

Virus Detection and Duration of Illness Among Patients With 2009 Pandemic Influenza A (H1N1) Virus Infection in Texas

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Knowledge from early outbreaks is limited regarding the virus detection and illness duration of the 2009 pandemic influenza A (H1N1) infections. During the period from April to May 2009 in Texas, we collected serial nasopharyngeal (NP) and stool specimens from 35 participants, testing by real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) and culture. The participants were aged 2 months to 71 years; 25 (71%) were under 18. The median duration of measured fever was 3.0 days and of virus detection in NP specimens was 4.2 days; however, few specimens were collected between days 5–9. The duration of virus detection (4.2 days) was similar to the duration of fever (3.5 days) (RR, 1.14; 95% CI, .66–1.95; $P = .8$), but was shorter than the duration of cough (11.0 days) (RR, .41; 95% CI, .24–.68; $P < .001$). We detected viral RNA in two participants' stools. All cultures were negative. This investigation suggests that the duration of virus detection was likely similar to the seasonal influenza virus.

In response to the rapid spread of a novel influenza virus, 2009 pandemic influenza A (H1N1) (pH1N1), across multiple continents, the World Health Organization raised the pandemic alert level to phase 6 on June 11, 2009 [1]; this was the first official influenza pandemic since 1968. During the early stages of the pandemic, infection control guidelines to minimize transmission recommended that persons with an influenza-like illness

(ILI), defined as the presence of fever with a cough or sore throat, stay home from school or work for 7 days or until the resolution of fever for at least 24 hours, whichever was longer [2]. This time period was chosen because results from experimental influenza virus infection among healthy volunteers indicated that for seasonal influenza, the duration of viral shedding is generally 5–7 days [3]. As more information on severity became available, the recommendation was changed to exclude persons from work or school for the duration of fever plus 24 hours after fever resolution in the absence of the use of fever-reducing medications.

Early reports of a high proportion of patients experiencing diarrhea while infected with pH1N1 [4] prompted the question of whether the 2009 pandemic influenza A (H1N1) virus is shed in human stool. The pH1N1 virus has been isolated from rectal swabs of experimentally

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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infected ferrets [5], and was detected in the stool of four persons with pH1N1 confirmed from respiratory secretions in Hong Kong [6]. Information on the duration of specific clinical symptoms and viral shedding patterns of the pH1N1 virus can inform infection control and community mitigation guidance efforts aimed at reducing the spread of influenza. We report results of an investigation of clinical signs and symptoms and virus detection by molecular methods conducted soon after the first recognition of pH1N1 in the United States.

METHODS

Investigation Population

We identified persons with laboratory-confirmed influenza A who were residents of Bexar, Guadalupe, and Comal counties, Texas, during 10 April–8 May 2009, through an active review of state and city influenza laboratory testing data. Beginning on 29 April 2009, all persons with laboratory specimens positive for nonsubtypable influenza A by real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) [7] were approached and asked to participate in an investigation of virus detection, with the goal of obtaining 40 participants. Enrolled participants were part of a larger investigation of household transmission of pH1N1 virus in San Antonio, Texas, in which persons with suspected ILI in schools, clinics, and households of infected persons were identified through intensive case-finding; NP and stool specimens from these persons were tested by rRT-PCR [8].

Household Visits

We visited participants at their home on the day of enrollment and every other day until 10 days after cessation of fever, and then at 2 weeks and 3 weeks after defervescence. For participants who reported never having a fever, we conducted follow-up based on the day of first symptom onset rather than the last day of fever. During the first visit, we recorded baseline demographic characteristics, antiviral treatment history, date of symptom onset (fever, cough, or sore throat), self-report of underlying medical conditions, and presence of immunosuppression (defined as the use of immunosuppressive medications within 7 days of illness onset or immunosuppressive medical conditions, such as human immunodeficiency virus infection; rheumatologic condition; hematopoietic stem-cell transplant recipient undergoing antirejection medication; solid-organ transplant recipient receiving antirejection medication; congenital immunodeficiency disorder; chemotherapy treatment for cancer; autoimmune conditions and treatments; and chronic corticosteroid use) [9]. At each subsequent visit, we asked participants about the presence of influenza-associated signs and symptoms and self-report of a measured fever ($\geq 38.0^{\circ}\text{C}$).

