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# Outbreaks of gastroenteritis linked to lettuce, Denmark, January 2010

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**At least 11 linked outbreaks of gastroenteritis with a total of 260 cases have occurred in Denmark in mid January 2010. Investigations showed that the outbreaks were caused by norovirus of several genotypes and by enterotoxigenic *Escherichia coli*. Lettuce of the lollo bionda type grown in France was found to be the vehicle.**

From 18 to 20 January 2010, a series of outbreaks of gastroenteritis were reported to Danish authorities. Outbreak investigations were initiated by the Danish food control authority in cooperation with Statens Serum Institut (SSI), the National Food Institute, the Food and Veterinary Administration as well as the medical officers and several clinical microbiological laboratories in Copenhagen. The epidemiological, microbiological and food investigation are still ongoing; here we report on the current status of the investigation of these outbreaks.

## Epidemiological examinations and findings

The link between lettuce and illness was discovered in the fourth week of January 2010 based on an analysis of five outbreaks. These outbreaks had been reported during week 3 to the regional food control authority, which covers the eastern part of Denmark. As of 8 February, 11 outbreaks have been included in the cluster. A further eight outbreaks in Denmark which are currently under investigation may also be associated with lettuce. Taken together, the 11 outbreaks comprised approximately 480 potentially exposed persons and approximately 260 cases with symptoms of gastroenteritis (see Table). The 11 outbreaks all took place in the eastern half of the country (on the islands of Funen and Zealand). Norovirus was initially suspected as the aetiology, but the Kaplan criteria were not fulfilled in all circumstances and attack rates were sometimes higher than expected for norovirus, indicating the possibility of the presence of more than one disease agent.

Although norovirus outbreaks are not rare in winter, such a high number of outbreaks was clearly above the seasonal average and initial investigations generally indicated a food source. All outbreaks occurred in groups of people (company employees, course attendees etc.) who had lunch delivered from catering companies. The food in each case included sandwiches or Danish-style open sandwiches (smørrebrød). Comparison of ingredient lists identified lettuce – which was often found in the sandwiches – as the only relevant common food item. All lettuce used by implicated catering companies was of the lollo bionda type and trace-back of the lettuce showed that in each outbreak one of two suppliers had been used. Both suppliers bought the lettuce from the same wholesaler who in turn bought it in France. The lollo bionda lettuce was reported to be produced in France, grown outdoors in the south-western part of the country.

Questionnaire studies were performed for several of the outbreaks. These showed a link between illness and consumption of sandwiches containing lettuce. In one larger group of people, a retrospective cohort study was performed. Eight different food items were available and were inquired about. Questionnaires were distributed to 60 persons of whom 44 responded; 34 reported to have been ill. Pooling of the three types of sandwiches that contained lettuce gave a relative risk of 6.2 (95% confidence interval: 1.0-38).

As described in the next section, enterotoxigenic *Escherichia coli* ETEC was found in cases of the outbreak cluster, but also in patients not part of any of the 11 known outbreaks. When interviewed, these patients were found to have been infected in Denmark (most ETEC infections in Denmark are believed to be travel-related). Onset dates ranged between 14 and 21 January 2010. Preliminary interviews suggested that these patients had also been infected through consumption of sandwiches made by catering companies. Possible additional outbreaks uncovered this way are

currently under investigation. Because of these results, we believe that the infections occurred predominantly in the form of outbreaks caused by whole-sale lettuce as opposed to lettuce sold in retail.

## Microbiological examinations and findings

Heads of lettuce could be collected from two of the implicated catering companies and were analysed for norovirus at the Danish National Food Institute. Norovirus of genogroup II was recovered from lettuce from one outbreak on 22 January. Subsequent analyses of remaining lettuce for the presence of *E. coli* were negative.

So far stool samples from 25 patients known to be part of one of the outbreaks have been examined for viruses at the SSI. Of these, 23 were positive: norovirus of genogroup I was found in two patients, genogroup II in 12 patients, and mixed infections with these viruses in nine patients. Preliminary sequencing results show the presence of at least three different genotypes. Results of the examination for other virus types are pending, but sapovirus has been detected in samples from two patients.

Initial analysis of the same samples for pathogenic bacteria (*Salmonella, Campylobacter, Shigella and Yersinia*) was negative, but examination for diarrhoeagenic *E. coli* revealed the presence of ETEC in 11 cases of 24 examined at the SSI. They were serotyped as *E. coli* O6:K15:H16 containing genes for both the LT and STh toxins. In addition to these cases, an unusually high number of 16 further ETEC patients was found through the routine diagnostics of stool samples performed at the SSI in January (see above); 15 of them were also found to be of serotype O6:K15:H16. Furthermore, *Staphylococcus aureus, Clostridium perfringens and Bacillus cereus* were recovered from two, three and two outbreak cases, respectively (of 24 examined).

## Control measures and European perspective

Based on the fact that lettuce of the same kind from the same supplier was present in all outbreaks, lettuce from the French supplier in question bought after 1 January 2010 was recalled from the Danish market on 22 January by order of the Danish Food and Veterinary Administration. A rapid alert (notification number 2010.0081) was issued on 25 January following the virological confirmation of norovirus in the lettuce. Trace-back indicated that only a small part of the incriminated batches had been sold through retail, most had been sold to catering companies and restaurants. The available evidence indicates that contaminated lettuce is no longer on the market in Denmark.

Two urgent inquiries were released through the European Centre for Disease Prevention and Control's food- and waterborne diseases network, mentioning the norovirus outbreaks (26 January) and the ETEC findings (28 January), and following this, the information was also distributed through the norovirus network formerly known as DIVINE-NET. In response, Norway reported having three outbreaks caused by lollo bionda lettuce. It appeared that part of two batches of lettuce which had caused disease in Denmark had been exported to Norway and that this was the direct cause of the Norwegian outbreaks. To our knowledge, no countries apart from Denmark and Norway have reported on outbreaks caused by lollo bionda lettuce. Information from French authorities on the possible cause of the contamination is pending.

## Discussion

Contaminated lettuce was shown to be the source of widespread illness of norovirus and ETEC in Denmark in January 2010. Epidemiological investigations of several outbreaks combined with trace-back analyses indicated that particular batches of lollo bionda lettuce were the source of the outbreaks. This was confirmed when norovirus was directly detected in the lettuce.

**Table**

Exposed persons (n=479) and cases (n=264) with gastroenteritis linked to lettuce, Denmark, January 2010

| Outbreak database number | Date of exposure | Date of discovery of outbreak | Number of exposed persons | Number of cases | Patients positive for norovirus | Patients positive for ETEC |
|---|---|---|---|---|---|---|
| 953 | 14 and 15 Jan | 18 Jan | 80 | 62 | Yes | No |
| 956 | 13 Jan | 18 Jan | 32 | 26 | Yes | Yes |
| 957 | 19 Jan | 19 Jan | 125[a] | 50[a] | Yes | Yes |
| 958 | 16 Jan | 19 Jan | 10 | 10 | Yes | Yes |
| 955 | 18 Jan | 20 Jan | 16 | 16 | No | No |
| 959 | 6 Jan | 11 Jan | 14 | 12 | Yes | No |
| 960 | 17 Jan | 20 Jan | 27 | 21 | Yes | Yes |
| 961 | 15 Jan | 26 Jan | 140 | 35 | No | No |
| 963 | 12 and 13 Jan | 20 Jan | 27 | 26 | No | No |
| 964 | 16 Jan | 20 Jan | 2 | 2 | No | No |
| 952 | 15 Jan | 18 Jan | 6 | 4 | Yes | Yes |

ETEC: enterotoxigenic E. coli.

Stool samples were not submitted for analysis in all outbreaks.

[a] Approximate numbers.

As several infectious agents have been detected in the samples taken from the cases, we believe that the lettuce was contaminated with multiple agents. How the lettuce became contaminated is as yet unknown, but it will be important to establish this in order to prevent similar outbreaks in the future. Since neither norovirus nor ETEC are zoonotic agents, we speculate that human faecal matter may have been the source of the contamination, possibly via contaminated water.

Neither norovirus nor ETEC are generally covered by routine analyses of stool samples from patients with gastroenteritis (in Denmark and other European countries). Surveillance for both agents is therefore incomplete and the extent of the infections may have been more widespread than what we describe here. Furthermore, both disease agents can be extremely difficult to detect in food. In this outbreak, the detection methods used for analyses for ETEC in the lettuce may not have been optimal, for instance the lettuce was stored frozen between the viral and the bacterial analyses. Norovirus is the disease agent giving rise to most food-borne outbreaks in Denmark [1]. However, series of linked norovirus outbreaks occur relatively rarely and ETEC outbreaks are also quite rare. The last large such outbreak occurred in 2006 and was also caused by imported fresh produce [2].

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# Impact of the 2009 influenza A(H1N1) pandemic wave on the pattern of hibernal respiratory virus epidemics, France, 2009

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**This short report based on clinical surveillance and laboratory data describes the circulation of rhinoviruses, influenza viruses and respiratory syncytial viruses (RSV) in France during the 2009-10 season compared with the previous winter season. The delayed circulation of RSV observed in 2009-10 compared with 2008-09 suggests that the early circulation of the 2009 pandemic influenza A(H1N1) viruses had an impact on the RSV epidemic.**

The emergence and spread of the 2009 pandemic influenza A(H1N1) virus during summer 2009 in northern and central America and in parts of Europe suggested that the epidemic wave would occur in Europe in September, according to weather conditions and social habits (start of the new school year). However, in early September 2009 while cases of clinical influenza-like illness (ILI) were increasing in France, Sweden and other European countries, the detection of 2009 pandemic influenza A(H1N1) remained sporadic.

