral shedding onset was –1.1 days (Interquartile range IQR –2.3, 0.4) for young children, –1 day (IQR –2.2, 0.5) for older children, and 0.2 days (IQR –1.2, 2.3) for adults.

Since no differences were found in the event times of viral shedding onset and cessation between males and females (SDC Figure 2), we included only age group and influenza type and subtype as covariates in our main analysis. There were no detectable differences in time from symptom onset to viral shedding onset between young and older children (acceleration factor AF 1.13; 95% credibility intervals C.I. 0.90, 2.10) (Appendix Table 1). Adults started shedding virus 53% later than young children (AF: 1.53, 95% C.I: 1.19, 2.10), i.e. approximately 2 days later. No significant differences were detected by influenza virus types/subtypes.

Time from illness onset to viral shedding cessation

Univariate analyses estimated the median time from symptom onset to viral shedding cessation was 3.1 days (IQR 2.2, 4.4) for young children, 2.3 days (IQR 1.6, 3.7) for older children and 2.7 days (IQR 1.9, 4.0) for adults.

Only 19% of subjects who had taken Oseltamivir completed a full course of medication (5 days). No significant association was found between Oseltamivir use, sex and viral shedding cessation (Appendix Figure 1 & Appendix Table 2). The estimated time from symptom onset to viral shedding cessation was 47% shorter for adults compared to young children (AF 0.53; 95% C.I. 0.42, 0.67), i.e. approximately 4 days shorter (Appendix Table 1). The estimated time was 20% shorter for older children compared to young children (AF 0.80; 95% C.I. 0.66, 0.96), i.e. approximately half a day shorter. Individuals who had influenza co-infection stopped shedding virus 56% later compared to A(H1N1)pdm09 infection alone (AF 1.56; 95% C.I. 1.04, 2.36).

Total viral shedding duration

Figure 1 shows the survival curves for influenza virus-specific shedding. Each panel represents the proportion of participants that were still rRT-PCR positive within 20 days of viral shedding onset. Viral shedding duration was shortest in adults, and older children had a tendency to shed the virus for a shorter duration compared to younger children. Sensitivity analyses including index cases and household contacts in separate models generated consistent results.

DISCUSSION

In our study, we found that children started to shed influenza virus earlier than adults and that pre-symptomatic viral shedding was frequently observed. Children also continued to shed virus longer than adults after illness onset. Previous systematic reviews did not find consistent results comparing viral shedding duration between children and adults^{6,7}. Our findings are consistent with two other cohort studies that included pre-symptomatic viral shedding in their analyses^{2,3}. Our analyses highlight a new finding that onset of rRT-PCR positivity occurred earlier in children compared to adults. Pre-existing immunity may determine early control of virus replication, and children often have lower pre-existing influenza-specific antibodies compared to adults. Further studies should examine the relationship of immune correlates of protection with influenza virus shedding to identify the biological mechanism that explains the observed age-related differences.

There are several limitations to our study. First, respiratory specimens were collected every 2 to 3 days. More frequent specimen collection may improve the accuracy of our estimates. The use of rRT-PCR positivity is not a perfect measure for infectiousness since non-viable influenza viruses can be detected. On the contrary, rRT-PCR might not be able to detect low-level virus shedding, leading to underestimation in shedding duration. The use of quantitative RT-PCR would have allowed us to characterize change in infectiousness during the course of infection. rRT-PCR status can be affected by the quality of respiratory specimens. We detected a high (16%) secondary attack rate of rRT-PCR-confirmed

infections among household contacts, which indicates our study was efficient in detecting viral shedding. It is possible that there were systematically longer delays in reporting illness onset for young children. Potential differences in shedding risk might exist between those who took Oseltamivir and those who did not, leading to spurious null association between Oseltamivir use and viral shedding. Lastly, our study may be underpowered to detect small differences between genders and influenza subtypes/types.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Persistence of viral shedding within 20 days of viral shedding onset