Specimen Collection

At each visit, we collected a nasopharyngeal (NP) swab and stool specimen. NP swabs were collected by using flocked swabs with flexible nylon minitips (COPAN Diagnostics, Inc., Murrieta, CA). Swabs were placed in 3.0 mL Remel MicroTest M4RT liquid viral transport media (Thermo Fisher Scientific/Remel Products, Lenexa, KS) or in similar influenza transport media produced and packaged by the Texas Department of State Health Services Laboratory Services Section, and stored at 4°C until shipped. We instructed participants on how to collect whole stool by using a Protocult sterile stool-collection device (Ability Building Center, Rochester, MN). Participants stored collected stool specimens either on foam ice packs or in their domestic refrigerator at 4°C until the next household visit. We shipped the specimens to the Centers for Disease Control and Prevention (CDC; Atlanta, GA) at 4°C within 24 hours of the household visit. At the CDC, stool and respiratory samples were stored at -70°C until thawed for extracts preparation.

Laboratory Testing

We extracted nucleic acid from NP and stool specimen preparations by using the MagNA Pure Compact instrument (Roche Applied Science, Indianapolis, IN) and tested them by semi-quantitative rRT-PCR on a 7500 Fast Dx Real-Time PCR instrument (Applied Biosystems, Inc., Foster City, CA) by using the following markers for pH1N1 virus: influenza A (Flu A), universal swine influenza A (Univ Sw) and swine H1 (SWH1) [7]. We defined a positive test as requiring <40 cycles to detect RNA by all three primer/probe sets, and quantified cycle-threshold values for each instance of virus detection by a primer/probe set. The protocol for testing respiratory specimens was adapted to test stool preparations. The stool preparations were made by thawing the stools and preparing a 10% suspension in phosphate-buffered saline (PBS).

Six of the original diagnostic specimens taken as part of clinical care were cultured in Madin-Darby canine kidney (MDCK) or egg cell cultures. All subsequently collected respiratory specimens that were positive by rRT-PCR for influenza A were inoculated onto MDCK cell cultures and followed daily for 8 days for any evidence of cytopathic effect [10]. At the CDC, respiratory specimens underwent two freeze–thaw cycles: one before PCR testing and one before culturing. Stool suspensions were maintained at 4°C while rRT-PCR data were obtained. Stool suspensions positive by rRT-PCR for any of the three markers were inoculated into cell cultures on the same day without additional freezing or thawing. Stool cultures were followed for 7–8 days for evidence of cytopathic effect. Two subsequent passages into fresh MDCK cells were performed before stools were determined negative. All stool cultures were tested for the presence of pH1N1 nucleic acid.

Data Analysis

We estimated the duration (in days) of influenza-associated signs and symptoms as the last date of reported symptoms minus illness onset date (defined as day 0). If the dates were the same, we called this one day of symptoms. We defined the presence of high-risk conditions for a severe influenza outcome as having one of the following conditions: pregnancy, asthma, chronic obstructive pulmonary disease, chronic lung disease, neurologic or neurodevelopmental conditions, heart disease, blood disorders, endocrine disorders, kidney disorders, liver disorders, metabolic disorders, and immunosuppressive conditions [11]. We defined the duration of viral detection as the number of days from illness onset until the day of the last positive rRT-PCR specimen before two consecutive negative specimens. If two specimens were not collected after the last positive one, we estimated duration until the day of the last positive specimen. We calculated a ratio of rates of ILI symptom cessation (fever, cough, and sore throat) for persons who did or did not receive antiviral medication (defined as receiving oseltamivir or zanamivir within 48 hours of symptom onset, irrespective of dose or duration of treatment) adjusted for age (0–18 years and ≥ 19 years). To assess the association between virus detection cessation and symptom cessation, we divided the incident rate of symptom (fever and cough) cessation by the incident rate of virus detection cessation, where a rate ratio (RR) = 1 indicated that shedding and symptom cessation occurred at the same rate, and $RR < 1$ indicated shedding cessation at a higher rate than symptoms. We compared Kaplan-Meier estimates for different groups using the LogRank test. We used STATA 10 (Stata Corp. College Station, TX) for all analysis.

