Influenza A(H1N1) outbreak in a long-term care facility for severely handicapped residents, Slovenia, March–April 2009

M Socan (maja.socan@ivz-rs.si), K Prosenc*, N Tjevž-Cizej*
1. Centre for Communicable Diseases, Institute of Public Health, Ljubljana, Slovenia
2. Laboratory for Virology, Centre for Communicable Diseases, Institute of Public Health, Ljubljana, Slovenia
3. Training, Occupational and Care Centre, Ig, Slovenia

Long-term care facilities are vulnerable to outbreaks of influenza. This report describes the response to such an outbreak in a long-term care facility for severely handicapped children and adults near Ljubljana, Slovenia, in March and April 2009. Of the 23 residents who lived in a unit of the facility, 10 fell ill with fever (≥37.5 °C) during a period of nine days. Probable and confirmed cases were residents who developed a fever after 24 March 2009. Respiratory symptoms were not included in the case definitions as some residents were unable to describe their symptoms due to their mental and/or physical impairment. Epidemiological data were collected and throat and nasal swabs taken. Influenza A virus was identified (without subtyping) and treatment with oseltamivir was given to patients with fever of no more than 48 hours’ duration. Oseltamivir was also given prophylactically to healthy residents and staff. Rigorous adherence to standard and droplet precautions was recommended by the regional institute of public health. Two days after respiratory and standard precautions have been strengthened, four more residents became ill. Viral subtyping showed that 12 of the 23 residents were infected with influenza virus A(H1N1); one had an influenza B virus infection. Of the 12 confirmed influenza A cases, 10 had been vaccinated with the seasonal influenza vaccine. Follow-up swabs were taken and were found to be still positive for influenza A virus in 6 of the 12 confirmed cases more then a week after illness onset. The virus was resistant to oseltamivir and susceptible to zanamivir. This influenza outbreak demonstrates the need for rapid typing and subtyping of influenza viruses for accurate diagnosis, treatment and chemoprophylaxis in special settings.

Introduction
Outbreaks of influenza in care facilities, such as nursing homes for elderly people and people with special needs, are common, despite good vaccination coverage among the residents [1] and a good match between the vaccine type and the viral strain [2]. According to the clinical practice guidelines of the Infectious Diseases Society of America, an epidemiological investigation should be carried out for such outbreaks and measures taken to prevent the spread of the influenza virus among the residents, as they often develop severe complications due to primary chronic illnesses or injuries [2]. To limit an outbreak, antiviral drugs need to be prescribed to those who are ill as well as to all (residents and staff) who still show no signs of illness [2]. The development of influenza A viral resistance to rimantadine and amantadine (M2 inhibitors) and the emergence of resistance of the seasonal influenza A(H1N1) virus to oseltamivir (neuraminidase inhibitor) narrow the therapeutic and prophylactic possibilities to control an outbreak [3].

In this report, we describe an influenza outbreak in March–April 2009 caused by the seasonal influenza A(H1N1) virus in a unit of long-term care facility for severely handicapped children and adults near Ljubljana, Slovenia. The facility consisted of five separate low-rise buildings, with 148 residents and 162 staff members (health and pedagogical workers) in total. There were also 21 non-residents who attended day-care services and some residents spent occasional weekends away from the facility. In the unit in which the March–April 2009 outbreak occurred, two to a maximum four residents shared the same bedroom. During the day, the residents were transported in specially adapted wheelchairs from their bedrooms to common rooms in the same unit.

An earlier influenza outbreak occurred on 17 February 2009 in a different unit of the care facility, which was reported to the regional institute of public health. Of the 21 residents, 16 became infected: five of them were hospitalised. The last patient with fever and respiratory symptoms fell ill on 24 February 2009. Throat and nasal swabs from the residents who were hospitalized were positive for influenza A(H3N2) virus. Of the 16 staff members, 10 reported a high fever (≥38 °C) with accompanying respiratory infection symptoms. No swabs were taken for virological analysis. Oseltamivir was used to treat the ill residents, while the healthy residents and staff were prescribed it prophylactically.
The outbreak did not spread in the same unit or to other groups of residents in the care facility.

