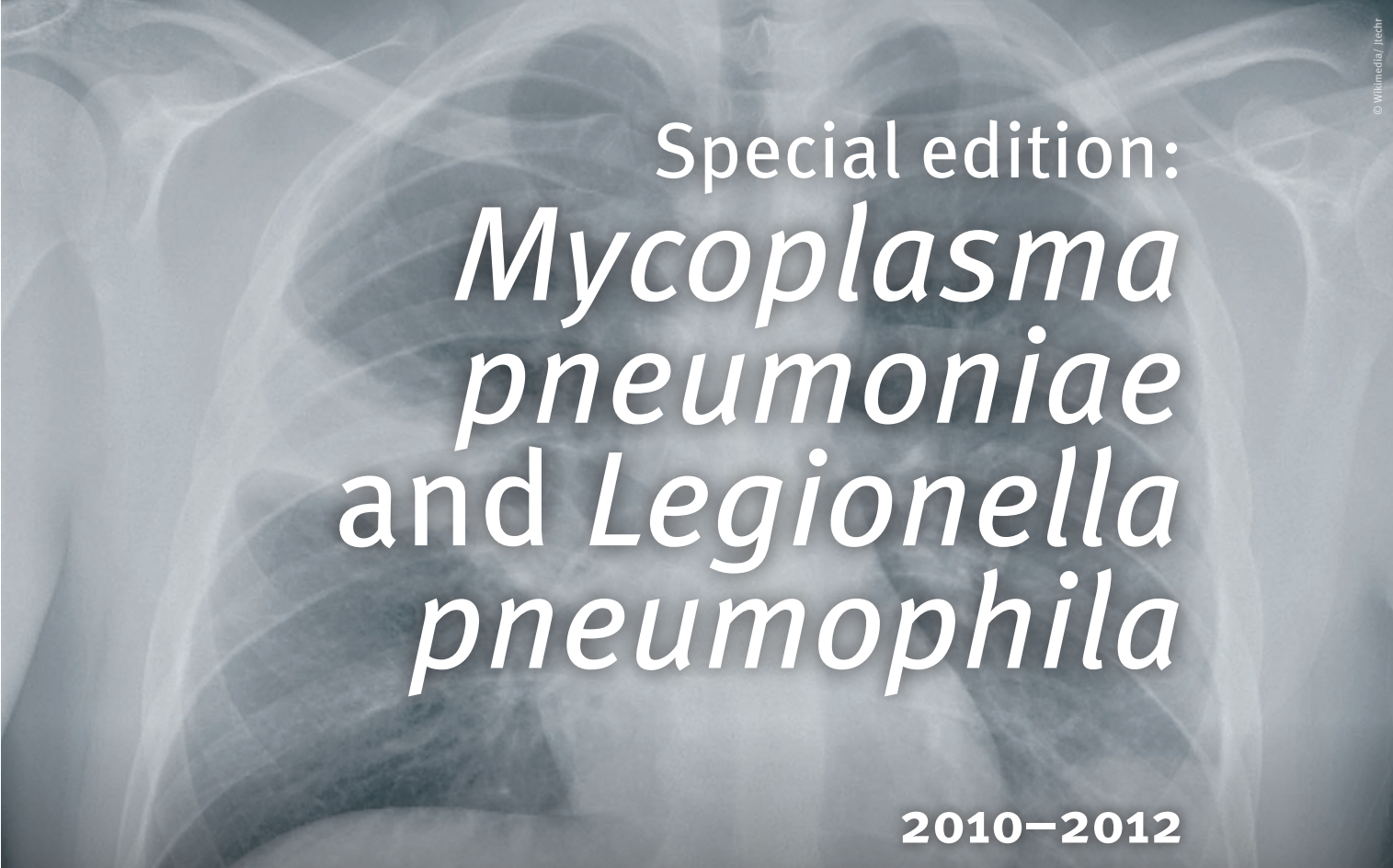




Eurosurveillance

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



Special edition:
Mycoplasma pneumoniae
and *Legionella pneumophila*

2010–2012

- Starting in late 2010, several European countries experienced outbreaks of *Mycoplasma pneumoniae* infection. In this issue, reports from 10 countries and a paper on the “Surveillance status and recent data for *Mycoplasma pneumoniae* infections in the European Union and European Economic Area” provide an overview of the situation in Europe and highlight some of the associated challenges related to surveillance including monitoring potentially emerging macrolide resistance.
- Also in this issue: several papers reporting outbreaks of legionellosis and a paper discussing wellness centres as potential sources of infections.

