Rapid communications

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This short communication hypothesises that rhinovirus epidemics occurring after start of school may interfere with the spread of influenza during the period when warm and humid climate decreases the influenza spread by aerosol. Limited laboratory data supporting this hypothesis are included in the article, but the report is written mainly to stimulate interest and research concerning the possibility that viral interaction may affect influenza epidemiology.

Modelling and prediction of the spread of influenza are important for rational decisions on how to handle epidemics and pandemics. Apart from immunity in the population, both climate and social behaviour seem to be important factors affecting the spread. Holiday time usually interrupts the spread [1]. In dry and cold weather the aerosol transmission of influenza is more efficient since the virus becomes stabilised by hardening of the lipid membrane, remains airborne for longer time and is spread to longer distances [2-3]. In warm and moist weather, droplet and possibly contact spread and inoculation by contaminated hands seem to become more important [4].

However, these factors do not explain all characteristics of the spread of the pandemic influenza A(H1N1) virus during 2009. In Sweden, and some other European countries, the spread increased after the end of the holidays, but after four weeks of increasing activity the spread suddenly declined, despite similar weather conditions and social behaviour (Figure 1) [5]. Limitation by herd immunity induced by the spread that actually took place is possible, but not very likely, as the reported number of infections and of influenza-like disease in total was rather low. Also, the experience from the United States and the United Kingdom, with considerable, though patchy, spread of the virus during late spring and summer, despite a climate unfavourable to influenza, makes it likely that the virus would have managed to reach a substantial peak in Sweden in early October, unless other factors than the weather affected the spread.

All cases of influenza were made reportable in Sweden on 13 May 2009. Samples were taken from all suspected cases until 16 July, when the strategy was changed from containment to mitigation. Figure 1 shows the number of laboratory-confirmed cases reported in Sweden according to the law. Influenza diagnoses reported from all Swedish laboratories during the past three seasons are included for comparison.

Since the number of samples sent for influenza analysis was increasing until week 36 [5] while the proportion of samples positive for pandemic H1N1 influenza was already decreasing (Table 1), we hypothesised that some other virus infection may have interfered with the spread of the influenza pandemic.

Laboratories in Sweden conducting extended viral diagnosis on samples sent for influenza examination were asked what viruses they found in the influenza-negative samples, and the answer was unanimous: rhinoviruses dominated, with sporadic findings of other respiratory viruses, such as enteroviruses and adenoviruses. We retrieved all data from one of the dominant laboratories, the microbiological laboratory at Karolinska University Hospital. All respiratory samples received are analysed by PCR for influenzavirus A and B, including pandemic influenza A(H1N1) virus, as well as for respiratory syncytial virus (RSV). Tests for a further thirteen viral pathogens are done if extended diagnoses is requested by the doctor submitting the sample [6]. The number of samples analysed between weeks 32 and 39 2009 at Karolinska University Hospital, as well as the results of the analyses, are shown in Tables 1 and 2. Extended PCR was only requested for samples that were negative for RSV and influenza. As shown in Figure 2, there was an increase in the proportion and number of rhinovirus diagnoses roughly in parallel with the decrease of influenza diagnoses.

A simple but likely explanation for the sudden interruption of the spread of influenza could thus be the increase in the spread of above all rhinoviruses. It is well known that a major rhinovirus epidemic always occurs soon after school has started [7]. The virus is spread mainly by contaminated hands [8], and has not been reported to be climate-dependent. Thus the spread of rhinoviruses may have had an advantage over influenza due to the mild and moist climate. Once a rhinovirus infection has become established, infected cells start producing interferon and other cytokines, similar to those produced by influenza [9]. This immune reaction causes the cells to enter an antiviral state. Though double infections occur, they are probably not common enough to maintain high level spread of both rhino and influenza viruses in the population.
Table 2

Number of samples examined for 13 viruses*, Karolinska University Hospital, Stockholm, August-September 2009 (n=401**)

<table>
<thead>
<tr>
<th>Week</th>
<th>32</th>
<th>33</th>
<th>34</th>
<th>35</th>
<th>36</th>
<th>37</th>
<th>38</th>
<th>39</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhinovirus, no. (%)</td>
<td>2 (6%)</td>
<td>2 (5%)</td>
<td>7 (19%)</td>
<td>6 (11%)</td>
<td>18 (25%)</td>
<td>16 (27%)</td>
<td>14 (27%)</td>
<td>9 (16%)</td>
</tr>
<tr>
<td>Picornaviruses not subtyped, no. (%)</td>
<td>0</td>
<td>2 (5%)</td>
<td>0</td>
<td>1 (2%)</td>
<td>4 (6%)</td>
<td>2 (3%)</td>
<td>1 (2%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>11 other viruses, no. (%)</td>
<td>1 (3%)</td>
<td>0</td>
<td>4 (11%)</td>
<td>4 (8%)</td>
<td>1 (1%)</td>
<td>1 (2%)</td>
<td>0</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Total no. examined**</td>
<td>35</td>
<td>38</td>
<td>36</td>
<td>53</td>
<td>71</td>
<td>60</td>
<td>51</td>
<td>57</td>
</tr>
</tbody>
</table>

*Rhinovirus, bocavirus, adenovirus, four types of human coronavirus, metapneumovirus, parainfluenzavirus types 1-3, non-subtyped picornaviruses, enteroviruses. Positive results for rhinovirus and non-subtyped picornaviruses, which could be rhinoviruses, are presented separately as numbers and percentages, the other viruses are summarised.

**A subset of samples from Table 1, which had tested negative for pandemic influenza A(H1N1), seasonal influenza and respiratory syncytial virus.

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Table 1

Number of samples examined with PCR for pandemic influenza A(H1N1) and number and proportion of positives*, Karolinska University Hospital, Stockholm, August-September 2009 (n=2,994)

<table>
<thead>
<tr>
<th>Week no. (2009)</th>
<th>32</th>
<th>33</th>
<th>34</th>
<th>35</th>
<th>36</th>
<th>37</th>
<th>38</th>
<th>39</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pandemic Influenza A(H1N1)-positives, no. (%)</td>
<td>10 (7%)</td>
<td>16 (11%)</td>
<td>38 (14%)</td>
<td>85 (19%)</td>
<td>61 (8%)</td>
<td>31 (5%)</td>
<td>24 (7%)</td>
<td>9 (3%)</td>
</tr>
<tr>
<td>Total no. examined</td>
<td>146</td>
<td>150</td>
<td>277</td>
<td>440</td>
<td>754</td>
<td>616</td>
<td>351</td>
<td>260</td>
</tr>
</tbody>
</table>

* Respiratory syncytial virus and seasonal influenza were also included in the examinations, with one positive each during the whole period.
Influenza surveillance with sentinel reporting normally does not start until week 40, and respiratory sampling for viral diagnostics is usually scarce during early autumn. For week 40, most Swedish sentinel doctors usually report zero cases of influenza-like illness (ILI), and we do not know whether the early autumn rhinovirus peak would have been reported as ILI in previous years even if reporting had been in place then. The reason for the large number of rhinovirus infections diagnosed in 2009 was most likely that people who got respiratory tract infections, who would not normally have visited a doctor, did so due to the fear of the pandemic influenza.

In conclusion, we hypothesise that a rhinovirus epidemic that occurred after the end of the summer holidays may have interfered with the spread of pandemic influenza during a period with warm and humid climate that decreases spread of influenza by aerosol. Although the laboratory data supporting this hypothesis are limited, it may stimulate research into the possibility that the interaction between different circulating viruses may affect influenza epidemiology.

We therefore suggest the following:

1. The epidemiology of influenza should be related to that of other respiratory viruses for improved understanding of the true epidemiological situation.
2. Surveillance of respiratory infections should be conducted throughout the year to create reliable baselines for ILI and acute respiratory infections, which are useful when a pandemic virus occurs that does not follow the usual pattern of spread.

References


Figure 2

Proportion of samples examined at Karolinska University Hospital, Stockholm, containing pandemic influenza A(H1N1) and rhinoviruses, August-September 2009
In 2009, to date 16 human cases of West Nile neuroinvasive disease (WNND) have been reported in Italy, in three regions: Veneto, Emilia-Romagna and Lombardia. The number of cases is higher compared with last year when nine cases were identified (eight cases of WNND and one case of West Nile fever) and the geographical distribution indicates spread from east to west.

**Introduction**

West Nile virus (WNV) infection is transmitted in natural cycles between birds and mosquitoes, particularly Culex spp. mosquitoes. Humans and horses are susceptible, dead-end hosts. Firstly identified in tropical Africa, WNV infection has been evidenced in northern Africa, Israel, India and Australia [1] and progressively spread in the Americas since 1999. WNV has been the cause of outbreaks and sporadic cases in central, eastern and Mediterranean Europe for more than 45 years.