Ethics Review

This investigation was part of the emergency public health practice response to the pandemic, and was reviewed by a human subjects coordinator at CDC and deemed not to be research in accordance with the federal human subjects protection regulations at 45 Code of Federal Regulations 46.101c and 46.102d and CDC's Guidelines for Defining Public Health Research and Public Health Non-Research. In addition, the privacy rule of the Health Insurance Portability and Accountability Act did not apply because the activity was part of an emergency public health response. Adults provided verbal consent for participation, and parents gave verbal consent for children aged < 18 years.

RESULTS

During April 28–May 24, 2009, we contacted 66 potential participants from the San Antonio area, of whom 42 (64%) agreed to participate (Figure 1). Thirty-five participants were confirmed as having the pH1N1 virus infection and were therefore included in the investigation. One participant provided stool

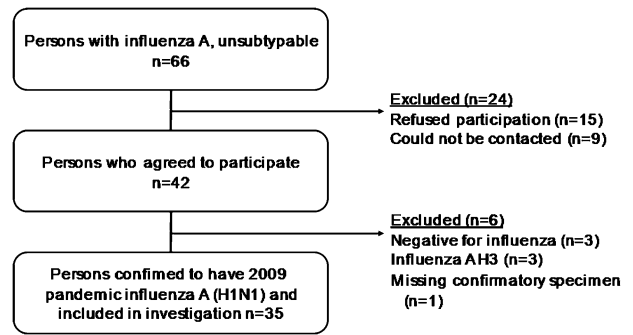


Figure 1. Enrollment of participants in an investigation of duration of illness and virus detection among persons with 2009 pandemic influenza A (H1N1) virus infection, San Antonio, Texas, 2009.

and NP specimens but no symptom data; one person provided stool but no NP specimen; and one person provided NP but no stool specimen. Participants were aged 2 months–71 years at enrollment, with 4 (11%) aged 0–4 years and 21 (60%) aged 5–17 years. Nine of 35 (26%) participants had a medical condition known to increase the risk of severe disease from influenza: asthma ($n = 2$), neurologic disorder ($n = 2$), atrial fibrillation ($n = 1$), diabetes mellitus ($n = 1$), chronic kidney disease ($n = 1$), pregnancy ($n = 1$), and immunosuppression ($n = 1$). Nineteen of 35 (54%) participants started antiviral therapy within 48 hours of illness onset: 16 (84.2%) with oseltamivir, 2 (10.5%) with zanamivir, and 1 (5.3%) with both. The median duration of treatment was 5 days (range, 3–6 days). One participant aged 71 years with a history of asthma and atrial fibrillation was hospitalized; no participants died.

Of 35 participants for whom symptom data were available (Table 1), the most frequently reported symptoms were cough (94%), subjective fever (91%), and documented fever $\geq 38.0^{\circ}\text{C}$ (80%). Where present, the symptoms with the longest median duration were cough (11.0 days; range, 1–20 days), rhinorrhea (median, 9.0 days; range, 1–24 days), and sore throat (median, 7.0 days; range, 2–20 days). Of 32 participants with subjective fever, the median fever duration was 3.5 days (range, 1–10 days); 28 of these persons had a measured fever $\geq 38.0^{\circ}\text{C}$ and the median fever duration was similar at 3.0 days (range, 1–10 days). The duration of symptoms did not differ significantly among persons who reported taking antiviral therapy, compared with persons who did not take antiviral therapy (Table 2).