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Lungs with community-acquired pneumonia pre-treatment

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Mycoplasma pneumoniae: now in the focus of clinicians and epidemiologists

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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 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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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

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).

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 (20.2–19.1)	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

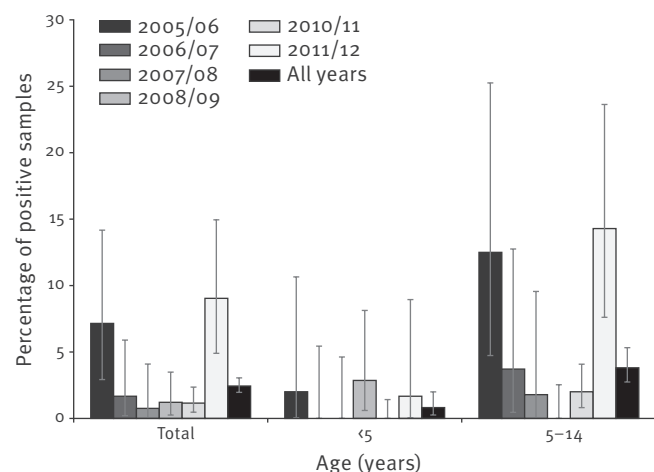
Percentage of *Mycoplasma pneumoniae*-positive samples from laboratory reports by age, England and Wales, 10 October (week 42) 2011–20 January (week 3) 2012 ($n=353$) and 1 January 1975 (week 1)–20 January (week 3) 2012 ($n=38,221$)^a

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.

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. Multilocus 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

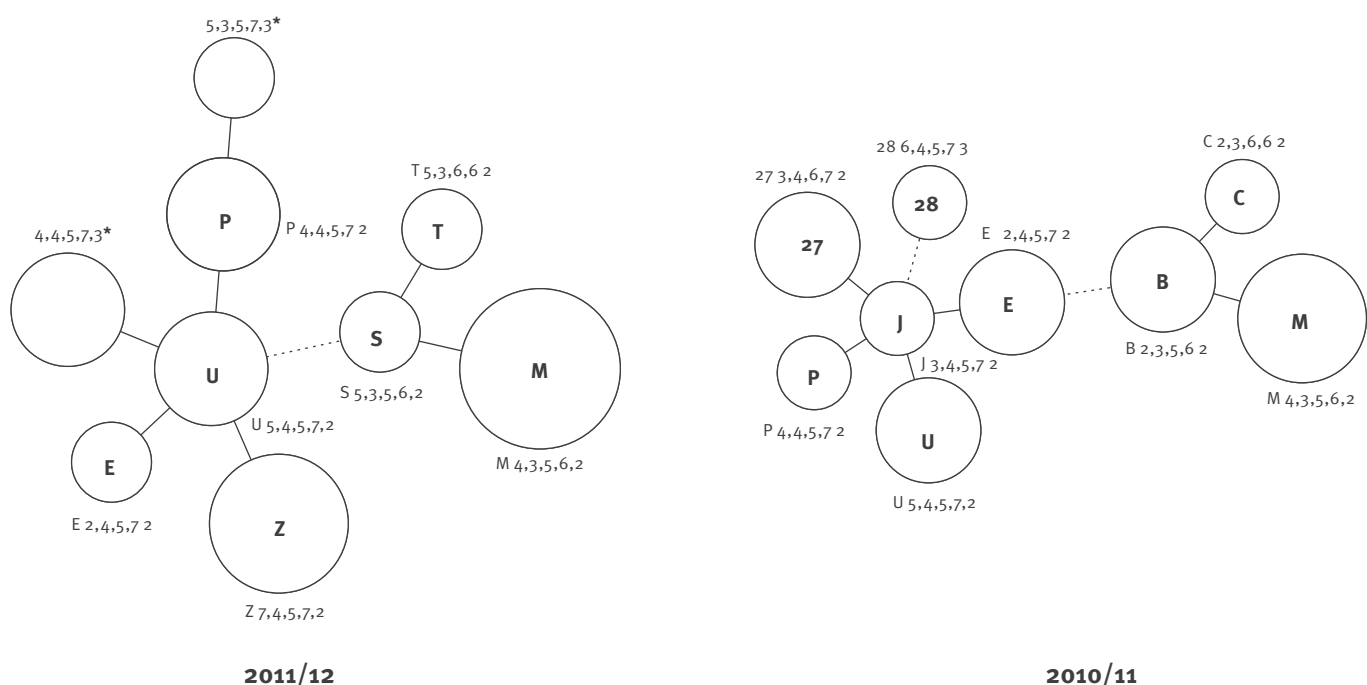
group were infected (14.3%), indicating at least one in seven children with respiratory signs attending general practitioners were infected with *M. pneumoniae*.

