



Eurosurveillance

Europe's journal on infectious disease epidemiology, prevention and control

Vol. 17 | Weekly issue 6 | 09 February 2012

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Mycoplasma pneumoniae: now in the focus of clinicians and epidemiologists

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Citation style for this article:

Jacobs E. *Mycoplasma pneumoniae*: now in the focus of clinicians and epidemiologists.

Euro Surveill. 2012;17(6):pii=20084. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20084>

Article published on 9 February 2012

Several northern European countries have experienced outbreaks of *Mycoplasma pneumoniae* infection in 2010 and 2011, as described in recent reports and in this issue. Such outbreaks appear with regular periodicity and have occupied clinicians and epidemiologists for many years.

Some 50 years ago, Chanock et al. [1] described an artificial medium that enabled the identification of the aetiological agent of an atypical pneumonia first reported 20 years earlier, which was first described as pleuropneumonia-like organisms (PPLO) and renamed as *Mycoplasma pneumoniae* [2]. More recently, genome analysis has revealed the bacterium's limited metabolism and biosynthesis of carbohydrates, proteins, nucleic acid and lipids, showing that the agent is well adapted to its only host, humans. We are, however, still unable to mimic the natural environment of *M. pneumoniae*: faster growth in culture media is needed for diagnostic purposes. It takes more than 10 days – in fact often up to three weeks – to grow *M. pneumoniae* from respiratory specimens taken from patients with an interstitial pneumonia. The organism can be cultured from samples taken in the acute phase of the infection, but because of the length of time needed, culture techniques have not been established in most bacteriological laboratories.

Lind et al. were the first in Europe to identify *M. pneumoniae* infection by detecting increases in *M. pneumoniae*-specific antibody titre, based at that time on cold agglutinin and complement fixation tests [3].

One striking aspect of *M. pneumoniae* infection is the periodicity of epidemics. The Danish seroepidemiological study of Lind et al., conducted over a 50-year period, showed between 1958 and 1973 an almost regular pattern of epidemics every four and a half years [3]. The authors suggested that herd immunity lasts about four years (range: 2–10) before people are again susceptible to infection with *M. pneumoniae*.

A prospective study of 4,532 outpatients in Germany aged at least 18 years with community-acquired pneumonia showed that *M. pneumoniae* was one of the major causative bacterial agents: 307 patients (6.8%) were *M. pneumoniae*-positive by real-time-PCR and/or positive for *M. pneumoniae*-specific IgM antibodies [4]. Some 72% of the patients with *M. pneumoniae* infection had only a mild pneumonia: this, combined with the number of days of hospitalisation required, might suggest a less severe pneumonia outcome in *M. pneumoniae* infections.

In many countries, clinicians had to treat patients with community-acquired pneumonia due to *M. pneumoniae* infection empirically during the whole acute phase because of the delay in the increase of antibody titres or because of the time needed for culture. Epidemiological studies were hampered for a long time because of these diagnostic difficulties. Consequently, *M. pneumoniae* was more or less ignored or in many countries 'a black box' in epidemiology because of the lack of diagnostic results. The situation changed, however, with the introduction of several molecular techniques, especially real-time PCR, into routine diagnosis [5]. Another advance has been the characterisation of different *M. pneumoniae* genotypes circulating in the human population. Clinical strains can be differentiated on basis of differences in the P1 adhesin gene or in the number of repetitive sequences at a given genomic locus using multilocus variable number tandem repeat analysis (MLVA) [6,7]. Both typing methods are not currently used routinely in epidemiological studies. However, typing will allow us to get more information about outbreaks of defined strains in different countries of Europe or even worldwide as well as information about changes in strains within a population. A long-term genotyping study from Japan [8] suggests that epidemics arise due to a change in the two main P1 types or even of because of further variants of P1 sequences, which were found recently [9,10].

MLVA allows greater discrimination between *M. pneumoniae* strains because of the very variable numbers

of repeats in the genome of different strains. It was used recently by Chalker et al. describing increased numbers of *M. pneumoniae* infections in England and Wales in 2011 and 2012 [11,12]. Outbreaks were seen in the years 1995, 1997/1998, 2002/2003, 2006 and a prepeak in 2010 before the outbreak in 2011. The peaking periods described showed all the characteristics of a *M. pneumoniae* epidemic, i.e. a broad 'shoulder', sometimes in two consecutive epidemic years with slightly fewer cases in summer than in later autumn and winter. Such a pattern was shown in Denmark for 2010 and 2011 [13].

Typing should answer the question, if such peaks could be attributed to different or to the same genotypes. Interestingly, Chalker et al. showed a small peak in 2010 before the outbreak in 2011. These findings suggest it will be necessary in the future to type more often strains from different countries and periods to answer the question of whether there is common epidemic spread of distinct genotypes in different countries of Europe. It is as yet unknown whether the recent epidemics in northern Europe [13-17] are caused by a common type strain.

Macrolide resistance has been described recently in Asia, with up to 90% of *M. pneumoniae* strains being resistant [18]. In the reports from the countries in northern Europe, no macrolide resistance was found in the tested strains except for Denmark, where 0.9% to 2.9% of strains were resistant. This is in accordance with data from France and Germany, where about 3% of strains were found to be resistant [19,20]. Particularly as a vaccine against *M. pneumoniae* is not yet available, macrolides – which are the only recommended therapy for children (whereas doxycycline and fluoroquinolones can be used for adults) – should be used carefully, as pointed out by Linde et al. in this issue [16]. It is not yet known whether the increased use of erythromycin in Norway at the end of 2011 [14] will induce more resistance. We should nevertheless be aware of possible macrolide resistance of *M. pneumoniae* during therapy even though this was not been seen in the paper by Uldum et al. [13]. The first two reports of emergence of macrolide-resistant *M. pneumoniae* during therapy were published last year by Cardinale et al. from Italy [21] and Averbuch et al. from Israel [22] in children with severe pneumonia. Such resistance may pose a major problem for clinicians, as certain antibiotics are not recommended for young children. In both cases, ciprofloxacin was given and the children were cured within a few days.

We now have the laboratory tools to detect *M. pneumoniae* within a day and also to identify possible macrolide resistance [20]. In order to aid clinicians, real-time PCR can be used, especially in the acute phase of infection, to diagnose *M. pneumoniae* in nasopharyngeal swabs or a provoked sputum [4]: this could become the gold standard for diagnosis. For more sophisticated studies, epidemiologists in Europe should come to an

agreement on standard sampling and a common typing method for *M. pneumoniae* strains.

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Increased detection of *Mycoplasma pneumoniae* infection in children in England and Wales, October 2011 to January 2012

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Citation style for this article:

Chalker VJ, Stocki T, Litt D, Bermingham A, Watson J, Fleming DM, Harrison TG. Increased detection of *Mycoplasma pneumoniae* infection in children in England and Wales, October 2011 to January 2012.

Euro Surveill. 2012;17(6):pii=20081. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20081>

Article published on 9 February 2012

Community surveillance data, based on quantitative real-time polymerase chain reaction analysis, showed that one in seven children aged 5–14 years with respiratory signs tested positive for *Mycoplasma pneumoniae* in England and Wales from October 2011 to January 2012 – a higher proportion than that seen in previous years. Multilocus variable number tandem repeat analysis indicates that at least seven known and two novel strain types were circulating in England and Wales during this period.

Recent reports indicate that an increased number of *Mycoplasma pneumoniae* infections have been detected in seven European countries including Denmark, Norway and Finland [1–4]. To determine the number of patients infected with *M. pneumoniae* in England and Wales and to see if the number had increased, compared with previous winters, community surveillance data and laboratory reports submitted to the Health Protection Agency (HPA) from 10th October (week 42) 2011 to 20th January (week 3) 2012 were reviewed. Our study shows an increase in the number of children with *M. pneumoniae* infection by PCR-based surveillance in the community during the study period.

Further analysis was carried out to determine which strains of *M. pneumoniae* were present in this period in the community surveillance samples, in addition to analysis of genetic markers for macrolide resistance.

Background

M. pneumoniae is a respiratory pathogen that is a common cause of pneumonia and may cause other serious sequelae such as encephalitis. The pathogen is found in all age groups, with higher prevalence in children aged 5–14 years [2,5].

In England and Wales, epidemic periods lasting on average 18 months have occurred at approximately four-yearly intervals [6]. In addition, low-level sporadic

infection occurs with seasonal peaks from December to February [5,6]. Since 2005, a community surveillance scheme for *M. pneumoniae* using quantitative real-time polymerase chain reaction (qPCR) analysis has been used to monitor *M. pneumoniae* infection in England and Wales [7]. Until 2010, this scheme was used for monitoring patients of all ages and from 2010 to date, for children aged under 16 years [7]. It is an extension of the virological community surveillance that is undertaken annually in England and Wales for a range of respiratory viruses including influenza virus, respiratory syncytial virus and human metapneumovirus [8]. Combined nasal and throat swabs were taken during the winter months (from October to March, 2005 to 2012, and throughout the recent influenza A(H1N1) pdm09 pandemic) from patients with respiratory symptoms including influenza-like illness, upper respiratory tract infection, lower respiratory tract infection, or fever or myalgia who attended general practitioner clinics [5]. Additional voluntarily submitted reports from regional laboratories and hospitals in England and Wales were collated by the Health Protection Agency (HPA) according to age and region to give an indication of the number of patients testing positive for *M. pneumoniae* by serological, molecular or culture tests each week.

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

Laboratory reports

The number of *M. pneumoniae*-positive laboratory reports submitted to the HPA during the study period (week 42 2011 to week 3 2012) varied from 11 to 36 per week, as shown in the four-weekly moving averages in [9]. From week 42 2011 to week 3 2012, a total of 353 reports were received, higher than the number in the same period in 2010 (week 42 2010 to week 3 2011), when 290 were received. Reports were received from all areas of England and Wales during this period (Table 1). The patients were of all ages, with the youngest

being less than one week old and the oldest 92 years of age (Table 2). This age profile of submitted *M. pneumoniae*-positive reports was very similar to that for all such reports received from week 1 1975 to week 3 2012.

Community surveillance

We carried out qPCR analysis on 144 anonymised combined nose and throat swabs taken as part of community surveillance from patients aged under 15 years with respiratory symptoms during October 2011 to January 2012 (a total of 144 swabs were taken during that time). Nucleic acid was extracted and stored as previously described before qPCR testing for the presence of the *M. pneumoniae* P1 gene [5,10].

A total of 13 of the samples (9.0%; 95% CI: 5.2–15.0) were *M. pneumoniae* positive. One in seven of the children aged 5–14 years (12/84) had detectable

TABLE 1

Percentage of *Mycoplasma pneumoniae*-positive samples from laboratory reports by region, England and Wales, 10 October (week 42) 2011–20 January (week 3) 2012 (n=353)

Region	Percentage of samples positive for <i>M. pneumoniae</i>	
	% (95% CI)	Number of positive samples
East Midlands	5.7 (3.7–8.7)	20
East	8.5 (6.0–11.9)	30
London	24.4 (20.2–29.1)	86
North East	6.0 (3.9–9.0)	21
North West	13.9 (10.6–17.9)	49
South East	4.0 (2.3–6.6)	14
South West	7.1 (4.8–10.3)	25
West Midlands	5.4 (3.4–8.3)	19
Wales	14.5 (11.1–18.5)	51
Yorkshire and Humberside	10.8 (7.9–14.5)	38

TABLE 2

Percentage of *Mycoplasma pneumoniae*-positive samples from laboratory reports by age, England and Wales, 10 October (week 42) 2011–20 January (week 3) 2012 (n=353) and 1 January 1975 (week 1)–20 January (week 3) 2012 (n=38,221)^a

Age in years	Percentage of samples positive for <i>M. pneumoniae</i>			
	Week 42 2011–week 3 2012		Week 1 1975–week 3 2012	
	% (95% CI)	Number of positive samples	% (95% CI)	Number of positive samples
<5	11.3 (8.4–15.1)	40	10.1 (7.3–13.4)	3,863
5–14	24.1 (19.9–28.8)	85	24.9 (20.1–29.5)	9,535
15–44	37.1 (32.2–42.3)	131	42.7 (37.8–47.7)	16,326
45–64	18.1 (14.4–22.5)	64	12.6 (9.6–16.3)	4,806
>65	9.4 (6.7–12.9)	33	5.1 (3.4–9.1)	1,957

^a Information about age was not available for all reports.

