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Increased incidence of *Mycoplasma pneumoniae* infection in England and Wales in 2010: multiocus variable number tandem repeat analysis typing and macrolide susceptibility

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An epidemic of *Mycoplasma pneumoniae* infection began in Denmark in late 2010. A similar increase in *M. pneumoniae* infections was noted in England and Wales in the same period, with a decline in early 2011. Multiocus variable number tandem repeat analysis typing and analysis of macrolide resistance markers indicate that at least nine known and two novel strain types were circulating in England and Wales during October 2010 to January 2011. There was no evidence of macrolide resistance.

After an epidemic of *Mycoplasma pneumoniae* infection in Denmark in late 2010 was reported, we found a similar increase in the number of *M. pneumoniae* infections in England and Wales in the same time period. By early 2011, the number of infections had fallen in England and Wales.

Background

M. pneumoniae is a common cause of pneumonia and is transmitted by aerosol or close contact. In England and Wales, the pathogen is found in all age groups, with higher prevalence in children aged 5–14 years [1]. Epidemic periods lasting on average 18 months have occurred at approximately four yearly intervals, as seen Denmark [2,3]. Epidemic periods follow the same pattern: sporadic infection occurs at a low level with seasonal peaks from December to February [1,2]. Recently, data were reported from Denmark indicating that a *M. pneumoniae* epidemic had started in October 2010 [3]. As previous epidemic periods in England and Wales have been synchronous with those in Denmark, we sought to determine whether an epidemic was also occurring in England and Wales. In these two countries of the United Kingdom, data submitted voluntarily from routine laboratory reports are collated by the Health Protection Agency (HPA) to give an indication of the number of patients testing positive by serological, molecular or culture tests for *M. pneumoniae* per week.

More recently, community surveillance data based on quantitative real-time polymerase chain reaction (qPCR) analysis have been used successfully to monitor *M. pneumoniae* infection in patients with respiratory symptoms – influenza-like illness, upper respiratory tract infection, lower respiratory tract infection, fever (›38.5 °C) or myalgia – attending general practitioner (GP) clinics (from 2005 to 2009) [1]. This was an extension to the virological community surveillance that is undertaken annually in England and Wales for a range of respiratory viruses including influenza virus, respiratory syncitial virus and human metapneumovirus [4].

To determine whether an *M. pneumoniae* epidemic was occurring in England and Wales, we reviewed the laboratory reports submitted to the HPA and, from October (week 40) 2010 to January (week 3) 2011, undertook qPCR-based community surveillance for *M. pneumoniae* infection in patients with respiratory symptoms attending GP clinics. Furthermore, to determine what strains of *M. pneumoniae* were circulating during this time, community surveillance samples and 10 additional respiratory samples (submitted to our laboratory by GPs and hospitals for routine testing) that were positive by qPCR were investigated to determine the type of infecting strain and whether there was any evidence of genetic markers for macrolide resistance. Resistance to macrolides is an increasing problem in Asia and has been found in the United States and some European countries [3].

Methods

We carried out qPCR anlysis of 1,221 anonymised combined nose and throat swabs taken from patients with respiratory symptoms during the winter months of 2010/11: October (week 40) 2010 to January (week 3) 2011. Nucleic acid was extracted and stored as previously described before qPCR testing for the presence of *M. pneumoniae* P1 and community-acquired respiratory distress toxin genes [1,5,6].

Samples that were positive by qPCR were examined for *M. pneumoniae* type and macrolide resistance. We also examined the additional 10 respiratory samples submitted to our laboratory for *M. pneumoniae* detection in this period.

Multiocus variable number tandem repeat analysis (MLVA) typing by fragment analysis has previously been used to type *M. pneumoniae* strains [7]. In this study, we used the same MLVA typing method for analysing nucleic acid extracts of clinical samples; culture isolation of *M. pneumoniae* was not undertaken. Putative novel MLVA profiles were given numerical designations, from MLVA-0027 onwards, to follow on from the known 26 MLVA types (MLVA-A to MLVA-Z) [7].

The possible presence of mutations in *M. pneumoniae* previously associated with macrolide resistance was examined by amplification and sequencing of a 720 base pair (bp) fragment of the 23S rRNA gene using MpnMR2063F (5'-ATCTCTTGACTGTCTCGGC-3') and MpnMR2617R (5'-TACAACTGGAGCATAAGAGGTG-3') primers [8].

Detection and analysis of *M. pneumoniae* in clinical samples

The number of *M. pneumoniae*-positive laboratory reports from regional laboratories and hospitals submitted to the HPA during the study period is shown in Figure 1: one report is received per patient and four-weekly moving averages are plotted. From week 40 of 2010 to week 3 of 2011, there were a total of 322 reports, the highest number since the previous peak of *M. pneumoniae* infections seen during the same sampling period in 2005 to 2006 (n=455). The mean number of reports received from 2006 to 2009 (from week 40 of one year to week 3 of next) was 234.

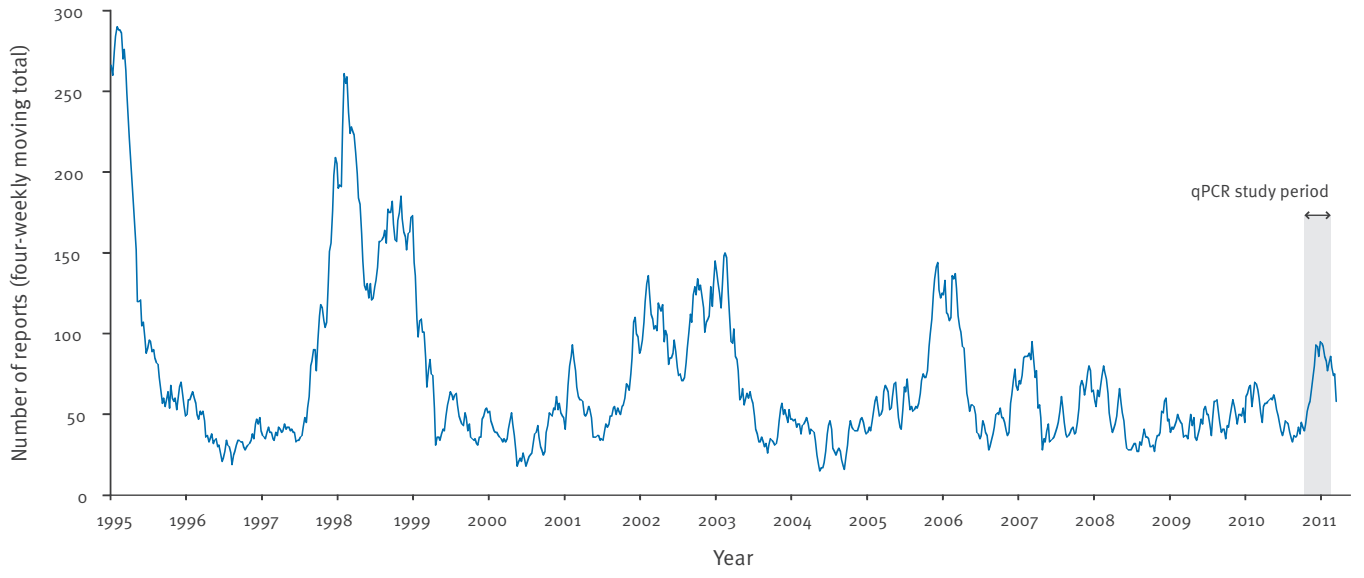
A total of 21 of 1,221 (1.7%; 95% CI: 1.1 to 2.6) community surveillance samples from week 40 of 2010 to week 3 of 2011 were *M. pneumoniae*-positive by qPCR. The percentage of positive cases per week is shown from 2005 to 2011 in the Table, showing an increase from October to December (weeks 40–49) 2010. Samples were more likely to be positive during this period in 2010 (18 of 629; 2.9%; 95% CI: 1.8 to 4.5) than in the following six weeks – December (week 50) 2010 to January (week 3) 2011 (3 of 592: 0.5%; 95% CI: 0.1 to 1.6; Fisher's exact test p=0.002). In November (weeks 44, 45 and 47) 2010, *M. pneumoniae* infections significantly increased in comparison with all previous weeks of sampling since 2005 (binomial probability test p=0.09, 0.07, 0.005, respectively).