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.

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

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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).

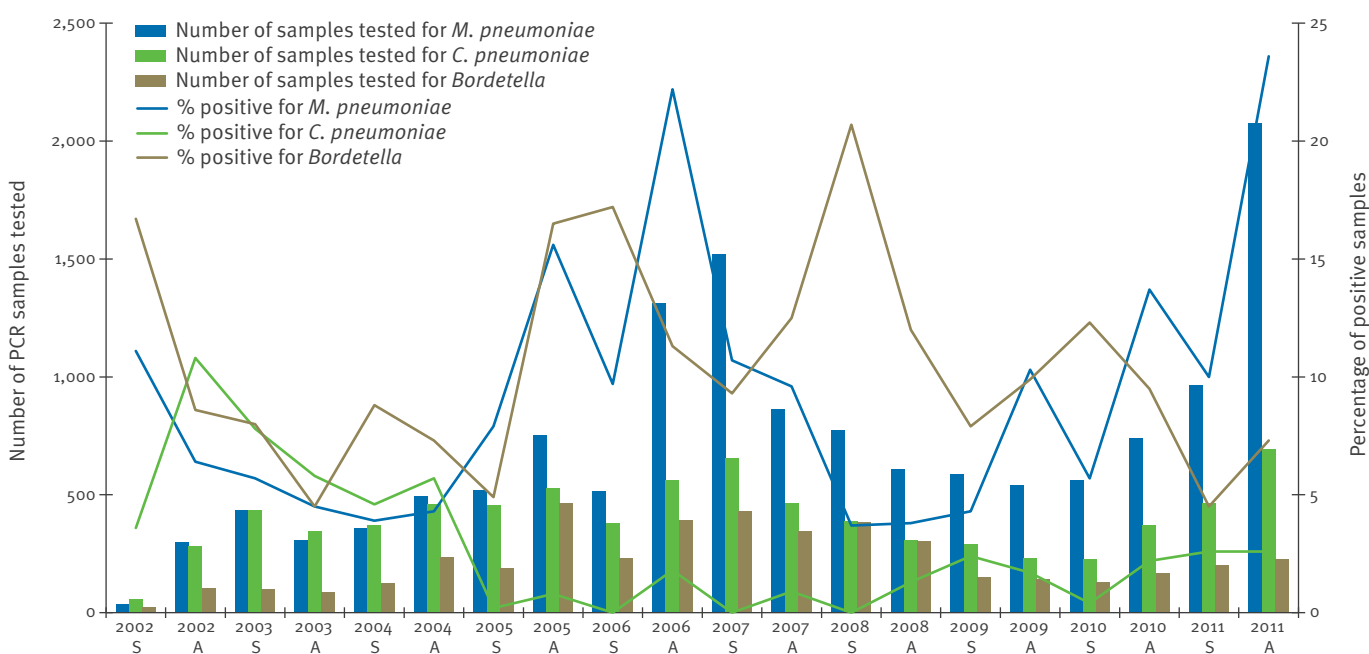
Discussion

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*, *Chlamydia pneumoniae* and *Bordetella pertussis/parapertussis* and the rate of positives per half year, Skövde, 2002–2011