The median time from symptom onset to collection of the first respiratory specimen after the initial rRT-PCR positive diagnostic specimen was 10 days (range, 5–20 days). The median duration between symptom onset and virus detection by rRT-PCR was 4.2 days (range, 1–19 days) (Figure 2). Duration of virus detection did not differ significantly for persons < 18 years old compared to persons ≥ 18 (RR, 1.24; 95% CI, 0.52–2.72), report of taking antiviral therapy (RR, 0.65; 95% CI, 0.31–1.40),

Table 1. Frequency and duration in days of signs and symptoms for 35 persons infected with 2009 pandemic influenza A (H1N1) virus, San Antonio, Texas, 2009

Symptom	Frequency (n = 35)		Symptom duration ^a Median days (range)	
	Number	Percent (%)	Median	Range
Cough	33	94	11.0	1–20
Subjective fever	32	91	3.5	1–10
Measured fever $\geq 38.0^{\circ}\text{C}$	28	80	3.0	1–10
Rhinitis	21	60	9.0	1–24
Headache	18	51	5.0	1–19
Sore throat	18	51	7.0	2–20
Muscle ache	15	43	4.0	1–10
Difficulty breathing	11	31	5.5	1–12
Vomiting	11	31	3.0	2–10
Diarrhea	7	20	5.0	1–10

^a Median and range are calculated from the onset of first symptom. Median and range values are for participants presenting with particular symptoms.

or presence of a high-risk medical condition (RR, 0.80; 95% CI, 0.34–2.00). The duration of virus detection (4.2 days) was similar to the duration of fever (3.5 days) (RR, 1.14; 95% CI, 0.66–1.95; $P = .8$), but was much shorter than for cough (11.0 days) (RR, 0.41; 95% CI, 0.24–0.68; $P < .001$) (Figure 3). Six (17%) of 35 initial diagnostic clinical specimens were submitted for culture confirmation; all 6 (100%) grew live virus (median, 2.5 days; range, 2–3 days from symptom onset). The virus could not be isolated in culture from the 9 rRT-PCR–positive NP specimens and 18 indeterminate NP specimens (positive for only 1–2 of 3 rRT-PCR assays). However, the follow-up samples all had high cycle-threshold values that indicated low viral load, and the first follow-up sample was collected a median of 10 days (range, 6–19 days) from illness onset.

Of 35 persons submitting stool specimens, 4 specimens were inconclusive for pH1N1 virus by rRT-PCR: 2 from a female,

aged 11 years, on days 14 and 26 after symptom onset (no virus detected on days 16 and 20) in the absence of vomiting or diarrhea, and 2 from a female, aged 71 years, on days 16 and 23 after symptom onset (first specimens taken) and with 5 days of diarrhea and 6 days of vomiting at the beginning of her illness. We were unable to isolate live virus in culture from the 4 stool specimens that were influenza A–positive by only rRT-PCR.

DISCUSSION

In this early investigation of virus detection and duration of illness from pH1N1, virus detection duration was found to be substantially shorter than cough, but similar in duration to fever. The median duration of virus detection was 4.2 days, which is similar to the findings from a systematic review of shedding studies of seasonal influenza strains of a median duration of virus detection of 4.7 days [3]. In our investigation, the median duration of measured fever was approximately 1 day shorter than the median duration of virus detection, and the survival curves for both the abatement of fever and virus detection followed a similar trajectory.