M. pneumoniae, whereas only one of the 60 children aged under 5 years was positive (Fisher's exact test $p=0.008$) (Figure 1).

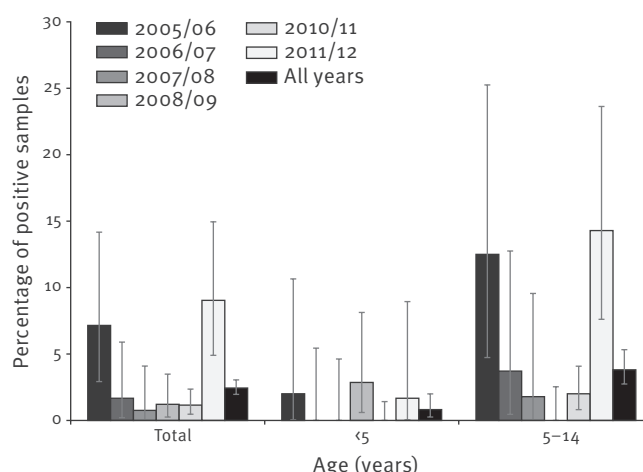
The percentage of positive cases per week (from week 42 to week 3 of the following year) for children aged under 15 years is shown for 2005 to 2012 in Table 3. This shows an increase from November 2011 to January 2012 (week 46 2011 to week 1 2012). Samples were more likely to be positive during this period in 2011/12 (13/91; 12.5%; 95% CI: 7.3–20.4) than in the previous four weeks (weeks 42–45 2011) and the following two weeks (weeks 2–3 2012) (0/40; 0%; 95% CI: 0.0–10.4; Fisher's exact test $p=0.02$).

In November 2011 (week 46), December 2011 (weeks 50 and 51) and January 2012 (week 1), the number of *M. pneumoniae* infections significantly increased in comparison with all previous weeks of sampling since 2005 (binomial probability test $p=0.00001$, 0.0007, 0.05 and 0.01, respectively).

The mean age of the 144 patients was 6.5 years (standard deviation (SD)±4.4; range: 0–14) with the majority of *M. pneumoniae*-positive patients being over 5 years-old (n=12 of 84). The mean age of the positive patients was 8.7 years (SD±2.6). Only one *M. pneumoniae*-positive patient was less than 5 years old (aged 4 years).

FIGURE 1

Percentage of clinical community surveillance samples from patients aged under 15 years positive for *Mycoplasma pneumoniae* determined by qPCR, England and Wales, October 2005–January 2012^a (total of 33 positive in 1,354 samples)



qPCR: quantitative real-time polymerase chain reaction.

The number of positive samples and total number of samples per year were 7 of 98 in 2005/06, 2 of 120 in 2006/07, 1 of 134 in 2007/08, 3 of 249 in 2008/09, 2009 not tested, 7 of 609 in 2010/11, 13 of 144 in 2011/12, giving a total of 33 positive in 1,354 samples for all years analysed. Error bars indicate the 95% CI for the percentages.

^a Excludes October (week 42) 2009 to January (week 3) 2010 when sampling was not performed due to the influenza A(H1N1)pdm09 pandemic.

Of the 144 patients analysed, 62 were male and 79 female (sex was not specified for three patients). Of the 13 *M. pneumoniae*-positive patients, 5 were male and 8 female.

M. pneumoniae type and macrolide resistance

Samples that were positive by qPCR were examined for *M. pneumoniae* type and macrolide resistance. Multicocus variable number tandem repeat analysis (MLVA) typing by fragment analysis, which has previously been used to type *M. pneumoniae* strains [7,11], was used to analyse nucleic acid extracts of clinical samples in our study; culture isolation of *M. pneumoniae* was not undertaken. MLVA typing was also performed on nine additional *M. pneumoniae*-positive respiratory samples that were submitted to the laboratory during October 2011 to January 2012. Genetic diversity was calculated using Hunter and Gastons variation of Simpson's diversity index [12].

The presence of mutations previously associated with macrolide resistance was examined by amplification and sequencing of a 720-base pair (bp) fragment of the 23S rRNA gene using the primers MpnMR2063F (5'-ATCTCTTGACTGTCTCGGC-3') and MpnMR2617R (5'-TACAACGGAGCATAAGAGGTG-3') [13].

MLVA analysis of eight of the 13 qPCR-positive community surveillance samples and the nine

M. pneumoniae-positive respiratory samples that were submitted to the laboratory during October 2011 to January 2012 showed a total of nine distinct strain types: seven of known MLVA type (type E (n=1), type M (n=4), type P (n=2), type S (n=1), type T (n=1), type U (n=2) and type Z (n=3)) and two putative novel types (profile 4,4,5,7,3 (n=2) and 5,3,5,7,3 (n=1)) (Figure 2). A full MLVA profile could not be obtained for the other five qPCR-positive community surveillance samples, probably because of the low levels of *M. pneumoniae* nucleic acid in these samples.

The strain type most frequently found in the 17 samples was MLVA-M (n=4), which was also the most prevalent strain type in England and Wales in 2010 and has been found in France (in 1997, 1999, 2000 and 2006), Germany (in 1995 and 2000) and Japan (in 2000 to 2003) [5,11]. Comparison of the Hunter–Gaston diversity index (DI) indicated that both populations in October to January 2010/11 and 2011/12 were similarly diverse (2010 DI: 0.93; 95% CI: 0.88–0.98, 2011 DI: 0.91, 95% CI: 0.85–0.97).

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 12 of the 13 qPCR-positive community surveillance samples. No mutations in these loci associated with macrolide resistance were identified in

TABLE 3

Percentage of clinical community surveillance samples positive for *Mycoplasma pneumoniae* determined by qPCR per week for children aged under 15 years, England and Wales, October (week 42)–January (week 3) 2005–2012^a (total of 33 positive in 1,354 samples)

Week Number	Percentage of samples positive for <i>M. pneumoniae</i>					
	2005/06	2006/07	2007/08	2008/09	2010/11	2011/12
	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)
42	0.0 (0.0–45.9)	0.0 (0.0–97.5)	0.0 (0.0–33.6)	0.0 (0.0–60.2)	0.0 (0.0–28.5)	0.0 (0.0–97.5)
43	0.0 (0.0–84.2)	0.0 (0.0–97.5)	0.0 (0.0–70.8)	20.0 (0.4–71.6)	0.0 (0.0–52.2)	0.0 (0.0–45.9)
44	0.0 (0.0–45.9)	0.0 (0.0–45.9)	0.0 (0.0–84.2)	0.0 (0.0–36.9)	13.3 (1.5–40.5)	0.0 (0.0–36.9)
45	14.3 (0.3–57.9)	0.0 (0.0–60.2)	0.0 (0.0–60.2)	12.5 (0.3–52.7)	0.0 (0.0–30.9)	0.0 (0.0–36.9)
46	0.0 (0.0–60.2)	12.5 (0.3–52.7)	0.0 (0.0–28.5)	0.0 (0.0–23.2)	0.0 (0.0–23.1)	25.0 (5.7–52.4)
47	0.0 (0.0–41.0)	0.0 (0.0–52.2)	0.0 (0.0–41.0)	0.0 (0.0–17.6)	5.9 (0.7–19.7)	7.7 (0.2–36.0)
48	0.0 (0.0–60.2)	0.0 (0.0–21.8)	0.0 (0.0–19.5)	0.0 (0.0–16.1)	0.0 (0.0–9.7)	0.0 (0.0–52.2)
49	25.0 (2.5–65.1)	0.0 (0.0–24.7)	0.0 (0.0–28.5)	0.0 (0.0–16.8)	2.1 (0.1–11.1)	0.0 (0.0–30.8)
50	14.3 (2.6–36.3)	0.0 (0.0–30.8)	4.8 (0.1–23.8)	2.4 (0.1–12.6)	0.0 (0.0–4.4)	16.7 (4.0–37.4)
51	9.0 (0.2–41.3)	0.0 (0.0–20.6)	0.0 (0.0–17.6)	0.0 (0.0–10.0)	0.0 (0.0–2.4)	10.5 (1.2–33.1)
52	0.0 (0.0–70.8)	0.0 (0.0–33.6)	0.0 (0.0–97.5)	0.0 (0.0–11.2)	0.0 (0.0–6.3)	15.4 (0.0–60.2)
1	0.0 (0.0–70.8)	0.0 (0.0–36.9)	0.0 (0.0–18.5)	0.0 (0.0–16.1)	0.0 (0.0–8.4)	18.2 (2.3–45.4)
2	0.0 (0.0–41.0)	7.7 (0.2–36.0)	0.0 (0.3–70.8)	0.0 (0.0–26.5)	2.1 (0.3–7.4)	0.0 (0.0–33.6)
3	0.0 (0.0–70.8)	0.0 (0.0–28.5)	0.0 (0.0–36.9)	0.0 (0.0–33.6)	0.0 (0.0–36.9)	0.0 (0.0–36.9)
All weeks	4.5 (2.9–15.1)	1.7 (0.2–5.9)	0.7 (0.0–4.1)	1.2 (0.2–3.5)	1.2 (0.5–2.4)	9.0 (5.2–15.0)
Number of positive samples/total number of samples	7/98	2/120	1/134	3/249	7/609	13/144

qPCR: quantitative real-time polymerase chain reaction.

Shaded cells represent weeks when *M. pneumoniae* was detected.

^a Excludes October (week 42) 2009 to January (week 3) 2010 when sampling was not performed due to the influenza A(H1N1)pdm09 pandemic.

these samples. For the remaining qPCR-positive community surveillance sample, sequence information could not be obtained, presumably due to low levels of *M. pneumoniae* nucleic acid.

Discussion

The level of *M. pneumoniae* infection in the qPCR-based community surveillance of children aged under 16 years from October 2011 to January 2012 was 9.0%, rising to 14.3% in the 5–14 year-olds. This is considerably higher than that in the same months from previous years from 2005 to 2011 (1.7%) [5]. Detectable *M. pneumoniae* infection was found by qPCR in children aged from 4 to 14 years and was absent from those aged under 4 years in the 2011/12 study period. As qPCR was not performed on specimens from adults, the level of adults with detectable *M. pneumoniae* DNA could not be ascertained. However, *M. pneumoniae*-positive laboratory reports collated from regional laboratories were received on adult patients during this period and the age profile was consistent with that of all reports received from 1975 to 2012.

The last period showing a large peak of detectable *M. pneumoniae* infection by qPCR was winter 2005/06, in which the infection was detected in 6% of 5–14 year-olds attending general practitioners with respiratory signs. In the study period reported here (winter 2011/12), an even greater number of children of this age

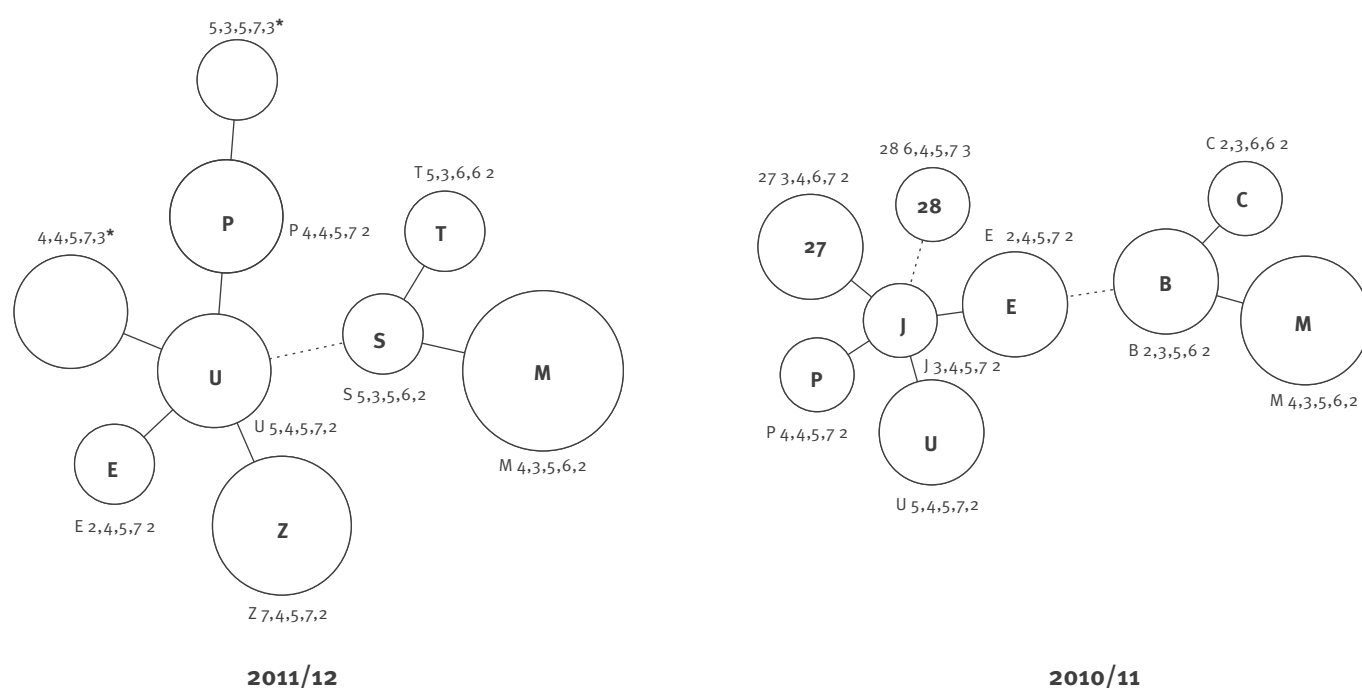
group were infected (14.3%), indicating at least one in seven children with respiratory signs attending general practitioners were infected with *M. pneumoniae*.