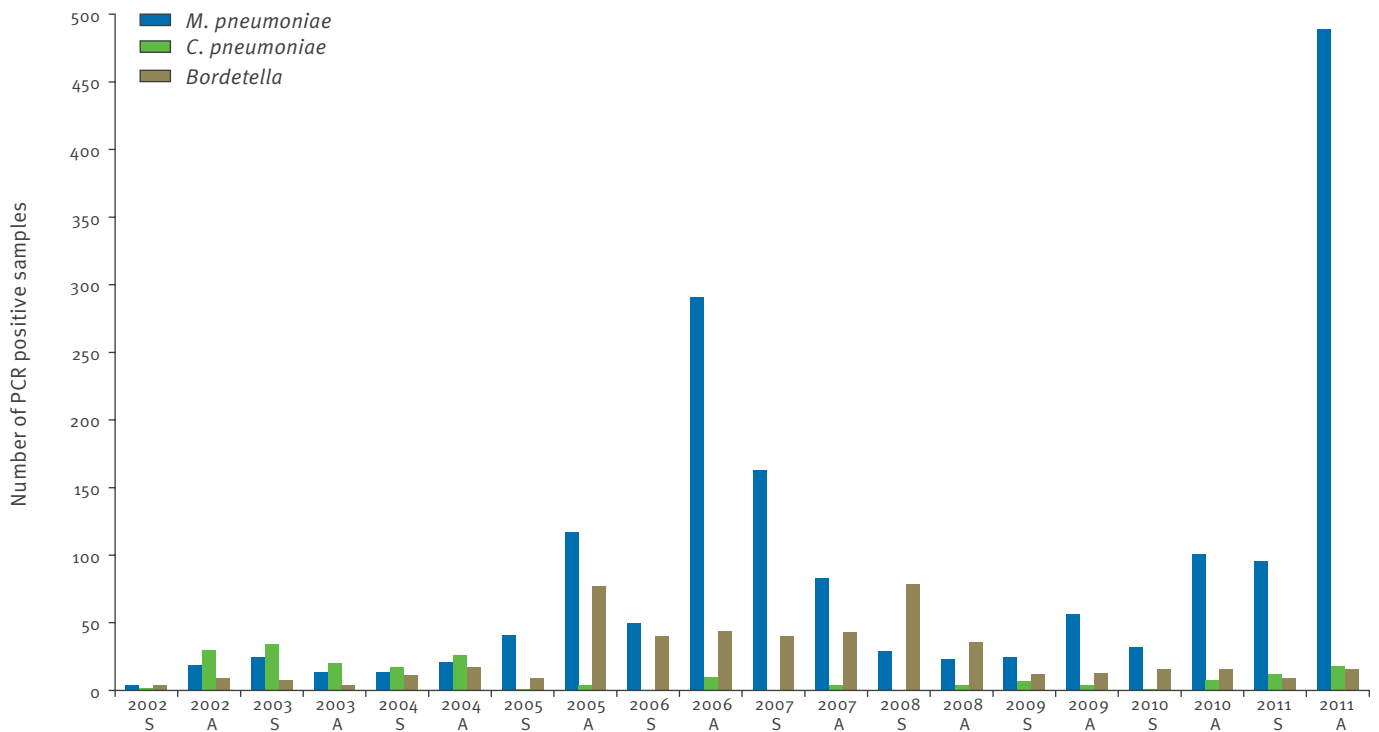
A: autumn; S: spring.