Our results of a median duration of virus detection of 4.2 days is either comparable to or slightly shorter than the duration of viral shedding or virus detection reported in other recent investigations. In Hong Kong, 22 patients with confirmed pH1N1 (all receiving oseltamivir 1–2 days after symptom onset) were determined to have a median duration of viral shedding of 4 days (range 1–5 days), which is similar to our findings of virus detection by rRT-PCR [6]. During an investigation of an outbreak of pH1N1 virus infection among trainees in the U.S. Air Force Academy, investigators isolated live virus in the majority of specimens taken from trainees in the first 3 days after symptom onset (91%) and in 24% of specimens taken from participants 7 days after symptom onset [12]. On the other hand, the duration of virus detection by rRT-PCR was measured in a Chinese cohort of 426 pH1N1 laboratory-confirmed

Table 2. Incident rate ratio of cessation of fever, cough, and sore throat by use of antiviral therapy, among participants with 2009 pandemic influenza A (H1N1) virus infection.

Symptom	Number reporting symptom	Used antiviral therapy ^a										Rate	95% CI	<i>P</i>
		Yes					No							
		Cases	Median duration in days (range)	Days of follow-up	Rate	Cases	Median duration in days (range)	Days of follow-up	Rate					
Fever	28	11	3.2 (1–7)	49	0.224	17	4.5 (2–10)	54	0.315	0.71	0.3–1.61	.13		
Cough	33	15	9.6 (1–19)	168	0.089	18	11.2 (2–20)	172	0.105	0.85	0.40–1.79	.43		
Sore throat	18	9	5.6 (2–12)	85	0.106	9	9.4 (4–20)	50	0.18	0.59	0.21–1.67	.09		

NOTE. *P* values based on LogRank test.

^a Antiviral therapy was defined as taking antiviral medication (oseltamivir or zanamivir) within 48 hours of symptom onset, irrespective of dose or duration of treatment.