In a similar period in 2010/11 (week 42 2010 to week 3 2011), 11 differing MLVA types were detected in 15 clinical samples with MLVA-M being the most prevalent in England and Wales [7]. Within the study period reported here (week 42 2011 to week 3 2012), seven MLVA types were identified, four of which were MLVA-M. The sample number is too low to specify the exact diversity of the population or to investigate the association of particular types with clinical severity. Nonetheless, it is interesting that clonal strains were not detected. Two putative new profiles were obtained but confirmation of these apparently novel MLVA types will require isolation of the strains.

The typing method used here was originally described by DéGrange et al., in which stability of five isolates was determined over 10 passages, indicating that the *M. pneumoniae* MLVA type is relatively stable [11]. Clonal spread of *M. pneumoniae* does occur, however. In fact, Pereyre et al., recently described the detection of *M. pneumoniae* MLVA-type 3,4,5,7,2 in seven children attending a primary school in France [14]. In our study, patients were from a variety of locations in England and Wales and, similar to our findings last year [5], the data do not support the hypothesis that

FIGURE 2

Minimum spanning trees for *Mycoplasma pneumoniae* MLVA types detected in England and Wales, October–January 2011/12 (n=17) and 2010/11 (n=16)



MLVA: Multilocus variable number tandem repeat analysis.

Trees were derived from the five MLVA alleles [11]. Each circle represents a unique MLVA type. The size of each circle illustrates the proportion of isolates with that MLVA type (the smallest circle in each tree represents one isolate). Solid lines separate single locus variants and dotted lines separate double locus variants. The asterisks mark the two putative novel MLVA types.

a single strain type of *M. pneumoniae* was responsible for this observed increase in infection in England and Wales. MLVA typing discriminates well between *M. pneumoniae*-positive specimens. In fact, there is a high diversity of types in the population and it does not appear that a few clonal types dominate in circulation. It would be of value to have a consistent typing methodology for *M. pneumoniae* strains in use internationally, with a database of types similar to those for other bacterial species. It would also be interesting to type strains from other countries during the same time period to determine how strains differ geographically during periods of increased infection.

Macrolide resistance is becoming an increasing problem in other countries [15]; despite the low sample number, no resistance was detected in any of the qPCR-positive samples from England and Wales analysed during the study period.

Acknowledgments

The authors would like to thank the Birmingham Research Unit of the Royal College of General Practitioners, HPA Influenza Group and Joy Field, HPA Health Protection Services.

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Antibiotic prescriptions and laboratory-confirmed cases of *Mycoplasma pneumoniae* during the epidemic in Sweden in 2011

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Citation style for this article:

Linde A, Ternhag A, Törner A, Claesson BE. Antibiotic prescriptions and laboratory-confirmed cases of *Mycoplasma pneumoniae* during the epidemic in Sweden in 2011.

Euro Surveill. 2012;17(6):pii=20082. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20082>

Article published on 9 February 2012

Swedish laboratories reported an increase of *Mycoplasma pneumoniae* during the autumn 2011. Data from the laboratory in Skövde, covering 12.9% of the Swedish population, indicate an approximate increase in the number of laboratory-confirmed cases in the whole country, from around 3,500 in 2009 to 11,100 in 2011. Antibiotics are recommended only for pneumonia, not bronchitis, but compared with the autumn 2009, 42,652 more prescriptions of doxycycline and macrolides were registered in the autumn 2011.

Introduction

Mycoplasma pneumoniae infections are not reportable in Sweden, but in the autumn 2010, the Swedish Institute for Communicable Disease Control (SMI) received informal information from several laboratories that the number of laboratory-confirmed diagnoses of *M. pneumoniae* had increased, and in 2011 an even greater increase was noted. However, reports from different laboratories were not comparable because information on methodology and/or total number of examined samples per population were missing.

The laboratory in Skövde covers 12.9% of the Swedish population. It has collected data from 2002 to 2011 on polymerase chain reaction (PCR) results and the total number of examined samples for bacteria causing protracted cough: *M. pneumoniae*, *Chlamydia pneumoniae* and *Bordetella pertussis/parapertussis*. In addition, it has collected data on *M. pneumoniae* IgM serology since 2006. Sampling of these cough pathogens was performed only for clinical purposes and the number of collected samples thus reflect provisional diagnoses or suspicions of the clinical doctor. We use the data from Skövde as a proxy to analyse the epidemic in Sweden as a whole.

The risk of antibiotic resistance due to overuse of antimicrobial drugs and the negligible benefit of

treating the mild symptoms caused by *Mycoplasma* [1] has prompted the Swedish strategic programme against antibiotic resistance (Strama) together with the Swedish Medical Product Agency [2], as well as other organisations in Europe [3], to issue strict recommendations for antibiotic treatment of *Mycoplasma* infections. The Strama recommendations have been described in three reports on *Mycoplasma* in the SMI weekly newsletter in 2010, 2011 and 2012 [4-6]. We therefore found it of interest to compare the increase in the use of penicillin V, generally recommended for treatment of pneumonia, with that of doxycycline and macrolides, recommended for atypical pneumonia, in relation to the ongoing epidemic. Further, we wanted to analyse the relation between the number of *M. pneumoniae*-positive samples and the number of antibiotic prescriptions and compare this with data recently published from Finland, Norway and Denmark [7-9] and for Europe [3].

Methods

The microbiology laboratory at Kärnsjukhuset in Skövde (Unilabs AB) serves 1,225,000 people in southern Sweden, which corresponds to 12.9% of the Swedish population.

Real-time PCRs were performed daily for *M. pneumoniae*, targeting a 76 bp region of the adhesion gene [10], for *C. pneumoniae*, targeting a 78 bp region of the MOMP gene [11] and for *B. pertussis* and *parapertussis*, targeting a 154 bp fragment of the IS481 gene and a 186 bp fragment of the IS1001 gene, respectively [12]. Sampling for pathogens in the lower respiratory tract was usually performed with ESswabs (Copan) from the retropharyngeal wall. An IgM assay (Ani Labsystems) was also used on request.

The SMI has been collecting national data on monthly antibiotic prescriptions every third month since 2007, using a nationwide data base (Concise, Apoteket

Service AB) covering all prescriptions from both outpatient and inpatient care. Data were aggregated to prescriptions per months.

Results

The number of samples examined by PCR for pathogens causing cough between 2002 and 2011 varied from 350 to 3,000 per year, with the highest level in 2011. The variation over time in the number of diagnoses and the positivity rate for each of the three agents is clear (Figure 1). The number of *M. pneumoniae* diagnoses increased from 2005 to 2007 and from 2010 to 2011, with peaks in 2006 and 2011 (Figure 2). In 2006 there were 341 PCR diagnoses of *M. pneumoniae*, and 585 in 2011, but the detection rate was 23% both years (Figures 1 and 2).

Of an additional 3,882 samples tested serologically, 660 were positive for *M. pneumoniae* IgM in 2011, with a positivity rate of 17%. If we allow a rough approximation for national comparisons, based on PCR and IgM results from Skövde, this corresponds to 117 confirmed diagnoses per 100,000 population in 2011, a total of around 11,000 cases for the whole of Sweden.

The use of penicillin V and doxycycline/macrolides decreased slightly during the five-year period from 2007 to 2011 (Figure 3). Comparing the non-epidemic period July to December 2009 with the epidemic period July to December 2011 the number of penicillin V prescriptions increased by approximately 9% (from 501,501 to 548,387). During the same time period the

number of doxycycline and macrolide prescriptions increased by 25% (from 218,694 to 272,515).

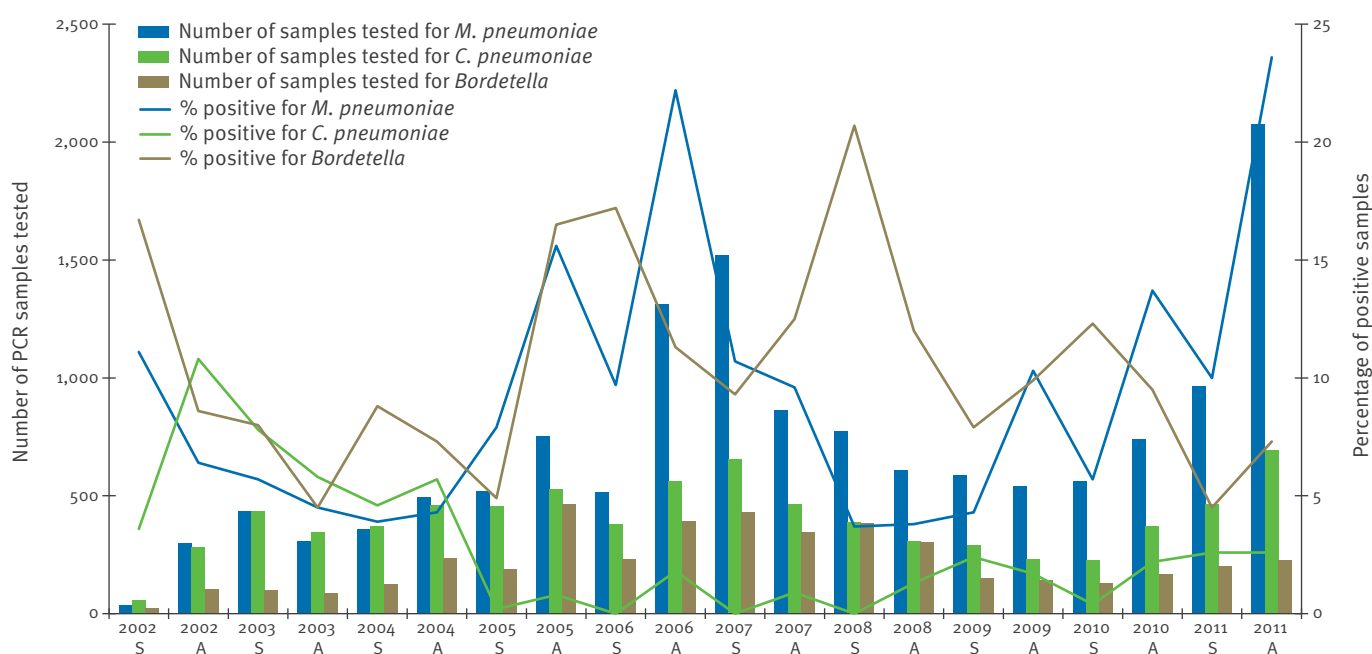
Discussion

To create standardised surveillance systems for various infectious diseases and syndromes, like the sentinel system for influenza, is presently not feasible. Multiplex laboratory analyses of relevant agents for specified clinical conditions such as cough could be a substitute system for early warning and estimation of the impact of epidemics, if appropriate data are systematically reported and analysed. The PCR diagnostics in Skövde reveal changes over time in the spread of all four microbes monitored, and so far the outbreaks of *Mycoplasma* have given rise to the largest epidemics. Similar increases in laboratory-verified *Mycoplasma* during 2011 were reported from laboratories all over Sweden. While this rate estimation for the country of around 120 per 100,000 population is very approximate, it is similar to those reported from the other Nordic countries [7-9], indicating that the epidemics have been of similar magnitude across these countries. However, epidemic differences do occur, the outbreak in Denmark during 2010 for instance seemed more intense than in Sweden, and the previous epidemic peaked in 2005 in Finland and in 2006 in Sweden.

