RESEARCH BRIEF

# Risk Factors For Prolonged Shedding of 2009 H1N1 Influenza Virus

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This retrospective study was conducted to estimate the shedding of 2009 H1N1 virus and the risk analysis by review of medical charts, laboratory and radiological findings of all inpatients with confirmed pandemic influenza A (H1N1) at a provincial pediatric hospital. A total of 41 cases attending the inpatient department between 15 November, 2009 to 14 December, 2009 were included. Prolonged and discontinuous shedding of 2009 H1N1 virus (median, 10days; range, 2 to 24 days) were detected by real-time RT-PCR. The interval from onset of symptom to the start of oseltamivir therapy was an independent risk factor for prolonged virus shedding.

**Key words**: *2009 H1N1 influenza; RT-PCR; Virus shedding.*

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The 2009 H1N1 influenza caused human infection in Mexico and the United States in late April 2009, and subsequently spread worldwide. Worldwide more than 214 countries and overseas territories or communities have reported laboratory confirmed cases of pandemic influenza H1N1 2009, including over 18,000 deaths [1].

The duration of virus shedding would provide important knowledge for epidemiological control, antiviral therapy and infection control measures. 2009 H1N1 influenza virus shedding in adults has been reported to range from 1 to 28 days, and median length varied from 3 to 6 days [2-5]. The duration of novel influenza virus shedding was associated with patients' age, immunologic status, receiving antivirus therapy and viral resistant mutation [2-5]. To date, there is little research on length of virus shedding and its risk factors in children.

## METHODS

This retrospective study was conducted by review of medical charts, and laboratory and radiological findings of all children admitted to the Children's Hospital of Zhejiang University Medical College with confirmed pandemic (H1N1) 2009. The study period was from 15 November, 2009 to 14 December, 2009. During this period all the children admitted to the hospital with a febrile or respiratory illness were tested for pandemic (H1N1) 2009 by real-time reverse transcriptase-polymerase chain reaction with primers made by the CDC lab. A national guideline, adapted from guidelines provided by the US Center for Disease Control and Prevention was used to direct the surveillance, severity of illness, diagnosis, and treatment of the disease. Patients whose first specimen was collected prior to antiviral therapy were included in this analysis, and their nasopharyngeal swab collection discontinued

after one to three consecutive negative results. The tests were done at a laboratory operated under the auspices of the Chinese Center for Disease Control and Prevention. The PCR products were sequenced for further confirmation with the use of the BigDye Terminator, version 3.1 Cycle sequencing Kit (Applied Biosystems) in accordance with the manufacturer's instructions. Specimens were collected from nasal pharyngeal swabs and had been collected every one or two days since the pandemic (H1N1) 2009 was confirmed. Epidemiological and clinical information collected were age, gender, pre-existing medical conditions, severity of illness, date of symptom onset, co-infections, specimen collection and antiviral therapy. The Research Ethics Board at Children's Hospital of Zhejiang University Medical College approved the study design. Statistical analysis was performed by binary logistic-regression analysis (Statistical package for social sciences, 15th version). *P* value <0.05 was considered significant.

## RESULTS

During the period from 15 November to 14 December 2009, 41 cases were enrolled in this study, of whom 10 (24%) were admitted to ICU and 22 were male (54%). The median age was 34 months (range: 1 month to 144 months). Thirty cases (73%) were under 59 months of age. ***Fig.*1** shows the age-specific number for confirmed hospitalized cases of 2009 H1N1 influenza, by month age groups, by risk factors for complications, including children younger than 5 years of age and those with underlying medical conditions including: asthma (5 cases), neurological and neurodevelopmental conditions (2 cases), chronic lung disease (0), heart disease (1 case), blood disorders (4 cases), endocrine disorders (0), kidney disorders (1 case), liver disorders (0), metabolic disorders (0), and deficiencies in immune function due to disease or medication (4 cases). 4 cases, 3 cases, 1 case and 2 cases with prolonged viral shedding had asthma, blood disorders, kidney disorders and neurological conditions, respectively.