It was first suggested by Linde *et al*. that rhinovirus epidemics may have interfered with the spread of pandemic influenza A(H1N1) and caused this delay [1]. Recently, we reported that the rhinovirus infections observed from early September to mid-October (Figure, panel B) appeared to reduce the statistical likelihood of pandemic influenza in the paediatric population [2]. These data support the hypothesis that the spread of the 2009 pandemic influenza A(H1N1) in France was delayed due to interaction between respiratory viruses at the beginning of autumn.

## Analysis of respiratory viruses circulating in 2008-9 and 2009-10

Recently, we wondered whether the first epidemic wave of 2009 pandemic influenza could have had an impact on the respiratory syncytial virus (RSV) epidemic [3]. As the winter epidemics 2009-10 have almost ended, a first analysis can be performed. The French Institut de Veille Sanitaire (InVS) manages the national surveillance data provided by two independent monitoring systems. Firstly, the sentinel networks ('réseau sentinelles'), collecting reports of clinical syndromes sent by volunteering general practitioners, and secondly, the Groupes Régionaux d'Observation de la Grippe (GROG), analysing samples collected from patients by a network of volunteering general practitioners.

The first cases of pandemic influenza were detected in France from early May 2009, but the pandemic wave began only in mid-October, in week 42, and peaked in mid-November 2009 in week 49, according to GROG data [4]. Seasonal influenza viruses were isolated sporadically and overall were entirely overshadowed by the pandemic virus: overall, only six influenza A(H3N2) isolates were detected between 1 September 2009 and the end of January 2010 compared with more than 12,800 isolates of 2009 pandemic influenza A(H1N1) virus (GROG unpublished data). At the end of December 2009, in week 52, when the influenza activity started to decline, the number of RSV cases peaked.

This late emergence of RSV is an unusual pattern compared with previous years. In the past four years, the RSV epidemics started in weeks 44-45 and peaked in weeks 48-49, whereas the seasonal influenza epidemics started later (GROG unpublished data). Moreover, InVS reports show that the 2009-10 RSV epidemic started more gradually with a delayed peak in the south of France and with a lower impact compared with the previous winter season [5,6]. The evolution of this pattern is supported by laboratory analyses of samples obtained from the emergency paediatric unit at 'Femme-Mère-Enfant' hospital in Lyon during the two last consecutive seasons (see Figure, panels A and B). The virological diagnosis was based on specific RT-PCR methods for the detection of influenza virus, rhinovirus and RSV as previously described [2].

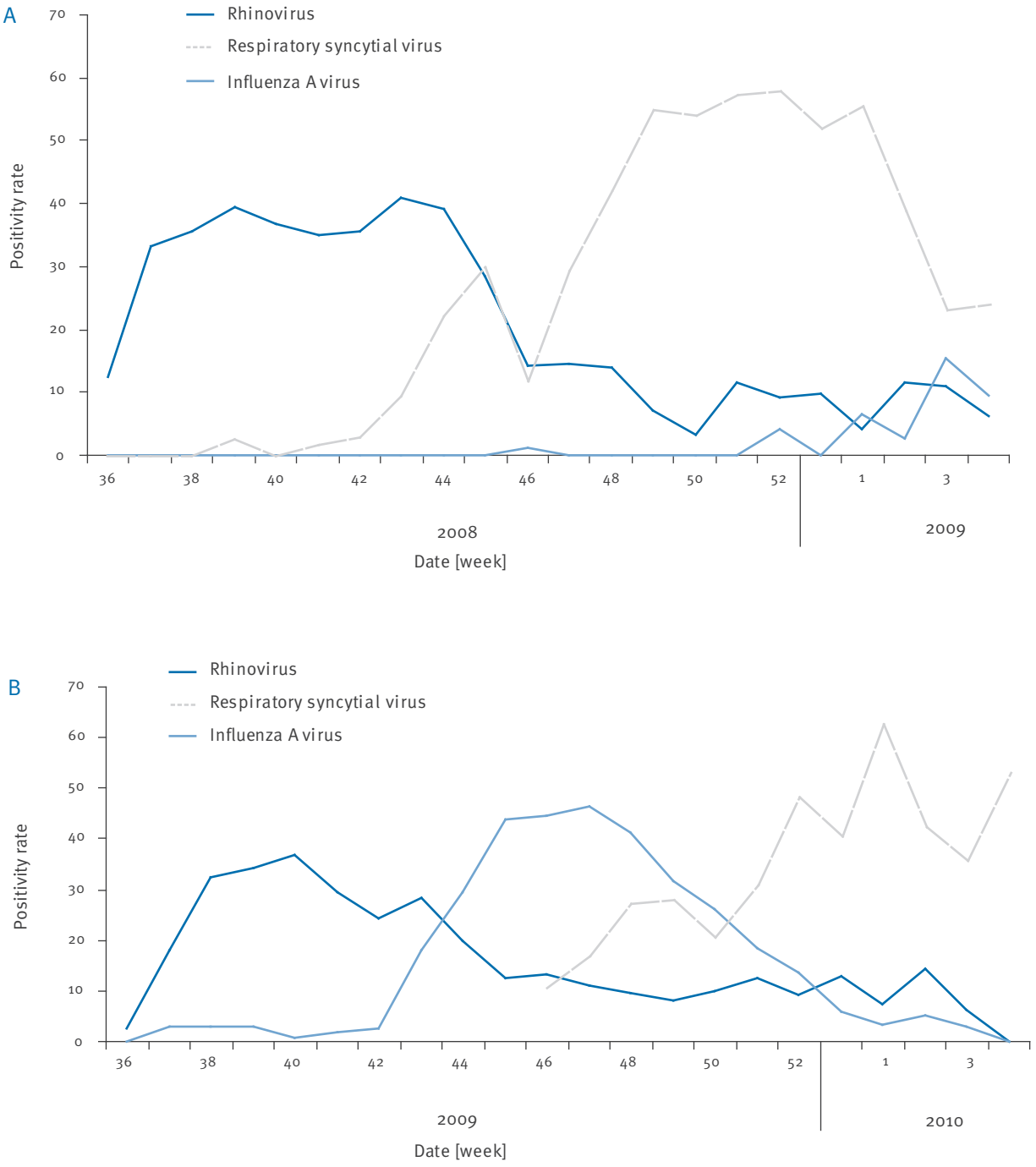
That the pandemic influenza in 2009 spread earlier than seasonal influenza in previous years was not unexpected, given the lack of immunity against the pandemic influenza virus in the majority of population. What is surprising, however, it the observation that the influenza pandemic wave of 2009 seems to have partially overcome the RSV epidemic. Which factors could have had this impact on the RSV epidemic? Several hypotheses can be suggested: weather conditions, increased hygiene measures implemented following the pandemic plan and viral interference.

## Conclusions

For the first time ever, the emergence and the spread of a pandemic wave were monitored using molecular techniques and modern surveillance schemes. This provided real-time information on the impact of the winter respiratory viruses, and was the source of changes in hygiene behaviour as a result of adopting mitigation measures. However, the pattern observed this winter 2009-10, with the almost complete disappearance of seasonal influenza viruses and the delayed and reduced RSV epidemic as opposed to an unchanged rhinovirus epidemic, emphasises how interactions

**Figure**

Positivity rates of laboratory-confirmed cases of rhinovirus, respiratory syncytical virus and influenza A virus during autumn and winter 2008-9 (A) and 2009-10 (B), in samples obtained from the emergency paediatric unit at 'Femme-Mère-Enfant' hospital in Lyon, France



between respiratory viruses can lead to changes in the circulation patterns and impact of different winter respiratory viruses. In addition, the implementation of mitigation measures and the changes in social behaviour in the context of the pandemic may also have played a role in this unusual pattern of hibernal respiratory virus epidemics.

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# Low acceptance of vaccination against the 2009 pandemic influenza A(H1N1) among healthcare workers in Greece

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**A questionnaire survey on the attitude of healthcare workers towards pandemic influenza vaccination showed low acceptance (17%) of the pandemic vaccine. Factors associated with vaccine uptake were acceptance of seasonal influenza vaccination, medical profession and age. The main reason for refusal of vaccination was fear of side effects, which was stronger in those who received information on the safety of the vaccine mainly from mass media.**

## Introduction

As of 31 January 2010, worldwide more than 209 countries and overseas territories or communities have reported laboratory-confirmed cases of 2009 pandemic influenza A(H1N1). In addition, at least 15,174 deaths related to this infection have been recorded [1]. In Europe, the pandemic is well past its peak and medium intensity transmission is now confined to five countries, all in eastern or south-eastern Europe. One of those countries is Greece, where the recorded number of fatal cases caused by 2009 pandemic influenza is currently 118 [2].

According to recommendations from the World Health Organization (WHO) all countries should immunise their healthcare workers as a first priority in order to protect the vital health infrastructure [3].

To our knowledge, information on healthcare workers' intention to take up vaccination against the 2009 pandemic influenza is sparse [4]. Consequently, the aim of our study was to investigate the attitude of healthcare workers towards this vaccine and possible factors associated with vaccine uptake.