The mean age of the patients was 19.8 years (standard deviation (SD)±19.4 years; range: 0–91 years). We detected no difference in age group affected by *M. pneumoniae* infection (Figure 2).

MLVA analysis of 10 of the 21 qPCR-positive community surveillance samples and the 10 additional concurrent respiratory samples showed a total of 11 distinct strain

Figure 1

Laboratory reports of *Mycoplasma pneumoniae* infection by date of report, England and Wales, January 1995–March 2011



qPCR: quantitative real-time polymerase chain reaction.

Four-weekly moving total of the number of reports collated by the Health Protection Agency Centre for Infections, including serological, molecular and culture test results. Report numbers per year are: 1,687 in 1995, 490 in 1996, 990 in 1997, 2,278 in 1998, 727 in 1999, 483 in 2000, 804 in 2001, 1,409 in 2002, 819 in 2003, 472 in 2004, 991 in 2005, 818 in 2006, 737 in 2007, 573 in 2008, 624 in 2009, 743 in 2010, 162 in 2011 (up to the first week of March), giving a total of 14,807 for all years), giving a total of 14,807 for all years. The arrowed line indicates the qPCR study period, October (week 40) 2010 to January (week 3) 2011, during which time 322 reports were received.

Source: [9].

types: nine of known types (MLVA-B, C, E, J, M, P, U, V and Z) and two putative novel types (termed MLVA-0027 profile 34672 and MLVA-0028 profile 64573). An MLVA profile could not be obtained for the other 11 qPCR-positive community surveillance samples, probably because of the low levels of *M. pneumoniae* nucleic acid in these samples.

The most prevalent strain type was MLVA-M (5 of 20), which had been found previously in France (in 1997, 1999, 2000 and 2006), Germany (in 1995 and 2000) and Japan (in 2000 to 2003) [7]. In our study, patients with this strain type had a cough (n=3), upper respiratory symptoms (n=1) or lobar pneumonia (n=1).

A full-length sequence of the 720 bp fragment of the 23S rRNA gene containing all four loci associated with macrolide resistance (2063, 2064, 2067 and 2618) was obtained from 14 of the 21 qPCR-positive community surveillance samples and the 10 additional respiratory samples. No mutations in these loci associated with macrolide resistance were identified in these samples. For the remaining seven qPCR-positive community surveillance samples, sequence information could not be obtained, presumably due to low levels of *M. pneumoniae* nucleic acid.

Discussion

The overall level of *M. pneumoniae* infection in the qPCR-based community surveillance of patients from October 2010 to January 2011 was low (1.7%) and was at a similar level to that found in the same months during 2005 to 2009 (1.7%) [1]. Detectable *M. pneumoniae* infection was found in all age groups; however, no significant difference in age group affected by *M. pneumoniae* infection was found over this time period, unlike the situation in 2005 to 2006. At that time, *M. pneumoniae* infections were mainly reported in children aged 5–14 years.

Samples from October to December (weeks 40–49) 2010 were more likely to be positive than those in the following six weeks. The increased incidence in this period in 2010 is consistent with a rise in the number of *M. pneumoniae* laboratory reports in the same period (Figure 1) and that seen in Denmark [3]. The increase in 2010 in the number of positive laboratory reports submitted to the HPA is four weeks later than that detected by qPCR, as the reports are mainly based on IgM serology, highlighting that data from laboratory reports collected by the HPA on *M. pneumoniae* infection in England and Wales lag a month behind actual infection in the population.

Table

Percentage of clinical community surveillance samples positive for *Mycoplasma pneumoniae*[a] per week, England and Wales, October (week 40) to January (week 3) of 2005–2011[b]

Week number	Percentage of samples positive for *M. pneumoniae*				
	2005/06	2006/07	2007/08	2008/09	2010/11
	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)
40	0.0 (0.0–28.5)	16.7 (0.4–64.1)	0.0 (0.0–100)	0.0 (0.0–60.2)	3.0 (0.1–15.8)
41	0.0 (0.0–16.8)	0.0 (0.0–52.2)	0.0 (0.0–70.8)	0.0 (0.0–84.2)	1.4 (0.0–7.8)
42	4.8 (0.0–23.8)	0.0 (0.0–60.2)	0.0 (0.0–12.8)	0.0 (0.0–60.2)	1.3 (0.0–6.9)
43	3.0 (0.1–15.8)	0.0 (0.0–14.2)	0.0 (0.0–13.2)	11.1 (0.3–48.2)	1.3 (0.0–7.0)
44	3.3 (0.1–17.2)	0.0 (0.0–10.0)	0.0 (0.0–14.8)	0.0 (0.0–60.2)	3.9 (0.8–11.0)
45	7.4 (0.8–24.3)	2.2 (0.1–11.5)	0.0 (0.0–12.3)	11.1 (0.3–48.2)	4.5 (0.9–12.7)
46	4.0 (0.1–20.4)	0.0 (0.0–7.5)	0.0 (0.0–8.0)	0.0 (0.0–20.6)	3.0 (0.4–10.4)
47	0.0 (0.0–14.8)	5.0 (0.6–16.9)	2.4 (0.1–12.9)	0.0 (0.0–30.8)	8.3 (2.2–20.0)
48	11.5 (2.2–30.2)	0.0 (0.0–5.7)	0.0 (0.0–6.5)	0.0 (0.0–14.8)	0.0 (0.0–7.4)
49	2.9 (0.1–14.9)	0.0 (0.0–6.3)	0.0 (0.0–5.6)	3.6 (0.1–18.3)	3.0 (0.4–10.5)
50	10.0 (1.9–26.5)	2.1 (0.1–11.3)	1.5 (0.0–8.0)	0.0 (0.0–8.0)	0.0 (0.0–3.6)
51	5.3 (0.6–17.7)	2.6 (0.1–13.8)	1.1 (0.0–6.0)	0.0 (0.0–8.6)	0.0 (0.0–1.8)
52	0.0 (0.0–41.0)	0.0 (0.0–8.2)	0.0 (0.0–5.4)	0.0 (0.0–17.6)	0.0 (0.0–4.9)
1	6.3 (0.8–20.8)	0.0 (0.0–5.1)	0.0 (0.0–3.1)	0.0 (0.0–18.5)	0.0 (0.0–5.9)
2	5.9 (1.2–16.2)	1.0 (0.0–5.6)	2.1 (0.3–7.5)	0.0 (0.0–33.6)	2.1 (0.4–6.1)
3	0.0 (0.0–8.8)	3.8 (0.8–10.6)	0.0 (0.0–4.9)	0.0 (0.0–33.6)	0.0 (0.0–36.9)
All weeks	**4.5 (2.6–6.8)**	**1.4 (0.7–2.6)**	**0.6 (0.2–1.4)**	**1.2 (0.2–3.5)**	**1.7 (1.0–2.6)**

