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# Viral shedding, clinical history and transmission of influenza

**Key Messages**

1. During influenza infections, most viral shedding occurs within a few days of illness onset.
2. Children may be more infectious than adults because they shed more virus.
3. The degree of viral shedding (infectiousness) correlates with symptoms and tympanic temperature.

## Introduction

Influenza is associated with significant morbidity and mortality through seasonal epidemics and occasional pandemics.[1] An accurate understanding of the effectiveness of intervention strategies through experimental studies and mathematical models often involves profiling of infectiousness.[2]

Data on viral shedding from the respiratory tract, corresponding time lines, and duration of clinical illness are available from volunteer challenge studies.[3] Nonetheless, whether these results can be generalised to natural infections is uncertain, because participants are usually young adults with low levels of pre-existing antibody to the influenza strain. Although there are studies on viral shedding in hospitalised patients,[4,5] studies on the patterns of viral shedding after naturally acquired infection in out-patients or the community are limited. We analysed the dynamics of clinical illness and viral shedding in subjects that acquired influenza virus infections in a community setting, and inferred infectiousness profiles.

## Methods

This study was conducted from September 2008 to August 2009. In 2008, we conducted a cluster-randomised controlled trial to study the efficacy of hand hygiene and face masks to prevent the transmission of influenza in households.[6] Subjects presented to out-patient clinics and private hospital emergency rooms with at least two symptoms (fever ≥37.8ºC, cough, sore throat, headache, runny nose, phlegm, muscle pain) were recruited. They also had to be (1) a Hong Kong resident, (2) with symptoms started in the preceding 48 hours, and (3) with two or more household members free of influenza-like illness in the preceding 2 weeks. If this index patient was found to be positive for influenza A or B virus infection following a rapid antigen test, every household member was followed up with a series of three home visits (each of which involved responses to a questionnaire) and nasal and throat swabs (NTS). Symptoms were self-recorded daily, and the body temperature was recorded using a digital tympanic thermometer.

A total of 3868 NTS specimens were collected over the course of the study. Specimens were tested by quantitative reverse transcription polymerase chain reaction (RT-PCR) to detect molecular viral loads and determine influenza A or B virus infection.[7] A subset of specimens were further tested to detect tissue culture infectious doses ($TCID_{50}$) and determine replicating viral loads by quantitative viral dilutions.[7]

There were three groups of symptoms: systemic symptoms (fever ≥37.8ºC, headache, myalgia), lower respiratory symptoms (cough, phlegm), and upper respiratory symptoms (sore throat, runny nose). Daily scores were tabulated by presence versus absence of each symptom and divided by the total number of symptoms in each group.[3] Trends in symptom scores and quantitative viral loads were plotted since the day of self-reported illness onset for index cases and since the day of onset of acute respiratory illness (ARI) for secondary cases.[8]

Viral shedding in secondary cases was considered representative of natural infections. We used a modelling approach to infer infectiousness from viral shedding. We used a Bayesian approach to fit lognormal, Weibull, and gamma-

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form parametric forms to the viral shedding trajectories and selected between models using the Bayesian information criterion.[9] All statistical analyses were conducted using R version 2.7.1 (R Development Core Team, Vienna, Austria)[10] and WinBUGS version 1.4.[11]

## Results

A total of 1015 household members from 322 households were followed up. All index subjects and 135 (13%) household members were confirmed by RT-PCR to have influenza virus infection. Among index cases, molecular viral shedding was highest on the day of symptom onset and gradually declined to undetectable levels within approximately 10 days (data not shown). The mean duration of shedding was 6 days. The dynamics of molecular viral shedding for influenza A and B virus infections were similar. Viral shedding was significantly higher in children than in adults with influenza A virus infections (data not shown).

Among secondary cases, 59 household members for whom the first NTS specimens collected were RT-PCR positive for influenza virus were excluded from analysis. An additional 17 household members were also excluded owing to the presence of ARI on the first home visit and RT-PCR confirmed influenza infection subsequently. Of the 59 secondary cases analysed, 16 were influenza A/H1N1 virus infections, 17 were influenza A/H3N2 infections, one was an influenza A/H1N1 and A/H3N2 coinfection, and the remaining 25 were influenza B virus infections. On the day of ARI onset, the most frequently reported symptoms were cough, nasal congestion/runny nose, and sore throat (Table). Thirty (51%) of them reported seeking medical care for their illness.