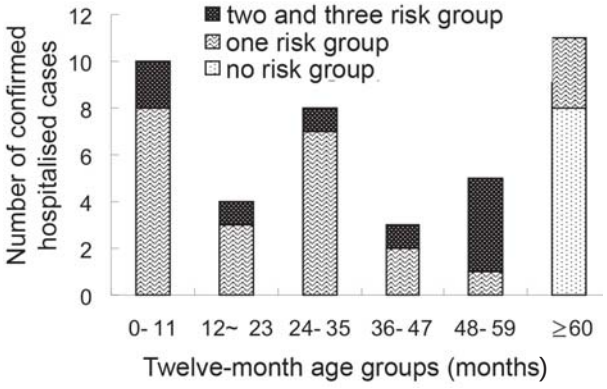


**FIG. 1.** *Age-specific cumulative number for confirmed hospitalized cases of pandemic influenza A (novel H1N1), by high risk factors for complications.*

Data on repeat RT PCR for novel H1N1 virus in pharyngeal swabs were available for all cases. The mean, median days of virus shedding were 11 days, 10 days respectively (range from 2 days to 24 days). For 6 ICU cases, we had the chance to monitor the viral shedding after the first one or two times of negative result, 4 of them transiently turned positive for one to two days, all of these four cases developed severe complications and 3 of them had co-infections; suggesting the virus shedding might be discontinuous. Twenty-one children (51%) received antiviral therapy 7 days after onset of symptom or later. ***Table* 1** shows the risk of viral shedding for ≥10 days. The binary logistic-regression analysis revealed only the interval from symptom onset to oseltamivir therapy was an independent risk factor for prolonged virus shedding (odds ratio, 8.4; *P*=0.006).

## DISCUSSION

At the late stage of the pandemic in Hangzhou city, the majority of patients had shifted to young children, this prolonged virus shedding in children was similar to the previous studies in seasonal influenza virus infection, it could persist for up to 21 days [6], viral load was found to be especially high in young children [7]. Children had longer pandemic (H1N1) 2009 virus shedding than adults [3,4], which provides information regarding virus-host interaction. The interval from onset of symptom to oseltamivir therapy was an independent risk factor for prolonged shedding, similar to the finding reported by Cao, *et al*. [3]. This prolonged virus shedding may be accounted for by delay in receiving oseltamivir therapy, for the drug can markedly reduce the replication of novel H1N1 virus in macrophage cells and dendritic cells [8]. The odds

**WHAT THIS STUDY ADDS?**

- The interval from onset of symptom to the start of oseltamivir therapy is an independent risk factor for prolonged virus shedding of 2009 H1N1 influenza virus.

ratio was higher in groups younger than 5 years old, male, ICU patients and immunodeficiency patients, but there were no significant difference on statistical analysis; the small sample size may be a reason.

The novel H1N1RT-PCR transiently turned positive after it had become negative in the some patients; suggesting the virus shedding was discontinuous. Co-infection and severe complication might be the clinical features of discontinuous shedding. Although a positive result of real-time RT-PCR testing does not necessarily indicate shedding of infective virus, PCR is more sensitive than culture for viral detection [4]. The extent of viral shedding would provide essential information in developing further study, such as detecting viable shedding for designing management polices in infection control.

Our study has several limitations. The sample size was small, we neither tested the viral load, nor did virus culture. We also did not test the resistant mutation strains.

**TABLE I** RISK OF VIRAL SHEDDING FOR 10 DAYS OR MORE

| *Variable* | *Length of viral shedding (days)* | | P *value* * |
|---|---|---|---|
| | ≥10 d *n*=22 | <10 d *n*=19 | |
| Age < 5y | 18 | 12 | 0.186 |
| Male | 14 | 8 | 0.171 |
| Immunodeficient | 3 | 1 | 0.762 |
| Fever | 17 | 17 | |
| ≥ 7 d Interval from symptom onset to oseltamivir therapy | 17 | 4 | 0.006 |
| ICU patients | 7 | 3 | 0.241 |
| Pyretolysis ≤ 24 hr after oseltamivir | 13 | 13 | |
| Clinical outcome | | | |
| Cure | 20 | 16 | |
| Improvement | 2 | 2 | |
| Death | 0 | 0 | |

** Data are from Binary logistic-regression analysis. Viral shedding was assessed on the basis of the results of Reverse-transcriptase-polymerase-chain –reaction testing.*

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