Limited novel influenza A (H1N1) 09 infection in travelling high-school tour group

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Background A single case of novel influenza A (H1N1) 09 infection was identified by PCR among a New Zealand high-school group that toured California in April 2009. Close monitoring of the tour group and their New Zealand contacts identified 11 other tour members with respiratory symptoms who were investigated. In all nine instances where nasopharyngeal swabs were indicated, tests were negative for novel influenza A (H1N1) 09 by PCR.

Objective To determine whether serology could identify any cases of novel influenza A (H1N1) 09 that had not been detected by PCR.

Methods Acute and convalescent serological testing for antibodies against pandemic (H1N1) 2009 and seasonal A (H1N1) influenza viruses using haemagglutination inhibition assays and microneutralisation assays.

Results Serological analysis of symptomatic tour members identified a further possible case of novel influenza A (H1N1) 09 infection. The possible case had not been tested by PCR because he or she had already received prophylaxis with oseltamivir.

Conclusions These findings suggest infection among tour group members was limited despite prolonged periods of close contact during travel. Furthermore, multiple public health interventions are likely to have effectively prevented an outbreak following the tour group’s return.

Keywords 2009 Pandemic influenza, contact tracing, haemagglutination inhibition assay, infection control, microneutralisation assay, public health.

Introduction

A high-school musical group of 53 Hawke’s Bay residents toured California during the week in which the World Health Organization announced detection of human infections with the novel influenza A (H1N1) 09 virus in the USA and Mexico.1 The group stayed at Culver City (20 April) before they were billeted with local families in Palm Springs (21–22 April) and Long Beach (23–26 April). Their shared itinerary during the week of 20–26 April included a public concert, visits to Disneyland and Universal Studios, and travel within California. On 26 April 2009 (United States time), they departed from Los Angeles to Auckland, New Zealand, sitting together in the same area of the aircraft for the twelve hour flight. Some members of the group were mildly unwell with symptoms of respiratory illness during the tour. On arrival at Auckland airport, on the morning of 28 April 2009 (New Zealand time, 21 hours ahead of Los Angeles), four tour members had symptoms, which did not meet the criteria for further investigation at the airport (see Table 1). They were cleared to rejoin the tour group and travel together by bus for six hours to Hawke’s Bay before disbanding. No respiratory precautions were taken during the bus trip.

Suspect case notifications for novel influenza A (H1N1) 09 infection initiated a Public Health Unit assessment of the whole tour group. Tour members with respiratory symptoms were offered diagnostic viral PCR, treated with oseltamivir, and advised to remain in isolation within a quarantined household. Blood samples were collected for possible serological testing. Asymptomatic tour members were monitored for symptoms by daily telephone call, offered oseltamivir prophylaxis and recommended to undergo household quarantine.

One tour member had novel influenza A virus (H1N1) 09 infection confirmed by PCR assay from a nasopharyngeal swab (NPS) taken on the day of arrival in New Zealand. Thus, the infectious period for this case is likely to have included the twelve hour return flight and the six hour bus journey.
A single confirmed case of novel influenza A (H1N1) 09 infection was unexpected given the tour group’s close contact during the case’s infectious period. Thus, we set out to determine whether serology could identify any cases of novel influenza A (H1N1) 09 that had not been detected by PCR.

**Methods**

Twelve tour members with respiratory illness in the week following return to New Zealand were offered acute and convalescent serological testing for novel influenza virus (H1N1) 09 antibodies. Acute serum samples were taken on the same day as the NPS. Convalescent serum samples were taken 7 weeks later. NPS were taken according the *National Laboratory Guidelines For Pandemic Influenza.*

All serological samples were frozen and transported to the WHO Collaborating Centre for Reference and Research on Influenza in Melbourne. Reactivity of sera against pandemic (H1N1) 2009 and seasonal A (H1N1) influenza viruses was measured using haemagglutination inhibition (HI) assays and microneutralisation (MN) assays.