Even with IgM results included, the estimated positivity rate for *M. pneumoniae* was slightly lower in Sweden than that reported from Finland and Norway [7,8]. The lower rate could be due to less intensive

FIGURE 1

Number of samples tested for *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae* and *Bordetella pertussis/parapertussis* and the rate of positives per half year, Skövde, 2002–2011



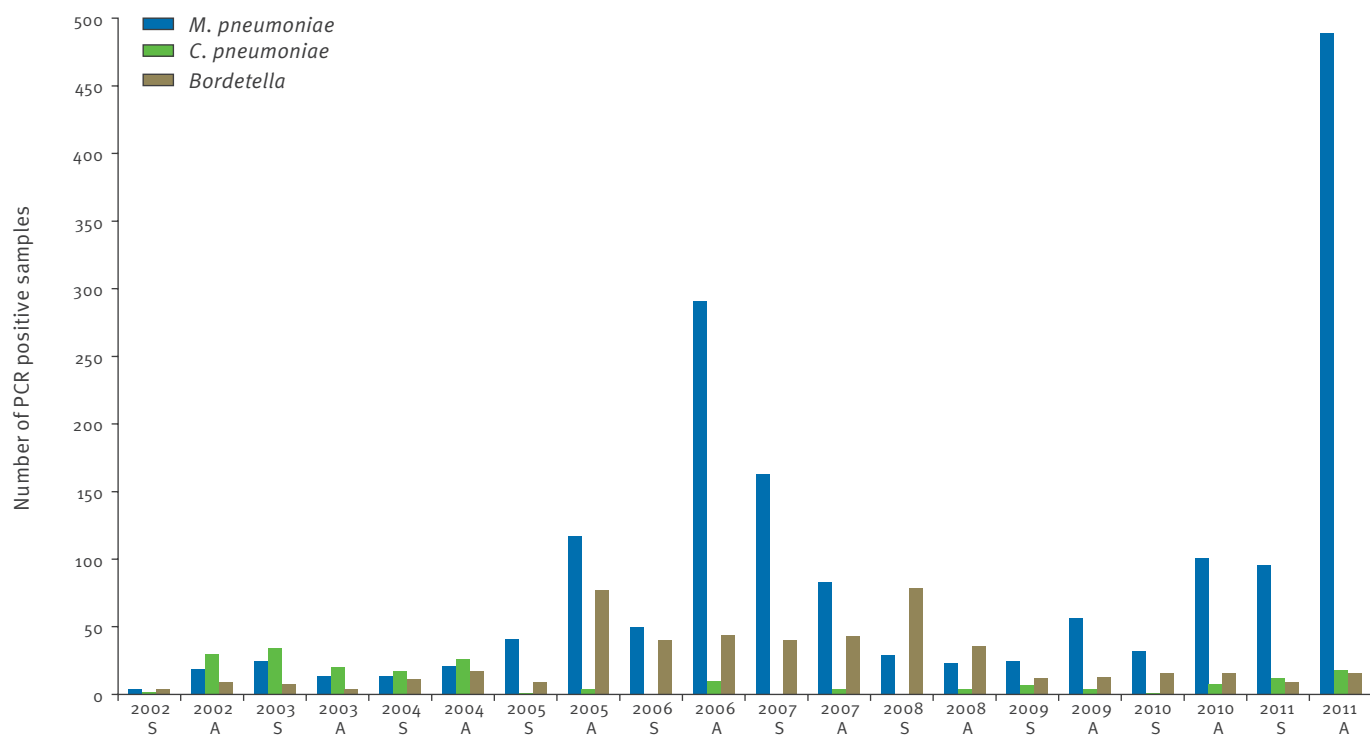
A: autumn; S: spring.

epidemic spread, less sampling, variations in the methods used for diagnosis or a combination of these factors. The laboratory confirmation of *Mycoplasma* has until recently rested largely on serology, and still does in Finland [7]. The IgM assays, however, were lacking in sensitivity [13], and collection of paired samples for

verification of the diagnosis is often not feasible. An excellent correlation between PCR for *Mycoplasma* and several commercial serology test systems has been shown [14], while only 30–40% of the patients had a positive IgM test at the first visit.

FIGURE 2

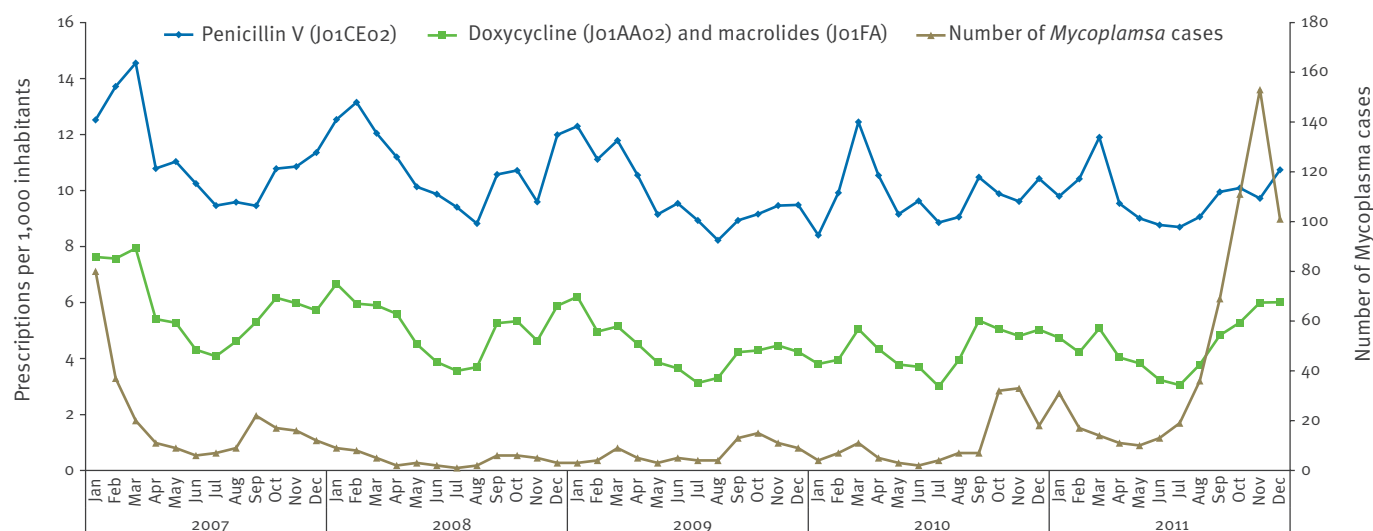
Number of samples positive for *Mycoplasma pneumoniae*, *Chlamydomphila pneumoniae* and *Bordetella pertussis/parapertussis* per half year, Skövde, 2002–2011



A: autumn; S: spring.

FIGURE 3

Prescriptions of penicillin V and macrolides/doxycycline per 1,000 inhabitants in Sweden, and monthly number of laboratory-confirmed diagnoses of *Mycoplasma pneumoniae* by PCR in Skövde, 2007–2011



The high rate of PCR positives (23%) may indicate a more selective sampling than in the other countries, but also that PCR is a more efficient test. However, although the total number of positive PCR samples was smaller in 2006, the rate of positive tests (23%) was as high during the peak in 2006 as in 2011, indicating that the intensity of the two epidemics may have been similar. This underlines the value of knowing the catchment population and number of samples examined in epidemiological analyses.

Approximately 90% of all antibiotics in Sweden are prescribed for outpatients (data provided by Concise, Apoteket Service AB) and 60% of these for respiratory tract infections [15]. A main indication for choosing doxycycline and macrolides is atypical pneumonia. It is plausible that the selective increase in prescriptions of doxycycline and macrolides, but not of penicillin V, in 2011 compared to 2009 could to a large extent be explained by variations in the incidence of *Mycoplasma* rather than the recurrent increase in lower respiratory tract infections seen every autumn. The prescriptions of doxycycline and macrolides in Sweden increased by 13% between October and November 2011, while the use of macrolides alone increased by approximately 125% in Norway during the same period [8]. Although the increase in prescriptions in Sweden was lower than in Norway, we believe that many patients with mild symptoms have been treated unnecessarily. To allow for rapid and correct guidance on the use of antibiotics at an early stage of epidemics of *M. pneumoniae* and possibly other causes of atypical pneumonia, structured laboratory reporting is desirable. A European consensus on indications for treatment should be sought, to limit the number of prescriptions for mild cases and thereby the antibiotic burden.

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A possible outbreak of hepatitis A associated with semi-dried tomatoes, England, July–November 2011

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Citation style for this article:

Carvalho C, Thomas HL, Balogun K, Tedder R, Pebody R, Ramsay M, Ngui SL. A possible outbreak of hepatitis A associated with semi-dried tomatoes, England, July–November 2011.

Euro Surveill. 2012;17(6):pii=20083. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20083>

Article published on 9 February 2012

In October 2011, two primary cases of hepatitis A virus (HAV) infection with identical HAV genotype IB strains to those seen in other outbreaks associated with semi-dried tomatoes were reported in England. Both cases had consumed semi-dried tomatoes. Epidemiological investigations revealed two additional cases of genotype IB strains with different sequences who also reported having consumed semi-dried tomatoes. In November, five cases of HAV infection with closely related strains were identified in the Netherlands. A foodborne multiple-strain outbreak is suspected.

In October 2011, two cases of hepatitis A with a genotype IB strain identical to that seen in a previous outbreak associated with consumption of semi-dried tomatoes, Hu/Netherlands/RIVM-006/2010 [1], were reported to the Health Protection Agency (HPA) in England [2]. The strain was identified based on 100% sequence identity over 505 base pairs of the VP1-2PA junction.

Neither of these patients had travelled to an endemic country within 50 days before the onset of symptoms [3] and both reported substantial consumption of semi-dried tomatoes (also known as sun-dried tomatoes). In this preliminary report of the ongoing investigation we highlight the finding of this rare hepatitis strain which may be related to the possible consumption of semi-dried tomatoes in at least two European countries, the United Kingdom (UK) and the Netherlands.

Background

To date, only one serotype of HAV has been identified worldwide [4]. However, sequence variability between HAV isolates from different parts of the world is substantial, which allows for HAV strains to be classified into different genotypes. Based on the sequencing of VP1-P2A protein regions, different HAV strains have been classified into six different genotypes designed

I–VI, Genotypes I, II and III have been further divided into subgenotypes A and B.

The Hu/Netherlands/RIVM-006/2010 strain, closely related to the strain detected in Australia in 2009 [5–7], was identified in 2010 in a hepatitis A outbreak in the Netherlands [1,8]. In 2010, three cases with that same strain were reported in England to the HPA, one in January, in a traveller returning from Amsterdam [2], and a further two in October [data not shown].

Epidemiological evidence has linked previous hepatitis A outbreaks to semi-dried tomato products [1,7,9], but the virus has only been isolated from samples of semi-dried tomatoes during an Australian outbreak in 2009 [7].

In 2011 the number of reported hepatitis A cases in England was below that reported for previous years. However, the finding of a rare HAV strain that had been associated with previous outbreaks in non travel-related cases in the Netherlands and in the UK, 2010, triggered an epidemiological investigation. The additional report of substantial semi-dried tomatoes consumption from two further cases with distinct strains (99.6% and 91.7% homology to the Hu/Netherlands/RIVM-006/2010 strain respectively) in the second part of 2011 raises the possibility that a single food source may be contaminated with more than one strain.

Simultaneously, a cluster of initially five cases with similar strains and exposure to semi-dried tomatoes was reported in the Netherlands [10] raising the concern that these events could be related to an internationally distributed food source.

Outbreak investigation

As a part of the ongoing investigation a case definition was developed. A case was described as any individual living in England with laboratory-confirmed

genotype IB hepatitis A infection, genotyped by the Virus Reference Department at the HPA Microbiology Services (MS) Colindale, London, with date of symptom onset from 1 July to 31 December 2011. Travel to a country where hepatitis A is endemic and contact with a laboratory-confirmed hepatitis A case within 50 days before the onset of symptoms, were considered exclusion criteria.