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

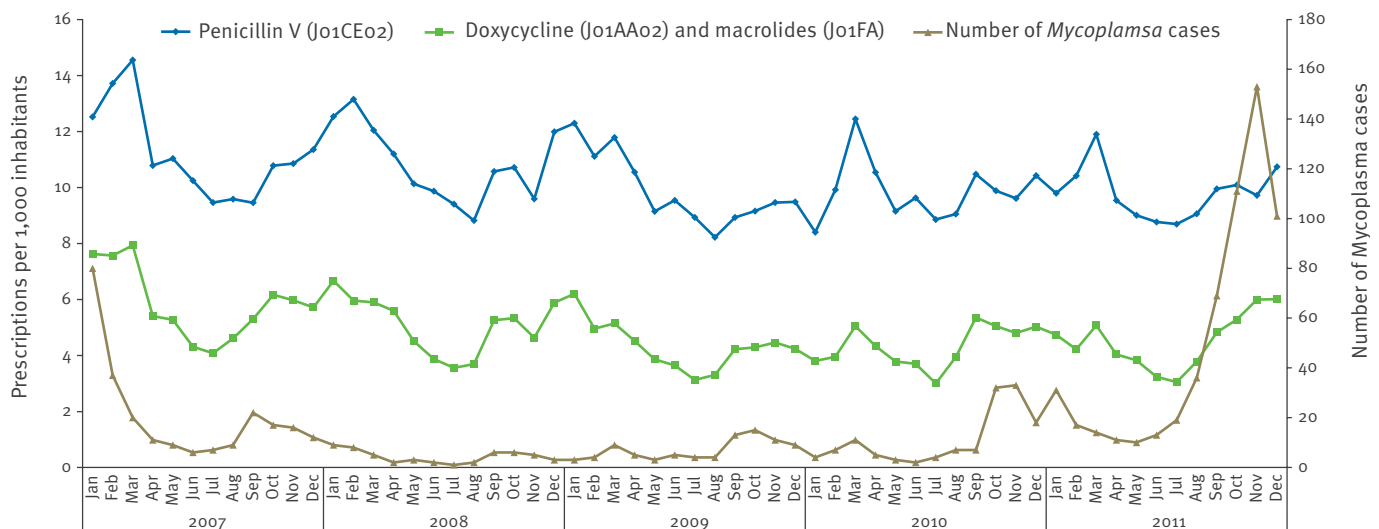
Number of samples positive for *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and *Bordetella pertussis/parapertussis* per half year, Skövde, 2002–2011



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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Surveillance status and recent data for *Mycoplasma pneumoniae* infections in the European Union and European Economic Area, January 2012

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In January 2012, the European Centre for Disease Prevention and Control (ECDC) conducted an email-based survey of European Union and European Economic Area countries to describe the existing surveillance activities for *Mycoplasma pneumoniae* infections, recent findings and existence of clinical guidelines for the treatment of *M. pneumoniae* infection. Of the 20 countries that participated in the survey, seven reported increases in *M. pneumoniae* infections observed during the autumn and winter of 2011.

In the first week of January 2012, the Norwegian Medicines Agency reported a likely shortage of erythromycin in the country following an unusually high number of mycoplasma infections [1]. Additional epidemic intelligence activities conducted at the European Centre for Disease Prevention and Control (ECDC) highlighted that similar increases in *M. pneumoniae* infections had been observed during the autumn of 2011 in various northern European countries, including Sweden, Denmark, Finland and the Netherlands [2-6].

With this epidemiological background and because *M. pneumoniae* infection is not notifiable at the European Union (EU) level, ECDC, in collaboration with EU and European Economic Area (EEA) Member States, conducted a brief survey among countries in order to verify whether unusual increases in reporting rates were recently observed, to describe existing *M. pneumoniae* surveillance activities and availability of guidelines for the treatment atypical pneumoniae which might include *M. pneumoniae* infections for clinicians in the country.

An email-based questionnaire was sent to EU/EEA Member States contact points (listed as Competent Bodies for Threat Detection) on 10 January 2012. Countries were asked to provide answers by the evening of 12 January 2012.

The questions asked in the email questionnaire are shown in the Box.