In secondary cases, peak molecular viral shedding for influenza A virus infections occurred on the day of ARI onset. Viral shedding declined steadily during the subsequent 7 days (Fig 1). The viral shedding patterns for influenza A/H1N1 and influenza A/H3N2 were similar (data not shown). Pre-ARI onset viral shedding was detected in four of the 15 subjects (27%; 95% confidence interval [CI], 8-55%). Viral shedding of influenza B virus was more variable over time and a clear peak was not recorded; pre-ARI shedding was detected on days 1 and 2 before ARI onset in four of the 14 subjects (29%; 95% CI, 8-58%). Influenza B viral shedding continued for about 6 days before declining to undetectable levels.

**Table. Symptoms of naturally acquired influenza A and B virus infections reported at the onset of acute respiratory illness**

| Symptom | No. (%) of patients | |
|---|---|---|
| | Influenza A (n=26) | Influenza B (n=18) |
| Runny nose or nasal congestion | 19 (73) | 11 (61) |
| Cough | 18 (69) | 14 (78) |
| Sore throat | 14 (54) | 7 (39) |
| Headache | 14 (54) | 5 (28) |
| Phlegm | 12 (46) | 5 (28) |
| Myalgia | 9 (35) | 6 (33) |
| Fever ≥37.8ºC | 8 (31) | 8 (44) |

For influenza A virus infections, the peak replicating viral load (assessed by viral culture) was on the day of ARI onset and declined steadily over the subsequent 5 days (Fig 1). For influenza B virus infections, $TCID_{50}$ levels initially peaked on the day of ARI onset and were more variable over time. Pre-ARI onset replicating viral load was detected in one adult with influenza A infection, and two adults and three children with influenza B virus infection. For both influenza A and B virus infections, the average symptom score and mean tympanic temperature peaked on the day of ARI onset, and steadily declined to nil after 3 to 5 days (Fig 1). Systemic symptoms resolved at a faster rate than respiratory symptoms (Fig 1).

Asymptomatic viral shedding was detected by RT-PCR in eight of the 59 secondary cases (14%; 95% CI, 6-25%); five and three cases were positive for influenza A and B viruses, respectively. In two of these eight, only the NTS specimen collected on the final home visit was positive. These may be cases of pre-ARI onset shedding, as subjects could develop symptoms after our follow ups. Viral shedding was detected in a further seven of 59 subjects that were subclinical, or reported just one symptom over the course of illness.

### *Infectiousness profiles*

A modified lognormal form provided a good fit to influenza A virus infection molecular viral shedding patterns. Assuming infectiousness as proportional to molecular viral shedding determined by RT-PCR, infectiousness was maximal within 2 to 3 days of the ARI onset. If infectiousness was assumed to be proportional to $\log_{10}$ molecular viral shedding or presence of detectable viral RNA, a longer duration could be inferred (Fig 2). Parametric forms did not offer any good fits to the influenza B molecular viral shedding patterns, or to replicating viral loads of influenza A or B assessed by viral culture.

## Discussion

Three different models have been used to describe infectiousness in influenza A virus infections over time. Assuming infectiousness is proportional to molecular viral shedding, most of the infectiousness is within 1 to 2 days (or 3 to 4 days) of the day of ARI onset (Fig 2). Considering the more rapid decline in replicating viral load compared to molecular viral shedding (Fig 1), the true duration of infectiousness may be overestimated by this method.