The cases were described in terms of time, place and person. Possible exposure to semi-dried tomato products was ascertained by staff of the local HPA Health Protection Unit (HPU), who used standard questionnaires in interviews with cases as part of their usual investigations of persons with hepatitis A. The HPUs provided the relevant exposure information to colleagues at the HPA national centre in Colindale, London.

Active case finding

All the HPUs in England were requested to report all cases of hepatitis A that had been identified between 1 July and 31 December 2011 to the national centre (HPA Colindale, London) and to provide appropriate information for each case. The United Kingdom (UK) national surveillance centres in Wales, Scotland and Northern Ireland were alerted.

Enhanced laboratory surveillance was instituted. Laboratories were requested to forward all hepatitis A serum samples taken during this period to the HPA Virus Reference Department in order to genotype and sequence samples that had not previously been analysed.

Laboratory surveillance data

In 2011, a total of 237 cases of laboratory confirmed hepatitis A were reported by laboratories from England, corresponding to an annual rate of 0.45 per 100,000 population. This number is well below the laboratory-confirmed rates observed during the five previous years (average 0.71 per 100,000) in England and almost eight times lower than the average rate in the European Union in 2009 [11].

Genotyping of hepatitis A viruses is not routinely performed in the UK. Local laboratories are not required to send samples to the Reference Laboratory at HPA MS Colindale, London, and tend to do so only when an outbreak is suspected. For cases from July to December 2011, 32 serum samples were received and genotyped by the Reference Laboratory at the HPA. Five of the 32 cases were genotyped as IA, 14 as IB (including the cases from October) and 13 as IIIA.

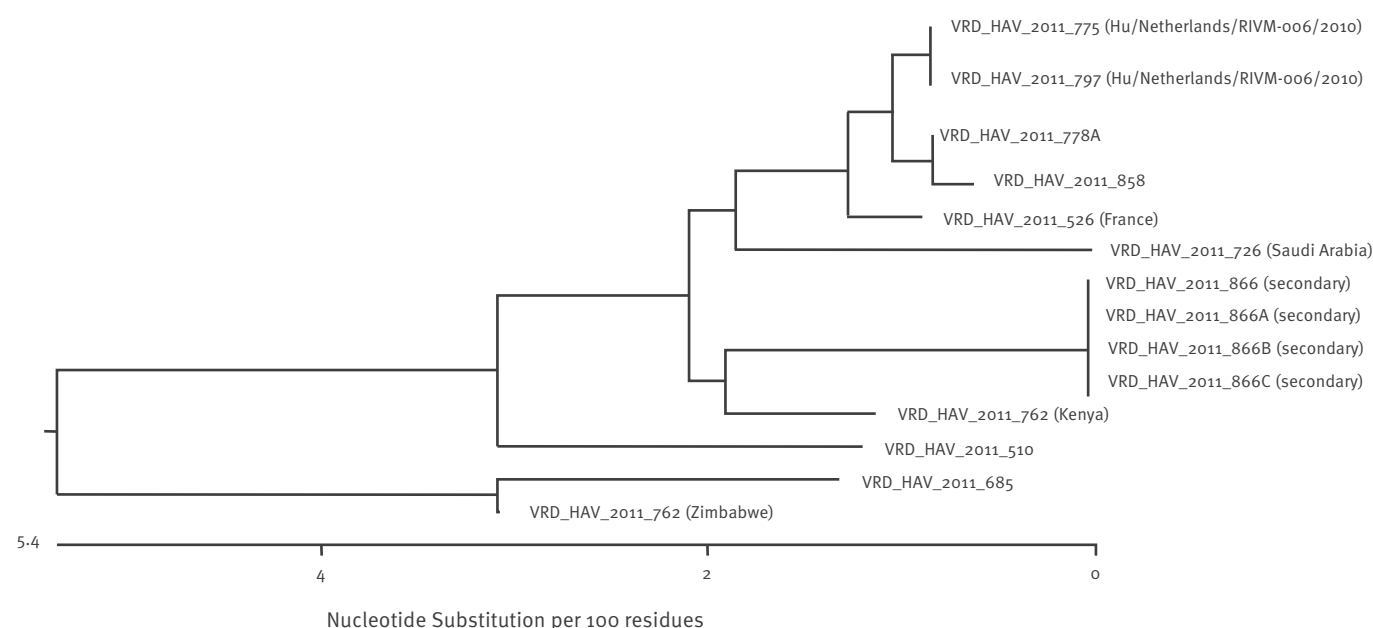
The two HAV genotype IB cases that triggered this investigation were identified as the Hu/Netherlands/RIVM-006/2010 strain based on 100% sequence identity over 505 base pairs of the VP1-2PA junction. A third case was identified based on a 99.6% sequence identity over the same region. The remaining HAV genotype IB strains included three primary non-travel related cases, one primary case with travel history to France, three cases with travel history to hepatitis A endemic countries and four secondary cases (Figure 1).

Epidemiological investigation

By the end of 2011, seven patients met the case definition. The onset of symptoms ranged from 6 July to 1 November 2011 (Figure 2).

FIGURE 1

Phylogenetic tree of hepatitis A virus sequences derived from genotype IB cases identified in England, July–December 2011



The cases resided in distinct areas of England, four in the East (around 5.5 million inhabitants), two in London (around 8 million inhabitants) and one in the Southwest (around 5 million inhabitants).

Four of the cases reported substantial consumption of semi-dried tomato products. In one additional case a

FIGURE 2

Cases of hepatitis A meeting the case definition, by month of onset of symptoms, and respective homology percentage to the Hu/Netherlands/RIVM-006/2010 strain, England July– December 2011 (n=7)

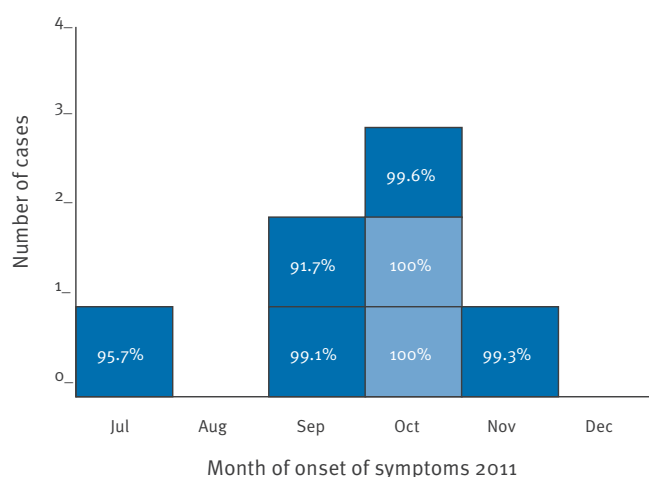


FIGURE 3

Geographical distribution of cases of hepatitis A meeting the case definition, England July–December 2011 (n=7)



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history of food consumption could not be collected, as the patient was a non-UK resident who had left the country prior to the notification being received, and in the two other cases the consumption of semi-dried tomatoes could not be clearly ascertained as they had not eaten semi-dried tomatoes as such, but had eaten products which could contain semi-dried tomatoes as an ingredient). The infecting HAV strains were related but differed between 0.4% and 8.3% of the base pairs compared with to the Hu/Netherlands/RIVM-006/2010 strain (Table).

Of the seven patients (two males and five females), four were admitted to hospital with symptomatic hepatitis A infection and were discharged some days later.

Potentially related outbreaks

From July to November 2011 the Hu/Netherlands/RIVM-006/2010 strain was identified in four patients of a cluster of seven reported in the Netherlands by the Dutch National Institute for Public Health and the Environment (RIVM) [10]. The HPA and RIVM are collaborating to identify the source of these clusters in both countries.

Discussion

Given the fact that the mutation rate of HAV is usually very low [12], the reported consumption of semi-dried tomatoes in patients with different strains suggests the possibility that semi-dried tomatoes could have been contaminated with multiple strains of the virus.

The UK sent a message via the Epidemic Intelligence Information System (EPIS) on 15 November 2011 and a joint message from the HPA and the RIVM was sent via the European Early Warning and Response System (EWRS) on the 21 November 2011, alerting countries to the cluster of cases.

In England an analytical case-control study will be initiated as soon as active case-finding is completed. Based on interviews with the first cases, the main

TABLE

Description of cases of hepatitis A meeting the case definition, England July–December 2011 (n=7)

Week of onset of symptoms	Age	Region	Lab reference	Strain homology ^a
27	24	East of England	2011_510	95.7%
35 ^b	10	South West	2011_526	99.1%
35 ^c	22	East of England	2011_685	91.7%
40 ^c	57	London	2011_775	100%
40 ^c	51	East of England	2011_797	100%
40 ^c	64	East of England	2011_778A	99.6%
44	18	London	2011_858	99.3%

^a Homology related to Hu/Netherlands/RIVM-006/2010 strain

^b Travel to France reported within the incubation period

^c Substantial exposure to semi-dried tomato products

hypothesis to be formally tested is that semi-dried tomato products are associated with primary sporadic (non-travel related) cases of hepatitis A diagnosed in England between July and December 2011. Exposure to other foods such as shellfish, berries, salads and raw vegetables, will be considered and explored. To compare consumption of specific food items in cases with consumption in a group of controls, four age- and residence area-matched controls will be assigned to each case: two patients with *Campylobacter* infection in the same month and two case-assigned controls. In order to reduce recall bias, food preferences and probable food consumption history will be collected.

The enhanced laboratory surveillance, in place since November 2011, should identify new cases more rapidly. Because no specific brands have been implicated to date, and given the fact that there are currently no validated HAV detection methods for environmental samples in the UK, no food samples have as yet been tested. The HPA is liaising with the UK Food Standards Agency.

A foodborne outbreak with multiple strains in at least two European countries is suspected and all those charged with investigation and control of hepatitis A may want to consider semi-dried tomatoes as a possible source in sporadic cases of hepatitis A in order to identify a possible common source.

Acknowledgments

The authors wish to thank all the Health Protection Units for providing line listings of reported cases of hepatitis A, and the various laboratories that submitted specimens and isolates to Microbiology Services Colindale. We would also like to thank the Food Standards Agency, especially Joanne Aish, for their involvement with this investigation. Many thanks to Helen Maguire, EPIET coordinator in the UK. Finally, we would like to thank our colleagues from RIVM, especially Linda Verhoef, Dominique Baas and Nelly Fournet for their close collaboration during this investigation.

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Another possible food-borne outbreak of hepatitis A in the Netherlands indicated by two closely related molecular sequences, July to October 2011

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Citation style for this article:

Fournet N, Baas D, van Pelt W, Swaan C, Ober HJ, Isken L, Cremer J, Friesema I, Vennema H, Boxman I, Koopmans M, Verhoef L. Another possible food-borne outbreak of hepatitis A in the Netherlands indicated by two closely related molecular sequences, July to October 2011. *Euro Surveill.* 2012;17(6):pii=20079. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20079>

Article published on 9 February 2012

In November 2011, a cluster of initially five cases of hepatitis A infection with closely related strains was identified in the Netherlands. England reported possibly related cases. Strains with identical sequences had been involved in previous outbreaks linked to semi-dried tomatoes. Investigation of the Dutch cluster suggested a link with ready-to-eat salads including those containing semi-dried tomatoes. Despite trace-back, a source was not identified. Vigilance is needed, and rapid sharing of data may help source-tracing.

In November 2011, a cluster of five cases of hepatitis A was identified through voluntary molecular surveillance in the Netherlands. It involved two highly similar strains of hepatitis A virus (HAV) genotype IB that were closely related to strains found in travellers from a specific region in the Middle East. Both strains were identical to strains found in earlier outbreaks in the Netherlands during the first half of 2010 [1,2]. One of the sequences was closely related to one found during an outbreak in Australia in 2009 [3] and the other sequence to one that caused an outbreak in France in 2010 [4,5]. These earlier outbreaks had all been epidemiologically linked to the consumption of semi-dried tomatoes. The Dutch cluster coincided with an urgent request from England in the European reporting system EPIS, concerning the same molecular sequence [6].

The overall number of the reported hepatitis A cases was not higher than expected for that time of year. However, since the cases were not epidemiologically linked, and molecular sequencing found that the strains were rare and identical to previous outbreaks, an outbreak investigation was initiated. Close collaboration was established with the Dutch Food Safety Authority

(NVWA) and the Health Protection Agency in England through telephone conferences to share updates and data [6]. With this article, we aim to alert other countries to be aware that this hepatitis strain and the contaminated product may be circulating, and to initiate source tracing if they detect the strain.