The number of positive samples and total number of samples per year are: 20 of 449 in 2005/06, 10 of 703 in 2006/07, 5 of 818 in 2007/08, 3 of 249 in 2008/09 and 21 of 1,221 in 2010/11, giving a total of 59 positive in 3,440 samples for all years.

[a] Determined by quantitative real-time PCR.

[b] Excludes October (week 40) 2009 to January (week 3) 2010 when sampling was not performed.

A total of 11 distinct MLVA types were identified during the study period, with MLVA-M being the most prevalent. Patients with this strain type did not all have the same symptoms or severity of infection and the sample number is too low to investigate the association of particular types with clinical severity. Two putative new profiles were obtained, in addition to nine known types. One of these, MLVA0027, was identified in two different samples. Confirmation of the novel MLVA types obtained will require isolation of the strains.

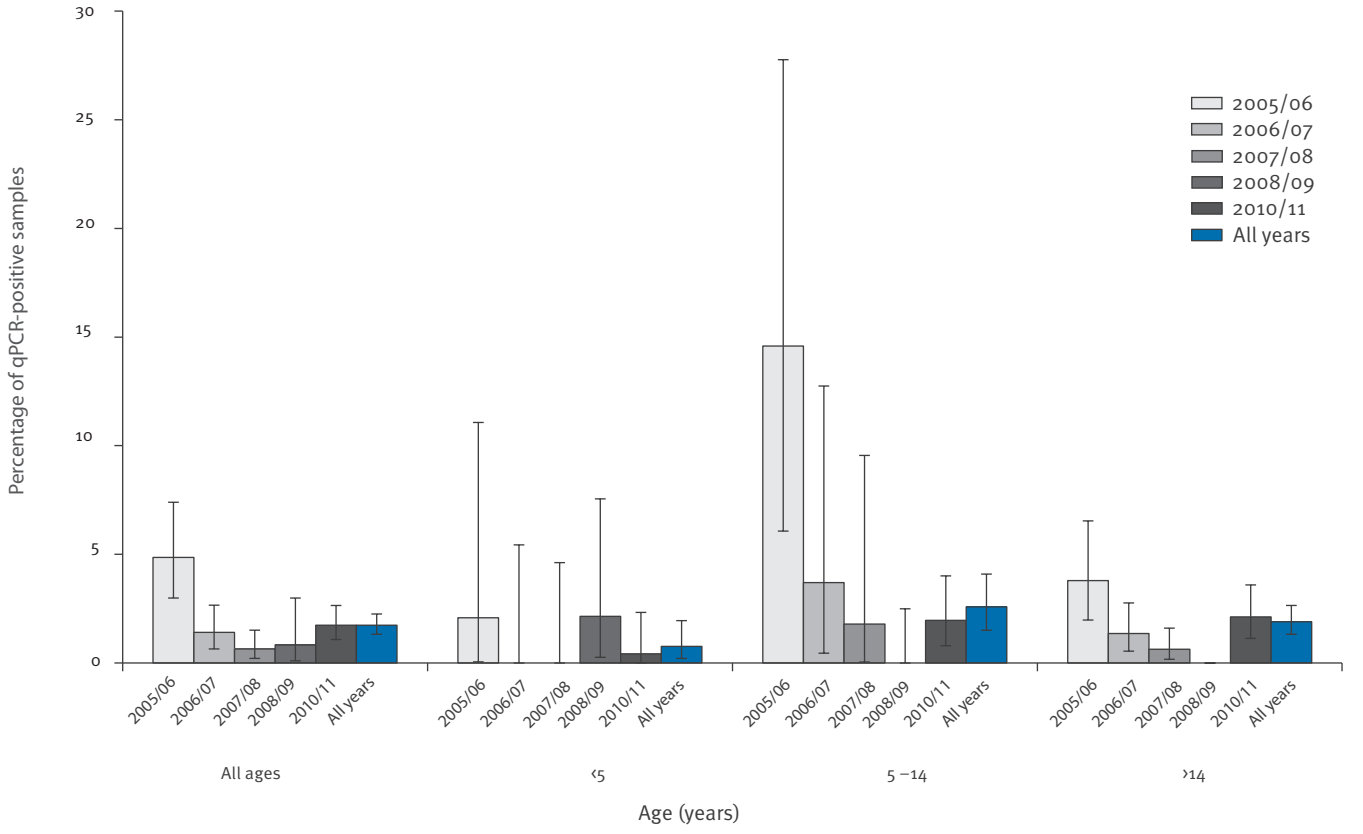
It is not known whether increases in incidence of *M. pneumoniae* infections are due to an increased incidence of an individual strain or a concurrent increased incidence of several strains. Speculation that a shift in P1 adhesin type may be the cause of epidemics has been disputed [7,10]. Evidence from our study does not support the hypothesis that a single strain type of *M. pneumoniae* was responsible for the observed increase in infection in England and Wales. Rather, a decline in immunity or increase of the immunologically naive population may have triggered the four-year cycle of epidemic periods. It would be interesting to type *M. pneumoniae* strains from Denmark and other countries during the same period to determine how strains differ geographically during periods of increased infection.

Macrolide-resistant *M. pneumoniae* is an increasing problem in Asia and has been seen in Europe and the United States; however, resistance remains uncommon in European countries (such as Denmark, France and Germany) [3]. Macrolide resistance was not identified in any of the qPCR-positive samples from England and Wales analysed during the study period.