Briefly, for the HI assay, sera were pre-treated with receptor destroying enzyme [RDE (II); Deka Seiken Co. Ltd., Tokyo, Japan], 1:5 (v/v), at 37°C overnight, then the enzyme inactivated by incubation with an equal volume of 1:1 54-4 mm tri-sodium citrate (Ajax Chemicals, Taren Point, New South Wales, Australia) at 56°C for 30 minutes. Intrazonal pool preparation of inactivated A/California/7/2009 virus (a gift of CSL Limited, Parkville, Victoria, Australia) was incubated with RDE-treated serum (1:1). Sera were titrated in twofold dilutions in PBS (1:10 to 1:1280), in duplicate. Haemagglutination was read using

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**Table 1. Results for tour members with reported respiratory illness**

| Tour member | Seasonal influenza vaccinated in 2009 | Symptom onset (New Zealand time) | Oseltamivir prophylaxis | NPS collection | A (H1N1) 2009 RNA | MN assay A (H1N1) seasonal | HI assay A (H1N1) 2009 | HI assay A (H1N1) seasonal | Seroconverted to A (H1N1) 09* |
|-------------|-------------------------------------|----------------------------------|-------------------------|----------------|--------------------|---------------------------|------------------------|---------------------------|-----------------------------|
| 1           | No                                  | 28 April                         | NO                      | 28-Apr         | Positive           | 29-Apr                    | 17-Jun                 | <40, <40                  | <40, <40                    | Yes                         |
| 2           | No                                  | 26 April                         | NO                      | 30-Apr         | Negative           | 30-Apr                    | 17-Jun                 | 40, 40                    | <40, <40                    | No                          |
| 3           | Yes                                 | 27 April**                       | YES                     | Not taken      | Not applicable     | 3-May                     | 17-Jun                 | 320, 320                  | 80, 160                    | Possibly***                 |
| 4           | Unknown                             | 26 April                         | NO                      | 1-May          | Negative           | 4-May                     | 17-Jun                 | 640, 640                  | <40, <40                    | No**                        |
| 5           | No                                  | 27 April**                       | NO                      | 4-May          | Negative           | 30-Apr                    | 17-Jun                 | <40, <40                  | <40, <40                    | No                          |
| 6*          | No                                  | 28 April                         | NO                      | 29-Apr         | Negative           | 21-May                    | 17-Jun                 | <40, 40                   | <40, <40                    | No                          |
| 7           | No                                  | 28 April                         | YES                     | Not taken      | Not applicable     | 29-Apr                    | 17-Jun                 | 640, 640                  | <40, <40                    | No                          |
| 8           | No                                  | 29 April                         | NO                      | 29-Apr         | Negative           | 29-Apr                    | 17-Jun                 | <40, <40                  | <40, <40                    | No                          |
| 9           | No                                  | 29 April                         | NO                      | 29-Apr         | Negative           | 29-Apr                    | 17-Jun                 | <40, <40                  | <40, <40                    | No                          |
| 10          | No                                   | 29 April                         | NO                      | 30-Apr         | Negative           | 29-Apr                    | 17-Jun                 | <40, <40                  | <40, <40                    | No                          |
| 11          | Unknown                             | 1 May                             | NO                      | 1-May          | Negative           | 1-May                     | 17-Jun                 | <40, <40                  | <40, <40                    | No***                       |
| 12†         | Yes                                 | 3 May                             | NO                      | 5-May          | Negative           | 5-May                     | 17-Jun                 | 320, 320                  | <40, <40                    | No                          |

HI, haemagglutination inhibition; MN, microneutralisation; NPS, nasopharyngeal swab. Suspect cases were defined as having recent onset of respiratory illness and travel in the last 7 days to an area of concern (Mexico, USA or Canada). Respiratory illness was defined as recent onset of at least two of the following: rhinorrhea or nasal congestion, sore throat, cough, fever 38°C.