In Italy, the first cases of equine WNV infection were detected in 1998, but no human cases were reported at that time [2]. The first human cases of WNV infection in Italy, including neuroinvasive forms, were identified in 2008 [3]. A total of nine human cases were reported by two regions: five confirmed cases of West Nile neuroinvasive disease (WNND) (four identified retrospectively) and one case of West Nile fever were recorded in Veneto, all in the province of Rovigo [4], and three confirmed WNND cases were detected in Emilia-Romagna [5,6].

**Table 1**

Case definition of West Nile neuroinvasive disease (WNND), surveillance programme in Veneto and Emilia-Romagna regions, Italy, 2008–2009

<table>
<thead>
<tr>
<th>Subjects</th>
<th>≥ 15 yr with fever ≥ 38.5°C and neurological symptoms (e.g., encephalitis, meningitis, Guillain-Barré syndrome or acute flaccid paralysis).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases were classified as:</td>
<td>Possible: clinical symptoms and aseptic CSF.</td>
</tr>
<tr>
<td></td>
<td>Probable: clinical symptoms and at least one of the following laboratory criteria:</td>
</tr>
<tr>
<td></td>
<td>- presence of IgM antibodies against WNV by ELISA;</td>
</tr>
<tr>
<td></td>
<td>- seroconversion by ELISA;</td>
</tr>
<tr>
<td></td>
<td>- fourfold increase of IgG antibodies against WNV in two consecutive samplings (&gt;5 days, preferably 15-20 days between the two samples) by ELISA.</td>
</tr>
<tr>
<td></td>
<td>Confirmed: clinical symptoms and at least one of the following laboratory criteria:</td>
</tr>
<tr>
<td></td>
<td>- isolation of WNV in blood or CSF;</td>
</tr>
<tr>
<td></td>
<td>- presence of IgM antibodies in CSF by ELISA;</td>
</tr>
<tr>
<td></td>
<td>- detection of WNV RNA by RT-PCR in blood or CSF;</td>
</tr>
<tr>
<td></td>
<td>- detection of increased levels of WNV IgM and IgG by ELISA and confirmed by PRNT.</td>
</tr>
</tbody>
</table>

Veneto and Emilia-Romagna implemented an active surveillance of farm workers that yielded a seroprevalence of 1.5% and 3.1% respectively [3-6]. In the Emilia-Romagna region, a seroprevalence study of blood donors was also performed, showing a seroprevalence of 0.7-0.8% [6]. Apart from human cases, equine WNV infections have also been detected in the same regions [6]. No human cases were described in other Italian regions during the summer of 2008.

Human cases of WNND reoccurred in the summer 2009. Hereby we briefly describe these cases and discuss possible implications for public health.

**WNND surveillance in Italy**
Following the identification of the first human cases of WNV infection in Italy in 2008, specific WNND surveillance systems were set up in the affected regions of Emilia-Romagna and Veneto. The case definitions used are presented in Table 1 [3,5]. Both systems collect data on human cases of WNND every year between 15 June and 31 October. In both regions animal and vector surveillance for WNV is also in place.

In Lombardia region, a surveillance system for neuroinvasive diseases has been in place since 2008. Cases from all age-groups are tested for a large panel of viruses and bacteria, including WNV. No cases of neuroinvasive disease due to WNV were detected in Lombardia in 2008.

In addition to surveillance of human cases, a national veterinary plan for WNV surveillance has been implemented since 2008 [7].

**Results**
A total of 16 confirmed cases of WNND were reported to the regional surveillance systems in three Italian regions between August and September 2009. Detailed information is presented in Table 2.

The distribution of human cases of WNND by month of symptom onset and geographical location in the years 2008 and 2009 is shown in Figure 1 and Figure 2 (A and B).

A detailed description of the epidemiological situation in the affected regions is reported below.

### Veneto
Since the end of August 2009, six human cases of WNND were reported to the regional surveillance system (Table 2). Five cases were observed in the area of Rovigo town and one case in the area between the provinces Rovigo and Venezia. The cases (four males and two females) were between 62 to 82 years old. Virus-specific IgM and IgG were detected in cerebrospinal fluid (CSF) and serum specimens by immunoglobulin M antibody (IgM) capture enzyme-linked immunosorbent assay (MAC-ELISA). The cerebrospinal fluid and serum specimens were obtained from the patients upon their first presentation to the clinic. Diagnosis was confirmed by the plaque-reduction neutralisation test (PRNT). All patients were hospitalised and they are still in critical condition. One patient from the province of Rovigo died.

### Emilia-Romagna
Since the end of August 2009, eight human cases of WNND were reported to the regional surveillance system in the provinces of Modena (one case), Ferrara (five cases), Imola (one case) and Bologna (one case). Of these, seven are in critical condition and one died. Ages of cases ranged from 62 to 78 years old. Virus-specific IgM and IgG were detected in cerebrospinal fluid (CSF) and serum specimens by immunoglobulin M antibody (IgM) capture enzyme-linked immunosorbent assay (MAC-ELISA). The cerebrospinal fluid and serum specimens were obtained from the patients upon their first presentation to the clinic. Diagnosis was confirmed by the plaque-reduction neutralisation test (PRNT). All patients were hospitalised and they are still in critical condition. One patient from the province of Rovigo died.

### Lombardia
Since September 2009, two confirmed cases of WNND were hospitalised in Emilia-Romagna region (Modena) and they are still in critical condition. The two cases were resident in Lombardia, in the province of Mantua bordering Emilia-Romagna region (Table 2). Virus-specific IgM and IgG were detected in CSF and serum specimens by MAC-ELISA and immunofluorescence assays (IFA). Diagnoses were confirmed by PCR. To date, 57 possible cases of WNND have been referred to the Regional Reference Centre for Microbiological Emergencies (CRREM) laboratory in Bologna and excluded after negative results of laboratory test.

**Table 2**
Confirmed cases of West Nile neuroinvasive disease (WNND) in Italy, August - September 2009 (n=16)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Province</th>
<th>Region</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>76</td>
<td>Rovigo</td>
<td>Veneto</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>78</td>
<td>Rovigo/Venezia</td>
<td>Veneto</td>
</tr>
<tr>
<td>(died)</td>
<td>M</td>
<td>82</td>
<td>Rovigo</td>
<td>Veneto</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>62</td>
<td>Rovigo</td>
<td>Veneto</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>78</td>
<td>Rovigo</td>
<td>Veneto</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>84</td>
<td>Rovigo</td>
<td>Veneto</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>73</td>
<td>Ferrara</td>
<td>Emilia Romagna</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>62</td>
<td>Ferrara</td>
<td>Emilia Romagna</td>
</tr>
<tr>
<td>(died)</td>
<td>M</td>
<td>72</td>
<td>Ferrara</td>
<td>Emilia Romagna</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>72</td>
<td>Ferrara</td>
<td>Emilia Romagna</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>68</td>
<td>Ferrara</td>
<td>Emilia Romagna</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>78</td>
<td>Bologna</td>
<td>Emilia Romagna</td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>77</td>
<td>Imola</td>
<td>Emilia Romagna</td>
</tr>
<tr>
<td>14</td>
<td>M</td>
<td>64</td>
<td>Modena</td>
<td>Emilia Romagna</td>
</tr>
<tr>
<td>15</td>
<td>F</td>
<td>72</td>
<td>Mantova</td>
<td>Lombardia</td>
</tr>
<tr>
<td>16</td>
<td>F</td>
<td>72</td>
<td>Mantova</td>
<td>Lombardia</td>
</tr>
</tbody>
</table>

**Figure 1**
Human cases of West Nile neuroinvasive disease (WNND) in Veneto and Emilia-Romagna regions, Italy, 2008-2009 (n=24)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Province</th>
<th>Region</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rovigo</td>
<td>Veneto</td>
</tr>
<tr>
<td>2</td>
<td>Rovigo/Venezia</td>
<td>Veneto</td>
</tr>
<tr>
<td>3</td>
<td>Rovigo</td>
<td>Veneto</td>
</tr>
<tr>
<td>4</td>
<td>Rovigo</td>
<td>Veneto</td>
</tr>
<tr>
<td>5</td>
<td>Rovigo</td>
<td>Veneto</td>
</tr>
<tr>
<td>6</td>
<td>Rovigo</td>
<td>Veneto</td>
</tr>
<tr>
<td>7</td>
<td>Ferrara</td>
<td>Emilia Romagna</td>
</tr>
<tr>
<td>8</td>
<td>Ferrara</td>
<td>Emilia Romagna</td>
</tr>
<tr>
<td>9</td>
<td>Ferrara</td>
<td>Emilia Romagna</td>
</tr>
<tr>
<td>10</td>
<td>Ferrara</td>
<td>Emilia Romagna</td>
</tr>
<tr>
<td>11</td>
<td>Ferrara</td>
<td>Emilia Romagna</td>
</tr>
<tr>
<td>12</td>
<td>Bologna</td>
<td>Emilia Romagna</td>
</tr>
<tr>
<td>13</td>
<td>Imola</td>
<td>Emilia Romagna</td>
</tr>
<tr>
<td>14</td>
<td>Modena</td>
<td>Emilia Romagna</td>
</tr>
<tr>
<td>15</td>
<td>Mantova</td>
<td>Lombardia</td>
</tr>
<tr>
<td>16</td>
<td>Mantova</td>
<td>Lombardia</td>
</tr>
</tbody>
</table>
Control measures implemented

Vector control measures consisted in regular mosquito spraying activities (adulticide and larvicide) especially at public events, in the affected regions. In addition, Emilia-Romagna region implemented public education messages on self-protection from mosquito bites on the region’s public health authority website.