HAV surveillance in the Netherlands

HAV infections are notifiable in the Netherlands and reported to the National Institute for Public Health and the Environment (RIVM) according to standardised criteria [7]. Sera from confirmed hepatitis A cases, if available, are sent to the RIVM by the laboratories. Viral RNA is extracted from IgM positive sera, and the 460 nt VP1/2A region is sequenced and compared to sequences recorded in the Dutch [8] and an international HAV sequence database of the Food-Borne Viruses in Europe (FBVE) network [9].

From July to October 2009 and July to October 2010, respectively, 66 and 111 hepatitis A cases were reported in the Dutch mandatory notification, of which 14 and 53 acquired their infection in the Netherlands. Over the same period in 2011, there were 68 overall hepatitis A cases of which 24 acquired their infection in the Netherlands.

Outbreak investigation

Confirmed cases were defined as laboratory-confirmed HAV cases who had no travel history to endemic countries, no male-to-male sexual contacts and was therefore considered as exposed to an unknown source in the Netherlands, who had a date of symptom onset from July to November 2011 and who was infected with the specific genotype IB strain identical to strains that

caused previous outbreaks in Europe and Australia in 2009–10 [1–5], i.e. Hu/Netherlands/RIVM-006/2010, closely related to Genbank accession number FJ687511, or Hu/Netherlands/RIVM-077/2010, closely related to strain FR-2010-LOUR, (GenBank Acc. No. GU646039). Cases related to primary cases and with onset of illness two weeks or more after the primary case were considered secondary cases. A probable case followed the same criteria but without laboratory confirmation.

Cases were contacted by the Dutch municipal health services and were requested to complete a questionnaire. The questionnaire included personal information like date of birth, sex, place of residence, vaccination status, symptoms, date of symptom onset and food history in the period two to six weeks before symptom onset. As recommended by the NVWA, questions on food consumption included a wide range of food products imported from the Middle East because of the similarity of the outbreak strain to strains in travellers from Middle East. The NVWA performed source tracing on the basis of questionnaire information, and tested the indicated food product for presence of hepatitis A RNA using methods described elsewhere [10].

The FBVE network [11] was alerted and asked to share sequences in the international database.

Description of the cases

By November, seven confirmed cases and one probable case had been identified in the Netherlands: two women and six men aged between 20 and 75 years. The date of onset of symptoms was between 29 July and 24 October 2011 (Figure). One of the seven confirmed cases was probably a secondary case (date of onset on 24 October 2011) because she was epidemiologically linked to the probable case with date of onset on 20 September 2011. Unfortunately, the serum sample of this probable case did not yield a PCR product, which may be explained by the fact that it was obtained on 4 November, i.e. 45 days after onset of disease. In a study by Tjon et al. sera were HAV RNA-positive for a median period of 42 days after onset of illness [12].

The HAV strain Hu/Netherlands/RIVM-006/2010 was found in the first four confirmed cases and the Hu/Netherlands/RIVM-077/2010 strain was found in the following three confirmed cases (Figure).

Six of seven confirmed cases completed the questionnaire, including the probable secondary confirmed case. All of them indicated the consumption of ready-to-eat (RTE) packaged salads obtained from two supermarket chains, however they did not, or not fully, specify the type of salad they had consumed. On the basis of initial reports from the Dutch municipal health services, 75% of the mentioned RTE packaged salads contained semi-dried tomatoes or dried tomatoes (tomato granulates). Nevertheless, only three of six cases specifically indicated to have consumed dried or semi-dried tomatoes. In November 2011, the NVWA systematically

sampled stocks of semi-dried and dried tomatoes to be used as ingredients in RTE salads and present in store houses of the suppliers of the two supermarket chains involved. A total of 114 food samples were tested, but in none of the samples HAV RNA could be detected. It remained unclear whether the tested batches were the same as those consumed by the patients. The NVWA worked in close collaboration with the Food Standards Agency (FSA) in England, but a common link in the supply chain to the Dutch and English supermarket chains could not be identified.

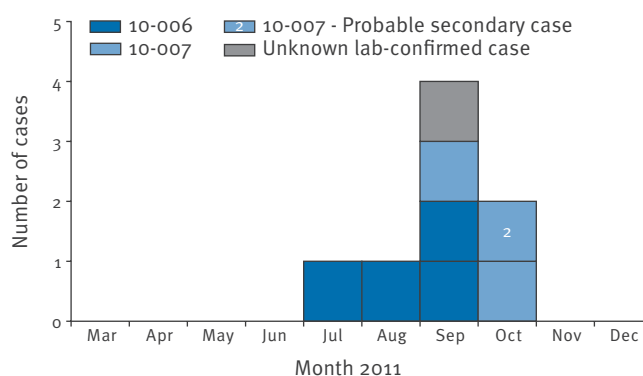
The international alert and the sharing of sequences in the international database resulted in a case reported from Australia who locally acquired an infection with an HAV strain identical to Hu/Netherlands/RIVM-006/2010 in a 311 nt sequence in the VP1/2A genomic region. For this case source tracing is currently ongoing.

Conclusion and recommendations

Seven confirmed hepatitis A cases and one probable case were identified in the Netherlands with HAV genotype IB strains identical or closely related to the ones found recently in England and Australia and in previous food-borne outbreaks in the Netherlands, Australia and France. All cases that completed the questionnaire indicated consumption of RTE salads, including those containing semi-dried tomatoes, during their incubation period. However, only three cases specifically indicated to have consumed semi-dried tomatoes. Because of the low number of cases, the long and variable incubation period, the long time between dates of onset of the first and of the last cases, and the complexity of the traceback, no common source could be identified. These were also the reasons for not including controls in our study. This might be considered a limitation,

FIGURE

Confirmed and probable cases of hepatitis A, indicated by two closely related molecular sequences (10-006^a and 10-077^b), notified in the Netherlands from March to December 2011 (n=8)



^a 10-006: Hu/Netherlands/RIVM-006/2010; strain detected in Netherlands 2010, closely related to strain detected in Australia 2009.

^b 10-077: Hu/Netherlands/RIVM-077/2010; strain detected in Netherlands 2010, closely related to strain detected in France 2009/10.

since we were not able to find an association to a single food product.

Until the time of publication of this report, no new cases have been reported. Nevertheless, we cannot exclude contaminated food items like RTE salads or semi-dried tomatoes being in circulation. Given the difficulty of identifying and tracing an international common food-borne source, combining information on all infected cases is of utmost importance. We are therefore interested in all cases infected with the HAV IB strains described here. The HAV database of the FBVE network can be used to compare sequences. Information on linked cases and requests for access to the database or assistance in source tracing activities can be addressed by email to fbve@rivm.nl.

Acknowledgments

We would like to thank the people working at the municipal health services for their extra efforts and help in source tracing, and the laboratories for sending in the sera to RIVM for molecular typing. We are grateful to the people working at the NVWA for their close collaboration in this investigation and their willingness to share their data internationally. Especially, we like to thank Jan van Kooij for his involvement in the initial phase of the outbreak, Gabriel Mainer Albiac for identifying food products derived from the specific geographical region, Geke Hägele and Nathalie te Loeke for the viral analyses of the many food samples. We like to thank the people from the HPA and FSA in England for their close collaboration throughout the outbreak investigation, especially Koye Balogun, Carlos Carvalho, Lucy Thomas, Siew Lin Ngui, and Joanne Aish. We would like to thank Michael Lyon for sharing the Australian sequence data in the international hepatitis A database.

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Outbreak of salmonellosis after a wedding party, Bavaria, Germany, summer 2010: the importance of implementing food safety concepts

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Citation style for this article:

von Wissmann B, Klinc C, Schulze R, Wolf A, Schreiner H, Rabsch W, Prager R, Hautmann W. Outbreak of salmonellosis after a wedding party, Bavaria, Germany, summer 2010: the importance of implementing food safety concepts.

Euro Surveill. 2012;17(6):pii=20076. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20076>

Article published on 9 February 2012

An outbreak of salmonellosis in the summer of 2010 after a wedding party in Bavaria, Germany, was investigated, to identify vehicles and source of the outbreak and any flaws in food safety procedures. A cohort study targeting all wedding participants was carried out in addition to laboratory and environmental investigations. The overall attack rate was 59% (52/88). A group of buffet dishes containing mayonnaise was associated with the highest relative risk: 6.6 (95% CI: 1.7–25.0). Subtyping of a subset of *Salmonella* isolates cultured from stool samples from wedding participants and catering staff and from food samples showed the isolates to be indistinguishable (*S. Enteritidis*, lyso-type 4/6, ribotype 3). European laws require food business operators to establish and implement food safety procedures. Investigation of the practices of the catering company revealed a lack of staff training and the absence of records of a food safety concept. Non-edible flower decorations in cold dishes were identified as a potential source of secondary contamination because their cultivation and processing are not subject to the food safety regulations minimising the risk of contamination of crops intended for human consumption. The outbreak demonstrated the importance of staff training and food safety concepts to identify and mitigate hazards to food safety.

Introduction

Salmonellosis is an important cause of food-borne gastroenteritis in Europe [1]. Prophylactic measures are aimed at all stages of food production from farm to fork and are set down in European laws governing food safety. Compliance of food business operators to the minimum hygiene regulations is checked through official controls. Additionally, food business operators are required to establish and operate food safety

procedures based on the hazard analysis and critical control points (HACCP) principles [2]. These principles form a flexible but structured approach to identifying hazards (e.g. risk of contamination) as well as control points (e.g. heating or cooling steps in the production or preparation of foods) that can mitigate these hazards. The procedures developed have to be documented by the food business operator and regular checks of the control measures have to be recorded (e.g. temperature checks to record the maintenance of the cold chain). While they do not replace official controls, the HACCP principles are central to the European concept of food safety by helping food business operators to attain a high standard of food safety. Investigations of food-borne outbreaks serve to identify the source of the outbreaks and may help to identify flaws or weak points in the planning and implementation of food hygiene measures. The results may also be used to augment general guidelines for good practice in hygiene and food safety.

Surveillance of salmonellosis in Germany is based on mandatory notification of laboratory-confirmed infections by the laboratory to the local health office [3]. Additionally, the local health office may be alerted to a cluster of clinical cases with a suspected common source by a clinician or an affected member of the public. The local health office further investigates the reported cases, carries out active case finding as part of the outbreak investigation and implements control measures. Case reports are forwarded to the federal and national level using an electronic database for notifiable infectious diseases. Isolated *Salmonella* strains can be sent to the German National Reference Centre for *Salmonella* and other Enteric pathogens at

the Robert Koch Institute for subtyping by classical and molecular methods.

In the summer of 2010, a local health office in northern Bavaria, Germany, was informed that approximately half of the 110 guests of a wedding that had taken place the preceding weekend had contracted gastroenteritis. At the wedding party, soup and a late-lunch buffet (served from 3 p.m.) and a cold dinner buffet (served from 10 p.m.) had been provided by an out-of-town caterer. In addition, a wedding cake made by a local bakery and a number of cakes and desserts contributed by different wedding guests were served by the catering staff at 20.00. The food served at the wedding was suspected to be the source of the outbreak. Initial laboratory results of stool samples of some guests who became ill indicated *Salmonella enterica* subsp. *enterica* serovar Enteritidis (*S. Enteritidis*) as the causative pathogen. The local health office, supported by the Bavarian Health and Food Safety Authority, initiated a comprehensive outbreak investigation including a cohort study, pathogen subtyping and an environmental investigation was conducted to identify the vehicle and source of the outbreak.

Methods

Cohort study

The study population for the cohort study was defined as all people who attended the wedding party and ate any of the served food. Completion of a written questionnaire was a prerequisite for individuals to be included in the cohort study. Catering personnel were excluded from the cohort study due to a perceived conflict of interest (the company was facing litigation).

Written questionnaire

A structured, written questionnaire and a stamped addressed envelope for its return were posted to all people who attended the wedding party and for whom a complete postal address could be obtained, two weeks after the wedding. The questionnaire included standardised questions relating to demography and the development of symptoms of salmonellosis, including date and time of onset, as used in previous outbreak investigations conducted by the Bavarian Health and Food Safety Authority. Questions on food consumption were adapted to refer to the specific food items served at the wedding party.