The mean serial interval for influenza infections is estimated to be 3.6 days,[8] whereas the incubation period is 1.5 to 2 days.[12] These estimates imply that the average time between ARI onset and transmission to a household contact is about 2 days, which is in line with the trend of

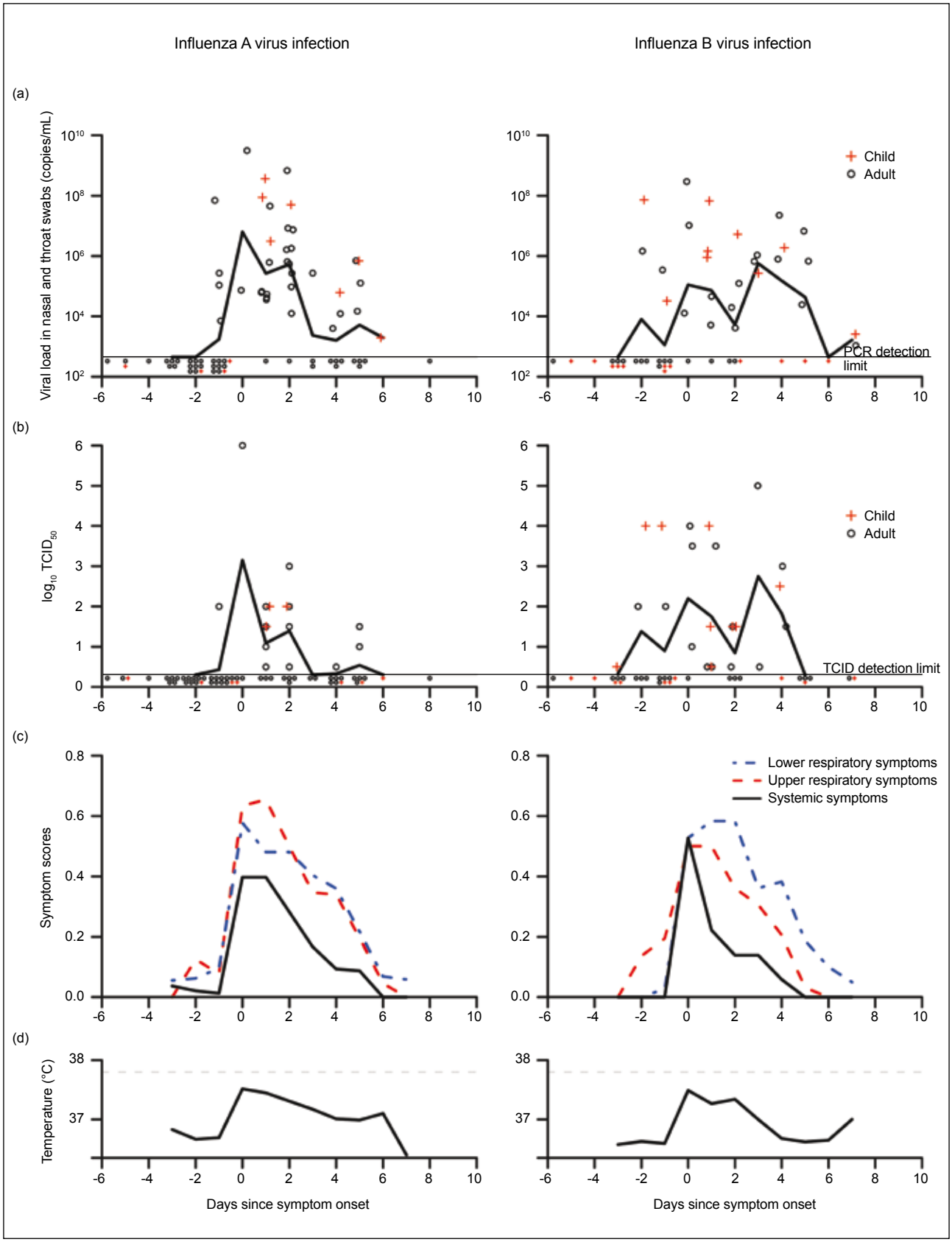


**Fig 1. Patterns of viral shedding and symptom scores in naturally acquired influenza A (n=26) and B (n=18) virus infections**
(a) The geometric mean viral shedding (the lower limit of detection of the RT-PCR assay is approximately 900 copies/mL), (b) the geometric mean tissue culture infectious dose ($TCID_{50}$), (c) symptom scores, and (d) the mean tympanic temperature