Regarding blood, tissue and organ safety, between 1 August and 30 October 2009, Italy applies nucleic acid amplification technology (NAT) screening on all blood donations from residents in the provinces of Ferrara (Emilia-Romagna), Rovigo (Veneto) and Mantua (Lombardy). The objective of this screening is to quantify the viral circulation in these provinces among blood donors and to ensure the early implementation of appropriate blood safety measures. The first NAT-positive blood donation is considered as a trigger to defer further donations from the province of residence of the donor, independent of the identification of human cases of WNND. In case of positivity, blood donors who have spent at least one night in affected provinces are deferred for 28 days. This policy is implemented nationwide.

Conclusions

The occurrence of human cases of WNND in Italy is indicative of the ongoing WNV activity. In Italy, the provinces of Ferrara (Emilia-Romagna), Rovigo (Veneto) and Mantua (Lombardy) are considered high risk areas of transmission of WNV, and equine cases of WNV infection were also confirmed there [8].

Compared to the summer of 2008, a larger geographical area was affected by WNV infection in 2009. In particular, the virus expanded its activity apparently moving from east to west. These changes were immediately detected by the public health authorities, which started the NAT screening of all blood donors in the newly affected provinces, in order not to defer donations from these areas. For this reason the exchange of data between human, animal and vector sector is crucial, as experienced in the Emilia-Romagna region where weekly reports with detailed description of WNV infections in humans, animals and vectors have been made since the beginning of 2009.

The national public health authorities are now considering the implementation of a nationwide enhanced human surveillance system in Italy, in order to include all those regions where the circulation of WNV has been reported (Emilia-Romagna, Lombardia, Veneto and Toscana) together with animal and vector surveillance [8].

Disseminating the information regarding the presence of WNV among clinicians could help public health authorities to rapidly identify new human cases of WNND, in order to implement control measures to reduce the transmission of the virus. This should be done in an integrated approach including veterinary and entomological surveillance in order to better monitor the situation in areas with favourable ecological conditions for WNV cycle.*

Acknowledgement:
The authors acknowledge the European Centre for Disease Prevention and Control (ECDC) for the conclusions formulated in the Threat Assessment on West Nile virus transmission with human cases in Italy.*

*Authors’ correction
On request of the authors, the last paragraph of the article was replaced and the acknowledgement was added on 9 October 2009.

References


The first two *Klebsiella pneumoniae* carbapenemase-producing (KPC) type 2 strains carrying ST258 were detected in Finland in June and early August 2009. They were found colonising two patients transferred from the Mediterranean; one patient referred from a hospital in Greece where isolates were first found in 2007 and another from Italy where the first isolates have been described only very recently.

**Case 1**

The first carbapenemase-producing *Klebsiella pneumoniae* (KPC) strains in Finland were detected this summer in two patients transferred to Helsinki University Central Hospital (HUCH) from Crete, Greece, and northwestern Italy, respectively. Case 1 was a patient transferred from Greece at the end of June 2009. In Greece, the patient was initially hospitalised at a ward but later transferred to the intensive care unit, due to pneumonia and acute myocardial ischemia. The clinical history upon referral to our hospital did not mention antibiotic treatment although it is highly probable that antibiotics were used when the patient was first admitted to hospital in Greece.

Since the patient arrived to the HUCH intensive care unit from a high risk epidemic area where carbapenemase-carrying strains are common, a stool sample was tested for extended spectrum beta-lactamase (ESBL) and carbapenem resistance, using ESBL Chrom-ID agar (bioMérieux, Marseille, France) detecting both ESBLs and AmpC at the HUCH laboratory. *Klebsiella pneumoniae* grew on this plate; from this isolate, a direct KPC PCR was done, and sequencing of the PCR product confirmed the gene to be *bla*KPC-2. Antibiotic susceptibility was tested using Etests (AB Biodisk, Solna, Sweden). The isolate was resistant or intermediately resistant to all antibiotics except trimethoprim-sulphamethoxazole and gentamicin (Table). Case 1 later died of multiorgan failure, not from infection related to the KPC strain.

**Case 2**

Case 2 was a patient transferred to Finland from north-western Italy in mid-August, after having been hospitalized for ten days during a trip due to seizures, unconsciousness and anaemia caused by an underlying alcohol-induced liver cirrhosis and total red cell aplasia. The clinical history upon referral to our hospital did not mention antibiotic treatment, however, it is highly probable that antibiotics were used at the hospital in Italy. The patient was found to have a chronic sacral wound from which a swab was taken and analysed at the HUCS laboratory.

*K. pneumoniae* grew on the culture plate and the isolate was further analysed as it showed high level resistance to all β-lactams, including carbapenems. It remained susceptible only to colistin and gentamicin (Table). The isolate was found to be positive for *bla*KPC-2 by PCR and sequencing. Case 2 later died from the multiple underlying conditions unrelated to the KPC strain.

### Table

Minimum inhibitory concentration (MIC) profiles of the carbapenem-producing *Klebsiella pneumoniae* isolates*, Finland, June-August 2009 (n=2)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Case 1</th>
<th>Case 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piperacillin/tazobactam</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>&gt;256</td>
<td>48</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>32</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Imipenem</td>
<td>8</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Meropenem</td>
<td>32</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Colistin</td>
<td>2</td>
<td>0.19</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Minocycline</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Amikacin</td>
<td>48</td>
<td>32</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>Trimethoprim/sulphamethoxazole</td>
<td>0.38</td>
<td>&gt;32</td>
</tr>
</tbody>
</table>

*Both isolates were also resistant to levofloxacin, cefpodoxime, cefpodoxime/clavulanic acid, ceftazidime/clavulanic acid and cefotaxime/clavulanic acid, tested using Oxoid disks (Oxoid, Basingstoke, UK).
Results

The isolates were sent for multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) typing to the National Institute for Health and Welfare, where the PCR results were confirmed. Both strains contained bla<sub>TEM-1</sub> and pyrosequencing identified an ESBL variant of bla<sub>SHV</sub> with Gly to Ser and Glu to Lys mutations at positions 238 and 240, respectively. PCR was negative for CTX-M, VIM-, IMP-, OXA-48 and GES-genes. Hydrolysis of imipenem was confirmed by spectrophotometric analysis of crude cell extracts. MLST [1] showed that both isolates belonged to the epidemic clone ST258. PFGE showed the strains to be somewhat similar (80%) to each other, as also found in other studies on this clone [2,3].

Conclusions

KPC-producing Klebsiella pneumoniae was first detected in North Carolina, USA, in 1996 [4]. After first only causing local epidemics on the east coast of the USA during the end of the 1990’s and at the beginning of the new millennium [5,6], the KPC epidemic now seems to be accelerating [7]. Both Finnish isolates belonged to the clone ST258, which has been shown to account for probably 70% of the KPC-positive K. pneumoniae isolates sent to the US Centers for Disease Control and Prevention (CDC) [2]. It has also been found in Norway and Sweden in patients transferred from Greece and Israel in 2007 [3], in an outbreak strain in Israel [2] as well as in isolates in Poland [8] and Italy [9]. No doubt, MLST of KPC strains from around the world will find that many older isolates also belong to this clone. The resistance varies between isolates of this clone; gentamicin is the only antibiotic effective against all isolates. The preparedness level in Finland was also increased at this time, by educating the clinical microbiology laboratories, and establishing reference methods.