## Methods

### Questionnaire

A structured, self-administered, anonymous questionnaire was distributed to a convenience sample of 441 healthcare workers in five public hospitals (one university hospital and four general hospitals) in the region of Thessaly, Greece. In particular, five healthcare workers invited all personnel at work during two consecutive days to participate in the study. The survey was conducted on 9 and 10 November 2009, one week before the official start of national vaccination campaign against the 2009 pandemic influenza A(H1N1) in Greece. The questionnaire included questions on demographics, willingness to accept seasonal influenza vaccination and willingness to accept the 2009 pandemic influenza vaccine. In the case of vaccination refusal the participants were requested to define the reason: lack of time, inertia; perception of not being at risk of serious illness, use of alternative drugs, fear about vaccine safety. In the case of fear about vaccine safety, the participant was requested to specify the concern given the following alternatives: Guillain-Barrés syndrome, systemic anaphylactic reaction, development of influenza illness, local side effects, other. Moreover, the healthcare workers were asked about their level of information on the safety of pandemic influenza A(H1N1) vaccines (no information/insufficient information, sufficient/very good information) and on their sources of information on influenza A(H1N1) vaccine safety: Internet, hospital infection control committee, National Hellenic Centre for Disease Control and Prevention (CDCP), medical journals/books, television and radio stations, newspapers/magazines, representatives of pharmaceutical companies. Finally, participants were asked to express their opinion on the value of vaccinations as an important means for the protection of public health, and in particular of healthcare workers.

### Statistical analysis

The collected answers were entered in a database created within Epi Info 2000 software. Absolute and relative frequencies were presented for qualitative variables, while quantitative variables were presented as mean (standard deviation). Chi-square test or Fischer's exact test was used for the univariate analysis of qualitative variables and Student's t-test or Mann Whitney test for quantitative variables. In order to identify factors

associated with vaccination uptake, logistic regression analysis was performed separately for seasonal and pandemic influenza vaccination. Statistical analysis was performed with Epi-Info software. Relative risk

**Table 1**

Characteristics of healthcare workers and attitudes towards vaccinations against seasonal influenza and the 2009 pandemic influenza A(H1N1), Thessaly, Greece, November 2009 (n=441)

| Characteristic | N/total (%) or mean (SD) |
|---|---|
| Sex | |
| Male | 150/437 (34.3) |
| Female | 287/437 (65.7) |
| Age (mean, SD) | 37.8 (9.97) |
| Educational level | |
| Lyceum | 44/430 (10.2) |
| Professional training | 25/430 (5.8) |
| Technological | 124/430 (28.8) |
| University | 202/430 (47.0) |
| Master/PhD | 35/430 (8.1) |
| Occupation | |
| Doctor | 215/435 (49.4) |
| Nurse | 169/435 (38.9) |
| Paramedic | 28/435 (6.4) |
| Other | 23/435 (5.3) |
| Years of work (mean, SD) | 13.34 (10.02) |
| Vaccinations are an important means of protecting public health, and in particular of healthcare workers: | |
| I agree | 378/390 (96.9) |
| I disagree | 12/390 (3.1) |
| My opinion on vaccination in general is: | |
| I agree | 354/370 (95.7) |
| I disagree | 16/370 (4.3) |
| Are you going to be vaccinated with seasonal influenza vaccine? | |
| Yes | 124/432 (28.7) |
| No | 308/432 (71.3) |
| If no, please specify: | |
| I do not have enough time | 8/308 (2.6) |
| Inertia | 13/308 (4.2) |
| Use of alternative drugs | 4/308 (1.3) |
| I am not at risk of serious illness | 133/308 (43.2) |
| Fear over vaccine safety | 134/308 (43.5) |
| If yes, please specify: | |
| Guillain-Barré Syndrome | 20/134 (14.9) |
| Anaphylactic reaction | 12/134 (9) |
| Influenza illness | 19/134 (14.2) |
| Local side effects | 9/134 (6.7) |
| Other | 3/134 (2.2) |
| Are you going to be vaccinated with the pandemic influenza vaccine? | |
| Yes | 72/424 (17.0) |
| No | 352/424 (83.0) |
| If no, please specify: | |
| I do not have enough time | 7/352 (2) |
| Inertia | 3/352 (0.9) |
| Use of alternative drugs | 5/352 (1.4) |

| | |
|---|---|
| I am not at risk of serious illness | 58/352 (16.5) |
| Fear over vaccine safety | 265/352 (75.3) |
| If yes, please specify: | |
| Guillain-Barré syndrome | 53/265 (20) |
| Anaphylactic reaction | 26/265 (9.8) |
| Influenza illness | 26/265 (9.8) |
| Local side effects | 9/265 (3.4) |
| Other | 24/265 (9.1) |
| My information about pandemic vaccine safety is | |
| No information/insufficient | 252/431 (58.5) |
| Sufficient/very good | 179/431 (41.5) |
| Sources of information | |
| Internet | 178/441 (40.4) |
| Hospital Infections Control Committee | 138/441 (31.3) |
| Hellenic Centre for Disease Control and Prevention | 94/441 (21.3) |
| Medical journals/books | 103/441 (23.4) |
| Pharmaceutical companies | 6/441 (1.4) |
| Television, radio stations | 226/441 (51.2) |
| Newspapers/magazines | 125/441 (28.3) |

N: number; SD: standard deviation.
Some questions were not answered by all participants (missing values).

## Table 2

Univariate analysis of acceptance of vaccination against 2009 pandemic influenza A(H1N1), healthcare workers, Thessaly, Greece, November 2009 (n=441)

| Factor | Acceptance of vaccination against 2009 pandemic influenza A(H1N1) | | |
|---|---|---|---|
| | N/total (%) | RR (95% CI) | P value |
| Age | | | |
| ≤ 38 years (reference value) | 29/131 (12.6) | 1.78(1.15-2.75) | 0.007 |
| › 38 years | 41/183 (22.4) | | |
| Sex | | | |
| Male | 43/147 (29.3) | 2.75 (1.79-4.21) | ‹0.001 |
| Female (reference value) | 29/273 (10.6) | | |
| Educational level | | | |
| Lyceum/professional training | 4/68 (5.9) | 0.30 (0.11-0.80) | 0.006 |
| University/ technological (reference value) | 67/345 (19.4) | | |
| Occupation | | | |
| Medical | 56/210 (26.7) | 6.30 (3.08-12.86) | ‹0.001 |
| Nursing/paramedical (reference value) | 8/189 (4.2) | | |
| Vaccinations are important for the protection of public health | | | |
| Yes | 70/364 (19.2) | 2.31 (0.35-15.25) | 0.34 |
| No (reference value) | 1/12 (8.33) | | |
| My opinion about vaccinations | | | |
| I agree | 69/341 (20.2) | 3.24 (0.48-21.85) | 0.168 |
| I disagree (reference value) | 1/16 (6.25) | | |
| Duration of employment | | | |
| ≤ 13 years (reference value) | 38/166 (22.9) | 1.69 (1.08-2.63) | 0.018 |
| › 13 years | 28/207 (13.5) | | |
| Acceptance of seasonal influenza vaccination | | | |
| Yes | 52/122 (42.6) | 6.68 (4.13-10.8) | ‹0.001 |
| No (reference value) | 19/298 (6.4) | | |

CI: confidence interval; N: number; RR: relative risk.

(RR), adjusted odds ratio (OR) and 95% confidence intervals (95% CI) were also calculated. The level of statistical significance was set at 0.05.

## Results

The demographic characteristics of the respondents are shown in Table 1. In total 441 questionnaires were returned. The number of missing values varied from question to question.

The overall acceptance of pandemic and seasonal influenza vaccines was 17% (95% CI: 13.6-21%) and 28.7% (95% CI: 24.5-33.3%), respectively. Moreover, 378 of 390 respondents (97%) stated that vaccinations are important for the protection of public health, and in particular of healthcare workers. The most common reason of refusing the pandemic influenza vaccine was fear about vaccine safety (75.3%), most frequently fear of the Guillain-Barrés syndrome. About 58.5% of the participants said that their information about pandemic influenza vaccine safety was insufficient (Table 1).

**Table 3**

Multivariate analysis of acceptance of vaccination against 2009 pandemic influenza A(H1N1), healthcare workers, Thessaly, Greece, November 2009 (n=441)

| Factor | Acceptance of vaccination against 2009 pandemic influenza A(H1N1) | |
|---|---|---|
| | OR (95% CI) | P value |
| Age group | | |
| >38 years | 2.28 (1.16-4.48) | 0.01 |
| ≤38 years (reference value) | 1.00 | |
| Sex | 0.78 (0.37-1.63) | 0.51 |
| Educational level | | |
| Lyceum/professional training (reference value) | 1.00 | 0.83 |
| University/technological | 1.19 (0.22-6.31) | |
| Occupation | | |
| Medical | 6.34 (2.31-17.4) | <0.001 |
| Nursing/paramedical (reference value) | 1.00 | |
| Acceptance of seasonal influenza vaccination | | |
| Yes | 10.2 (5.1-20.4) | <0.001 |
| No (reference value) | 1.00 | |

CI: confidence interval; OR: odds ratio.