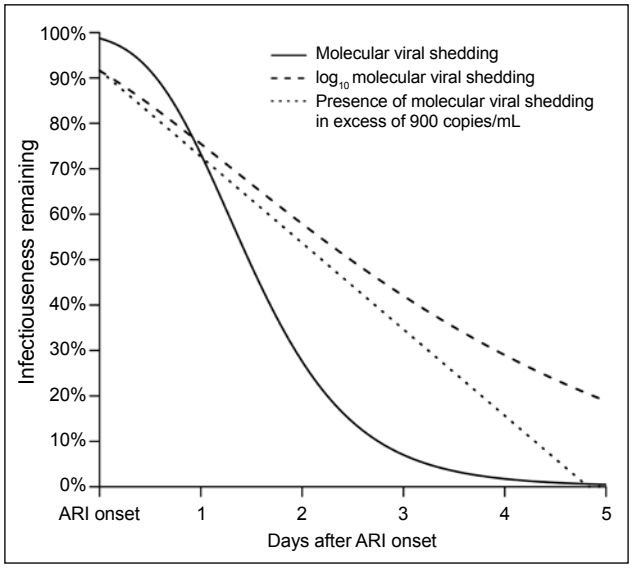


**Fig 2. Infectiousness since the onset of acute respiratory illness (ARI) in the course of naturally acquired influenza A virus infection**

Infectiousness is assumed to be proportional to molecular viral shedding, $\log_{10}$ molecular viral shedding, and presence of molecular viral shedding in excess of 900 copies/mL under the fitted curve

infectiousness over time based on molecular viral shedding and $\log_{10}$ viral shedding.

The trends in viral shedding and average symptom scores in secondary cases were consistent with findings in the volunteer study.[3] Viral shedding for influenza A virus infections peaked at approximately the same time as ARI onset before subsiding. In a small proportion of cases, viral shedding was detected prior to ARI onset. The daily replicating viral load (assessed by $TCID_{50}$) subsided at a faster rate than viral shedding (measured by RT-PCR). For influenza B infections, the patterns of viral shedding over the course of illness was more variable but consistent with data from the volunteer study, showing sustained shedding from the time of ARI onset for approximately 5 days.[3]

In secondary cases, systemic symptoms decline more quickly than respiratory symptoms for both influenza A and B virus infections.[3] The more rapid decline of systemic symptoms (specifically fever, its profile is similar to viral shedding) can be attributed to the controlled decrease in immune response, as the virus is gradually cleared from the body.[13]

Among the 59 household contacts with RT-PCR–confirmed infection, eight (14%; 95% CI, 6-25%) did not report any symptoms, and 15 (25%; 95% CI, 15-38%) were either asymptomatic or subclinical. Previous experimental infectiousness studies found a frequency of asymptomatic infection higher than the upper bound of our results[3] and similarly in longitudinal studies that examined paired pre- and post-season serology in household contacts.[14] Our study might have failed to detect infected subjects that were either shedding lower quantities of virus or shedding virus for a very short duration, and proportionally more of these subjects may be asymptomatic or subclinical.[3,15] It is unclear whether asymptomatic individuals have the potential to transmit influenza virus.[16] However, mathematical models typically assume that 33% to 50% of all infections are asymptomatic or subclinical, and the transmission potential of these subjects is half of that of symptomatic individuals.[2,17] Our results suggest that asymptomatic infections may be less important epidemiologically than previously thought.

The small sample size limited the ability to analyse the association between viral shedding and age or other characteristics, to characterise the patterns of viral shedding in secondary cases, and to ascertain an accurate proportion of asymptomatic and subclinical cases. In addition, our recruitment criteria and study design restricted recruitment to households without any illness during recruitment of the index subjects, possibly biasing the recruited households to those with a lower risk of infection or illness.[6] Besides, this study was not designed to address the degree to which asymptomatic or subclinical cases are responsible for transmission in the community. Addition of serological evidence to our findings would have been valuable, and further studies should consider the inclusion of such testing.

## Conclusions

Viral shedding determined by RT-PCR and $TCID_{50}$ in natural community influenza virus infections peaks around the day of symptom onset. The trend of viral shedding closely matches the trends of the average symptom score and mean tympanic temperature suggesting that infectiousness is likely to be correlated with illness severity, and that asymptomatic persons may be less important in influenza transmission than previously thought. The greatest infectiousness of influenza A virus is within 1 to 2 days following ARI onset. Individuals should take protective measures against transmission while they have febrile illness, and if possible while any symptoms persist.

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