Currently there is no compulsory screening programme for carbapenemase-producing pathogens at national level in Finland, but the authors would strongly recommend that patients transferred from abroad should be screened. Chromogenic ESBL-selective plates seem to be a fast, simple and presumably sensitive tool to detect carriers of multiresistant gram-negative bacteria for this purpose. In addition to ESBLs they detect KPC strains, and in our experience possibly also metallo-beta-lactamase-carrying strains. At least stool/rectal samples should be tested, preferably also swabs from the oropharynx and axillae [11].

Fortunately, the largest tertiary care hospital in Finland stayed alert to the threat of KPC colonised patients and was thus able to detect these two strains. The epidemic spread of carbapenemase-carrying strains from colonised patients is well-documented [12], and should be taken seriously.

References

In the last week of March 2009, five measles cases among students of an anthroposophic school were reported to the public health authorities in the Austrian province of Styria where only five cases had been reported in the whole of 2008. A descriptive epidemiological investigation of the measles outbreak was performed. Between 2 March and 10 May 2009, 37 cases of measles were identified in Styria: 33 confirmed outbreak cases and four probable outbreak cases. The measles outbreak spread from the general population (12 cases) to an anthroposophic community (25 cases). Cases outside of the anthroposophic community were mostly over 10 years of age (10/12). Thirty-five cases were unvaccinated, and two of the 37 had received one dose of measles, mumps, rubella vaccine. Following a measles outbreak in Salzburg in 2008 with 394 cases, this outbreak reemphasizes the continued need for additional vaccination campaigns in population groups over the age of 10 years.

Introduction

In the last week of March 2009, five measles cases were reported to public health authorities in the Austrian province of Styria (total population: 1.2 million). All cases were pupils of an anthroposophic school (total school population: 305). No measles cases had been reported in the two previous months in Austria. In 2008, five cases had been reported in Styria during the whole year.

A bivalent measles, mumps (MM) vaccine was introduced in Austria in 1974 as part of the national childhood immunisation programme. This was replaced in 1994 by a trivalent measles, mumps, rubella (MMR) vaccine (two-dose regimen with the first dose at 15 months and the second dose at six years of age) [1]. The Ministry of Health estimates the average measles vaccine coverage with at least one dose for the birth cohorts 1997–2007 to be 84% [2]. Measles vaccination is not mandatory in Austria for enrolling a child in school.

The World Health Organization (WHO) set the year 2010 as the target for elimination of measles in the European Region [3]. Between 2004 and 2007, Austria was considered a low to moderate incidence country, according to the criteria of EUVAC.NET (< 1/100,000 population/year) [4]. In 2008, a measles outbreak with at least 394 cases in the Austrian province of Salzburg, linked to the anthroposophic community, changed Austria’s status to a high incidence country [5].

The aim of the outbreak investigation was to describe the outbreak by person, place and time and to identify the proportion of cases who were vaccinated.

Methods

A descriptive epidemiological outbreak investigation was performed. Case data on demographics, date of rash onset, clinical symptoms, past history of contact with a known measles case, vaccination status, and disease outcome were assessed by telephone interviews.

A confirmed outbreak case was defined as a patient with a generalised macular-papular rash with fever accompanied by at least one of the following clinical signs: cough, coryza, or conjunctivitis, who fulfilled one of the criteria of a laboratory-confirmed measles infection as described elsewhere [6] or who was epidemiologically linked to a laboratory-confirmed measles infection within 7–21 days prior to rash onset, who fell sick after 1 March 2009, and was resident in the Austrian province of Styria. A probable outbreak case was defined as a patient who fulfilled the clinical criteria of measles, who fell sick after 1 March 2009, and was resident in the Austrian province of Styria.

Active case finding was conducted among contact persons of the measles cases who were notified to the district public health authorities. Infection with measles virus was defined as laboratory-confirmed if at least one of the following three laboratory criteria was fulfilled: detection of measles virus-specific IgM, detection of measles virus RNA in clinical specimens as described by El Mubarak et al. [7] and genotyping as described by Santibanez et al. [8] were performed by the Austrian National Reference Centre for Measles.

Results

Thirty-seven cases fulfilled the outbreak case definition. Of these, 33 were confirmed and four were probable cases. Nine of the 11 laboratory-tested cases were confirmed for measles virus infection. The measles virus RNA from two outbreak case specimens was partially sequenced and was genotype H1. The outbreak affected four of the 17 public health districts of Styria between 2 March (week 10) and 10 May 2009 (week 19), and peaked with eight cases with onset of symptoms in week 17 (20-
Between March and May 2009, 11 unrelated measles cases were reported in the other eight Austrian provinces. The figure shows the outbreak cases by week of rash onset according to the outbreak case classification.

Of the thirty-five cases, 25 belonged to the anthroposophic community, including 12 pupils of the anthroposophic school - giving a school attack rate of 12/305 (3.9%) - four household members, and nine acquaintances. A likely source was identified as one of the first four anthroposophic community cases (including two cases in pupils), who fell sick at the same time. This anthroposophic case was a pupil who had visited a billiard pub within the three weeks prior to his rash onset. An earlier case from the general population had also reported having visited the same pub. This is one probable route which enabled the measles virus to spread from the general population to the susceptible anthroposophic community.

Among the cases belonging to the anthroposophic community, the age group of 5-9 year-olds was most affected with 14 of 25 cases. Among the cases in the general population, the age group of 10-14 year-olds was most affected, with five of 12 cases (Table). Most of the cases from the general population were over 10 years old (10/12).

The symptoms most commonly reported by all 35 cases were fever (n=35), cough (n=34), conjunctivitis (n=34) and cold-like symptoms (n=28). Two measles cases reported having otitis media. Two cases were hospitalised during the course of the infection for five and eight days, respectively. All cases recovered.

None of the 37 outbreak cases had received both doses of MMR vaccine. Two cases had received one vaccine dose of MMR. Both belonged to the 12 cases in the general population. All cases in the anthroposophic community and ten cases in the general population were completely unvaccinated (Table).

The anthroposophic school was closed for two weeks and cases were asked to stay at home for the period of communicability (at least four days after the onset of the rash). An MMR post-exposure prophylaxis was offered free of charge to susceptible contacts of outbreak cases.

**Discussion**

We report a measles outbreak, which began in the general population in week 10 of 2009 and spread to an anthroposophic school in week 13. In a measles outbreak in 2008 involving 397 cases, the attack rate in the affected anthroposophic school was 44% (150/340 pupils), significantly higher than the 3.9% attack rate in the general population. A likely source was identified as one of the first three anthroposophic community cases (including two cases in pupils), who fell sick at the same time. This anthroposophic case was a pupil who had visited a billiard pub within the three weeks prior to his rash onset. An earlier case from the general population had also reported having visited the same pub. This is one probable route which enabled the measles virus to spread from the general population to the susceptible anthroposophic community.

**Table**

<table>
<thead>
<tr>
<th>Case characteristics</th>
<th>N&lt;sub&gt;total&lt;/sub&gt; = 37</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex ratio (m:f)</strong></td>
<td>2.1:1</td>
</tr>
<tr>
<td><strong>Group A</strong></td>
<td>12</td>
</tr>
<tr>
<td><strong>Group B</strong></td>
<td>25</td>
</tr>
<tr>
<td><strong>Age distribution</strong></td>
<td></td>
</tr>
<tr>
<td>0-4</td>
<td>0</td>
</tr>
<tr>
<td>5-9</td>
<td>1</td>
</tr>
<tr>
<td>10-14</td>
<td>5</td>
</tr>
<tr>
<td>15-19</td>
<td>8</td>
</tr>
<tr>
<td>20-24</td>
<td>0</td>
</tr>
<tr>
<td>25-29</td>
<td>0</td>
</tr>
<tr>
<td>30-34</td>
<td>0</td>
</tr>
<tr>
<td>35-39</td>
<td>1</td>
</tr>
<tr>
<td><strong>Clinical symptoms</strong></td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>35</td>
</tr>
<tr>
<td>Cough</td>
<td>34</td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>34</td>
</tr>
<tr>
<td>Cold</td>
<td>28</td>
</tr>
<tr>
<td>Otitis media</td>
<td>2</td>
</tr>
<tr>
<td>Hospitalisation</td>
<td>2</td>
</tr>
<tr>
<td>Laboratory-confirmed cases/tested</td>
<td>9/11</td>
</tr>
<tr>
<td>Measles virus RNA positive/tested</td>
<td>2/9</td>
</tr>
<tr>
<td>Measles virus-specific IgM positive/tested</td>
<td>9/9</td>
</tr>
</tbody>
</table>

**Figure**

Measles cases by week of rash onset, Styria, Austria, March-May 2009 (n=35*)

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*Thirty-five of the 37 outbreak cases were accessible for telephone interviews.*
observed here. Assuming similar low vaccination coverage in the anthroposophic community as observed in the 2008 measles outbreak, the low attack rate in this outbreak was likely due to the prompt two-week closure of the anthroposophic school and the prompt isolation of cases at home for the period of communicability. The supplementary province-wide MMR vaccination campaign addressing the 15-25 years age group in the general population was implemented as a consequence of an outbreak affecting Austrian provinces other than Styria in 2008. In the first six months of 2008, 5,335 first doses (5.1% of those administered within the age group of 7–25 years) were administered, which is more than the number of first doses administered during the first half of 2009 (i.e. the period of the described measles outbreak) [unpublished data]. A concurrent rubella outbreak (ongoing since October 2008) may have also contributed to raise awareness for contagious rash diseases, which probably led to an early case presentation and case isolation [9].