Case definition

A clinical case of salmonellosis was a person who had eaten at the wedding party and who developed at least one symptom of salmonellosis (nausea, vomiting, stomach cramps, diarrhoea (three or more loose stools in 24 hours), pyrexia (body temperature above 38.5 °C)) between six hours after the start of the buffet (which began at 3 p.m.) and 72 hours after the end of the buffet (the end was considered to be at 00.30 a.m., when the caterers left). Laboratory confirmation of a salmonella infection was obtained by the isolation of *Salmonella* species from a stool sample taken as part

of the outbreak investigation, irrespective of the presence or absence of symptoms of salmonellosis. People with clinical and/or laboratory-confirmed salmonella infections were considered as salmonellosis cases in the cohort study.

Statistical analysis

For the descriptive analysis of cases and non-cases in the cohort study, continuous variables were summarised using median and range. Categorical variables were presented using absolute numbers and percentages. Where appropriate, a binomial 95% confidence interval (CI) was calculated for the percentages. The univariable association between case status and exposure to individual dishes was tested using the chi-square test or Fisher's exact test where appropriate. The effect of each exposure variable was quantified by estimation of the relative risk and the respective 95% CI. When the numbers were small, exact Poisson regression was used for estimation. Multivariable analysis was performed using a generalised linear model with log link and binomial error distribution. Exposures with a p value of <0.1 were included in the multivariable model. The multivariable model was constructed by first including all variables with a p value of <0.1 and then dropping variables manually in a backwards elimination procedure based on the Wald test. Statistical significance was assumed when $p < 0.05$ and the 95% CI of the relative risk did not include 1. When interpretation of the p value and the 95% CI were contradictory, the more conservative interpretation (not statistically significant) was reported. Data analysis was carried out in STATA 10.1.

Grouping for analysis purposes

Dishes served at the wedding party were grouped by the four different courses served: soup, lunch buffet, cakes and dinner buffet. Further groupings of the food items were created for analysis according to common ingredients (e.g. one group for all meat dishes that were served in a cream sauce, another group for all dishes containing mayonnaise) or according to the type of food or preparation and the perceived risk of contamination (e.g. all tinned food items were put into one group, as their probability of contamination with *Salmonella* was similarly low). A Cochran-Armitage test for trend was performed for the association between the risk of infection and the number of dishes containing mayonnaise consumed, to assess the dose-response.

Environmental investigation

Although the catering staff were excluded from the cohort study, all eight staff members (one of whom was not present at the wedding party but was involved in preparation of the dishes) were contacted by telephone by the local health office on the day the office was notified about the outbreak (four days after the wedding event), to ensure that individuals with symptoms of salmonellosis did not continue to handle food and to determine whether any of the catering staff had displayed symptoms of gastroenteritis before the

wedding event. The catering company's records for staff hygiene training as well as their records for food temperature controls (as an indicator variable for the implementation of HACCP procedures) were checked. The kitchen facilities of the venue of the wedding party were inspected and swabs of the work surfaces were sent for laboratory testing. The premises of the catering company and their facilities were inspected by the responsible local veterinary office and swabs of the work surfaces were submitted for laboratory testing.

All wedding guests were contacted by the local health office by telephone four days after the wedding to identify clinical cases of salmonellosis and to exclude such person with salmonellosis working in the food industry from handling food until they were proven to be no longer infected. Food leftovers taken home by wedding guests were submitted for laboratory diagnosis, as no food items were left at the party venue or at the caterer's facilities.

Laboratory diagnosis

Stool samples

Stool samples from all catering personnel (n=8) were submitted to the local health office and tested for *Salmonella* and *Shigella* (by culture) and for norovirus (by polymerase chain reaction (PCR)) in a medical laboratory. Positive samples were forwarded to the National Reference Centre for *Salmonella* and other Enteric pathogens at the Robert Koch Institute.

Stool samples from wedding guests were submitted to medical laboratories as part of the clinical diagnosis by their general practitioners (GPs). The samples were tested for *Salmonella* and *Shigella* (by culture) and for other pathogens (*Campylobacter* and *Yersinia enterocolitica* (by culture) and norovirus (by PCR)), depending on the request submitted by the GP. For a number of samples, *Salmonella* isolates were serotyped (depending on the laboratory). At the request of the local health office, *Salmonella*-positive samples or isolates were forwarded to the National Reference Centre if the material was still available.

Food samples and swabs

Food samples and swabs of the kitchen facilities at the wedding party venue, as well as swabs of the caterer's kitchen facilities were tested for *Salmonella* (by culture) and norovirus (by PCR). *Salmonella* isolates were serotyped and forwarded to the National Reference Centre.

Subtyping of isolates

At the National Reference Centre, a subsample of the *Salmonella* isolates from stool samples and food samples were phage typed and ribotyped. The routine phage typing of *S. Enteritidis* was performed according to the typing system of Ward et al. [4] as well as that of Lalko and László [5], e.g. phage type (PT) 4/6, i.e. phage type 4 according to Ward et al. and 6 according to Lalko and Laszlo. The ribotyping was performed according to Liebana et al. [6] and Rabsch et al. [7].

Results

Response to questionnaire

Questionnaires were sent to all wedding guests (including the bride and groom) for whom a complete postal address was obtained (n=100). Of these, 91 returned their completed questionnaires.

Among the 19 people for whom questionnaire data were not available (10 people for whom no valid address was available, nine people who did not return the questionnaire), five stated during their telephone interview with the local health office that they had had symptoms of gastroenteritis. Exact date and time of symptom onset for these people were not known. For one of the five, laboratory diagnosis confirmed infection with *S. Enteritidis*.

Descriptive statistics

Among the 91 respondents, 52 matched the case definition: 22 had laboratory-confirmed infection and symptoms of salmonellosis, one had a laboratory-confirmed infection without symptoms and 29 had symptoms and no laboratory confirmation.

A further three of the respondents had symptoms of salmonellosis with laboratory confirmation, but as the time of onset was outside the period defined in the case definition they were excluded from the analysis. All further analysis therefore relates to 88 respondents, of whom 52 were classified as cases and 36 as non-cases (attack rate: 59% (95% CI: 48–70)).

Of the 52 cases, 35 were female and 17 were male. The attack rate was lower among the male (17/37; 46% (95% CI: 30–63)) than the female wedding guests (35/51; 69% (95% CI: 54–81)) (chi-square test $p = 0.04$). Information on age was unavailable for two of the 88 respondents: the median age of the remaining 86 respondents was 39 years (range: 2–70). Table 1 shows the distribution of cases and attack rate by age group.

The majority of cases had diarrhoea (49/52), stomach cramps (43/52), headache (36/52), joint and muscle ache (32/52) and nausea (29/52). Fever and

TABLE 1

Cases of salmonellosis after a wedding party and attack rate by age group, Bavaria, Germany, summer 2010 (n=50)

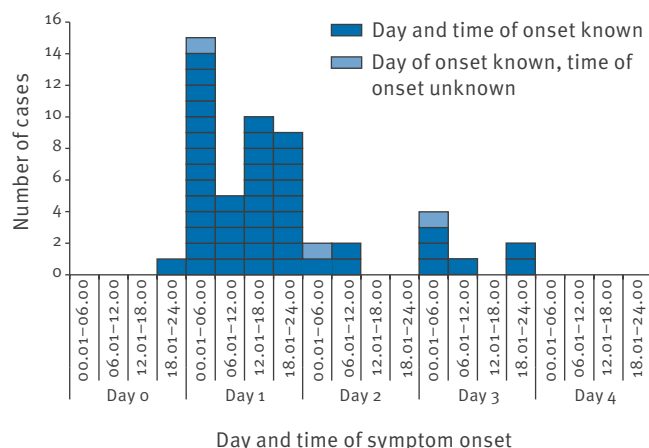
Age group (years)	Number of respondents (n = 86) ^a	Number of cases (n = 50) ^a	Attack rate % (95% CI)
0–4	4	1	25 (1–81)
5–17	1	1	100 (3–100)
18–40	40	25	63 (46–77)
41–60	32	17	53 (35–71)
61–80	9	6	67 (30–93)

^a Age was unknown for two cases among the 88 wedding guests who responded to the questionnaire.

vomiting was experienced by 28 and 21 of the 52 cases, respectively.

FIGURE

Cases of salmonellosis after a wedding party by day and time of symptom onset, Bavaria, Germany, summer 2010 (n=51)^a



Day 0 = day of the wedding party.

^a One case with laboratory-confirmed infection but without symptoms was excluded.

A total of 10 cases with a median age of 36 years (range: 23–71) were hospitalised between 2 and 10 days (total number of hospital days: 55) due to gastroenteritis. The distribution of cases by day and time of symptom onset is shown in the Figure.

When day of onset was known, but time of onset was unknown (n=3), the cases were systematically attributed to the time period of 00.01–06.00 for the appropriate date.)

Analytical statistics

In univariable analysis, the exposure to 18 out of 63 individual dishes was associated with a significantly increased relative risk of salmonellosis (Table 2). Only the following three items were associated with a relative risk >2: ‘wedding rings’ (pasta, rice, carrots and mayonnaise), prawn salad (prawns, tomatoes and mayonnaise) and Greek noodles (rice noodles and tomatoes).

When the dishes were grouped according to the four different courses served at the wedding party (soup, lunch buffet, cake, dinner buffet), only exposure to the lunch buffet was significantly associated with infection (Fisher’s exact test $p < 0.01$). As there were no cases

TABLE 2

Univariable analysis of dishes served at a wedding party, consumption of which was significantly associated with onset of salmonellosis^a, Bavaria, Germany, summer 2010

Dish	Exposed			Not exposed			Relative risk (95% CI)	Chi-square test p value
	Number exposed	Number of cases	Attack rate as percentage	Number not exposed	Number of cases	Attack rate as percentage		
Lunch buffet								
'Wedding rings' (pasta, rice, carrots, mayonnaise)	15	15	100	66	32	49	2.06 (1.61–2.64)	< 0.001
Glass noodle salad	18	17	94	58	28	48	1.96 (1.47–2.61)	< 0.001
Prawn salad	15	14	93	55	23	42	2.23 (1.59–3.14)	< 0.001
Layered salad with herring	27	23	85	55	25	46	1.87 (1.35–2.61)	0.001
Greek noodles (rice noodles and tomatoes)	22	19	86	54	23	43	2.03 (1.43–2.88)	0.001
Stuffed mushrooms	29	24	83	48	20	42	1.99 (1.37–2.89)	0.001
Fried aubergines	10	10	100	67	35	52	1.91 (1.52–2.41)	0.004
Cheese salad with mayonnaise and garlic	8	8	100	66	32	49	2.06 (1.61–2.64)	0.006
Stuffed tomatoes	19	16	84	58	29	50	1.68 (1.22–2.33)	0.014
Caesar salad	8	7	88	57	25	44	2.00 (1.35–2.96)	0.027
Peppers	14	12	86	66	34	52	1.66 (1.21–2.28)	0.034
Olives	20	16	80	59	30	51	1.57 (1.13–2.20)	0.035
Broccoli-cauliflower cheese	38	27	71	42	20	48	1.49 (1.02–2.17)	0.042
Garlic mushrooms	23	17	74	52	24	46	1.60 (1.09–2.34)	0.043
Grilled turkey fillet with cream sauce	28	20	71	42	19	45	1.58 (1.05–2.37)	0.049
Dinner buffet								
Tomatoes and mozzarella	29	22	76	51	24	47	1.61 (1.13–2.30)	0.018
Shrimps with cream	13	11	85	61	29	48	1.78 (1.25–2.53)	0.029
Cold trout with slices of lemon	22	17	77	60	31	52	1.50 (1.07–2.09)	0.045

^a $p < 0.05$; 95% CI did not include 1.

among the people who did not partake of the lunch buffet, a relative risk of 4.49 (95% CI: 0.82–∞) for exposure to dishes of the lunch buffet was estimated by exact Poisson regression.

When all dishes were grouped according to common ingredients and type of preparation, two groups of dishes served as part of the lunch buffet and four groups of dishes served at the dinner buffet were associated with a significantly increased relative risk of infection (Table 3). Notably the group of dishes served at lunch that contained mayonnaise was associated with the highest relative risk of 7.83 (95% CI: 2.08–29.51). Exposure to at least one dish of the group of dishes containing mayonnaise could account for 44 out of the 46 cases who could be unambiguously classified as exposed or non-exposed (for six cases and two non-cases, unambiguous classification was not possible as they were uncertain about their consumption of one dish in this group). Consumption of an increasing number of dishes containing mayonnaise was associated with an increasing risk of infection (Table 4); this trend was statistically significant (Cochran-Armitage test for trend $p < 0.001$).