**Table 4**

Source of information and fear over 2009 pandemic influenza A(H1N1) vaccine safety, healthcare workers, Thessaly, Greece, November 2009 (n=441)

| | N (%) | RR (95% CI) | P value |
|---|---|---|---|
| My information about the safety of vaccines against pandemic influenza A(H1N1) is | | | |
| Sufficient/very good | 95/179 (53.1) | 0.75 (0.64-0.89) | <0.001 |
| No information/insufficient information | 176/252 (69.8) | | |
| **Source of information** | | | |
| Internet | | | |
| Yes | 100/178 (56.2) | 0.83 (0.71-0.97) | 0.017 |
| No | 177/263 (67.3) | | |
| Hospital Infection Control Committee | | | |
| Yes | 93/138 (67.4) | 1.10 (0.95-1.28) | 0.179 |
| No | 184/303 (60.7) | | |

| | | | |
|---|---|---|---|
| Hellenic Centre for Disease Control and Prevention | | | |
| Yes | 43/94 (45.7) | 0.67 (0.53-0.85) | <0.001 |
| No | 234/347 (67.4) | | |
| Medical journals/books | | | |
| Yes | 56/103 (54.4) | 0.83 (0.68-1.00) | 0.042 |
| No | 221/338 (65.4) | | |
| Pharmaceutical companies | | | |
| Yes | 3/6 (50.0) | 0.79 (0.35-1.77) | 0.513 |
| No | 274/435 (63.0) | | |
| Television/radio stations | | | |
| Yes | 157/226 (69.5) | 1.24 (1.07-1.44) | 0.003 |
| No | 120/215 (55.8) | | |
| Newspapers/magazines | | | |
| Yes | 86/125 (68.8) | 1.13 (0.98-1.31) | 0.101 |
| No | 191/316 (60.4) | | |

CI: confidence interval; N: number; RR: relative risk.

## Table 5

Acceptance of vaccination against 2009 pandemic influenza A(H1N1) and source of information, healthcare workers, Thessaly, Greece, November 2009 (n=441)

| Factor | Acceptance of vaccination against 2009 pandemic influenza A(H1N1) | | |
|---|---|---|---|
| **Source of information** | **N (%)** | **RR (95% CI)** | **P value** |
| Internet | | | |
| Yes | 44/174 (25.3) | 2.25 (1.46-3.47) | <0.001 |
| No | 28/222 (255) | | |
| Hospital Infection Control Committee | | | |
| Yes | 25/130 (19.2) | 1.20 (0.77-1.86) | 0.411 |
| No | 47/294 (16.0) | | |
| Hellenic Centre for Disease Control and Prevention | | | |
| Yes | 30/92 (32.6) | 2.57 (1.71-3.87) | <0.001 |
| No | 42/332 (12.7) | | |
| Medical journals/books | | | |
| Yes | 29/99 (29.3) | 2.21 (1.46-3.34) | <0.001 |
| No | 43/325 (13.2) | | |
| Pharmaceutical industry | | | |
| Yes | 1/6 (16.7) | 0.98 (0.16-5.94) | 0.98 |
| No | 71/418 (17.0) | | |
| Television/radio stations | | | |
| Yes | 26/218 (11.9) | 0.53 (0.34-0.83) | 0.004 |
| No | 46/206 (22.3) | | |
| Newspapers/magazines | | | |
| Yes | 16/122 (13.1) | 0.70 (0.42-1.18) | 0.1777 |
| No | 56/302 (18.5) | | |

CI: confidence interval; N: number; RR: relative risk.

The most frequent source of information on vaccine safety was television and radio stations (51.2%) followed by the internet (40.4%), hospital infectious control committee (31.3%), and newspapers/magazines (28.3%). Univariate analysis showed that sex, age, educational level, occupation, duration of employment, and acceptance of seasonal influenza vaccination were significantly associated with acceptance of the pandemic influenza vaccine (Table 2).

Healthcare workers who had a positive attitude towards seasonal influenza vaccination had a higher rate of acceptance of the pandemic influenza vaccine than colleagues who refused the seasonal influenza vaccine (RR: 6.3; 95%CI: 3.08-12.86). Multivariate analysis revealed that occupation (OR: 6.34; 95% CI: 2.31-17.4), acceptance of seasonal influenza vaccination (OR: 10.2; 95% CI: 5.1-20.4) and age (OR: 2.28; 95% CI: 1.16-4.48) were independently associated with the acceptance of pandemic influenza vaccination (Table 3).

In order to explore the impact of information on the fear of side-effects, univariate analysis was performed (Table 4). It documented that healthcare workers with sufficient/very good information about safety of the pandemic influenza vaccine had a lower risk of reporting fear over vaccine safety than colleagues with insufficient information (RR: 0.75; 95% CI: 0.64-0.89). Further analysis revealed an impact of the information source on the reporting of fear of side effects. In particular, healthcare workers who had received information about pandemic influenza vaccine safety from television and radio stations demonstrated an increased risk of reporting negative attitude towards the vaccination due to fear of side effects (RR: 1.24; 95% CI: 1.07-1.44), while healthcare workers who received information on the vaccine's safety from medical journals, the internet, hospital infection control committees, and the CDCP had a significantly decreased risk of reporting fear over vaccine safety (Table 4).

The impact of the source of information on acceptance of the pandemic influenza vaccine is presented in Table 5. Interestingly, participants who received information on vaccine safety from the CDCP, medical journals and the internet documented a higher probability for vaccination acceptance.

Multivariate analysis (results not shown) revealed an independent association of source of information on vaccine safety with acceptance of pandemic influenza vaccination. In particular, information sources like the CDCP, and medical journals were independently associated with the probability of accepting pandemic influenza vaccination (OR: 2.36; 95% CI:1.32-4.12 for CCPD; OR:2.13; 95% CI:1.20-3.80 for medical journals). In contrast, information on vaccine safety related to mass media and particularly to television and radio stations was independently associated with a decreased probability for accepting the vaccination (OR: 0.53; 95% CI:0.31-0.93).

Regarding seasonal influenza vaccination, our study revealed an acceptance rate of 28.7%. Multivariate analysis indicated that only age was independently associated with the likelihood of accepting seasonal influenza vaccination (OR: 1.62; 95% CI: 1.02-2.56) (Table 6).

## Discussion

Our study revealed a low acceptance (17%) of vaccination against the 2009 pandemic influenza among Greek healthcare workers. There is some evidence that the willingness of European healthcare workers to be vaccinated with seasonal influenza vaccine is poor, ranging from 14% in the United Kingdom to 48% in France [5].

**Table 6**

Multivariate analysis of acceptance of seasonal influenza vaccination, healthcare workers, Thessaly, Greece, November 2009 (n=441)

| Factor | Vaccination acceptance | |
|---|---|---|
| | OR (95% CI) | P value |
| **Age group** | | |
| ›38 years | 1.62 (1.02-2.56) | 0.037 |
| ≤38 years (reference value) | | |
| **Sex** | 0.65 (0.38-1.09) | 0.106 |
| **Educational level** | | |
| Lyceum/ professional training (reference value) | 1.36 (0.62-2.95) | 0.430 |
| University/technological | | |
| **Occupation** | | |
| Nursing/paramedical (reference value) | 1.59 (0.90-2.82) | 0.107 |
| Medical | | |

CI: confidence interval; OR: odds ratio

Strong independent positive determinants for accepting the pandemic influenza vaccine were acceptance of seasonal influenza vaccination and medical profession. These findings are line with a previous study conducted in Hong-Kong [4]. The main reason for the low acceptance of the vaccine - apart from the perception that the 2009 pandemic influenza A(H1N1) is not a serious illness - was the fear of adverse effects and in particular Guillaine-Barrés syndrome. Nevertheless, it is of interest that 48.3% of the participants did not specify which side effect they feared. Fear of vaccine-related side effects was dependent on the source of information on vaccine safety and especially pronounced in those receiving information from television and radio stations, reflecting the fact that mass media play a disproportionate role in the information sources on the safety of pandemic influenza vaccines.

Multivariate analysis identified a positive attitude towards seasonal influenza vaccination as the strongest determinant for accepting the pandemic influenza vaccine. Similar observations have been made in other studies on influenza A(H5N1) [6] and pandemic influenza A(H1N1) vaccines [4]. Compared with nurses and paramedics, medical doctors had a sixfold higher rate of acceptance the pandemic vaccine, although even this rate of 27% was suboptimal. These findings are in line with a study conducted in Hong Kong and highlight the necessity to target nurses and paramedics with information to change their attitude towards this vaccination [4].

Acceptance of the pandemic vaccine also increased with age. This is in part explained by the fact that it was shown to be independently associated with the acceptance of seasonal influenza vaccination, which increases with age. The uptake of the seasonal influenza vaccination in our study was 28.7%, considerably higher than that of the pandemic vaccine, but not satisfactory. Previous studies have also recorded low coverage with seasonal influenza and hepatitis B vaccination in healthcare workers in Greece [7,8].

Our study has the limitation of being a cross-sectional questionnaire study, and some information bias could have occurred. We believe that the acceptance rate the pandemic influenza vaccine found in our study could be overestimated given that healthcare workers who were not interested in the vaccination may not have been motivated to participate in the survey. On the other hand, healthcare workers who believe that influenza vaccination is an obvious solution may also have been less inclined to participate than persons who are concerned over vaccine safety. An additional limitation is the sampling method (convenience sample). However, we believe that the figures reported here are a satisfactory reflection of the intentions of Greek healthcare workers regarding pandemic influenza vaccination, given that our sample included staff from both university and general hospitals and that Thessaly is a large region in Greece, with almost 8% of the country's population. At least one hospital from each of the four prefectures of the region was included in the study, the sample could therefore be considered as geographically representative. Furthermore, unpublished data from a general hospital in Athens indicated acceptance rates similar to those provided by our study.

## Conclusion

The low acceptance rate of the pandemic vaccine among Greek healthcare workers is alarming given that they are used as an example for their patients and the public [9]. Vaccination is important in order to keep the healthcare system operating at maximum capacity during a pandemic [10]. Policy makers in Greece, and maybe in other countries in Europe could consider our findings in order to improve the vaccination strategy for healthcare workers in future vaccination campaigns.