Combating measles is still a high public health priority in Europe [10]. In Austria, a mumps outbreak in 2006, a measles outbreak in 2008, and a rubella outbreak in 2008-2009 have shown a clear shift of the age distribution of the cases to those older than ten years [1,5,10]. The age groups most affected were: 16-30 year-olds (mumps), 10-19 year-olds (measles), and 15-24 year-olds (rubella) [1,5,10]. The current outbreak of measles, in which the over 10 year-olds accounted for 10 of the 12 cases in the general population, justifies the introduction of supplementary MMR vaccination campaigns targeting the over 10 year-olds in Styria. Based on the vaccination register in Styria [unpublished data], an average vaccination coverage of 90% was reported for the birth cohorts 1999-2008.

Age group specific seroprevalence surveys could provide the required comprehensive information for designing supplementary age group-targeted vaccination campaigns Austria-wide. In neighbouring Germany, adolescents are often not fully vaccinated or unvaccinated [11]. Coverage is still insufficient to achieve wide enough herd immunity for measles elimination in central Europe. Continuing with suboptimal vaccination coverage in certain population groups such as the adolescents endangers the possibility of achieving the 2010 target for measles and rubella elimination in the WHO European Region.

References
Respiratory syncytial virus (RSV) surveillance is important to get insight into the burden of disease and epidemic pattern of RSV infection. This information is useful for healthcare resource allocation as well as the timing of preventive messages and palivizumab prophylaxis. For influenza surveillance the European Influenza Surveillance Scheme (EISS) was established in 1996, but no surveillance platform is available for RSV. To improve surveillance an RSV Task Group was established in 2003 and recommendations for RSV surveillance were developed. By 2008, progress was made for four out of six recommendations: the number of European countries testing specimens for RSV increased from six to fourteen; nose and/or throat swabs were generally used for detection of influenza and RSV; a total of 25 laboratories performed molecular testing for diagnosis and participated in a quality control assessment for RSV with an overall good performance; four of the ten countries that joined EISS in 2004 started reporting RSV detections in addition to influenza in the period 2004-8. Limited progress was achieved for standardising methods and the development of a sentinel surveillance system of representative hospitals. Improving RSV surveillance is possible by further harmonising the data collection and increased reporting of RSV.

**Introduction**

Respiratory syncytial virus (RSV) is the most important viral agent causing severe respiratory disease in young children [1-3]. RSV is also being recognised as a significant pathogen in adults [2,4] causing moderately severe respiratory disease especially in the elderly [5,6]. Influenza is widely recognised as a major cause of morbidity and mortality in humans [7,8]. Since RSV and influenza virus infections are associated with similar clinical symptoms [9] and frequently co-circulate around the same time of the year, there is substantial potential for confusion regarding the cause of influenza-like illness [10].

Influenza and RSV account for similar numbers of deaths in children and their impact varies by winter and age group. RSV is associated with more deaths than influenza in children aged 1-12 months [11]. Excess deaths due to RSV and influenza virus infection have also been reported for the elderly population [5,8]. When comparing cause-specific mortality due to influenza virus and RSV infection in all ages, it has been estimated that most deaths were associated with influenza A(H3N2) viruses, followed by RSV, influenza B, and influenza A(H1N1) [8].

While influenza is on the list of communicable diseases that must be covered by the European Community network for surveillance, RSV is not on this list [12]. Nonetheless, RSV causes considerable burden of disease and RSV surveillance is important for determining the burden of illness in all age groups and in defining seasonality and epidemic pattern. This facilitates the preparation of hospital settings to receive more children and to define the timing of the start of palivizumab prophylaxis [13]. Palivizumab can be administered as passive immunoprophylaxis and is the only strategy that has been demonstrated to reduce RSV hospitalisations in high-risk children [14]. For real-time influenza surveillance the European Influenza Surveillance Scheme (EISS), a collaborative multinational project, was established in 1996 [15], but no such scheme was available for other respiratory viruses including RSV. Since RSV and influenza infections typically occur in the winter, EISS made it possible to report RSV detections into the EISS database, on a voluntary basis, from 1996 until September 2008.

In 2003 an RSV Task Group was established within EISS to explore the possibility to design a comprehensive RSV surveillance scheme within the EISS framework. This Task Group was composed of four epidemiologists and two virologists. Three meetings were organised between July 2003 and January 2006 and updates on the activities were presented to the EISS group during the EISS Annual Meetings. A retrospective analysis was carried out. Additionally, RSV surveillance recommendations were published in 2006 [16], and are presented below:

1. Specimens collected as part of an influenza surveillance programme should also be tested for RSV.
2. Both combined nose/throat swabs and nasal pharyngeal aspirates are acceptable for RSV diagnosis.
3. The application of molecular techniques such as real time PCR in the diagnosis of respiratory disease has been demonstrated and we advocate this technique for RSV detection.
4. Further developments are encouraged on the use of standardised methods and laboratory techniques.
5. The development of a sentinel approach of representative hospitals should be considered.
6. New countries joining EISS are encouraged to integrate RSV surveillance alongside influenza surveillance.

Our objective was to assess whether the RSV reporting within EISS in the period 2004-2008 complied with these surveillance recommendations, and to describe the detection and reporting of seasonal influenza and RSV infections in six selected countries in Europe.

**Methods**

**Data collection in EISS**

EISS was based on an integrated clinical and virological surveillance model. Sentinel primary care physicians reported weekly the number of new cases of influenza-like illness and/or acute respiratory infections and obtained respiratory specimens from a sample of patients for laboratory testing. The specimens were tested for influenza and in several countries for RSV as well. Weekly consultation rates and laboratory test results were entered by the national surveillance networks into the EISS database via an internet-based system [17]. Non-sentinel, mainly hospital-based data for influenza and RSV were also collected, but will not be presented in this paper.

Since September 2008, European influenza surveillance has been carried out by the European Centre for Disease Prevention and Control (ECDC) and involves all 27 European Union Member States and Norway. Three other countries, Serbia, Switzerland and Ukraine, are reporting data to World Health Organization (WHO) Regional Office for Europe.

This paper presents a descriptive study. Surveillance data for seven winter seasons (2001-2 to 2007-8; week 40-20) in the EISS database were screened for RSV detections by country. The database containing virological detections of RSV and influenza was downloaded by September 2008. An RSV reporting country was defined as a country that reported at least 10 sentinel specimens positive for RSV from 2001-2008. With this method the progress for recommendation 1 and 6 could be assessed. For the other

### Table 1

Reporting of respiratory syncytial virus (RSV) and influenza data to the European Influenza Surveillance Scheme (EISS) in the period 2001-2008

<table>
<thead>
<tr>
<th>Season</th>
<th>Number of countries reporting RSV*</th>
<th>Number of countries reporting influenza</th>
<th>Number of RSV detections</th>
<th>Number of influenza detections</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001-2</td>
<td>6</td>
<td>18</td>
<td>203</td>
<td>2276</td>
</tr>
<tr>
<td>2002-3</td>
<td>8</td>
<td>19</td>
<td>335</td>
<td>3787</td>
</tr>
<tr>
<td>2003-4</td>
<td>12</td>
<td>22</td>
<td>143</td>
<td>2732</td>
</tr>
<tr>
<td>2004-5</td>
<td>12</td>
<td>23</td>
<td>557</td>
<td>5483</td>
</tr>
<tr>
<td>2005-6</td>
<td>14</td>
<td>28</td>
<td>803</td>
<td>3171</td>
</tr>
<tr>
<td>2006-7</td>
<td>14</td>
<td>30</td>
<td>888</td>
<td>5077</td>
</tr>
<tr>
<td>2007-8</td>
<td>13</td>
<td>31</td>
<td>929</td>
<td>5076</td>
</tr>
</tbody>
</table>

*Countries reporting RSV:

2001-2: CZ, FR, DE, SI, CH, UK-E, UK-S.
2002-3: CZ, FR, DE, NL, SK, SI, CH, UK-E, UK-S.
2003-4: CZ, FR, DE, NL, SK, SI, CH, UK-E, UK-S.
2004-5: AT, CZ, DK, FR, DE, IT, LU, PL, RO, SI, CH, UK-E, UK-S.
2005-6: AT, CZ, DK, EE, FI, FR, DE, IT, LU, NL, PL, RO, SI, UK-E, UK-S.
2006-7: AT, CZ, DK, EE, FI, FR, DE, IT, LU, NL, PL, RO, SI, UK-E, UK-S.
2007-8: AT, HR, CZ, DK, EE, FI, FR, DE, LU, NL, PL, SI, UK-E, UK-S.