About a month later, a second outbreak occurred, beginning on 24 March 2009. This time, 10 of the 23 residents of a second, spatially separated unit fell ill with fever during a nine-day period; some residents also had respiratory symptoms. The attending physician notified epidemiologists at the regional institute of public health on 2 April 2009 and an epidemiological investigation was started. The evolution of the outbreak was followed: clinical data were collected and repeat swabs taken from all the residents (symptomatic and asymptomatic) to enable the early detection of possible infection among still asymptomatic individuals.

Methods

Case definitions

We used the following definitions in our investigations:

- **Confirmed case**: a resident who fell ill with fever (≥37.5 °C) and in whom the presence of influenza viral RNA was confirmed by real-time reverse-transcription polymerase chain reaction (rt-RT-PCR). The European Union definition of influenza requires at least one further symptom, in addition to acute onset of fever, such as a cough, sore throat or breathing difficulties to be present [4]. In this investigation, however, we used a modified definition of influenza, as some of the residents were incapable of describing their symptoms, due to mental and/or physical impairment.

- **Probable case**: a resident from the group who had fever (≥37.5 °C), no virological confirmation of influenza virus infection, but was epidemiologically linked to a confirmed case.

Nursing staff measured the body temperature of residents daily (part of routine care) and recorded the patients’ symptoms (when possible), as well as signs of infection observed in patients.

Virological analysis

Throat and nasal swabs were tested for influenza viral RNA from patients with symptoms of influenza-like illness. After influenza A was identified as the causative agent of the illness, repeat swabs were taken from all the residents (symptomatic and asymptomatic). The follow-up swabs were taken on 6, 15 and 22 April 2009, regardless of the results from the preceding sample.

The swabs were tested at the National Influenza Centre (Laboratory for Virology, National Institute of Public Health) by rt-RT-PCR (QIAGEN OneStep RT-PCR Kit, ref. no. 210212), using reagents and oligonucleotide primers and probes as described in the literature [5–7], with some modifications (O. Hungnes, personal communication). Influenza A and B viruses were detected in a single PCR reaction. In samples positive for influenza A, additional PCR reactions were carried out to detect subtypes H1 and H3.

Most of the influenza A (H1) viruses were tested for sensitivity to the antiviral drug oseltamivir at the Health Protection Agency (a WHO influenza reference centre) in London, United Kingdom, by pyrosequencing of the neuraminidase gene [8]. This detects the mutation that causes viral resistance to oseltamivir – a histidine-to-tyrosine substitution at position 275 (H257Y) in the neuraminidase.

Results

Epidemiological information

At the time of the outbreak, there were 23 residents in the care facility unit: their ages ranged from nine to 34 years (mean: 21.7 years, median: 22 years); 12 of the 23 residents were male. All but two of the residents had been vaccinated with the seasonal influenza vaccine. A total of 14 (10 men, four women; mean age: 17.7 years, median: 17 years) fell ill with fever: in 12, body temperature was ≥38 °C; in two, it was between 37.5 °C and 37.9 °C. The first confirmed case fell ill on 24 March 2009 and the last on 3 April 2009 (Figure).

The rise in body temperature persisted from one to a maximum of nine days (mean: 3.5 days, median: 3.5 days). Of the 14 patients, five had a fever only, with no additional signs observed by staff. One or more additional symptoms were recorded in nine patients: six had a cough, four had a runny nose, two had a sore throat and/or myalgia.

A total of 12 patients were confirmed cases, according to the definition chosen for this investigation. Two patients were probable cases: the first was a male resident whose initial swab was negative for influenza A viral RNA, but influenza B viral RNA was detected in the second sample taken 16 days after the beginning of his illness. The second probable case was a female resident with fever lasting for two days, but whose swab (taken shortly after the fever abated) was negative. Further samples could not be obtained as she temporarily left the care facility.
The attack rate was higher for males than females: of the 12 confirmed cases, nine were male, three were female (attack rates of 75% and 27% respectively). We could not find any explanation for the observed difference.

On 3 April 2009, the day after notification of the outbreak, type A influenza virus was confirmed. However, the subtyping results were not available. If not more than 48 hours had passed since the appearance of symptoms, confirmed cases (n=3) were treated twice daily with 75 mg oseltamivir. The nine residents who remained asymptomatic (two men, seven women; mean age: 28.8 years, median: 28 years) were given oseltamivir prophylactically for 12 days. All asymptomatic residents had been vaccinated against influenza in November 2008.