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# Household transmissibility and other characteristics of seasonal oseltamivir-resistant influenza A(H1N1) viruses, Germany, 2007-8

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**During the influenza season 2007-8, the proportion of seasonal influenza A(H1N1) viruses resistant to the neuraminidase inhibitor oseltamivir increased worldwide. We conducted an investigation to compare patients infected with oseltamivir-resistant (ose-R) and oseltamivir- susceptible (ose-S) influenza A(H1N1) viruses regarding risk factors for resistance and the capability to transmit in the household setting. Within a cohort of 396 laboratory confirmed influenza patients from sentinel physicians we conducted a nested case-control study among patients infected with A(H1N1). Thirty patients in the cohort were infected with influenza B, none with influenza A(H3N2) and 366 with A(H1N1). Of the 366 A(H1N1) viruses 52 (14%) were ose-R. Demographic characteristics, oseltamivir exposure, travel history and outcome were not significantly different between ose-S and ose-R patients. Among 133 households in the nested case-control study, secondary household attack rates in households with ose-R cases and households with ose-S cases were similar (23 versus 26%; p-value=0.54). Ose-R household status and occurrence of secondary cases were associated with an odds ratio of 0.85 (95% confidence interval 0.38-1.88). We conclude that seasonal ose-R influenza A(H1N1) viruses have transmitted well in the household setting.**

## Introduction

The neuraminidase inhibitors zanamivir and oseltamivir became available for the treatment and prophylaxis of influenza in 1999. Before the beginning of the influenza season 2007-8 in the northern hemisphere monitoring systems had identified resistance to oseltamivir in influenza viruses in less than 1%, and resistance to zanamivir had been detected even less often [1,2]. Higher rates of resistance to oseltamivir were only reported in children in Japan (16%, 18%), where weight-based dosage is lower than approved in Europe and may have led to increased resistance rates [3,4]. Studies in ferrets showed that resistant viruses were in general less virulent and less transmissible in comparison to susceptible viruses [5-8].

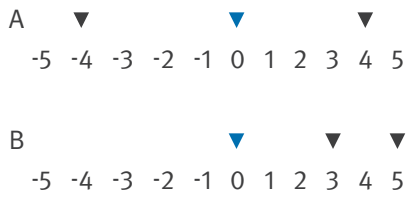
In November 2007, Norway reported an unusually high proportion of seasonal influenza A(H1N1) viruses resistant to the neuraminidase inhibitor oseltamivir (ose-R). Soon after, other countries in Europe and the US also detected ose-R viruses [9]. Sequence analysis of viruses identified a substitution of tyrosine instead of histidine at residue 274 (H274Y in the N2 numbering) which conferred reduced drug sensitivity (IC50) of the viral enzyme neuraminidase. Susceptibility to zanamivir was maintained. In Europe, the weighted average proportion of ose-R among influenza A(H1N1) viruses increased over time from near zero in week 40 (2007) to 56% in week 19 (2008) [9]. When the season 2007-8 had subsided, 22 (73%) of 30 countries who had tested for oseltamivir resistance, had detected ose-R in A(H1N1) viruses (median among countries: 10%; range: 0 - 67%) [10]. In addition, countries of the southern hemisphere reported the occurrence of ose-R influenza A(H1N1) viruses during their 2008 influenza season. In some of them the proportion of resistant viruses exceeded that found in European countries, for example in South Africa (100%; 225 of 225) [11], and Australia (80%; 47/59) [12]. During the influenza season 2008-9 close to 100% resistance was reported from European countries [13].

In March 2008, the European Centre for Disease Prevention and Control (ECDC) called a meeting with several European countries, to discuss the most salient questions around the new phenomenon. Following this, we launched an investigation (i) to compare the clinical characteristics and outcome of patients infected with ose-R and ose-S influenza A(H1N1) viruses, (ii) to investigate if – prior to the sample having been taken – patients with ose-R A(H1N1) viruses had been treated with oseltamivir more frequently than patients with ose-S A(H1N1) virus infections, (iii) to investigate if the

occurrence of ose-R A(H1N1) virus infections was associated with exposure to an influenza-infected person in the household who was treated with oseltamivir, (iv) to examine if patients infected with ose-R A(H1N1) viruses were more likely to have had travelled abroad prior to infection more frequently compared with patients with ose-S A(H1N1) viruses, and (v) to explore the transmissibility of ose-R A(H1N1) viruses in comparison to ose-S A/H1N1 viruses in the household setting.

**Figure 1**

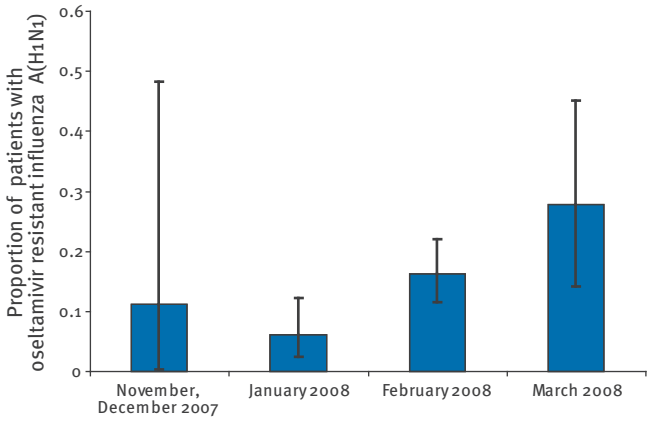
Transmission of seasonal influenza A(H1N1) viruses in two exemplary households (A and B), study on transmission of oseltamivir-resistant seasonal influenza A(H1N1) viruses in household settings, Germany 2008




The arrow heads indicate the onset of influenza-like illnes of household contacts (black arrows) and of the sentinel cases (laboratory confirmed; blue arrows). Day 0: onset of illness of sentinel cases.

**Figure 2**

Proportion of seasonal influenza A(H1N1) viruses resistant to oseltamivir among patient samples taken by sentinel physicians, Germany, influenza season 2007-8




# Methods

## Cohort study

We used data from 396 laboratory confirmed influenza patients for whom samples (nose and/or throat swabs) were sent for laboratory investigation to the National Reference Centre for Influenza (NRCI) at the Robert Koch Institute (RKI, German national public health institute), Berlin by sentinel physicians cooperating with the German network for influenza surveillance (Arbeitsgemeinschaft Influenza; www.influenza.rki.de/agi). These patients are referred to as "sentinel cases". Together with the samples, information is collected routinely on the date of illness onset, age, sex, location of the treating physician, presenting symptoms, influenza vaccination status, and willingness to be contacted by the RKI and telephone number in case of a positive reply.

## Nested case-control study

To obtain information on pre-existing medical conditions, travel history, intake of oseltamivir prior to taking the sample, exposure to oseltamivir through a household contact, complications or outcome (otitis, pneumonia, hospitalisation, death, duration of sick leave and number of days confined to bed), household size and the occurrence of influenza-like illness (ILI) in the household on the same day or five days before or after onset of illness in the sentinel case, we attempted to contact (i) all sentinel cases with an ose-R A(H1N1) virus infection, and (ii) a subset of sentinel cases infected with ose-S A(H1N1) viruses. This subset consisted of patients who had previously agreed to be contacted and had provided their telephone number. Interviewers conducted a questionnaire with the respective patients or, in the case of minors, their guardians. Interviewees were blinded to the susceptibility status of the virus of the sentinel case. Households were contacted between one and five months after occurrence of the laboratory confirmed household case.

Data were entered into a Microsoft Access database (Microsoft Corp., Redmond, WA, USA). Analysis was performed using STATA version 10.1 (STATA Corp., College Station, TX, USA). For categorical variables we calculated univariate odds ratios and p-values using Fisher's exact test. Numerical variables were analysed using a ranksum test.

**Table 1**

Patients with oseltamivir-susceptible and oseltamivir-resistant seasonal influenza A(H1N1) viruses by age group, cohort of patients attended by sentinel physicians (sentinel cases), Germany, influenza season 2007-8 (n=358)

| | Number of patients infected with oseltamivir-susceptible influenza A(H1N1) viruses (%) | Number of patients infected with oseltamivir-resistant influenza A(H1N1) viruses (%) | Total |
|---|---|---|---|
| 0-4 years | 52 (88%) | 7 (12%) | 59 |
| 5-14 years | 133 (87%) | 19 (13%) | 152 |
| 15-34 years | 65 (83%) | 13 (17%) | 78 |
| 35 years and older | 57 (83%) | 12 (17%) | 69 |
| **Total** | **307 (86%)** | **51 (14%)** | **358** |

For the analysis of the likelihood to transmit the virus within the household we conducted a multilevel analysis with levels person and household. In this context we defined the following terms:

*Household transmission period (HTP):* period from five days before until five days after the illness onset in sentinel cases, in total 11 days.

*Household transmission:* occurrence of at least one secondary case within the HTP after a primary case of ILI or laboratory-confirmed influenza (Figure 1).

*Influenza-like illness (ILI) in a household contact:* illness in a household contact of the sentinel case during the HTP with (i) subjective feeling of having fever; and/or (ii) cough and (myalgia or headache).