Abbreviations: Austria (AT), Croatia (HR), Czech Republic (CZ), Denmark (DK), Estonia (EE), Finland (FI), France (FR), Germany (DE), Italy (IT), Luxembourg (LU), the Netherlands (NL), Poland (PL), Romania (RO), Slovenia (SI), Slovakia (SK), Switzerland (CH), UK-England (UK-E), UK-Scotland (UK-S).

### Table 2

Number of sentinel influenza and respiratory syncytial virus (RSV) detections by country in the period 2001-2008

<table>
<thead>
<tr>
<th>Country</th>
<th>Number of RSV detections per season mean (range)</th>
<th>Number of influenza detections per season mean (range)</th>
<th>Total number of RSV and influenza detections mean (range)</th>
<th>Percentage of RSV cases [%] (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Czech Republic</td>
<td>18 (5-30)</td>
<td>206 (83-311)</td>
<td>223 (102-327)</td>
<td>8 (% 3-19)</td>
</tr>
<tr>
<td>France</td>
<td>145 (47-227)</td>
<td>1053 (824-1374)</td>
<td>1198 (947-1601)</td>
<td>12 (% 4-18)</td>
</tr>
<tr>
<td>Germany</td>
<td>43 (12-138)</td>
<td>1129 (553-2145)</td>
<td>1172 (564-2172)</td>
<td>4 (% 1-10)</td>
</tr>
<tr>
<td>The Netherlands**</td>
<td>12 (1-19)</td>
<td>121 (15-142)</td>
<td>131 (16-153)</td>
<td>4 (% 0-16)</td>
</tr>
<tr>
<td>Slovenia</td>
<td>6 (1-12)</td>
<td>101 (69-132)</td>
<td>106 (77-135)</td>
<td>5 (% 1-12)</td>
</tr>
<tr>
<td>UK-England</td>
<td>44 (14-125)</td>
<td>231 (82-432)</td>
<td>275 (107-477)</td>
<td>16 (% 8-56)</td>
</tr>
<tr>
<td>UK-Scotland</td>
<td>23 (14-35)</td>
<td>101 (31-193)</td>
<td>123 (50-220)</td>
<td>18 (% 11-38)</td>
</tr>
</tbody>
</table>

* The percentage of RSV cases in relation to the total number of samples that tested positive for either influenza or RSV.
** No RSV detections were reported for the Netherlands in the winters of 2001-2 and 2004-5.
recommendations the progress was summarised by collecting relevant data from inventories and a quality control assessment.

RSV detections: six countries
Country selection
Data from the Czech Republic, France, Germany, Netherlands, Slovenia and the United Kingdom (UK) (represented by England and Scotland) were assessed to describe the RSV surveillance in these countries. All had reported data for at least five winter seasons. Sentinel primary care physicians included general practitioners (GPs) in the Czech Republic and the Netherlands, and GPs and paediatricians in the Czech Republic, France, and Germany, and GPs, paediatricians and specialists in Slovenia. The sentinel doctors represented 1-5% of all physicians working in the country.

Case definition
Data on new cases were based on reporting of consultations for influenza-like illness (ILI) in the Netherlands, Slovenia and United Kingdom. Consultations for acute respiratory infections (ARI) were collected in France and Germany. From 2001-2 to 2004-5 the Czech Republic reported the number of new cases of ARI, and from 2005-6 onwards they reported cases of ILI in addition to ARI [18]. Case definitions for ARI and ILI differed slightly between countries [19]. The type of specimen that was collected (nose and/or throat swab) as well as transport conditions were similar [20]. Samples were generally collected within five days after onset of symptoms and systematically tested for both influenza virus and RSV in all countries. In Germany, only specimens of children aged 0-3 years were tested for RSV. Cases were defined positive for RSV or influenza when at least one laboratory test yielded a positive result. Between-country comparisons will not be made due to methodological differences.

Results
Recommendation 1
Specimens collected as part of an influenza surveillance programme should also be tested for RSV.

Seventeen countries had reported RSV detections in the period 2001-2008: Austria, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Italy, Luxembourg, Netherlands, Poland, Romania, Slovenia, Slovakia, Switzerland, UK- England and Scotland. Since England and Scotland have their own sentinel surveillance systems, these are presented separately in this paper. The number of countries reporting influenza data increased from 18 in 2001-2 to 31 in the winter of 2007-8 (Table 1).

In 2001-2 only six countries reported RSV detections in addition to influenza, but their number gradually increased, particularly around 2003-4, among both countries that had participated since 2001 and new members (see also results for recommendation 6). From 2005-6 no further increase in the number of countries reporting RSV was observed (Table 1).

Recommendation 2
Both combined nose/throat swabs and nasal pharyngeal aspirates are acceptable for RSV diagnosis.

Different types of specimens are used for detection of influenza and RSV [21]. Generally the nasopharyngeal aspirates have a high sensitivity, and are often used in a hospital setting. Easier to use and less painful are nasal/nasopharyngeal swabs [22]. An inventory carried out in 2002 indicated that in sentinel surveillance systems in Europe nose and/or throat swabs were taken [20]. Twelve out of 20 national networks collected combined nose/throat swabs. The remaining networks collected either nasopharyngeal, nasal, or throat swabs. In addition, three networks took blood samples and one network obtained nasal aspirates [20]. Since all countries had already used the recommended type of respiratory sample and fulfilled the recommendation, no progress was assessed after 2002.

Recommendation 3
The application of molecular techniques such as real time polymerase chain reaction (PCR) in the diagnosis of respiratory disease has been demonstrated and this technique is advocated for RSV detection.

In 2006, laboratories were invited to participate in a quality control study for molecular methods. Of the 33 laboratories participating in EISS, 25 performed this technique with an overall performance of 88% correct results [23]. The majority (22 out of 25) of laboratories used an in-house molecular assay. In particular, real time PCR and nested PCR assays provided the highest performance scores (93% correct score; range 70-100) and were used in 19 laboratories. Three laboratories used commercial assays and the percentage of correct results ranged from 50% to 80% [23].

Recommendation 4
Further developments in the use of standardised methods and laboratory techniques are encouraged.

Limited progress was made in standardising methods. Only for influenza, not RSV, laboratory protocols were shared and standardised reagents were made available via the EISS website. However, with the application of molecular methods, as indicated in recommendation 3, and quality control assessment of this method, the quality of laboratory testing of RSV is ascertained.

Recommendation 5
The development of a sentinel system of representative hospitals should be considered.

No efforts were made to develop a European sentinel surveillance system consisting of representative hospitals, though national initiatives may have been undertaken. For example, a laboratory-based surveillance for RSV involving different hospital laboratories in Slovenia was implemented in 2006 [24].

Recommendation 6
We recommend the new networks joining EISS to integrate RSV surveillance alongside influenza.

Ten new countries became members of EISS between 2004 and 2008: Austria, Bulgaria, Croatia, Estonia, Finland, Cyprus, Greece, Hungary, Ukraine and Serbia [25]. Of these, four countries followed the recommendation and started reporting RSV data (Table 1).

RSV detections: six countries
To illustrate the data that were collected by EISS, we present the results of RSV detections for six countries. All countries reported at least five seasons of data, which provided insight in the occurrence of RSV in these countries. RSV and influenza detections are
presented in Table 2. The percentage of RSV-positive specimens largely differed by season, e.g. from 3% to 19% in the Czech Republic (Table 2). For all seasons and countries together the percentage of RSV-positive specimens varied from 4% in Germany and the Netherlands to 16-18% in the United Kingdom. RSV activity usually started a few weeks before the onset of influenza activity (data not shown). The data collected are useful to describe the seasonality of RSV and show that RSV is detected in patients with ILI and/or ARI.