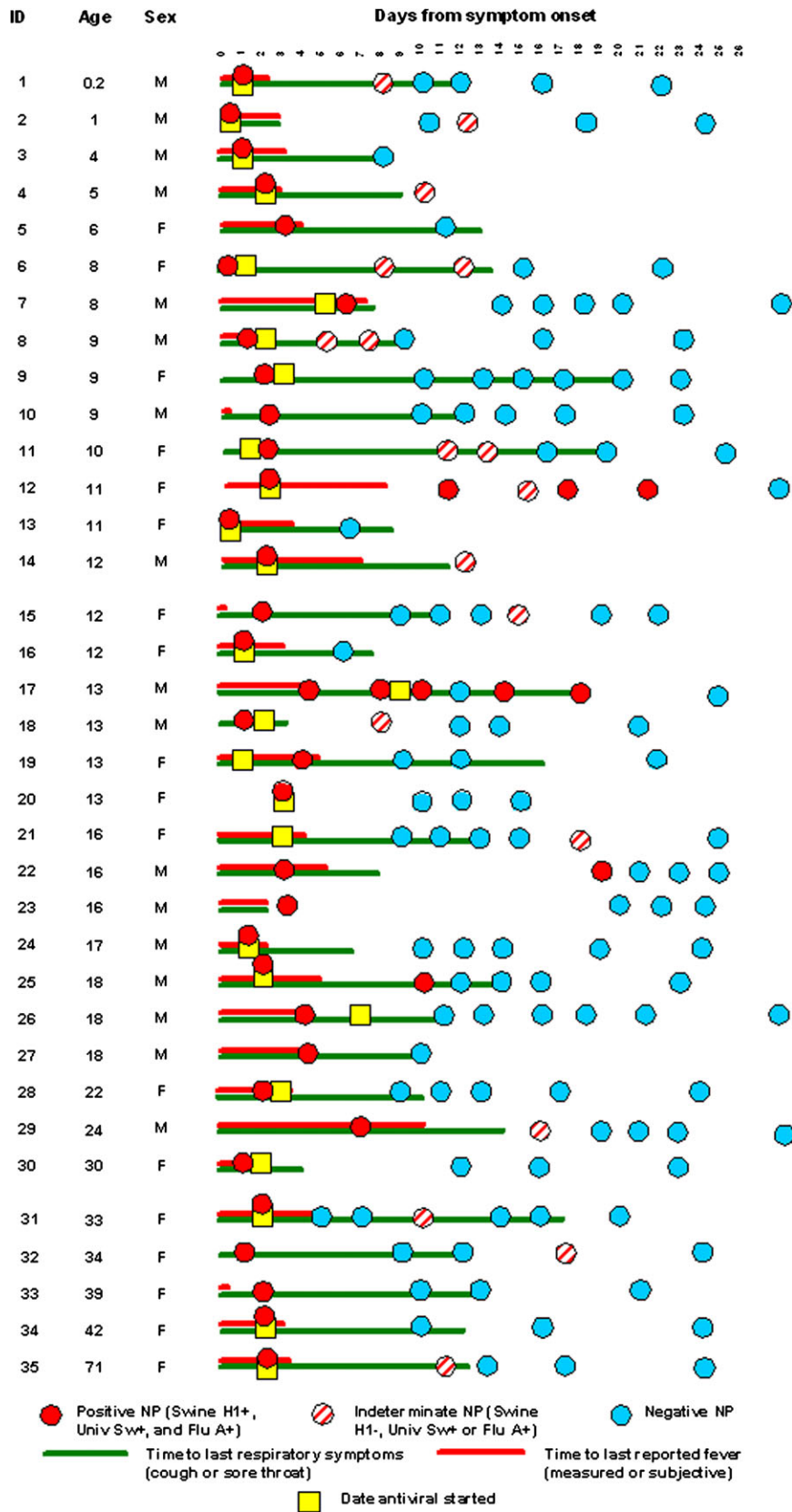


Figure 2. Duration in days of fever, respiratory symptoms, and virus detection, by molecular methods, by patient infected with 2009 pandemic influenza A (H1N1) virus, San Antonio, Texas, 2009.

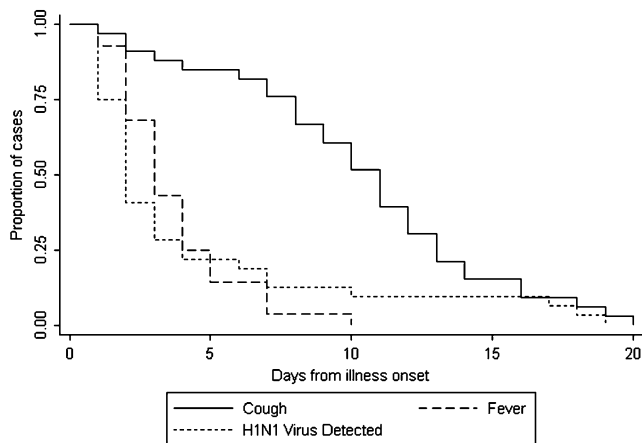


Figure 3. Kaplan-Meier plot of cessation of virus detection by molecular methods and cessation of reported fever and cough among 35 persons with 2009 pandemic influenza A (H1N1) virus infection, San Antonio, Texas, 2009.

patients to be 6.0 days [13]. Compared to our findings, fever and cough were less frequently reported in China (67.4% for fever and 69.5% for cough), the median fever duration was the same (3 days), and the median cough duration (6 days) was longer, but the difference in duration of fever and cough was far less pronounced. Finally, in Singapore, investigators studied 70 patients hospitalized with pH1N1 virus infection and reported a mean duration of viral shedding of 6 +/- 2 days after illness onset (4 days +/- 2 days after admission and persisting after 7 days in 37% of patients) [14]. The mean duration of fever was 1.3 +/- .6 days and the mean duration of respiratory symptoms was 3.9 +/- 1.8 days, but fever had no correlation with viral shedding, while duration of respiratory symptoms was moderately correlated (Spearman rank correlation, $q = 0.37$).