**Table 2**

Age, sex, vaccination status and symptoms of patients with seasonal influenza A(H1N1) viruses by sensitivity to oseltamivir, cohort of patients attended by sentinel physicians (sentinel cases), Germany, influenza season 2007-8 (n=343)

| | Variable present | | Variable not present | | Risk ratio | 95%CI | Fisher exact test; p-value |
|---|---|---|---|---|---|---|---|
| | Number of cases | Number of ose-R (%) | Number of cases | Number of ose-R (%) | | | |
| Age (>14 years) | 147 | 25 (17%) | 211 | 26 (12%) | 1.38 | [0.83–2.29] | 0.22 |
| Male sex | 192 | 32 (17%) | 171 | 20 (12%) | 1.43 | [0.85–2.40] | 0.23 |
| Vaccination | 17 | 2 (12%) | 340 | 49 (14%) | 0.82 | [0.22–3.08] | 1.00 |
| Symptoms | | | | | | | |
| Acute onset | 352 | 50 (14%) | 6 | 1 (17%) | 0.85 | [0.14–5.19] | 1.00 |
| Cough | 336 | 49 (15%) | 19 | 1 (5%) | 2.77 | [0.40–19.00] | 0.49 |
| Fever | 353 | 51 (14%) | 9 | 1 (11%) | 1.3 | [0.20–8.40] | 1.00 |
| Muscle, limb, body pain | 330 | 49 (15%) | 13 | 2 (15%) | 0.97 | [0.26–3.54] | 1.00 |

CI: confidence interval; ose-R: oseltamivir resistant seasonal influenza A(H1N1) viruses; ose-S: oseltamivir-susceptible seasonal influenza A(H1N1) viruses.

**Table 3**

Associations of pre-existing medical conditions, risk factors and complications or outcome variables with oseltamivir susceptibility status of seasonal influenza A(H1N1) cases, nested case-control study, Germany, influenza season 2007-8

| Exposure | Patients with ose-R virus infection | | | | Patients with ose-S virus infection | | | | OR | 95% CI | Fisher exact test; p-value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Number of cases | Variable present | Variable present % | Median (IQR) | Number of cases | Variable present | Variable present % | Median (IQR) | | | |
| Pre-existing medical conditions | | | | | | | | | | | |
| Diabetes | 38 | 1 | 3% | | 95 | 0 | 0% | | - | [0.00–∞] | 0.29 |
| Chronic heart disease | 38 | 0 | 0% | | 95 | 0 | 0% | | - | - | - |
| Chronic lung disease | 38 | 3 | 8% | | 95 | 3 | 3% | | 2.63 | [0.33–20.40] | 0.35 |
| Chronic immuno-suppression | 38 | 0 | 0% | | 95 | 0 | 0% | | - | - | - |
| Risk factors | | | | | | | | | | | |
| Travel history | 38 | 1 | 3% | | 95 | 2 | 2% | | 1.26 | [0.02–24.78] | 1.00 |
| Oseltamivir treatment or prophylaxis before sample was taken | 37 | 0 | 0% | | 93 | 1 | 0% | | 0 | [0.00–∞] | 1.00 |
| Exposure to oseltamivir through household contact | 37 | 0 | 0% | | 95 | 1 | 0% | | 0 | [0.00–∞] | 1.00 |
| Complications or outcome variables | | | | | | | | | | | |
| Otitis | 38 | 0 | 0% | | 95 | 4 | 4% | | 0 | [0.00–2.38] | 0.58 |
| Pneumonia | 38 | 2 | 5% | | 94 | 0 | 0% | | - | [1.32–∞] | 0.08 |
| Hospitalisation | 38 | 0 | 0% | | 95 | 0 | 0% | | - | - | - |
| Death | 38 | 0 | 0% | | 95 | 0 | 0% | | - | - | - |
| Duration of sick leave in days [a] | 11 | | | 7 (6–14) | 26 | | | 7 (7–10) | | | 0.74 |
| Number of days confined to bed [a] | 38 | | | 3.5 (2–7) | 93 | | | 3 (2–5) | | | 0.12 |

CI: confidence interval; IQR: interquartile range; OR: odds ratio; ose-R: oseltamivir resistant seasonal influenza A(H1N1) viruses; ose-S: oseltamivir susceptible seasonal influenza A(H1N1) viruses.

[a] For continuous variables a ranksum test was used.

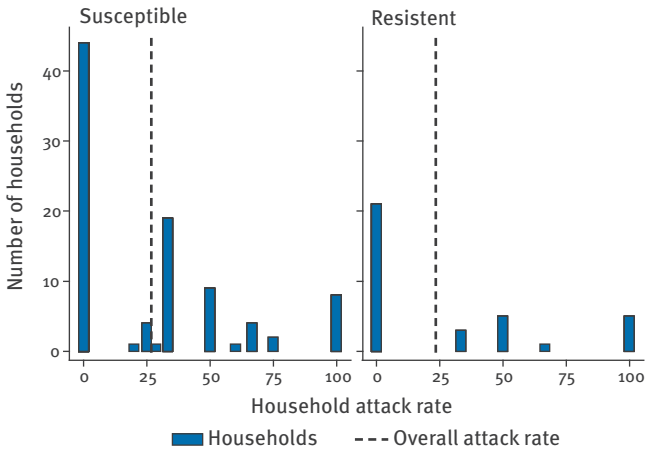
*Primary case:* first case in the household during the HTP with either ILI or laboratory-confirmed influenza. *Secondary case:* occurrence of at least one other case following the primary case during the HTP.

Different from the sentinel cases, additional household cases were identified through interviews only and were not laboratory tested for influenza. We assumed that (i) within the HTP resistance status did not change, i.e. we applied the resistance status of the sentinel case also to other household contacts if they became cases, and that (ii) within the HTP secondary cases occurred only from infection within the household and not from the community.

On household level we used as explanatory variables household size and age and sex of the primary case. Moreover, treatment of this patient with oseltamivir, whether the influenza virus causing infection was ose-R, and date of illness onset were used as additional explanatory variables.

**Figure 3**

Frequency distribution of secondary household attack rates by oseltamivir susceptibility status of the households, nested case-control study, Germany, influenza season 2007-8 (n=128 households)




## Laboratory testing

Susceptibility testing to oseltamivir was conducted at the NRCI using either a genotypic test for the H274Y-mutation, and/or the phenotypic neuraminidase susceptibility analysis, as described previously [14].

# Results

## Cohort study

Of the 396 patients with laboratory confirmed influenza infection, 366 (92%) were infected with seasonal influenza A(H1N1), none with A(H3N2) and 30 (8%) with influenza B. None of the influenza B viruses were ose-R. Further analysis was restricted to 366 sentinel cases with influenza A(H1N1). Of these, age was known for 358 (98%) patients, ranging from one to 78 years and patients were categorised in the following age groups: 0–4 years (n= 59; 17%), 5–14 years (n= 152; 42%), 15–34 years (n=78; 22%), 35 years or older (n= 69; 19%). Sex was known for 363 (99%) patients, 192 (47%) were male, 171 (53%) female. Onset of symptoms was between 6 December 2007 and 19 March 2008; 87% of cases occurred in January and February. Patient samples came from all 16 German states except one (Mecklenburg-Western Pomerania).

Overall, 52 (14%) patients were infected with an ose-R virus. The proportion of patients with ose-R virus infections rose over time (p-value = 0.02) and reached 28% in March 2008 (Figure 2).

There were no significant differences in age or sex between patients with and without ose-R virus infections, even though the proportion of patients infected with ose-R viruses increased slightly with age (Table 1). Also regarding symptoms and vaccination status there was no statistically significant difference (Table 2).

**Table 4**

Univariate and multivariate analysis of explanatory variables for secondary household cases with influenza-like illness[a] (multilevel model, see text), nested case-control study, Germany, influenza season 2007-8

| | Univariate | | | Multivariate | | |
|---|---|---|---|---|---|---|
| | OR | 95% CI | p-value | OR | 95% CI | p-value |
| Oseltamivir resistance of primary case | 0.85 | 0.38–1.88 | 0.69 | 0.72 | 0.31–1.69 | 0.45 |
| Treatment of primary case with oseltamivir | 0.68 | 0.31–1.51 | 0.34 | 0.62 | 0.28–1.40 | 0.25 |
| Male sex of primary case | 0.83 | 0.42–1.63 | 0.59 | 1.12 | 0.56–2.25 | 0.75 |
| Date of symptom onset of the primary case | 0.99 | 0.97–1.01 | 0.33 | 0.99 | 0.97–1.01 | 0.36 |
| Household with two persons | 2.43 | 0.76–7.79 | 0.13 | 3.94 | 1.05–14.82 | 0.04 |
| Age group of primary case | | | | | | |
| 0-4 years | 0.63 | 0.19–2.04 | 0.44 | 0.56 | 0.16–1.99 | 0.37 |
| 5-14 years | 0.67 | 0.26–1.74 | 0.41 | 0.85 | 0.32–2.28 | 0.75 |
| 15-34 years | 0.38 | 0.12–1.27 | 0.12 | 0.53 | 0.16–1.74 | 0.29 |
| 35 years and older | 1 | (Reference) | - | 1 | (Reference) | - |

CI: confidence interval; OR: odds ratio.

[a] All cases in the period of five days before until five days after onset of disease of sentinel case, in total period of 11 days.

## Nested case-control study

Of 52 ose-R patients, 38 (73%) could be contacted for a telephone interview either because they had agreed to be contacted (n=22) or were asked by their physician to get in contact with us (n=16). Of 105 ose-S patients who were willing to be contacted by telephone we reached 95 (90%). No statistically significant difference regarding age or sex was found between patients who had agreed to be contacted by our institute and those who did not. Therefore, we included 133 patients (38 ose-R, 95 ose-S) in the calculations for the nested case-control study.