In addition to chemoprophylaxis, adherence to standard and droplet precautions was intensified. A total of 23 staff members cared for the residents daily: none had been vaccinated against influenza. Two nurses fell ill with high fever >38.0 °C and cough before the outbreak but no swabs were taken. The other 21 staff members received oseltamivir prophylactically for 12 days, as for the asymptomatic residents.

### Virological data

Throat and nasal swabs were taken from residents on four occasions (in 2009): 2 or 3 April (n=7), 6 April (n=23), 15 April (n=22) and 22 April (n=21) (Table).

In the first swab collection, all seven samples were positive for influenza A(H1) virus, 10 of 23 from the second swab batch and one of 22 from the third. All 21 samples from the fourth batch were confirmed negative.

Influenza A viral RNA was detected in the 12 confirmed cases even after body temperature returned to normal. In six cases, the virus could still be detected seven or more days after illness onset; in one case, even the third sample (taken 18 days after illness onset) was positive (Table). None of the patients was immunocompromised.

All samples that were positive for influenza A were also tested for the H275Y mutation, which causes resistance to oseltamivir. The mutation was detected in samples

<table>
<thead>
<tr>
<th>ID no.</th>
<th>Sex/age (years)</th>
<th>Date of illness onset, in 2009</th>
<th>Duration of fever (days)</th>
<th>Treatment or prophylaxis with oseltamivir</th>
<th>Date treatment or prophylaxis started, in 2009</th>
<th>Throat/nasal swab results, in 2009 (number of days from illness onset to taking of swab)</th>
<th>H275Y mutation detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F/19</td>
<td>24 March</td>
<td>9</td>
<td>none</td>
<td>–</td>
<td>NA pos (14) neg (23) neg (30) ND</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>M/17</td>
<td>27 March</td>
<td>3</td>
<td>none</td>
<td>–</td>
<td>NA pos (11) neg (20) neg (27) ND</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>M/28</td>
<td>29 March</td>
<td>3</td>
<td>none</td>
<td>–</td>
<td>pos (6) pos (9) neg (18) neg (25) yes</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>M/23</td>
<td>29 March</td>
<td>5</td>
<td>none</td>
<td>–</td>
<td>pos (6) neg (9) neg (18) neg (25) yes</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>M/29</td>
<td>29 March</td>
<td>4</td>
<td>none</td>
<td>–</td>
<td>pos (5) pos (9) pos (18) neg (25) yes</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>M/13</td>
<td>30 March</td>
<td>4</td>
<td>none</td>
<td>–</td>
<td>NA pos (8) neg (17) neg (24) ND</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>M/27</td>
<td>30 March</td>
<td>7</td>
<td>none</td>
<td>–</td>
<td>pos (5) neg (8) neg (17) NA ND</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>M/30*</td>
<td>31 March</td>
<td>3</td>
<td>none</td>
<td>–</td>
<td>pos (3) pos (7) neg (16) neg (23) yes</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>M/17</td>
<td>31 March</td>
<td>2</td>
<td>none</td>
<td>–</td>
<td>NA neg (7) B (16) neg (23) –</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>F/19</td>
<td>1 April</td>
<td>2</td>
<td>none</td>
<td>–</td>
<td>NA neg (6) NA NA –</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>M/22</td>
<td>2 April</td>
<td>4</td>
<td>treatment</td>
<td>3 April</td>
<td>pos (2) pos (5) neg (14) neg (21) yes</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>F/17*</td>
<td>2 April</td>
<td>2</td>
<td>treatment</td>
<td>3 April</td>
<td>pos (2) pos (5) neg (14) neg (21) yes</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>F/29</td>
<td>2 April</td>
<td>1</td>
<td>none</td>
<td>–</td>
<td>NA pos (5) neg (14) neg (21) yes</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>M/28</td>
<td>3 April</td>
<td>4</td>
<td>treatment</td>
<td>3 April</td>
<td>NA pos (4) neg (13) neg (20) yes</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>F/25</td>
<td>asymptomatic</td>
<td>–</td>
<td>prophylaxis</td>
<td>3 April</td>
<td>NA neg (3) neg (9) neg –</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>F/26</td>
<td>asymptomatic</td>
<td>–</td>
<td>prophylaxis</td>
<td>3 April</td>
<td>NA neg (3) neg (9) neg –</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>M/17</td>
<td>asymptomatic</td>
<td>–</td>
<td>prophylaxis</td>
<td>3 April</td>
<td>NA neg (3) neg (9) neg –</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>M/12</td>
<td>asymptomatic</td>
<td>–</td>
<td>prophylaxis</td>
<td>3 April</td>
<td>NA neg (3) neg (9) neg –</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>F/20</td>
<td>asymptomatic</td>
<td>–</td>
<td>prophylaxis</td>
<td>3 April</td>
<td>NA neg (3) neg (9) neg –</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>F/21</td>
<td>asymptomatic</td>
<td>–</td>
<td>prophylaxis</td>
<td>6 April</td>
<td>NA neg (3) neg (9) neg –</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>F/15</td>
<td>asymptomatic</td>
<td>–</td>
<td>prophylaxis</td>
<td>5 April</td>
<td>NA neg (3) neg (9) neg –</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>F/34</td>
<td>asymptomatic</td>
<td>–</td>
<td>prophylaxis</td>
<td>3 April</td>
<td>NA neg (3) neg (9) neg –</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>F/22</td>
<td>asymptomatic</td>
<td>–</td>
<td>prophylaxis</td>
<td>3 April</td>
<td>NA neg (3) neg (9) neg –</td>
<td></td>
</tr>
</tbody>
</table>