Our study shows that qPCR based surveillance of *M. pneumoniae* infections in the community is invaluable, allowing rapid detection of infection in the population and contributing timely data on infecting strain characteristics, diversity and antimicrobial resistance.

Figure 2

Percentage of clinical community surveillance samples positive for *Mycoplasma pneumoniae*[a] by age group, England and Wales, October (week 40) 2005 to January (week 3) 2011[b]



qPCR: quantitative real-time polymerase chain reaction.

The number of positive samples and total number of samples per year are: 20 of 412 in 2005/06, 9 of 638 in 2006/07, 5 of 769 in 2007/08, 2 of 239 in 2008/09, 21 of 239 in 2010/11, giving a total of 57 positive in 3,265 samples for all years. Information on the age was not available for all patients. The error bars indicate 95% confidence intervals.

[a] Determined by qPCR.

[b] Excludes October (week 40) 2009 to January (week 3) 2010 when sampling was not performed.

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Diphtheria in the south of France, March 2011

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In March 2011, a 40 year-old French man was diagnosed with diphtheria caused by toxigenic *Corynebacterium diphtheriae*. Fifty-three close contacts were identified from whom throat samples were analysed. *C. diphtheriae* was found only in the asymptomatic partner of the index case. The two cases had travelled in Spain during the incubation period of the index case. Investigation around the second case identified 13 new close contacts. None of them was found to be infected.

Case report

On 14 March 2011, the French Institute for Public Health Surveillance (Institut de Veille Sanitaire, InVS) was informed by the Regional Health Agency of Languedoc-Rousillon that *Corynebacterium diphtheriae* had been isolated from a patient with angina and pseudomembranes since 7 March. The patient had no history of vaccination, and no history of recent travel to an endemic area. He had visited his General Practitioner on 8 March, who performed a throat swab and prescribed oral antibiotic treatment with cefuroxime 500 mg daily. On 12 March, *C. diphtheriae* was isolated by a local laboratory from the throat swab. The patient was immediately advised to attend a hospital emergency department. He did not present any signs suggestive of severe disease. Antibiotic treatment was changed to roxithromycin, 300 mg daily for 14 days after receipt of the microbiological results and it was agreed that the patient could stay at home but had to remain in isolation and wear a protective mask in the event of receiving visitors. Diphtheria antitoxin was not given, as the interval between the onset of the disease and the date of availability of the serum was too long, and the case did not have any signs of systemic affection. A laboratory follow-up test six days after the start of treatment with roxithromycin was negative for *C. diphtheriae*.

Contact tracing

Immediately upon receipt of the positive results from the local laboratory, on 12 March, an investigation was conducted by the local health authorities to identify the source of infection, trace contacts and to implement control measures. The investigation followed the French national guidelines for diphtheria case management [1].

Fifty-three close contacts were identified around the index case. These were close friends or work colleagues (n=2), healthcare workers (n=15) and patients waiting with the case in the same room of the emergency department and not wearing protective masks (n=36). Contacts were contacted and physically examined, and were all offered throat swabs and antibiotic prophylaxis. All close contacts agreed to have their samples taken and all were negative except that of the index case's partner who was identified as an asymptomatic carrier of *C. diphtheriae* from a throat swab taken 11 days after the onset of disease of the index case. This second case had been vaccinated with diphtheria, tetanus, and pertussis (DTP) vaccine in 2006 and received azythromycin 500 mg per day for three consecutive days and one dose of booster vaccine for diphtheria.

Around this second case, 13 co-workers were identified as close contacts. They were also offered testing and prophlaxis and laboratory results were negative for all 13 contacts. Prophylaxis recommended to all persons in close contact with the two cases, was azythromycin 500 mg per day for three consecutive days and one dose of booster vaccine for diphtheria, unless they could document a history of full vaccination (three doses) with a booster within less than five years.

International notification

In the course of the investigation, patient history revealed that, from 3 to 6 March, the two cases had travelled together in Spain. They did not report any specific close contacts during this trip. However, they had both participated in an international gathering (Carnival of Sitges) between 5 and 6 March. The national Spanish Health Authorities were informed by the French Health Authorities about the two diphteria cases and a notification through the European Union's (EU) Early Warning and Response System (EWRS) was performed on 24 March. No cases of diphtheria have been reported by the local Spanish Health Authorities.

Laboratory investigation at the National Reference Centre

On 9 March, a culture seed, on a Columbia CNA agar + 5% sheep blood plate and two throat samples from the index case were sent to the National Reference Centre (NRC) where they were analysed for toxigenic corynebacteria and where a PCR assay for the detection of diphtheria toxin gene (*tox*) was performed. On 16 March, the NRC confirmed *C. diphtheriae* carrying the *tox* gene in these samples. The sample used for molecular analyses and for seeding a new culture was the first from Columbia CNA agar + 5% sheep blood plate. The throat swabs were keept at –20°C.

Throat samples from identified contacts were sent to the NRC from 16 March onwards where they were processed in a similar fashion than the samples from the index case.

The non-production of diphtheria toxin in the index case and in a second case identified during the contact tracing, as well as the isolates' sensitivity to antibiotics, in particular macrolides, was confirmed only 12 days after the detection of the *C. diphtheriae tox* gene because the purification of the isolate from the contaminated culture received was difficult. The two isolates from the two respective cases were *C. diphtheriae* biovar *mitis*. The molecular typing was recently performed by multilocus sequence typing (MLST) and both isolates have the same sequence type (ST), ST (212) which has not been described in the literature so far. The Elek test was negative for both.

Background and epidemiological situation in France

Diphtheria can result in an acute bacterial toxic infection of the upper respiratory tract or in cutaneous lesions. It is caused by toxin-producing *C. diphtheriae*, *C. ulcerans*, and *C. pseudotuberculosis*. The infection is characterised by a sore throat with an adherent pseudomembrane on the tonsils, pharynx or nasal cavity. The severity of the infection is related to obstruction of the upper respiratory tract and the dissemination of diphtheria toxin, which may cause myocardial and neurological lesions. Diphtheria is transmitted by aerosol secretions and/or contact with skin lesions. It can also be transmitted from asymptomatic individuals who may carry the bacteria for several weeks.

Toxigenic diphtheria is a mandatory notifiable disease in France and all cases supected on clinical grounds (angina with pseudomembranes, or cutaneous lesions with pseudomembranes) must be notified without delay to the Regional Health Agency if a *Corynebacterium* is isolated. Since 2003, the case definition of confirmed cases also includes *C. ulcerans* harbouring the *tox* gene [2]. Due to widespread immunisation, there were less than five cases notified per year in the 1980s (Figure). In addition, 18 indigenous cases of diphtheria *C. ulcerans* harbouring the *tox* gene (*tox*-positive) have been reported in France since the early 2000s. The last indigenous cases of infection with *C. diphtheriae* were reported in France in 1989. Four imported cases were notified between 2002 and 2010 [3].