Odds ratios and p-values for pre-existing medical conditions, travel history, exposure to oseltamivir (patient him/herself or through household contact), complications and outcome are displayed in Table 3. Overall, 39 (34%) of 114 were treated with oseltamivir. No patient with ose-R influenza and only one patient with ose-S influenza had taken oseltamivir before the respiratory sample was taken. Similarly, no patient with an ose-R infection and one patient with an ose-S infection was exposed to a household contact who had taken oseltamivir. Two cases of pneumonia occurred among patients with ose-R influenza, none among infected with ose-S viruses. The number of days that patients were bedridden was higher in patients with an ose-R virus infection (mean 4.6 days versus 3.4 days; p-value = 0.12). When this variable was stratified by age and sex there was no difference among the groups except for males less than five years old, where the median for patients with ose-R viruses was five (interquartile range, IQR: 1.5–7; n=4) and the median for patients with ose-S viruses 0.5 days (IQR: 0–2; n=6).

## Household transmission

Information on household size was available for 132 (99%) of 133 households. The median number of persons per household was four (IQR: 3–4; n=132). The number of household members in ose-S and ose-R households did not differ significantly (p-value = 0.2). Overall, the secondary attack rate in households was 25.4% (89/350); in households with ose-S patients (ose-S household) it was 26.2% (71/271) compared with 22.8% (18/79) in ose-R households (p-value = 0.54) (Figure 3). In univariate analysis ose-R of the household was not significantly associated with household transmission (Table 4), neither were date of infection, treatment of the primary case with oseltamivir, living in a two-person household or male sex of the primary case. Furthermore, there was also no trend (in terms of increasing or decreasing odds ratios) associated with increasing age of the primary case. In multivariate analysis none of the above variables except living in a two-person household were significantly associated with increased household transmission. The variance of the random effect for household was estimated as 0.86 (0.27–2.78), and this model was significantly better fitting the data than a usual logistic regression model that does not account for the household structure (p-value < 0.01).

## Discussion

Our study took place during the influenza season 2007-8 and focuses on the seasonal influenza A(H1N1) virus circulating at the time, i.e. before the appearance of the 2009 pandemic influenza A(H1N1) virus. Nevertheless, the conclusions that can be drawn from our study are of importance also in the context of the 2009 pandemic influenza. Using household based data we demonstrate formally and convincingly that the ose-R seasonal influenza A(H1N1) virus of that time was capable of being transmitted to the same extent as the ose-S virus. The 2009 pandemic virus is also a subtype A(H1N1) virus. It is therefore possible at any time that the pandemic virus may become resistant to oseltamivir while maintaining transmissibility and pathogenicity similar to the seasonal A(H1N1) virus in the influenza seasons 2007-8 and 2008-9.

The occurrence of secondary cases in households of patients with an ose-R infection in our study can be interpreted as evidence of transmission of ose-R virus in the household setting. In addition, as secondary household attack rates and the odds for a secondary case were similar in ose-R and ose-S households there is evidence that ose-R and ose-S viruses do not differ considerably in their capacity to transmit within the household setting. Analysis of demographic characteristics and other factors related to the primary case or household showed that treatment of the primary case with oseltamivir did not inhibit transmission significantly. As the proportion of ose-R viruses increased over time, it was interesting to know if date of infection was associated with an increased transmission probability. However, we did not find any time trend in this regard. Although the power of our study was too low to show any difference (if it exists), the point estimates and p-values of investigated factors did not indicate that they are of relevance. Nevertheless, if transmission is as likely for ose-R viruses as it is for ose-S viruses it remains unclear why the proportion of ose-R A(H1N1) viruses has increased over the course of the 2007-8 season not only in Germany, but also in other countries in Europe [15]. As we have measured transmission in households only it is possible that differences in transmission in the community account for the increasing dominance of resistant viruses.

Although van der Vries *et al.* have described a fatal case of ose-R A(H1N1) infection in a man with chronic lymphocytic leukemia [16], systematic comparisons of the outcome of ose-S and ose-R infections in European countries and the United States (US) have not suggested a difference in clinical outcome [17-19]. Similarly we also found no different pathogenicity of ose-R viruses compared with ose-S viruses, when measured by complications (otitis, pneumonia) or outcome.

Oseltamivir resistance was also not associated with exposure to oseltamivir, neither through treatment or prophylaxis before sampling of the sentinel case nor through exposure from any of his/her household

contacts. Thus, our data do not indicate that drug pressure has led to the emergence of ose-R viruses in individual patients. This finding is consistent with patient-level data from the US [18] and ecological state or nation-level data from the US and Europe, respectively, where increased ose-R rates in influenza A(H1N1) viruses were not associated with increased levels of prescriptions of oseltamivir [18,20].

Lastly, in our data there was no sign that travel history was associated with the emergence of oseltamivir resistance. As most sequenced European ose-R A(H1N1) viruses are closely related and belong to a separate group, distinct from that of ose-S viruses, it is likely that they originate from a single variant [9] which at some point before the start of the season had been imported, from an unknown location, and was transmitted in the community afterwards. We would therefore expect to find no association with foreign travel.

Our study has some limitations. Additional household cases were not laboratory confirmed but were identified through a symptom-based unspecific case definition only. This may have led to over- or underestimation of the true number of additional household infections. However, it is unlikely that information bias has occurred because this limitation applies equally to ose-S and ose-R households. The time interval between disease of the sentinel case and interview was variable and sometimes long. This may have reduced the ability of interviewees to remember details asked in the questionnaire. Again, this did not happen differentially in one or another group and should have therefore not resulted in distorted effect measures.

In conclusion, analysis of our data from the influenza season 2007-8 suggests that there is no indication of an association of oseltamivir exposure and/or use and the occurrence of ose-R seasonal influenza A(H1N1) viruses. Ose-R viruses seem to be as pathogenic as ose-S viruses. We have found evidence of and have quantified transmission of ose-R A(H1N1) viruses in the household setting and its degree is comparable to that in ose-S viruses. This information is important to understand the epidemiology of ose-R viruses, but more work needs to be done to fully comprehend the reasons for the increase of the prevalence of ose-R among A(H1N1) viruses over the two influenza seasons 2007-8 and 2008-9.

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# Microbiological and molecular investigation of an increase of human listeriosis in Belgium, 2006-2007

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**In Belgium, the majority of cases of listeriosis are sporadic cases. In this study we present evidence for an episode of listeriosis: a time-linked cluster of cases that occurred in 2006 and 2007, and the identification of identical strains. The episode involved 11 patients, infected with *Listeria monocytogenes* of serovar 4b. The source of infection was not detected.**

## Introduction

*Listeria monocytogenes* is a gram-positive intracellular food-borne pathogen. In some groups (immunosuppressed people, neonates, pregnant women and their unborn children) it can be an important cause of life-threatening bacteraemia and meningitis. Because listeriosis has a long incubation time (three to 60 days), it is often difficult to trace the source of infection. This explains why the vast majority of cases are notified as single cases. Nevertheless some well-documented outbreaks of listeriosis have been reported from Finland [1], France [2], Switzerland [3], the United Kingdom (UK) [4] and United States (US) [5]. In Belgium, one outbreak of listeriosis has been described in 2001 [6]; data from one additional study were inconclusive [7]. In the present study, 36 clinical human strains from hospital laboratories and five food isolates were characterised by serotyping, metal resistance typing and pulsed-field gel electrophoresis (PFGE). This combination provided us with the opportunity to link strains from 11 sporadic cases.

## Methods

Patients were not systematically interviewed about their food habits; clinical data were provided by clinical laboratories.

### Identification and molecular typing

Since 1966, the Belgian Listeria Reference Centre (BLRC) has received hospital-isolated strains of *L. monocytogenes* on a voluntary basis. Strain identification is carried out with the api Listeria kit (bioMérieux, France). All strains are serotyped according to a standard protocol [8] using a commercial agglutination test (Denka Seiken, Tokyo, Japan) for somatic (O) and flagellar (H) antigens. Strain susceptibility to arsenic and cadmium is determined according to McLauchlin *et al.* [9]. Molecular typing is performed if a cluster of isolates is suspected based on geographical considerations, on the occurrence of multiple cases within a short period of time or a cluster of isolates with identical serotype.

PFGE is done following the US PulseNet protocol [10] after DNA digestion with *Apa*I and *Asc*I. Analysis of the banding pattern is performed with the ImageMaster video documentation system (Amersham Pharmacia Biotech) and Fingerprinting II Informatix software (Bio-Rad). DNA extraction for Random Amplified of Polymorphic DNA (RAPD) is done with the QI Amp DNA Mini Kit; primer HLWL 74 and the PCR conditions have been described by Wernars *et al.* [11].

### Isolation of *Listeria monocytogenes* from food

25 g of food sample were added to 225 ml of Half Fraser broth for 24 h at 30°C, followed by the inoculation of 0.1 ml into 10 ml of Frazer broth and incubation for 24 h at 37°C. Following enrichment, 500 µl of the culture was used in the VIDAS LMo2 test (bioMérieux). If positive, 10 µl of the Fraser enrichment broth were subcultured on two media: RAPID L. Mono (Bio-rad) and ALOA (bioMérieux). Colonies characteristic of *L. monocytogenes* were further confirmed with the api Listeria kit.

## Results

BLRC receives annually between 30 and 68 strains of human clinical listeriosis cases, representing an annual incidence of three to six cases per million inhabitants. This incidence is comparable to the rates reported by other industrialised countries.

In 2006, 56 clinical strains were received: 19 of them were of serovar 4b. Monthly baseline isolations of serovar 4b varied between none and two. However, in October and November 2006, five and six strains were isolated, respectively, suggesting a possible episode of listeriosis (Figure 1). These 11 isolates were first differentiated by metal resistance typing. Three metal resistance types were found: arsenic sensitive – cadmium resistant (SR) (one strain), arsenic and cadmium resistant (RR) (two strains), and arsenic and cadmium

sensitive (SS) (eight strains). It was clear that the episodic strain was of SS phenotype.