Disease background information

Mycoplasma pneumoniae, a bacterium lacking a cell wall, is a major cause of respiratory disease in humans. Infection can lead to prolonged carriage and therefore serve as a reservoir for the spread of the pathogen to others [7]. It is transmitted from person-to-person by respiratory droplets and its incubation period varies from one to three weeks, although it can be as short as four days [8]. *M. pneumoniae* infections tend to be endemic, punctuated by epidemics at four-to-seven-year intervals [9,10]. Climate, seasonality and geographical location are not thought to be of major importance, although in North America most epidemics usually begin during summer, peak in late autumn/

Box

Email questionnaire regarding *Mycoplasma pneumoniae* infection sent to EU/EEA countries, January 2012

1. Do you have MP surveillance ongoing in any form in your country?
2. If yes, please describe briefly which sources of information (including diagnostic tests, hospital-based/laboratory based, sentinel hospitals or standardised etc) are used by the ongoing surveillance in your country and whether there have been any major changes in the system in 2010 and 2011.
3. If you do have some form of MP surveillance, could you indicate whether you have seen any significant increases (or decreases) this autumn and winter or in previous years?
4. Do you have existing national guidance for clinicians on the treatment atypical pneumonia, including infections with MP?
5. Do you have existing national guidance for handling outbreaks of atypical pneumonia, including with MP in institutional settings?

EEA: European Economic Area; EU: European Union;
MP: *Mycoplasma pneumoniae*.

early winter and fade out during winter [8,11]. However, this pattern seems to differ between continents [8,11].

M. pneumoniae infects the upper and lower respiratory tracts in children and adults and is one of the aetiological agents of community-acquired pneumonia [11,12]. Studies have shown that it can cause up to 40% of community-acquired pneumonia and 18% of hospitalisations in children [13]. Most *M. pneumoniae* infections lead to overt clinical disease and although these infections are often self-limiting, 1–5% of cases may require hospitalisation. The most prominent symptoms are malaise, fever, headache and cough and in children aged less than five years, coryza and wheezing [13]. *M. pneumoniae* infection can also result in extrapulmonary manifestations, which can be present before, after or even in the absence of respiratory symptoms and have been reported with varying rates. Extrapulmonary manifestations of infection are rare, but when they occur can affect the central nervous system (including encephalitis and cranial nerve palsies) [11,14] and can also result in dermatological, haematological and cardiac manifestations [13].

Diagnostic testing for *M. pneumoniae* includes, among others, polymerase chain reaction (PCR) and serological assays, each with varying sensitivities and specificities and limited standardisation between testing protocols [15,16]. PCR is the preferred method in some countries [17]; however, no testing method has proven reliable in the context of an outbreak [14]. Surveillance data for *M. pneumoniae* infections are likely to be underestimated because of the challenges in diagnosis as well as the fact that in many cases, the infection is often subclinical and usually dealt with in outpatient settings.

National and international guidelines are available for the management of community-acquired pneumonia, including for those caused by *M. pneumoniae*. Therapeutic decision-making is up to the clinical judgement of the treating physician based on clinical presentation, co-morbidities, risk factors, assessment of pneumonia severity and the available evidence-based guidelines. Effective antibacterial agents for the treatment of *M. pneumoniae* include macrolides, tetracyclines and fluoroquinolones. Prudent use of antibiotics is urged for all cases of *M. pneumoniae* infection because of worldwide reports of macrolide resistance. Moreover, it is suggested that treating clinicians be vigilant when prescribing macrolides for suspected or confirmed cases, particularly in areas with high rates of macrolide resistance, as treatment might fail in patients infected with macrolide-resistant isolates.

Recent studies on previous outbreaks in both community and institutional settings have been published from Denmark [9], England and Wales [18], Finland [19], France [20], Italy [21], the Netherlands [7] and Scotland [22].

Survey findings

Of the 30 countries contacted, 20 replied to the questionnaire (response rate: 67%). Of those that replied, 13 reported having some type of surveillance activities providing data to monitor *M. pneumoniae* infections. Table 1 summarises the situation in 2011 and in previous seasons as well as surveillance activities. Seven countries had no available data that could be used to indicate changes in reporting rates for *M. pneumoniae* infections during 2011 compared with previous seasons. Of the 13 countries monitoring *M. pneumoniae*, seven indicated observing an increase compared with 2010 while six indicated no such increase (Belgium, Malta, Portugal, Slovakia, Slovenia and Spain). Of these six, Slovenia reported that reporting rates for *M. pneumoniae* infections were higher in the autumn of 2010 compared with the same period in 2011.