*Seroconverted is defined as a fourfold rise in antibody titre by either HI assay or MN assay against pandemic A (H1N1) 09 between the paired serum samples, or as a higher antibody titre by either HI assay or MN assay compared to background levels generally found in the age group for the single serum samples.

**Took several days to develop symptoms, which met the case definition.

Serum samples were taken less than 7 days from symptom onset, and this may not be sufficient time for a serological response to be detected.

Not a suspect case: had one respiratory symptom and other flu-like symptoms not included in case definition.
Limited novel influenza A in travelling tour group

1% turkey red blood cells. Titres were expressed as the reciprocal of the highest dilution of serum where haemagglutination was prevented.

All samples were also tested by MN assay. Undiluted serum was inactivated at 56°C for 30 minutes. Heat-treated serum (twofold dilutions from 1:10) and 100 tissue culture infective dose (TCID)_{50} A/Auckland/1/2009 (novel H1N1 09) or A/Fukushima/141/2006 (A/H1N1) (1:1) were incubated at 35°C for 1 hour, in duplicate. The virus/serum mix was incubated on washed confluent (90%) monolayers of Madin-Darby canine kidney cells in 96-well plates at 35°C, 5% CO₂ for 2 hours. The virus/serum mix was replaced with foetal calf serum (FCS)-free tissue culture medium supplemented with 4 μg/ml trypsin and the cells were incubated at 35°C, 5% CO₂. Four days later, supernatant from each well was assayed for virus by haemagglutination assay with 1% turkey RBC. Titres were expressed as the reciprocal of the highest dilution of serum where haemagglutination was prevented.

Results were interpreted in conjunction with seasonal influenza vaccination history, the clinical history and the timing of collection of NPS for PCR analysis.

Results

Twelve members of the 53 person tour group (23%) reported illness with respiratory symptoms in the week following return from California. Ten tour members met the suspect case definition of two symptoms, whilst the remaining two (tour members 6 and 12) only showed one respiratory symptom and other flu-like symptoms not included in the case definition (eg: myalgia, headache, abdominal pain) (Table 1). There was no history of contact with suspected or confirmed cases of influenza A (H1N1) 09 infection. The median age of affected tour members was 15 years (range 13–54 years).

Oseltamivir treatment was given to eleven of the twelve symptomatic tour members, ten of whom completed treatment. Oseltamivir prophylaxis was given to 63 people (with compliance monitoring). They were asymptomatic members of the tour group or household contacts of symptomatic tour group members. Four other people were offered prophylaxis but declined and were placed in 7 days isolation. Monitoring of all these contacts in New Zealand did not identify any other suspect cases.

Two tour members were not tested by PCR because they had already taken oseltamivir prophylaxis prior to developing symptoms, which met the case definition (Table 1). All other NPS taken from suspect cases, upon presentation of symptoms, tested negative for the novel influenza virus. This testing was limited however, as NPS were taken up to 1 week after symptom onset when viral shedding may no longer be detectable.

Infection with influenza A (H1N1) 09 was also assessed by seroconversion. A total of 19 serum samples were obtained from the twelve tour members reporting illness (Table 1). Sera from seven members were paired acute and convalescent bleeds, whilst only one serum sample was collected from the remaining five tour members: three acute and two convalescent samples. Seroconversion was defined as a four-fold increase in antibody titre by either HI or MN assay, with a minimum titre of 40 in the convalescent bleed in both assays, similar to other studies.5,6 Where only one serum sample was available, an indicated estimate of infection was made by comparing the single titre with historical background titres for this age group. Of the seven sets of paired sera, only tour member 1, the index case, met this definition of seroconversion to the novel A (H1N1) 09 virus, demonstrating infection with this strain. No significant change in HI or MN titre to A (H1N1) 09 was detected in tour member 2. Of the tour members who provided a single serum sample, moderate levels of antibodies to A (H1N1) 09 were also detected by HI and MN assay for tour member 3. Five tour members (3, 5, 7, 8, 12) had high neutralising antibody titres to seasonal influenza A (H1N1) virus. Two of these tour members (3 and 12) had recent history of influenza vaccination, whilst the remaining three (5, 7 and 8) had no recent history of influenza vaccination and are likely to have had recent infections with seasonal influenza A (H1N1).