Despite a high vaccination coverage in infants (98–99%), immunisation coverage remains insufficient in adults in France especially after fifty years-old [4]. This mainly reflects the fact that booster diphtheria vaccination was only introduced in the immunisation schedule in 2006 [5].

Discussion and conclusion

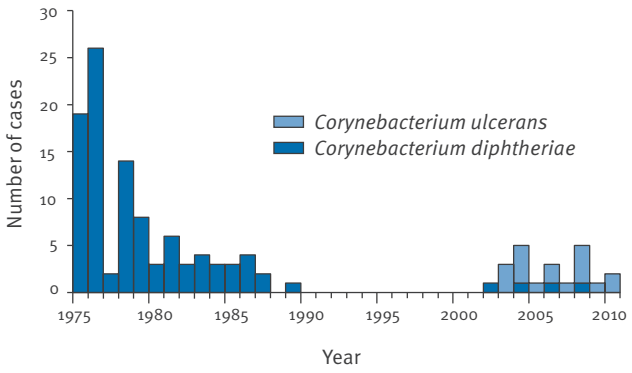
Two epidemiologically linked cases of toxigenic *C. diphtheriae* infections were identified in March 2011 in France. This is the first notification of *C. diphtheriae*, with no travel history to an endemic area, in France since 1989. Among the patients' contacts in France, no additional cases were identified suggesting an absence of local transmission. Both cases had travelled to Spain where they had attended the Carnival of Sitges. Transmission during this international event is possible, especially considering the interval between the event and the onset of disease in the index case, which was within the usual incubation period of diphtheria (2 to 5 days) [6]. The second case may have been infected at the same time, or have been infected by the index case. We can also not rule out that the asymptomatic case was the first infected.

The investigation followed national guidelines [1], identifying all persons in close contact with the cases during the incubation period. The main difficulty was to detect close contacts among the patients who stayed several hours in the same waiting room as the case inside the emergency department. This led to a large number of people being considered as contacts, and this could have been avoided with more appropriate case management. This placed a significant workload on the local hospital, local health authorities, and the NRC, with logistical constraints for collecting and processing samples.

Microbiological investigation was complicated because a contaminated isolate delayed the Elek test. However,

Figure

Number of laboratory confirmed cases of toxigenic diphtheria, France, 1975–2010



detection of the *tox* gene was performed the day the sample was received with the identification of the bacteria the next morning, indicating that molecular identifications are very useful to quickly confirm the infection.

Effective cooperation between the different partners involved in the investigation and implementation of control measures allowed the successful management of this event. It reminds us of the need to maintain vigilance regarding the possible diagnosis of diphtheria even in the absence of recent travel in endemic areas. The greatest challenges are retaining and developing clinical awareness, microbiological skills and surveillance systems among EU Member States. It also emphasises the need for a high vaccine coverage in the adult population [7].

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Yersinia enterocolitica O:9 infections associated with bagged salad mix in Norway, February to April 2011

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In March 2011, the Norwegian Institute of Public Health identified a possible outbreak involving 21 cases of *Yersinia enterocolitica* O:9 infection with similar MLVA-profiles. Preliminary results of epidemiological and microbiological investigations indicate bagged salad mix containing radicchio rosso (also known as Italian chicory) as a possible source. As a result of the investigation, bagged salad mixes of a specific brand were voluntarily withdrawn from the market by the producer.

Introduction

In March 2011, the Department of Infectious Disease Epidemiology at the Norwegian Institute of Public Health (NIPH) was informed by the National Reference Laboratory (NRL) for enteropathogenic bacteria of an unusually high number of *Yersinia enterocolitica* serotype O:9 isolates from geographically disparate areas in Norway. After being notified of five cases of *Y. enterocolitica* O:9, which is rare in Norway, a multidisciplinary investigation team was established on 18 March 2011 to find the source and prevent further illness.

Yersiniosis is a mandatorily notifiable disease and the fourth most commonly reported cause of bacterial diarrhoeal disease in Norway [1]. In the past 10 years, between 80 and 150 cases of yersiniosis were reported annually. More than 98% of yersiniosis cases in Norway are due to serotype O:3, which is also the dominant cause of yersiniosis in Europe, Japan, Canada and parts of the United States [2].

Descriptive epidemiology

A confirmed case was defined as an individual with laboratory-confirmed *Y. enterocolitica* O:9 infection with the outbreak MLVA-profile identified between 1 January and 5 May 2011. By 5 May, the reference laboratory had registered 21 cases with the outbreak strain of *Y. enterocolitica*. Of the 21 confirmed cases, 15 were female and six were male. The age range of patients was from 10 to 63 years with a median age range of 30-39 years (Figure 1).

Cases occurred in geographically disparate areas of the country, across ten different municipalities (Figure 2).

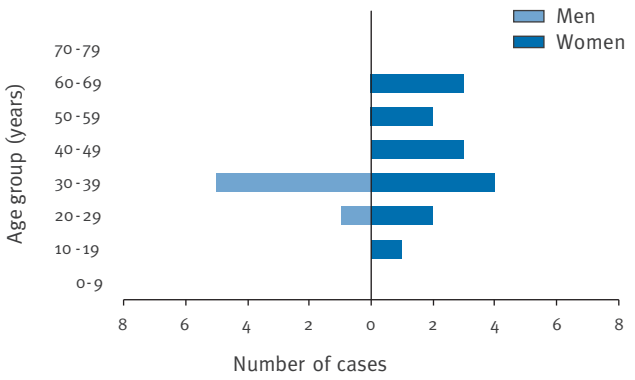
For four patients, the date of symptom onset was unavailable and the date of positive microbiological sample was used for the epidemic curve (Figure 3). Between week 6 (7–13 February) and week 11 (14–20 March), 17 patients with positive microbiological samples became ill.

Epidemiological investigation

When there are outbreaks in Norway where the cases are geographically widespread, the NIPH is responsible for coordinating the outbreak investigation. As is often done in foodborne outbreaks in Norway, after being notified of a microbiologically confirmed outbreak case, the NIPH contacted the respective municipal doctor and asked them to contact the patient in order to get consent for the district Food Safety Authority office to visit the home, collect food samples and conduct an interview. The first seven cases were interviewed using a trawling questionnaire, designed to collect information on food consumption in the seven days prior to onset of symptoms, animal contact and environmental exposures, as well as clinical and demographic

Figure 1

Age and sex distribution of cases of *Yersinia* O:9 infection, Norway, February–April 2011 (n=21)