None of the responding countries reported major recent changes in the existing surveillance systems that would account for the observed increases. However, Sweden did highlight that awareness of *M. pneumoniae* among clinicians may be higher during this winter season, which may have resulted in more testing. Also, the widespread use of PCR for testing might have had an impact on current surveillance data.

With respect to which methods were used for laboratory diagnosis of *M. pneumoniae*, ten countries were able to provide some information. Five of these countries (the Netherlands, Norway, Spain, Sweden and the United Kingdom) reported using a mixture of serology and PCR. The Czech Republic and Portugal used mainly serological tests. Denmark and Slovenia reported data for samples confirmed by PCR and Finland reported using serology, PCR or culture for the diagnosis of *M. pneumoniae*.

A total of 15 countries reported some form of guidance available for clinicians for the treatment of atypical pneumonia, including *M. pneumoniae* infection; 10 countries have guidelines that are considered national (Table 2). Six reported the existence of guidelines that can be used in institutional outbreaks. Even though none are specific for *M. pneumoniae* infection, these guidelines would be applied in the occurrence of an outbreak of *M. pneumoniae* infection in institutional settings.

Limitations of the study

This survey was conducted as a part of epidemic intelligence activities conducted at the EU level. The questions included were not comprehensive enough to provide a complete and detailed overview of the functioning of the surveillance systems for *M. pneumoniae* infection in all countries. Details of diagnostic tests used, indicators for surveillance, frequency of surveillance, implicated stakeholders, etc. are therefore missing from this report. Furthermore, as clinical data and type of diagnostic test used for the diagnosis of each case were also not provided in the responses to

the survey, we have not been able to provide a direct comparison of such data between countries in this report. Additionally, given the short deadline, it may have been difficult for several countries to collect the relevant information in time.

Conclusion

As expected, surveillance for *M. pneumoniae* infections across responding EU/EEA countries is highly variable in terms of data collected and methods of laboratory detection of cases. For this reason, comparisons of surveillance data from different countries have limitations. However, information from predominantly northern European countries (Denmark, Finland, the Netherlands, Norway, Sweden, United Kingdom) and the Czech Republic does suggest that the autumn of 2011 had an increase of *M. pneumoniae* infections reported through the existing surveillance systems. Data from Denmark as presented earlier and in this issue [9,23] and Sweden [24] suggests that the epidemic wave started in 2010. With the results from our study, however, we cannot assess whether the

reported increases fit into the expected four- to seven-year epidemic waves even though this seems to be indicated by data from Finland, Norway and Denmark in this issue [23,25,26].

Available data seem to suggest that Member States from southern Europe are not yet facing an increase as important as that reported in the northern countries. Increasing awareness among healthcare providers in countries not yet heavily affected could strengthen surveillance activities and ensure timely diagnosis and appropriate treatment of the disease in affected patients. It would be interesting to analyse whether in the countries where increases in *M. pneumoniae* infection rates were reported, similar increases or concurrent decreases in reporting rates for other respiratory pathogens took place during the same time period. However, this was beyond the scope of this assessment.

For the responding countries for which information was available, it is clear that all treating clinicians

TABLE 1

Availability of surveillance data for *Mycoplasma pneumoniae* infection and comparison with 2010, EU/EEA countries, January 2012

Country	Data available on <i>M. pneumoniae</i> infections	Increase compared with 2010	Comments
Czech Republic	Yes	Yes	Numbers stable but percentage of positive samples 35% in 2011 compared with 21% during the same period in 2010.
Denmark	Yes	Yes	Almost twice as many samples were investigated in 2011 compared with 2010, but the proportion of <i>M. pneumoniae</i> -positive samples remained the same. An epidemic was also seen in 2010 [9].
Finland	Yes	Yes	Increase in <i>M. pneumoniae</i> infections reported since October 2010.
The Netherlands	Yes	Yes	Important increase in <i>M. pneumoniae</i> infection reports in autumn 2011, similar to previous epidemics in 2002 and 2005.
Norway	Yes	Yes	Increase in <i>M. pneumoniae</i> -positive samples since September 2011. Last epidemic reported in 2005/06 season.
Portugal	Yes	No	Retrospective data of discharged hospitalised cases, although underestimates, suggests a mean of 100 cases of <i>M. pneumoniae</i> infection per year based on laboratory results (serology), with no changes in the last 10 years.
Sweden	Yes	Yes	All time high in <i>M. pneumoniae</i> infection reports during autumn 2011.
United Kingdom ^a	Yes	Yes	Increase in <i>M. pneumoniae</i> infection reports since end of 2011, in line with reports during previous seasons.
Belgium	Yes	No	No observed increase.
Malta	Yes	No	No observed increase.
Slovakia	Yes	No	No observed increase.
Slovenia	Yes	No	Decrease compared with 2010.
Spain	Yes	No	No observed increase.
Cyprus	No	–	–
France	No	–	–
Greece	No	–	–
Hungary	No	–	–
Ireland	No	–	–
Poland	No	–	–
Romania	No	–	–