**Discussion and conclusion**

Progress in RSV surveillance was made in the period 2001-2008, with the obvious increase in the number of reporting countries during the time the RSV Task Group was active, between 2003-2006. Progress was made particularly in terms of the number of countries testing specimens for RSV and the use of molecular techniques. The results for the six countries that had reported at least five years of data showed that RSV surveillance and reporting is feasible in Europe. The overall percentage of RSV-positive specimens for the Czech Republic, France, Germany, Netherlands, Slovenia and the UK amounted to 4-18% indicating that a substantial number of patients who consulted their sentinel physician with influenza-like illness or acute respiratory infection actually had an RSV infection. The EISS surveillance is real time and therefore can be relevant for timing of the influenza and RSV peak and providing insight into the morbidity and seasonality of these respiratory illnesses.

Limited progress was made for recommendation 4 on the use of standardised laboratory methods. With the use of mainly in-house developed methods that perform well [23], the standardising of methods was not further explored. The rationale was that standardising methods is important and is encouraged by sharing protocols, but more important is the ability of the laboratory test to correctly identify RSV. Furthermore, limited progress was made for recommendation 5 on the development of a sentinel approach of hospitals. This recommendation was ranked as a lower priority because non-sentinel data from hospitals are currently being collected. The non-sentinel data could be used for the future establishment of a sentinel laboratory monitoring system and would then need to be assessed for representativeness and quality of data collection.

In this paper we presented data on sentinel RSV and influenza detections. Relatively low numbers of positive RSV tests were reported and this is therefore a limitation. In addition to sentinel data, RSV reports from non-sentinel sources, mainly derived from hospitalised infants are also available and these can provide insight into the epidemic peak of RSV during wintertime. We think that both sources of data are important and complement each other. Sentinel data highlights the occurrence of RSV in the community, where it is an important confounder in influenza surveillance. And hospital-based data present the circulation of RSV in more severe cases and high-risk groups.

The limitations of the sentinel influenza surveillance carried out by EISS are related to differences in case definitions [19], sampling guidelines and laboratory techniques among the different countries [20]. Some difficulty in obtaining swabs from all age groups has been reported, especially for young children in the Netherlands and the elderly in the Netherlands and France [16]. Another limitation is that we could not further investigate other possible causes of respiratory infections such as rhinovirus, adenovirus and coronavirus [26,27] and human metapneumovirus [28]. Country resources however may limit the extension of testing for other viruses in addition to influenza and RSV. Furthermore, no comparison regarding the occurrence of RSV and influenza between the different countries could be made because of differences in data collection procedures and laboratory methods. Additionally, differences in healthcare seeking behaviour may influence the findings between countries.

Currently diagnostic specimens are collected from patients presenting with ILI or ARI. Although ILI and/or ARI case definitions have been used for the detection of influenza for many years, this may not be the optimal clinical indicator for RSV. To investigate the clinical impact and determine the burden of illness of RSV one should extend the diagnostic categories to include acute bronchitis and otitis media [29]. This may become feasible with the movement towards sentinel networks based on electronic data.

We presented the progress in RSV surveillance based on an influenza surveillance network and data collected for six countries. This illustrated the feasibility of reporting RSV data and showed that a proportion of about 4-18% of the patients were infected with RSV. Sentinel monitoring of RSV and influenza virus is important and may even be extended to other respiratory viruses as the development of multiplex PCR [30] facilitates the detection of other causative agents of respiratory illness. All countries are encouraged to test their specimens for RSV and improvements can be made as less than half of the countries participating in EISS had reported these data. Furthermore, swabbing procedures should be further harmonised and regular quality control of laboratory methods should be performed. When these criteria are met, surveillance of RSV and influenza virus will contribute to a better insight into the burden of respiratory diseases and may be used by healthcare organisations to decide on the timing of palivizumab prophylaxis for RSV in Europe. Overall, this paper illustrated that an existing influenza surveillance system can be relatively easily broadened to include the surveillance of RSV and may be extended to other viruses in the future.

**Acknowledgements**

The members of the RSV Task Group were: Helena Rebelo de Andrade (Instituto Nacional de Saúde, Lisbon, Portugal), Brunhilde Schweiger (Robert Koch Institute, Berlin, Germany), Lisa Domegan (Health Protection Surveillance Centre, Dublin, Ireland), Dougas Fleming (Royal College of General Practitioners, Birmingham, United Kingdom), Anne Mosnier (Open-Rome, Paris, France; chairperson of the Task Group), Maja Socan (National Institute of Public Health, Ljubljana, Slovenia).

We thank the countries that participated in EISS reporting RSV and influenza data between 1996 and 2008 and we thank all sentinel practitioners that participated in the study. Without their efforts the surveillance by EISS would not be possible.

This work was supported by H. Hoffmann-La Roche Ltd, Sanofi Pasteur and Sanofi Pasteur MSD via the European Influenza Surveillance Scheme. None of the supporting parties was involved in the data analysis and reporting. All authors declare they have no conflicting or dual interests.

**References**


**Surveillance and outbreak reports**

**Legionnaires’ disease cluster linked to a metal product aqueous pre-treatment process, Staffordshire, England, May 2008**

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In May 2008, a report of two workers from the same construction equipment manufacturing plant who were admitted to hospital with Legionnaires’ disease confirmed by urine antigen prompted an outbreak investigation. Both cases were middle aged men, smokers, and with no travel, leisure or other common community exposure to *Legionella* sources. There were no wet cooling towers at the plant or in the surrounding area. No increase in respiratory disease or worker absenteeism occurred at the plant during the preceding month. Wider case ascertainment including alerts to hospitals and medical practitioners yielded no further cases. The environmental investigation (and sampling of water systems for *Legionella*) identified a *Legionella pneumophila* serogroup 1 (Mab 2b) count of >3.0x10^4 cfu/l in water samples from an aqueous metal pre-treatment tunnel, which generates profuse water aerosol. Drainage, cleaning and biocide treatment using thiazalone eliminated *Legionella* from the system.

**Introduction**

Legionnaires’ disease is an atypical pneumonic illness caused by the inhalation of aerosolised *Legionella* bacteria. These bacteria are found naturally in environmental water sources usually in low numbers. Multiplication of this organism is favoured when water is stagnant and warm. Poorly maintained aerosol-generating devices and water systems such as wet cooling towers, and spa pools are well documented sources of Legionnaires’ disease [1]. Aside from travel exposure, the majority of cases and clusters of Legionnaires’ disease in Europe are associated with community sources, mainly cooling towers and spa pools. Direct links with industrial manufacturing processes are less common [2,3].

On 15 May 2008, public health authorities in the West Midlands, England, were notified of two confirmed cases of Legionnaires’ disease, admitted to the same hospital on the previous day. Both cases worked on the production line at the same construction and agricultural equipment manufacturing plant (plant X). The local health protection unit declared this a presumptive Legionnaires’ disease cluster and led an outbreak control team to investigate common infection sources at work and in the community. This paper describes the disease cluster, the environmental investigation and the control measures implemented.

**Methods**

A confirmed case of Legionnaires’ disease was defined as a person working at plant X who had clinical symptoms of pneumonia, was confirmed radiologically and by laboratory evidence of infection with *Legionella pneumophila* serogroup 1 (Lp-1), with onset of symptoms after 22 April 2008. Laboratory confirmation consisted of detection of Lp-1 antigen in urine.

Searching for additional cases included a review of worker sickness absenteeism and reports of respiratory illness at plant X during the preceding month. The occupational health service at the plant informed the work force of potential risks and advised early reporting of respiratory symptoms. All workers with onset of respiratory symptoms after 22 April 2008 were urgently investigated and offered a urine antigen test. In addition, clinicians and microbiologists at local medical referral centres and hospitals, as well as neighbouring health protection units were alerted.

The cases and their close family members were interviewed in hospital shortly after admission using a standardised questionnaire to elicit demographic details, clinical history, risk factors for Legionnaires’ disease, and sources of potential *Legionella* exposure during the previous 14 days. Details were obtained regarding travel (abroad and locally), recreational activities (water exposure, spa pool exposure), hospital admissions, domestic risk factors, and occupational activities.

Environmental health and safety officials undertook an environmental investigation and risk assessment including a review of local wet cooling towers, and a description of water systems at the plant with collection of water samples for *Legionella* culture and isolation.