B: influenza B virus detected; F: female; H: histidine; ID no. identity number; M: male; NA: not available; ND: not determined, due to insufficient viral RNA; neg = negative; pos: positive; Y: tyrosine.

* Patients not vaccinated against influenza.

Table

Residents’ data, influenza A (H1N1) outbreak, long-term care facility for severely handicapped residents, Slovenia, March–April 2009 (n=23)
from eight confirmed cases. However, in samples from the other four cases, there was insufficient viral RNA for pyrosequencing (Table).

Discussion
In Slovenia, influenza virus infection was confirmed in 556 patients in the 2008–9 season (from week 40 in 2008 to week 20 in 2009), within the virological sentinel and non-sentinel surveillance system network. A total of 493 (88.6%) of these patients were positive for influenza A(H3N2) virus. Only 33 (6%) were positive for influenza B virus and influenza A(H1N1) virus was seen in just two (0.4%); 28 (5%) of influenza A viruses were not subtyped [9].

In the March–April 2009 influenza outbreak in the care facility, an epidemiological investigation of the outbreak was launched 10 days after the first patient started showing symptoms. The main reason why the outbreak had not been reported sooner was the clinical impression that it was not influenza. This arose from the experience of the first outbreak in the same care facility, which was caused by influenza A(H3N2) virus. That outbreak, in February 2009, took place in a different building and affected staff and residents, who had similar intellectual and motor impairments as the residents in the March–April outbreak. The patients in the first outbreak suffered more severe disease: five of the 16 affected residents had to be hospitalised, due to respiratory problems and/or uncontrollable symptomatic epilepsy in their febrile state. In the March–April outbreak, the affected residents, who were infected with influenza A, experienced less severe disease, had shorter febrile illness and suffered no serious complications; none needed hospital care. The clinical findings in the two outbreaks are in agreement with the general experience that influenza A(H3) infection is more severe and causes more complications than influenza A(H1). One of the indicators of illness severity is excess mortality, which is higher during influenza A(H3) seasons [10].

Before we received the influenza A virus subtyping results, we assumed that the March–April outbreak was caused by influenza A(H3N2) virus. This was based on the fact that this was the predominant circulating virus and, moreover, had been the causative agent in the previous outbreak at the same institution. In this second outbreak, influenza A virus was confirmed and was later subtyped as H1N1. Until this second outbreak, there had been only two laboratory-confirmed cases of infection with A(H1N1) virus in the 2008–9 season in Slovenia and there were no data on drug resistance of these viral isolates. As the National Reference Laboratory does not have the necessary equipment to assess influenza virus resistance to antiviral drugs, in the 2007–8 season, the Health Protection Agency in London, UK, analysed 28 Slovenian influenza A virus isolates and detected the H275Y mutation in only one of the isolates.