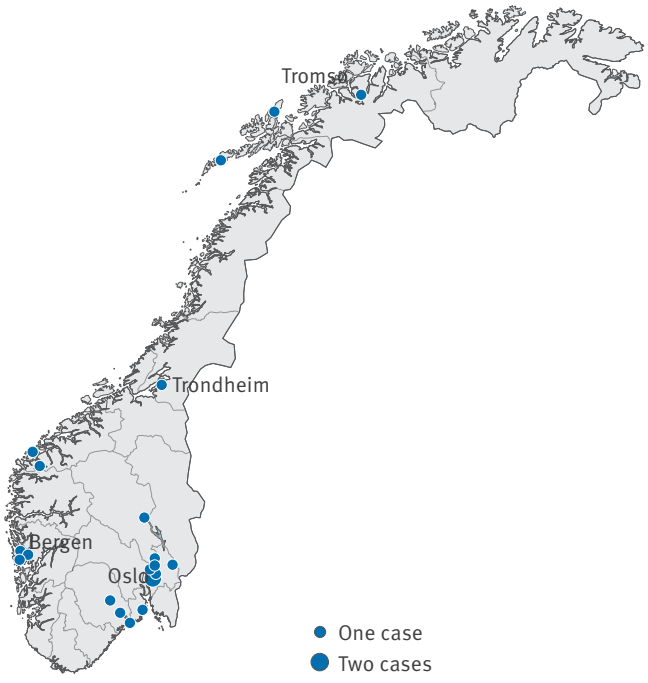


information. Following these interviews, the questionnaire was shortened to focus on categories of foods of most interest, and used to conduct a case-control study. In particular, from the trawling interviews, bagged salad mix was suspected as the source of infection. The case-control study was conducted in week 13 (28 March–1 April 2011). At that time, nine patients had been interviewed using the shortened questionnaire. In order to ensure enough statistical power in the case-control study given the small number of cases, three controls for each case were selected from the national population register. Controls and cases were matched by age, sex and municipality of residence. Potential controls were excluded if they reported having had diarrhoea during the last 14 days.

The results from the trawling interviews revealed that limited number of cases had consumed pork products. Salad mix and arugula were consumed by a notable number of cases, with at least four specifically stating they had consumed a specific brand of salad mix containing arugula. Preliminary results of the case-control study corroborate the hypothesis of bagged salad mix as the suspected source. Among the nine cases, six had eaten bagged salad mix in the week prior to onset of illness compared with three of 25 controls (matched odds ratio (mOR):13.7; 95% confidence interval (CI): 1.6–116.3). We included eight significant food items in a conditional multivariate logistic regression model. A forward selection procedure was used by starting with the most significant item and including the other items one by one. The only food item which remained significant in the model was the bagged salad mix.

Figure 2

Geographical distribution of cases of *Yersinia* O:9 infection, Norway, February-April 2011 (n=21)



International notifications

On 26 April 2011 the NIPH sent a message via the European Centre for Disease Prevention and Control (ECDC) Epidemic Intelligence Information System asking whether other countries had also experienced an increase in cases of *Y. enterocolitica*. The Norwegian Food Safety Authority sent a notification through the Rapid Alert System for Food and Feed (RASFF) on 15 April 2011. International requests for information produced no reports of similar yersiniosis outbreaks in European countries. However, it is possible that few countries routinely perform serotyping of *Y. enterocolitica*.

Microbiological investigation

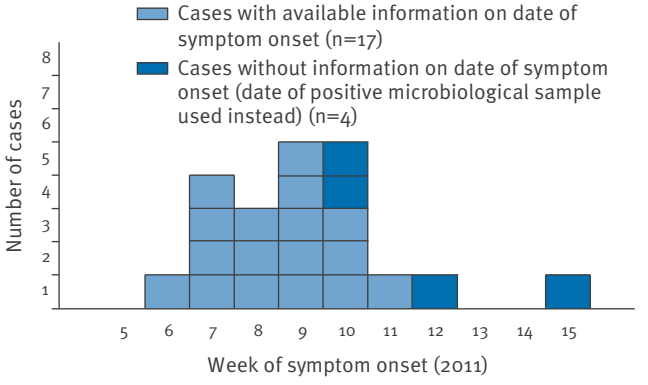
At the NIPH-located NRL all isolates of *Y. enterocolitica* from human patients are routinely characterised phenotypically, biotyped and serogrouped against O:3 and O:9 as well as a range of other serogroups. The *Y. enterocolitica* isolates were MLVA-typed by the method described by Gierczyński et al. [3], locally adjusted to capillary electrophoresis.

Food samples were sent to the Norwegian Veterinary Institute for analysis. A total of 61 samples consisting of two chicken meat products, two pork products and 57 diverse salad products and bagged salad mix products were collected from patient homes, retail and the company producing the bagged salad mix products. All products were analysed according to NMKL 117B, an adaptation of ISO 10273. Additionally, samples were cold enriched for 21 days according to NMKL 117. All enriched broths and colonies isolated were further examined for the *ail* gene, an indicator for pathogenic *Y. enterocolitica*, using PCR (NMKL 163, Part A (1998)). PCR positive colonies were characterised by biochemical reactions and their serogroup was determined.

Diverse *Yersinia* spp. including enterocolitica were isolated from 11 of the salad products of which two were consistently positive by PCR. These strains were

Figure 3

Cases of *Yersinia* O:9 infection by week of symptom onset, Norway, 7 February–20 March 2011 (n=21[a])



[a]Including the four cases for which information on date of symptom onset was not available.

isolated from one particular salad type, radicchio rosso, imported from Italy, and mixed salad products, which also contain radicchio rosso. However, these isolates were not serogroup O:9.

Discussion and conclusion

The geographically widespread occurrence of the yersiniosis cases and the illness onset dates indicate that the suspected source of infection is likely a product that was widely distributed but available only for a relatively short period of time. In addition, the number of female cases compared to male cases indicated that the source was a food product more commonly consumed by women. Radicchio rosso is the only variety of salad included in the suspected bagged salad mixes that keeps long enough to fit with the duration of this outbreak. Radicchio rosso is stored at –1°C before it is supplied to the market. The storage conditions may increase growth of *Y. enterocolitica* as this bacterium is able to grow down to –2°C.

Yersiniosis outbreaks are often associated with consumption of pork, as the pig is the only animal consumed by humans which regularly harbours the pathogenic serovars O:3 and O:9 [2]. Although most cases of yersiniosis in Norway are sporadic, there have been several previous outbreaks, including an outbreak of *Y. enterocolitica* O:9 in 2005-2006 due to a Norwegian ready-to-eat pork product ('sylte') [4]. Published literature on yersiniosis outbreaks linked to salad and/or fresh vegetables is limited. Although previous outbreaks of *Salmonella*, *Shigella* and *Escherichia coli* in Norway have been linked to the consumption of fresh vegetables [5-8], this is the first outbreak of yersiniosis in Norway to be linked to consumption of vegetables.

As of 5 May 2011, no new outbreak cases have been reported. The supplier voluntarily withdrew suspected bagged salad mixes containing radicchio rosso from the market based on the information collected through the interviews, case-control study preliminary results and positive PCR results, as well as their own risk assessments. Information obtained through RASFF indicates that while the exporter of radicchio rosso implicated in this outbreak also sends the product to the United Kingdom, the batch in question was only distributed in Norway. Although the epidemiological evidence incriminates bagged salad mix, the ongoing trace-back investigation of the product has been complicated. *Yersinia* is challenging to cultivate from food products [9] and microbiological testing is also still in progress.