Laboratory confirmation of clinical cases used *Legionella* urine antigen Binax NOW rapid immunochromatographic assay for the qualitative detection of *L. pneumophila* serogroup 1 antigen in urine samples [4]. Isolation and typing of environmental *Legionella* consisted of concentrating 1 litre water samples by membrane filtration and elution of the deposit. The deposit was heat- and acid-treated to reduce unwanted bacterial growth. Treated and untreated
portions of the deposit were inoculated onto selective buffered charcoal yeast extract agar containing cysteine and iron [5].

**Results**

Two confirmed cases (cases A and B) were admitted to hospital on 14 May 2008 with clinical pneumonia. Symptom onset had been on 6 and 8 May 2008, respectively. Both cases were 40-50 year-old men with a history of heavy cigarette smoking. They responded well to standard treatment, did not require mechanical ventilation, and were discharged from hospital after eight days. Attempts at sputum sample collection were unsuccessful and clinical Lp-1 isolation was therefore not possible.

The cases lived in different towns (9 miles apart) and drove to work using different routes. Both had not travelled locally, within the country or abroad in the preceding two months, and had no exposure to common domestic, leisure and community aerosolised water sources. Both were full-time production line workers at plant X but were not close friends and had no contact outside of work. They reported working on different stages of the production line approximately 20 metres apart.

Plant X has a workforce of 642 people and is situated in a semi-rural town in a district of approximately 500,000 residents. Case searching at the plant did not yield any further cases. No increase in absenteeism was detected at the plant during the six months prior to identification of the two cases. Fourteen workers were identified who had been absent from work in the previous four weeks, of which 11 reported respiratory symptoms. None of these had clinical pneumonia or were admitted to hospital, and all tested urine antigen-negative for Lp-1. The two confirmed Legionnaires’ disease cases did not represent an increase in notifications above the average of two cases (range: 0-9) per year that occurred in the prior 14 years in this district. A review of all industry-linked Legionnaires’ disease reports in this district since 1994 identified only two cases but their exact exposure could not be identified.

The plant has a basic rectangular floor plan, housing a comprehensive production line and small administrative section. No wet cooling or air conditioning systems are used at the plant. The plant used four water systems:

1. Two independent domestic type hot and cold water systems supplying the restroom and changing facilities. These systems had been drained in April 2008, were regularly monitored, and had no stagnant water sections.
2. A paint mist trap in an unheated spray paint booth. Here a below ground-water jet traps paint mist under negative pressure to an extraction stack. The water is at ambient temperature.
3. An aqueous metal pre-treatment tunnel. Steel parts on a monorail move through a degreasing and rinsing tunnel in preparation (pre-treatment) for painting. The system has a complex network of pipelines and tanks providing jet spraying of parts with solutions (including alkaline degreaser and an acidic phosphate solution) and water (which has a pH neutralising effect) at successive stages inside a tunnel.

Different solutions and water are drawn from their respective tanks by pumps and fed to spray nozzles inside the tunnel. There are six pre-treatment stages: a cleaning stage followed by two water rinses, then a ‘keying chemical’ stage with a further two water rinses. Each stage has its respective supply and collection tank. The chemical tanks were heated to 55-60 °C. The water for rinsing is mains-fed and supplies four unheated water tanks (volume of each tank: 8,000 to 15,000 litres) at 25-38 °C. The brushes covering the conveying raling were missing and there was no local extraction for the tunnel. Aerosols were visibly leaking from the gap of the conveying raling and the large openings at the entrance and exit of the tunnel.

Prior to this incident, the aqueous pre-treatment process had not been risk-assessed as a source of *Legionella* organisms and potential human exposure. No management system (protocol) for monitoring (including *Legionella* sampling), disinfecting and cleaning the water systems was in place.

Case A worked on the assembly production line, and Case B worked at the aqueous pre-treatment and powder coating section. Case A walked past the pre-treatment plant a number of times daily to an adjoining factory exit where he smoked.

Baseline sampling and culture of all water systems (a, b, and c) was undertaken on 16 May 2008. No *Legionella* was isolated from the domestic hot and cold water system (a) or the paint mist water trap system (b). Water samples from the aqueous pre-treatment system (c) contained *L. pneumophila* serogroup1 (Mab 2b) at a count of >3.0x10^4 colony-forming units (cfu)/l.

Drainage and cleaning of the aqueous pre-treatment system (c) and the domestic-type hot and cold water system (a) were undertaken during the initial two weeks following the detection of the two cases, followed by chlorine dioxide shock treatment of the pre-treatment system. For maintenance, biocide treatment with thiazalone was preferred over chlorine and other halogen-based products, as these may interact with degreasing chemicals, causing corrosion and affecting product quality. The subsequent dosing regime was reviewed regularly and modified until a suitable balance was achieved, taking into account the short half life of thiazalone. During plant shut down at each weekend, all tanks were completely drained and cleaned.

Subsequent water samples from the water tanks supplying the metal pre-treatment process (c) yielded *L. pneumophila* serogroup 1 (Mab2b) in diminishing numbers over a four week period, leading to eradication on 20 June 2008.

**Discussion and conclusions**

We report on two epidemiologically linked Legionnaires’ disease cases with likely occupational exposure to an aqueous pre-treatment system in a construction equipment manufacturing plant. The aqueous pre-treatment system carried the highest risk as a probable source of infection because of the isolation of *L. pneumophila* serogroup 1 from the water and associated aerosolisation. Because clinical samples were not available for further typing and matching to Lp-1 isolated from the water samples, definitive causality could not be established. Future investigations should therefore prioritise obtaining clinical isolates to confirm the aqueous pre-treatment system as the source of infection. The domestic systems (a) were reasonably controlled, and the paint-mists water trap system (b)
had a Legionella-inhibitory temperature (below 15 °C) with water aerosols under suction. Therefore, the risk of human exposure from those systems is low.

No prior risk assessment of the aqueous pre-treatment system had been undertaken at the plant. Immediate and medium-term control measures (water sampling, biociding, cleaning/drainage) were effective in controlling Legionella growth and preventing further cases of Legionnaires’ disease.

Legionnaires’ disease clusters have been reported from industrial settings with workers exposed to sources of aerosolised water, including from biological treatment plants in the pulp and paper industry [6], contaminated metal-working fluids in the automotive industry [7], factories that use water to cool moulded plastics [8], and waste water treatment facilities [9]. Aqueous cleaners are generally believed to present a low risk to workers’ health and gained popularity in industry as degreasing of metal parts by organic solvents was gradually phased out [10]. To the best of our knowledge this is the first report implicating an aqueous metal pre-treatment plant as a possible source of Legionella linked to a cluster of Legionnaires’ disease.

Aqueous pre-treatment systems are prone to Legionella growth due to favourable water temperature, the presence of nutrients such as rusts and dirt from metal parts, convoluted surfaces that favour biofilm development, and recirculation of the water. Since the report of these two cases, five similar aqueous pre-treatment systems have been inspected by the United Kingdom’s Health and Safety Executive, and Legionella has been isolated in four. A cleaning and disinfection regime similar to the one reported here was implemented and has prevented further growth of Legionella. The findings of this subsequent investigation are being submitted for publication.

Significantly, aqueous pre-treatment systems generate profuse water aerosol, and preventing escape may prove complex. Assessing the risks for Legionnaires’ disease in similar systems, common in the metal manufacturing industry, is recommended.

Acknowledgements
We are grateful for the support we received from the local environmental health departments, the Health Protection Agency laboratories, and the infectious disease team at the University Hospital North Staffordshire.

References
Google Flu Trends, a tool that estimates the level of influenza activity in near real-time using aggregated search queries, has been released for 14 countries in Europe on 8 October 2009 by Google.org, the philanthropic arm of Google [1]. In total Google Flu Trends covers information for 20 countries worldwide and it is available in 37 languages. It aims to complement traditional influenza surveillance systems used by the public health community such as the European Centre for Disease Prevention and Control’s (ECDC) European Influenza Surveillance Network (EISN). For 12 of the 14 European countries, Google developed models to track influenza using historical influenza data, provided in the public domain by EISN. For two countries the models are “experimental”, meaning there were not enough historic data for the validation process described in the scientific publication where Google Flu Trends used influenza-like illness (ILI) data provided by the United States’ Centers for Disease Control and Prevention’s (CDC) Influenza Sentinel Provider Surveillance Network [2]. Google Flu Trends is a complementary tool to traditional surveillance systems which offer more specific and detailed information regarding influenza in a population. Traditional systems take time to collect, validate and release data, while Google search queries can be counted immediately and trends can be updated daily. Google Flu Trends aims to be a supplementary tool in the surveillance of influenza that can provide additional information both for public health officials and the public when making informed decisions about preparing for the influenza season.

References