The confirmed case developed symptoms one hour after arrival in Auckland on 28 April 2009, suggesting infection in California. Symptoms on the day of onset included a significant cough, fever, coryza, sore throat, headache and myalgia. No special measures were taken during the bus trip to reduce aerosol transmission of respiratory secretions. Upon arriving in Hawke’s Bay, the case proceeded immediately to a consultation in primary care where NPSs were taken and oseltamivir was prescribed. The confirmed case completed 5 days of oseltamivir treatment and 7 days of isolation/household quarantine. Household contacts were prescribed post-exposure prophylaxis and placed in quarantine.

The possible case developed rhinorrhoea/nasal congestion and headache on 27 April 2009, 1 day prior to arrival in New Zealand. These symptoms did not meet the case definition, and as a contact of a suspect case, prophylactic oseltamivir was delivered on 30 April 2009. On day 4 of prophylactic treatment, the possible case developed fever, coryza, sore throat and vomiting. Full oseltamivir treatment was initiated, and quarantine was upgraded to isolation.

Discussion

Novel influenza virus (H1N1) 09 infection among tour group members could easily have led to rapid transmission of disease within the group and the wider school community. An estimated 1-96 secondary cases were generated from each
primary case early in New Zealand’s first pandemic wave (the reproduction estimate).⁷ Reproduction estimates in other countries range from 1.2 to 2.4,⁸–¹³ and reached 2.8 for a subanalysis of persons under 20 years of age.¹⁰ Certainly rapid transmission amongst students was commonly observed internationally.¹⁴–²²

The risk of inter-student transmission was also elevated by close contact during extended travel with newly symptomatic cases. This is the period when viral load in aerosolised respiratory secretions should be highest.²³ Remarkably, no evidence of transmission within the student group during return travel to New Zealand or after arrival has been identified after close monitoring and serological investigation of those with respiratory symptoms, although transmission may have occurred to asymptomatic tour party members who did not receive serological testing.

Kar-Purkayastha and Ingram et al.¹⁸ also found no evidence of transmission during bus travel in their extensive investigation of a school-based outbreak in England. The period of travel in their investigation was however significantly shorter (30 minutes) than the travel reported here.

Our findings suggest that the combined interventions of isolation, household quarantine, active monitoring and oseltamivir treatment and prophylaxis have successfully contributed towards preventing an outbreak in the community upon the tour members’ return. This is consistent with the report by Kimberlin et al.,²⁴ of effective novel influenza A (H1N1) 09 infection containment at a summer camp.

Limitations of the study include the possibility that some tour members did not disclose illness and that asymptomatic infection occurred. We also cannot exclude the possibility that other passengers on the flight were infected and then travelled to destinations other than Hawke’s Bay. In addition, NPS collection was delayed after symptom onset in almost half of the patients, which may reduce virus detection, yet the absence of seroconversion in these patients suggests that infection with influenza A (H1N1) 09 did not occur. Furthermore, it has been demonstrated that approximately 90% patients with known influenza A (H1N1) 09 infection had seroconverted at least 3 weeks after symptom onset.³ However, it has been suggested that oseltamivir treatment may suppress serological responses, especially in mild or asymptomatic cases,²⁵ thus, an under-estimate of H1N1 09 infection in this tour group cannot be discounted. The absence of further confirmed cases in Hawke’s Bay until mid-June does, however, add weight to the suggestion that an outbreak was prevented.

Conclusion

This study describes limited novel influenza A (H1N1) 09 infection among a large high-school tour group. Limited evidence of transmission was identified despite prolonged close contact during travel. These findings differ from other reports of rapid transmission within school communities. Effective public health intervention is likely to have played a role in preventing an outbreak in the community.

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