After obtaining the subtype results of the isolates in the March–April 2009 outbreak, treatment and prophylaxis with oseltamivir could have been suspended, as it was likely that the virus was resistant to it. At that time, data showed that influenza A(H3N2) viral resistance to oseltamivir in countries of the WHO European Region was 90–100% [11]. Result from the Health Protection Agency revealed the presence of the H275Y mutation in viral isolates from eight of the 12 confirmed cases in the March–April 2009 outbreak (Table) and resistance to oseltamivir was confirmed on 22 April 2009. Given these results, and the fact that influenza A(H3N2) viruses circulating in Europe are almost exclusively resistant to oseltamivir [3], we can assume that the resistance of the influenza A(H1N1) viruses in the outbreak was not a result of treatment.

Three influenza A viruses were tested for zanamivir sensitivity at the Health Protection Agency, London, UK, and no resistance was found (data not shown). However, in this outbreak, either treatment or prophylaxis with zanamivir was not initiated as none of the residents was free of charge, the nursing and pedagogical protection after vaccination. Although the vaccine was offered free of charge, the nursing and pedagogical staff had not been vaccinated, which does not comply with national recommendations. Two healthcare workers fell ill with fever and cough before the outbreak, but it is not known whether they had influenza. If they did, it is possible that they may have been the source of infection for the residents. It is also possible that a visitor brought the virus into the facility.

Given the resistance of the influenza A(H1N1) viral isolates in this outbreak, it is unlikely that oseltamivir given as treatment and prophylactically had any impact on the course of illness in those affected and the mitigation of the outbreak. It is more likely that confirmation that this was an influenza outbreak contributed to
stricter following of standard and droplet precautions, stopping further the spread of the virus.

According to recommendations from the United States Centers for Disease Prevention and Control for preventing the spread of influenza virus in acute care facilities, standard and droplet isolation should be adopted for patients for five days after the onset of their illness [14]. Studies involving healthy young volunteers showed that secretion of influenza virus lasts approximately five days and ceases when the symptoms start to disappear: after intranasal inoculation of the virus, secretion lasted for seven days at most [15]. The results of such studies are probably an approximation of what happens after natural influenza virus infection. Intranasal virus application is different from natural infection, which can be a consequence of indirect contact with infected respiratory secretions. After natural infection, children and people with immunity impairment secrete influenza virus longer than immunocompetent adults do [16,17]. In our investigation, most of the cases had viral RNA in their swabs after more than five days since the onset of illness, even after the symptoms disappeared. A semiquantitative method (observing cycle threshold values of rt-RT-PCR results) demonstrated that the concentration of the influenza virus reduced with time and, consequently, the possibility of transfer to other people (data not shown). The question remains as to how long the residents who recovered from influenza were still able to spread the virus within their community in their facility. Perhaps we only detected the inactive viral RNA. In addition, there is also the question of whether five days after the onset of symptoms is really an appropriate time interval for standard/droplet isolation in such an institution or longer period should be recommended [2].

In conclusion, rapid detection, reporting of and response to influenza outbreaks in long-term care facilities must be emphasised [18]. Influenza cannot be distinguished from other respiratory infections on a clinical basis: virological diagnosis is required. In our investigation, most of the cases had viral RNA in their swabs after more than five days since the onset of illness, even after the symptoms disappeared. A semiquantitative method (observing cycle threshold values of rt-RT-PCR results) demonstrated that the concentration of the influenza virus reduced with time and, consequently, the possibility of transfer to other people (data not shown). The question remains as to how long the residents who recovered from influenza were still able to spread the virus within their community in their facility. Perhaps we only detected the inactive viral RNA. In addition, there is also the question of whether five days after the onset of symptoms is really an appropriate time interval for standard/droplet isolation in such an institution or longer period should be recommended [2].

References


Acknowledgements

We sincerely thank Angie Lackenby from the Health Protection Agency, London, UK, for carrying out the antiviral susceptibility tests for this study.