The difference in relative risk of infection between male and female participants was no longer significant

after adjustment for the consumption of lunch dishes containing mayonnaise (data not shown).

In the multivariable analysis, the following three groups of dishes remained significantly associated with infection and were retained in the final model: the group of dishes that contained mayonnaise served at lunch, those containing mayonnaise served at dinner and the sauces served at lunch (Table 5).

Environmental investigation

Seven people from the catering company were present at the wedding party: one additional person was involved only in the food preparation at the caterer's facilities. Of the seven at the wedding party, one developed symptoms of salmonellosis on the return journey after the event and a further five became ill the following day. Only the driver did not have any symptoms of salmonellosis. All seven stated that they had eaten various dishes from the buffet as well as different cakes provided by wedding guests.

According to the caterer, only the meat dishes were prepared at the caterer's facilities in advance. All sauces, antipasti and salads were made from commercially produced ingredients and were prepared at the wedding party venue. Food items that required cooling

TABLE 3

Univariable analysis of groups of dishes served at a wedding party, consumption of which was significantly associated with onset of salmonellosis^a, Bavaria, Germany, summer 2010

Group of dishes	Exposed			Not exposed			Relative risk (95% CI)	Chi-square test p value
	Number exposed	Number of cases	Attack rate as percentage	Number not exposed	Number of cases	Attack rate as percentage		
Lunch: dishes containing mayonnaise	59	44	75	21	2	10	7.83 (2.08–29.51)	<0.001
Lunch: sauces	15	12	80	58	28	48	1.66 (1.15–2.39)	0.041
Dinner: dishes containing mayonnaise	25	20	80	50	22	44	1.82 (1.26–2.63)	0.003
Dinner: vegetables	38	28	74	43	19	44	1.67 (1.13–2.45)	0.013
Dinner: shellfish	33	25	76	50	24	48	1.58 (1.12–2.23)	0.013
Dinner: other seafood	36	26	72	41	18	44	1.65 (1.10–2.46)	0.020

^a $p < 0.05$; 95% CI did not include 1.

TABLE 4

Association between the number of dishes containing mayonnaise consumed per respondent and the risk of salmonellosis after a wedding party, Bavaria, Germany, summer 2010

Number of dishes containing mayonnaise consumed per respondent	Number of respondents ^a (n=80)	Number of cases (n=46)	Relative risk (95% CI)	Wald test p value
0	21	2	1 Reference	–
1	19	9	4.97 (1.22–20.19)	0.025
2	19	15	8.29 (2.17–31.61)	0.002
3	8	7	9.19 (2.40–35.23)	0.001
≥3	13	13	10.99 (2.94–41.07)	<0.001

^a Eight respondents, for whom classification was not possible as they were uncertain about their consumption of one dish in this group, were excluded from the analysis.

were transported in cooling boxes and stored in cooling units at the venue. At the venue, the lunch buffet was set up for six hours, between 2 p.m. (the buffet was set up one hour before it was available to guests) and 8 p.m. No checks were carried out of holding temperatures of the warm or cold dishes. Salads and other cold dishes were not cooled during this period. Photographs of the buffet showed that a number of the cold dishes were decorated with non-edible flowers (such as roses), which were inserted into the food. The flowers had been purchased at a wholesale retailer. Cakes supplied by a number of wedding guests were stored without cooling until they were served at 20:00. The dishes of the dinner buffet were not cooled. They were first served at 10 p.m. It is unclear for how long the dishes of the dinner buffet were served; however, it is known that the catering personnel departed at 00:30.

Inspection of the catering facilities and interview of the catering staff revealed a number of shortcomings contravening European food hygiene regulations. The facilities used by the caterer were not registered with the local authorities. There were no records of the required staff training on food hygiene. No temperature controls of cooling devices or transport boxes were carried out, nor were temperatures monitored during preparation or serving of warm dishes. There were no records of HACCP concept planning or implementation. The company was banned from catering until proof of changes in their practices had been provided to the local authorities.

Inspection of the kitchen facilities at the wedding party venue did not identify any deficiencies.

No food samples could be secured from either the venue or the caterer. However, four food samples, each consisting of a mixture of dishes, were provided for laboratory analysis by wedding guests who had taken home leftovers from the buffet.

TABLE 5

Multivariable analysis of groups of dishes served at a wedding party, consumption of which was significantly associated with onset of salmonellosis^a, Bavaria, Germany, summer 2010

Variable	Relative risk (95% CI)	Wald test p value
Lunch: dishes containing mayonnaise		
0	1 Reference	–
1	6.61 (1.74–24.99)	0.005
Dinner: dishes containing mayonnaise		
0	1 Reference	–
1	1.42 (1.04–1.93)	0.027
Lunch: sauces		
0	1 Reference	–
1	1.27 (1.04–1.56)	0.021

^a p<0.05; 95% CI did not include 1.

Laboratory results

A total of 38 of the respondents stated in their questionnaire that they had submitted a stool sample for laboratory analysis via their GP or at hospital. *Salmonella* species were isolated from stool samples from 26 of them. For 24 of these isolates, *S. Enteritidis* was identified as the serovar.

Stool samples from all eight of the catering personnel were positive for *Salmonella*.

Swabs from both the wedding party venue and the catering company's kitchen facilities were negative. In two of the four food samples tested (one contained several meat dishes served at the lunch buffet, the other contained several seafood dishes served at the dinner buffet), *S. Enteritidis* was isolated in a standard 25 g of sample material.

At the National Reference Centre, eight isolates from the respondents, two isolates from catering personnel and two isolates from the food samples were characterised. All 12 isolates were identified as *S. Enteritidis*, phage type 4/6. Molecular subtyping of these *S. Enteritidis* strains by *PstI-SphI* ribotyping showed that all strains possessed an indistinguishable ribotype pattern: ribotype 3 according to the National Reference Centre arbitrary designation of ribotypes of *S. Enteritidis* PT 4/6. (To date, the National Reference Centre has identified 37 different ribotype patterns among isolates of phage type 4/6 (data not shown))

Discussion

This paper describes the investigation of an outbreak of *S. Enteritidis* PT4/6 amongst the guests of a wedding party. The cohort study showed that a variety of dishes were associated with a significantly increased risk of infection: in particular consumption of a group of lunch dishes containing mayonnaise was associated with a high relative risk. Despite the constraint of a two-week delay between the wedding party and the questionnaires being sent out, participants appeared to recollect well which dishes they had consumed. All respondents were contacted by the local health office by telephone within four days of the wedding, which meant that both cases and non-cases were made equally aware of the outbreak close to the event, reducing the risk of recall bias.

The isolation of *S. Enteritidis* from two of the food samples at the wedding party was judged to show that the food served posed a health risk, as all the food items were ready for consumption without requiring further preparation or heating. The isolation of indistinguishable *Salmonella* strains from the food samples as well as from stool samples of respondents and catering personnel supported the hypothesis that the outbreak was food-borne. Besides the use of classical methods (serotyping and phage typing), molecular methods such as *PstI-SphI* ribotyping are essential for identifying genomic polymorphism, in order to differentiate

between individual strains of the highly clonal serovar Enteritidis and thus confirm a common source of the pathogen.

There are several possibilities for the source of the *Salmonella* contamination in this outbreak. Mayonnaise is a well-recognised vehicle of contamination when raw egg is used as an ingredient [8-12]. However, in this outbreak all cold dishes and salads were made from commercially prepared ingredients. As commercially produced mayonnaise and sauces are conventionally based on pasteurised ingredients, it is unlikely that they would be the primary source of contamination. Commercial mayonnaise by itself is also not suitable for *Salmonella* propagation, due to its low pH adjusted by acetic acid. However, addition of mayonnaise to other salad ingredients may alter the overall acetic acid concentration of the mixture, thus providing a suitable base for proliferation once the pathogen has been introduced by secondary contamination [13].

None of the catering staff said they had symptoms of salmonellosis before the wedding party. However, shedding of pathogens in the prodromal phase of infection, as well as asymptomatic excretion of *Salmonella*, has been reported in the literature [14,15]. Thus the possibility of food contamination by an asymptomatic shedder among the catering personnel cannot be excluded.

Contact during storage or transport between raw ingredients that may have been contaminated (such as meats or fish) and dishes ready for consumption may have been another possible source of cross contamination. Interestingly, a number of the cold dishes and salads (including the 'wedding rings') and a number of other dishes also containing mayonnaise served at the lunch and dinner buffets had been decorated with flowers. European food safety laws state that food contamination during primary production must be minimised by controlling potential contamination in soil, water and fertiliser. Cultivation of non-edible flowers, however, is not subject to these laws and flowers are therefore at increased risk of pathogen contamination. The flower decoration in a variety of cold dishes and salads may have served as the source of secondary *Salmonella* contamination in this outbreak. Unfortunately, it was not possible to test this hypothesis as only some of the dishes could be clearly identified in photographs of the buffets and there were no other records of which dishes had been decorated with flowers nor was it possible to obtain this information from the catering staff. Unavailability of systematic samples of all the dishes, or ingredients, which could have helped to elucidate the source of contamination, posed a further limitation to the outbreak investigation.

The environmental investigation revealed a number of infringements of food safety regulations, including a lack of staff training and the absence of records of a food safety concept according to the HACCP principles.

Lack of temperature controls for food storage and transport as well as prolonged presentation of buffet dishes at room temperature provided ideal conditions for pathogen proliferation, regardless of the primary source of contamination.

While they do not replace official controls, the HACCP principles are central to the European concept of food safety by helping food business operators to attain a high standard of food safety. Successful implementation of procedures based on the HACCP principles requires the full cooperation and commitment of food business employees. Adequate training of personnel is central to achieving this goal [2]. The outbreak investigated in this study demonstrates the consequences of lack of staff training and the failure to identify hazards to food safety, as well as failure to implement control measures to mitigate such hazards. The use of flowers as food decoration demonstrated insufficient understanding of the potential for contamination through products that are not intended for food production and therefore not subject to food hygiene regulations.

Intelligently implemented food hygiene concepts not only benefit the consumer but are also very much in the interest of the food business operator, whose business can be threatened by food-borne outbreaks. Initial hygiene and food safety training for food business operators should therefore also explain microbiological principles underlying food safety practices in order to equip the businesses with the required background knowledge and motivation to design and implement an intelligent food safety/HACCP concept, including the consideration and identification of potential sources of contamination. Explicit mention of the dangers of the use of non-edible flowers for decoration should be considered in guides to good practice, which are a valuable instrument to aid food business operators with compliance with food hygiene rules and with the application of the HACCP principles.

Acknowledgments

The authors would like to thank all their colleagues at the local health offices and local veterinary offices in Ansbach, Kassel and Werra-Meissner for their help and collaboration in the outbreak investigation. We thank S. Kulbe and D. Busse for their skilful assistance in phage typing and B. Knüppel for ribotyping. The authors would also like to thank M. Dehnert, I. Karagiannis and K. Alpers at the Robert Koch Institute for their advice throughout the investigation.

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Call for expression of interest for the cohort 2012 of EPIET Member State track

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Citation style for this article:

Eurosurveillance editorial team. Call for expression of interest for the cohort 2012 of EPIET Member State track.

Euro Surveill. 2012;17(6):pii=20080. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20080>

Article published on 9 February 2012

The call for expression of interest for the cohort 2012 of EPIET Member State track (EPIET MS-Track) is now open. European Union (EU) Member States with an acknowledged EPIET training site can express their interest in having a fellow trained. The deadline for expression of interest is 26 February 2012. For the 2012 cohort ECDC will be co-funding 12 MS-Track fellowships.

For those Member States which do not yet have an accredited EPIET training site, visits will be organised to those countries interested in training an EPIET-MS Fellow, to appraise their potential availability as training sites.

Since 2011 the Member State track of EPIET provides training in EU/European Economic Area country of origin. It addresses the need of strengthening the field epidemiology capacity in the Member States that have not yet benefited from the EPIET Programme. In 2011 seven fellows participated in the MS-Track programme and the EPIET Training Site Forum (ETSF) requested that more fellowships be made available. The response of the European Centre for Disease Prevention and Control (ECDC) to this request was to increase in the number of MS-Track seats offered from seven to 12.

For more information and the application form see the ECDC and the EPIET websites.