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An outbreak of *Salmonella* Typhimurium traced back to salami, Denmark, April to June 2010

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Between April and June 2010, a small national outbreak of *Salmonella* Typhimurium with a particular multilocus variable-number tandem repeat analysis (MLVA) type was identified in Denmark through laboratory-based surveillance. The outbreak involved twenty cases, primarily living within the greater Copenhagen area. Half of the cases were children aged ten years or younger and 12 were male; three cases were hospitalised. A matched case-control study showed a strong link between illness and eating a particular salami product containing pork and venison, matched odds ratio (mOR):150, confidence interval (CI): 19–1,600. The salami had been produced in Germany. Microbiological confirmation in food samples was sought but not obtained. Danish consumers were notified that they should return or dispose of any packages from the suspected salami batch. Because the salami product had potentially been sold in other European countries, the European Centre for Disease Prevention and Control urgent enquiry and Rapid Alert System for Food and Feed systems were used to highlight the possibility of outbreaks in these countries. Case-control studies are a strong tool in some outbreak investigations and evidence from such studies may give sufficient information to recall a food product.

Introduction

Salmonella enterica is the second most common cause of bacterial gastroenteritis in Denmark. A series of national control and intervention programmes have reduced the annual incidence to less than 30 cases per 100,000 population in 2010 [1] compared to 96 in 1997 [2]. However, imported products, including products from other European Union (EU) Member States, are not monitored within the Danish national programmes, but are rather tested for *Salmonella* by random sampling at import and, less often, during retail product control. Ready-to-eat products sold in Denmark are required to be free of *Salmonella*.

The circulation of *Salmonella* serovars in humans and animals in Denmark is monitored by the mandatory national human laboratory surveillance system and by analysing data derived from isolates from animals and food items of animal origin as part of the control programmes. In 2010, 33% (521) of all registered human cases in Denmark were caused by *S.* Typhimurium [1]. Human isolates are sent from clinical laboratories to Statens Serum Institut (SSI) for typing, which for *S.* Typhimurium isolates include multilocus variable-number tandem repeat analysis (MLVA). MLVA is a typing method which has been shown to have good discriminatory power within *S.* Typhimurium [3]. Clusters of *S.* Typhimurium patient-isolates with identical MLVA profiles are treated as potential outbreaks. Routine MLVA typing is now standard practise for surveillance of human *S.* Typhimurium infections in Denmark. This allows the detection of outbreaks that would otherwise have remained undiscovered.

On 15 April 2010, a cluster of 11 cases with identical *S.* Typhimurium MLVA profiles was detected, with all cases notified in April. Cases were invited to respond to a hypothesis-generating questionnaire by telephone. Two affected families independently indicated having consumed a certain unusual type of salami, which led to a working hypothesis that consumption of this salami product was associated with infection with *S.* Typhimurium. Here we describe the investigations undertaken to confirm this hypothesis, identify the source of the outbreak and to trace-back the suspected product.

Methods

MLVA was performed using primers described by Lindstedt et al [4] in the widely accepted method for *S.* Typhimurium. Of the five loci, STTR9 and STTR6 were labelled with 6-FAM, STTR5 and STTR3 with HEX and STTR with NED [5]. The primers were used in a single multiplex PCR followed by detection on an ABI310 [6].

Phage typing was undertaken following the Anderson typing scheme [7].

Case definition and case-control study

For this investigation, a case was defined as a person residing in Denmark, who became ill with symptoms of gastroenteritis (diarrhoea and/or vomiting) after 1 April 2010, whose culture results yielded the outbreak strain and who had not travelled abroad between 25 March and 14 June. The outbreak strain was defined as *S.* Typhimurium having MLVA profile 3-14-12-NA-211.

Following initial hypothesis-generating patient interviews using a standard *S.* Typhimurium trawling questionnaire, a case-control study was initiated on 14 June, immediately after the discovery that a salami product may have been the source of infection. Controls were selected from the Danish population registry [8], matched by municipality, sex, and date of birth. To create a more robust statistical analysis, three controls were identified and interviewed for each case. Participants were interviewed by phone using a tailored questionnaire focusing on consumption of various types of meats, cold cuts, places where food was bought, as well as other exposures. Controls who experienced symptoms of gastroenteritis (diarrhoea and/or vomiting) or who had travelled outside Denmark during April and May 2010 were excluded.

Statistical analysis

Data from case and control questionnaires were entered into an EpiData database [9]. Statistical analyses were conducted in STATA 10 (StataCorp, TX). In order to examine relationships between each exposure and disease, odds ratios (ORs), matched odds ratios (mORs) and 95% confidence intervals (CI) were calculated.

International aspects

On 15 June, an urgent enquiry was published through the European Centre for Disease Prevention and Control (ECDC) Epidemic Intelligence Information System (EPIS) and on 16 June a Rapid Alert System for Food and Feed (RASFF) notification was issued. The RASFF notification led to inspection by the German food authorities of the factory where the salami was produced.

Results

During the time of the outbreak, 5 April to 6 June, 20 patient isolates (one isolate per patient) with the specific MLVA type were found over a period of nine weeks (Figure).

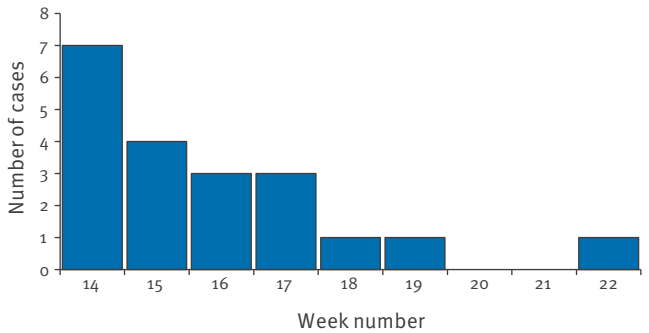
The median age of the patients was 20 years (range 1–69). Half of the cases (10) were children aged 11 years or younger and twelve were male. Three cases were hospitalised from symptoms caused by the infection, but no deaths occurred. The majority of patients (12 of 20) lived within the greater Copenhagen area which covers about 34% of the Danish population of approximately 5.5 million.

Trawling interviews using a generic *Salmonella* outbreak questionnaire led to a hypothesis concerning salami, when the majority of interviewed cases reported using the same supermarket chain when buying food and two families with cases mentioned having bought the same specific type of salami in that chain. The salami in question contained venison (meat from deer) and was traced back to a German producer which manufactures ready-to-eat products for the above-mentioned Danish supermarket chain. Considering the disease onset dates and the shelf life of the salami (one and a half months), it was determined that the potentially contaminated packages of salami were from a single batch labelled with a use-by-date between 6 April and 16 June 2010.