Significant interest surrounds the role of neuraminidase inhibitors (oseltamivir and zanamivir) in reducing the duration of illness and duration or quantity of viral shedding [3, 15–25]. Clinical trials and volunteer challenge studies of seasonal influenza strains have demonstrated a reduction in symptom duration among persons treated with neuraminidase inhibitors [3, 17, 19, 20, 22, 24, 25]. Our analysis did not demonstrate a difference in symptom duration, but suggested a trend toward a reduction in symptom duration with antiviral therapy. In an investigation of 384 index outpatients (identified as influenza A-positive by rapid antigen test results in 2007 and 2008) and their household contacts, antiviral therapy was found to be effective in reducing symptom duration among persons with influenza A virus infection (9 days without antiviral therapy versus 11 days with antiviral therapy), but the effect on household transmission was inconclusive [26]. Similarly, investigators in Singapore found that early initiation of oseltamivir therapy (days 1–3 of illness) significantly reduced the duration of pH1N1 viral shedding (median 5 days in person receiving oseltamivir 1–3 days after

symptom onset as compared to median 8.5 days in persons receiving oseltamivir ≥ 5 days after symptom onset) [14].

We identified viral nucleic acid by rRT-PCR in the stool from two participants, but were unable to culture a live virus. In the absence of accepted standard methods for culturing influenza virus in human stool, we used the 10% stool preparation (prepared for rRT-PCR), similar to the preparation used in testing avian H5N1 infectivity [27]. Preliminary data provided evidence that this type of preparation permits virus culture; pH1N1 virus inoculated into a negative stool pool was cultured (data not shown). Although we do not present compelling evidence for frequent virus detection in stool samples, stool might be a potential source of virus and has been detected in the stool of 4 patients in Hong Kong (and isolated by culture in 1 patient) [6], which highlights the importance of sound hand hygiene practices among patients and caregivers.

Our investigation was subject to several limitations. The primary limitation was that few specimens were collected during days 5–9 after illness onset, which other investigations have shown to be an important endpoint for pH1N1 shedding. This made precise interpretation of a shedding timeframe difficult, and limited the confidence of estimating an endpoint for virus detection. This uncertainty means that more persons probably shed virus beyond 24 hours after fever subsided, which has been shown in multiple investigations and studies. The few positive pH1N1 cultures from specimens collected on day 8 or later in investigations reported from the U.S. Air Force and Hong Kong (3 out of 53 in Air Force and 1 out of 21 in Hong Kong) [6, 12] suggest that prolonged shedding beyond one week in healthy persons is uncommon, as do the modest time intervals for shedding of seasonal influenza strains [3, 6, 15, 17–25]. In addition, the number of participants in our investigation was too small to reach conclusions regarding variations in virus detection by age or by antiviral treatment status. Additional investigations, including larger numbers of participants, might help to better describe these relationships.

Another limitation was that the nasopharyngeal specimens were subject to 2 freeze-thaw cycles, which might have reduced the yield of viral culture. This limitation raises questions about the true meaning of our negative respiratory culture data. All 6 diagnostic NP specimens taken 2–3 days after symptom onset and cultured as part of the clinical diagnosis grew live virus (not subject to two freeze-thaw cycles). The other 29 initial diagnostic NP specimens could not be attained for diagnostic culture because of the resource demands at the outset of the pandemic. However, since all subsequent specimens sent for culture were obtained ≥ 6 days (median, 10 days) after symptom onset, it is possible that these truly did not have viable viruses.

In summary, we found that fever cessation was a better clinical predictor of cessation of virus shedding than cough, which occurred several days beyond virus detection. The 2009

pH1N1 virus RNA was identified in a few stool samples, but no culture-positive virus was identified. Prospective studies are needed to ascertain the relationships between influenza symptoms and viral shedding, the risk for virus shedding in stool, the effects of neuraminidase inhibitors on the duration of virus shedding, and the possible differences in shedding patterns among children compared with adults.

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