**Figure 1**

Clinical isolates of *Listeria monocytogenes* serovar 4b received at the Belgian Listeria Reference Centre, by month, 2006 (n=19)

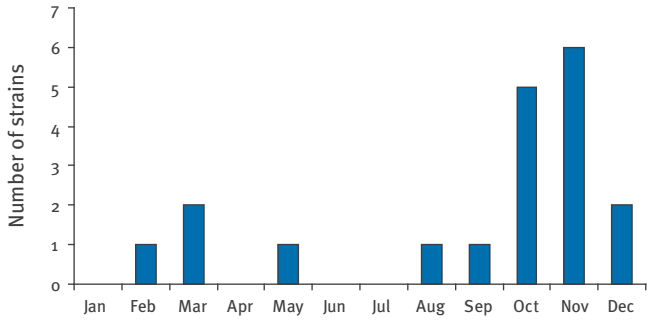



**Figure 2**

Pulsed-field gel electrophoresis *Asc*I and *Apa*I profiles of *Listeria monocytogenes* pulsovar A

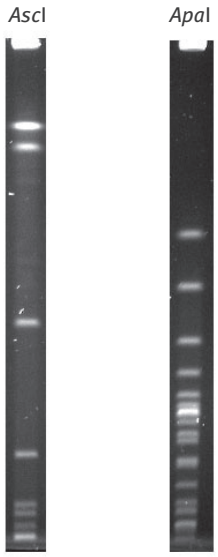
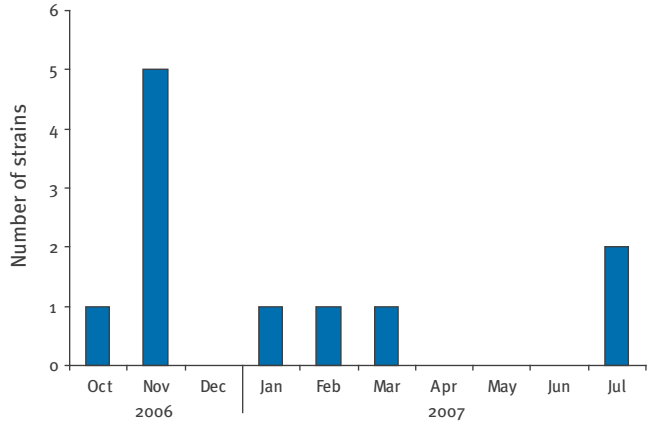



**Figure 3**

Monthly distribution of the episodic *Listeria monocytogenes* strain (4b,SS, pulsovar A), Belgium, October 2006-July 2007 (n=11)




The eight strains of phenotype 4b SS were further subtyped by PFGE. They belonged to three different pulsovars, with pulsovar A represented by six strains (Figure 2). The remaining two strains belonged to a different pulsovar. One of these pulsovars (B) was phylogenetic related to pulsovar A with a similarity index of 92,31% (Fingerprinting II Informatix software application).

To evaluate the duration of the episode, the remaining strains of phenotype 4b – SS from 2006 and those isolated in 2007 were subtyped with PFGE. Another five strains (all from 2007) were found with a profile indistinguishable from the episodic strain. The rate of isolation in 2007 was irregular: January (one strain), February (one strain), March (one strain), July (two strains) (Figure 3). The last two cases were geographically linked, indicating a local extension of the episode. Before the onset of the episode, strains with pulsovar A were very uncommon: only one strain was identified in 2005.

A total of 11 cases appear to have been involved in this episode (six in 2006 and five in 2007). The patients' characteristics are outlined in the Table. Of the positive cultures of these patients, nine were from blood and two from cerebrospinal fluid; four cases were pregnant women or newborns. One pregnant woman had a twin stillbirth. The episode was not geographically clustered, as the isolates were received from three different regions in Belgium: Flanders, Wallonia and Brussels.

The episode was first recognised by the BLRC in November 2006. Only on four occasions were patients asked about their food habits. No standardised questionnaire was used. Suspected food samples were taken from the patients' refrigerators or from the same batch of the suspected food at the retail level. Smoked salmon was sampled because in a case of preterm birth, the mother remembered having eaten smoked salmon. Raw beef brains were the only suspected food item in a case of septicaemia. The woman with the twin stillbirth reported having eaten pre-packed lasagne; this food item was suspected after some of her housemates presented with gastroenteritis. However, *L. monocytogenes* could not be detected in any of these samples.

As the contamination source was not found, another line of investigation was followed. We searched the BLRC food isolations collection for a strain with the same characteristics as the episodic strain. Among the food isolates received at BLRC in 2006 and 2007, only six strains were of phenotype 4b-SS. They were subtyped with RAPD. Two strains isolated from poultry preparations matched the episodic strain RADP profile. However, these strains were of a different pulsovar, which excludes them from being involved in the episode.

## Discussion

In Belgium, the majority of listeriosis cases are reported as single cases. Clinical laboratories mandatorily report cases to the community health authorities. However, these authorities rarely receive information on strain characteristics, which does not facilitate linking sporadic cases. Clinical laboratories may submit their isolates to the BLRC. It is estimated that the BLRC receives approximately 70% of the total number of clinical isolates in Belgium. As the BLRC performs strain typing, clusters of identical strains are easily recognised.

The combination of serotyping, metal resistance typing and PFGE led to the identification of 11 identical isolates. The episodic strain was of serovar 4b, sensitive to arsenic and cadmium and belonged to pulsovar A. Six of them were isolated within a period of a few weeks which is exceptional for a small country like Belgium. Besides the cluster isolations in 2006, the episodic strain was isolated from a further five patients in 2007, indicating a long extension of the episode which went on until July 2007.

The source of contamination was not detected. Two factors may have contributed to this failure: no systematic interviewing of the patients and unsuccessful food sampling. During this episode only four patients were contacted by community health inspectors and only three different food samples were taken which proved to be negative for *L. monocytogenes* in 25 g. On the other hand, the episodic strain was not present in the BLRC collection of food strains, which therefore could not provide a clue for potential suspected food items to be investigated.

Not finding the implicated food is not exceptional. In 1987, 23 cases of listeriosis in the UK were attributed to a strain of unusual serotype designated 4b(X); the implicated food was not identified [12]. In the Netherlands, cluster analysis based on serotyping and PFGE showed one cluster of 15 listeriosis cases without the identification of a clear source of infection [13].

Serovar 4b is not unusual. In Europe and North-America, most published outbreaks of listeriosis in the past 20 years have involved 4b [14]. In addition, strains of serovar 4b tend to be overrepresented in perinatal listeriosis, suggesting that they may have special virulence attributes for pregnancy and breach of the blood-placenta barrier. In the cluster described here, four of the 11 cases were pregnancy-related.

It is presumed that the episodic strain was particularly virulent because it involved a relatively high number of pregnancy-related cases and meningitis cases, four of 11 and two of 11 respectively. According to annual data from the BLRC, strains from cases with maternal-neonatal listeriosis represent 10% of the total number of clinical strains; a similar proportion is observed for cases with meningitis.

It is generally accepted that persons with an underlying disease are more susceptible to contracting listeriosis. In this episode, information from only three patients was available. As it not mandatory for clinical laboratories to report to the BLRC, a lot of epidemiological data are ignored.

During this episode of listeriosis, we received a human *Listeria* strain closely related to the episodic strain. This strain was of serovar 4b, sensitive to arsenic and cadmium but represented another pulsovar (B). Pulsovar B differs from pulsovar A by only three bands in the *Apa*I profile. According to Tenover *et al*. [15], two profiles that differ by only three bands are considered as closely related. However, the similarity index between the two profiles was 92.31% and thus lower than 95%, the minimum level for highly related strains. Therefore this strain was excluded from the episode.

**Table**

Patients' characteristics of the listeriosis episode, Belgium, October 2006-July 2007 (n=11)

| Isolation date | Region | Age (years) | Sex | Isolation site | Symptoms | Underlying disease |
|---|---|---|---|---|---|---|
| October 2006 | Flanders | 48 | Female | Blood | Septicaemia | NA |
| October 2006 | Flanders | 52 | Female | CSF | Meningitis/coma | Lupus |
| November 2006 | Brussles | 65 | Male | CSF | Meningitis | NA |
| November 2006 | Wallonia | 81 | Male | Blood | NA | Heart disease |
| November 2006 | Wallonia | Neonate | Female | Blood | Preterm birth | NA |
| November 2006 | Flanders | Neonate | Male | Blood and ear | Septicaemia/preterm birth | NA |
| January 2007 | Flanders | 58 | Male | Blood | Septicaemia | NA |
| February 2007 | Flanders | 36 | Female | Blood | Septicaemia/twin stillbirth | NA |
| March 2007 | Flanders | Neonate | Female | Blood | Septicaemia | NA |
| July 2007 | Wallonia | 59 | Male | Blood | NA | Cancer |
| July 2007 | Wallonia | 54 | Female | Blood | Septicaemia | NA |

CSF: cerebrospinal fluid; NA: not available.

This episode would have passed unnoticed had not the BLRC performed strain typing. Efficient monitoring of listeriosis requires systematic interviewing of the patients using a standardised questionnaire. Close cooperation between community health inspectors, the Belgian Federal Agency for the Safety of the Food Chain (FASFC) and the BLRC would result in a rapid linking of sporadic cases and enhance the chance of finding the infection source in outbreaks.

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