For the case-control study, 17 of the 20 identified cases and 79 controls were interviewed. A total of 16 controls were excluded from the analysis; five due to symptoms of gastrointestinal illness and 11 due to having travelled abroad. Consuming Brand X salami with smoked venison and pork was strongly associated with illness (Table). All cases but two reported having consumed this salami during the week before illness onset (mOR: 150; 95% CI: 19–1,600; P<0.0001). Illness was also significantly associated with consumption of salami

Figure

Cases of *Salmonella* Typhimurium with the outbreak MLVA type, by week of disease onset, Denmark, 5 April–6 June 2010 (n=20)



Table

Results of single-variate analysis of selected exposures from case-control study, Denmark 2010 (n=80)

Exposure	Cases n/N	Controls n/N	Matched Odds Ratio (95% CI)
Pork	2/17	29/63	0.2 (0.03–0.7)
Chicken	8/17	39/63	0.5 (0.2–1.6)
Turkey	2/17	8/63	1.3 (0.2–6.1)
Beef	7/17	29/63	2.0 (0.2–95.8)
Sliced rolled meat	2/17	4/63	1.1 (0.3–4.1)
Salami	15/17	34/63	6.4 (1.3–61.1)
Beef salami	4/17	17/63	0.8 (0.2–3.2)
Game salami	14/17	3/63	93 (13.9–723.9)
Brand X smoked deer and pork salami	15/17	3/63	150 (18.8–1626)

Multiple exposures to food items were possible.

in general (mOR: 6.4; 95% CI: 1.3–61) and answering yes to having consumed salami containing game meat (mOR: 93; 95% CI: 14–720) (Table).

The outbreak strain was initially phage typed to be a mixture of the biphasic *S.* Typhimurium DT120 and DT7 with resistance to ampicillin, streptomycin, sulphamethoxazole and tetracycline. Separate phage typing of several isolates at the World Health Organisation Collaborative Centre for phage typing of *Salmonella* (Health Protection Agency, Colindale, United Kingdom [10]), later confirmed that the type was actually DT193 with several non-specific reactions. These results were obtained after the EPIS and RASFF notifications had been issued. In contrast, the MLVA type was highly characteristic and non-varied and thus the case definition was purely based on the MLVA results. All patient isolates in the outbreak had the same MLVA type.

At the time of the discovery, the salami batch, which had most likely caused the outbreak, had passed its expiry date and therefore no recalls were made. Additionally, it was not possible for the regional food authorities to obtain any salami for microbiological examination and thus no samples of salami from the suspected batch were available for microbiological testing. The inspection by German authorities of the factory where the salami was produced did not identify problems in the factory nor a contamination of the meat in question. The Danish supermarket chain selling the salami also operates in other European countries, however investigation by the Danish Veterinary and Food Administration indicated that the particular salami from the same producer had only been sold in Denmark and Germany. There was no indication that the implicated batch of salami had also been sold in Germany, although similar products from the same producer were available in German stores of the same supermarket chain. The batch-focused food trace back yielded no information if and how the deer meat salami sold in German stores was related to the Danish batch.

Danish food authorities issued a warning for consumers to return or dispose of any packages of Brand X smoked deer and pork salami with a use-by-date between 6 April and 16 June 2010.

Discussion

A small national outbreak of *S.* Typhimurium DT193 was identified through laboratory-based surveillance using MLVA typing. The most likely conclusion, based on the findings of the case-control study, disease onset dates as well as product supply and distribution, was that the outbreak was caused by specific sliced salami containing smoked deer and pork meat. When the suspected outbreak source was detected in June, new cases had not been identified for several weeks. It was concluded that a single batch of infected salami had caused the outbreak. Survival of *Salmonella* in ready-to-eat products has the potential to cause illness and salami has on several occasions been identified as the food vehicle for *S.* Typhimurium [11-13]. A recent multistate outbreak of S. Montevideo in the United States was shown to have been caused by salami products containing contaminated red and black pepper, additionally highlighting the importance of post-processing contamination of ready-to-eat products [14].

Following the EPIS urgent enquiry, Germany reviewed the *S.* Typhimurium situation during April and May 2010. MLVA typing is not routinely performed in Germany; thus it would only be possible to detect a corresponding outbreak by looking at *S.* Typhimurium case numbers in general. During the time in question, a similar number of cases of *S.* Typhimurium were notified from Germany as a whole compared to April and May in previous years (1,479 vs a mean of 1,489 in 2007-9). However, restricting the comparison to only those six federal states where stores from the implicated Danish supermarket chain are widespread, a 24% increase in *S.* Typhimurium cases was observed, compared to the mean of 2007-9 (645 vs 519 cases).This excess was mainly in adult males above 18 years old and strongest between 12 and 18 April. Due to the time elapsed, these cases were not investigated further.

The observed regional increase in German *S.* Typhimurium cases, restricted to states with branches of the Danish supermarket chain, is suggestive of a link to the Danish outbreak. Differences in age distribution between the German and the Danish cases may be explained by the fact that food preferences for such an unusual sausage product may vary geographically. Without MLVA confirmation, we cannot be sure that the German regional increase was due to the same type of *S.* Typhimurium.

No further cases matching the outbreak profile were reported from Europe.

The investigations described in this paper highlight the usefulness of typing methods in combination with simple case-control studies to detect an outbreak and identify possible sources of infection. Although no samples were available for testing, it was concluded that the smoked pork and deer meat salami was the likely source of infection in this outbreak, solely based on the case-control investigation results. Vehicle identification was aided by the unusual type of salami, which made it stand out in the memories of the patients.

The international dimension of this outbreak in Denmark is not only demonstrated by the fact that the salami at the source was produced in Germany, but also because the Danish supermarket chain selling the salami operates in other European countries, including Germany and England. Therefore the ECDC urgent enquiry and the RASSF systems were used to highlight the potential for similar outbreaks in these countries. It is not unlikely that the particular salami had caused cases of salmonellosis in countries other than Denmark, however, the use of different national subtyping systems

made it difficult to detect such cases by the Danish definition in these countries. This is potentially a serious problem in a multi-national outbreak situation. To rapidly determine if disease outbreaks in several countries are caused by the same strain, methods for molecular typing should be standardised throughout the EU. Ammon and Tauxe [15] highlight the need for developing a consensus about which methods to use, their application in all laboratories as well as implementing additional methods, such as resistance testing and pulsed-field gel electrophoresis, in particular situations. Another potential challenge within the EU is the investigation of a food outbreak source by microbiology and trace-back in situations where the food is produced in a different country from the one where the outbreak takes place. Encouragingly, however, in this outbreak a food investigation was made by German authorities as a result of an RASSF notification from another country, based purely on the results of a case-control study, even though there were no confirmed cases in Germany.

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