EEA: European Economic Area; EU: European Union.
a England, Wales and Scotland.

TABLE 2

Existence and details of clinical guidelines available in EU/EEA countries for treatment of *Mycoplasma pneumoniae* infection, January 2012

Country	Guidelines available	Details on available guidelines
Belgium	Yes	Case treatment: recommendations on treatment of lower respiratory infections from the Belgian Antibiotic Policy Coordination Committee (BAPCOC) [http://www.bapcoc.be/].
Czech Republic	Yes	Case treatment: (i) standards for the usage of antibiotics [http://www.cls.cz/dalsi-odborne-projekty/]; (ii) specific guidelines for diagnostics and treatment of pneumonia in adults [http://www.pneumologie.cz/].
Denmark	Yes	Case treatment: hospital-specific guidelines in addition to guidelines from Statens Serum Institut [http://www.ssi.dk/].
Finland	Yes	Case treatment: national guidance for treatment of pneumonia, including <i>M. pneumoniae</i> infection and other atypical pneumonia.
France	Yes	Case treatment: recommendations on treatment of lower respiratory infections from the French Agency for the Safety of Health Products (Afssaps) [http://www.afssaps.fr/content/download/26334/348020/version/7/file/map-infections-respiratoires-basses-adultes.pdf]. Institutional settings: national recommendations for treatment of lower respiratory infections in homes for the elderly by the Ministry of Health [http://www.sante.gouv.fr/].
Greece	Yes	Case treatment: national treatment guidelines exist on the management of community-acquired pneumonia, which include atypical pneumonia and infections with <i>M. pneumoniae</i> by the Hellenic Centre for Disease Control and Prevention (KEELPNO) and the Hellenic Society of Infectious Diseases [http://www.keelpno.gr/]. Institutional settings: KEELPNO has guidance for handling airborne infections in institutional settings [http://www.keelpno.gr/].
Hungary	Yes	Case treatment: national guidance exists, but does not address the newer diagnostic methods (e.g. PCR).
Ireland	Yes	Case treatment: Hospitals used their own guidelines for treatment of community-acquired pneumonia based on the latest guidelines from the British Thoracic Society, European Respiratory Society and the Infectious Disease Society of America. In children, the Paediatric Infectious Disease Society guidelines for community-acquired pneumonia in children are usually followed.
Malta	Yes	Case treatment: national guidelines have recently been published.
The Netherlands	Yes	Case treatment: (i) National Institute for Public Health and the Environment (RIVM): guideline specific for <i>M. pneumoniae</i> infection; (ii) Dutch College of General Practitioners: guideline for standard 'acute cough'. This includes case treatment of community-acquired pneumonia by general practitioners; (iii) Dutch Working Party on Antibiotic Policy (SWAB): guideline on the management of community-acquired pneumonia in adults [http://www.swab.nl/swab/cms3.nsf/uploads/6929745C8C9BE541C125794900720B77/\$FILE/CAP_SWAB_Nov14-def.pdf]. Institutional settings: guidelines for infectious respiratory disease outbreak management, but not specific for <i>M. pneumoniae</i> infection.
Norway	Yes	Case treatment: National guidelines on which antibiotics to use.
Portugal	Yes	Case treatment: recommendations of the National Society of Pneumologists for treatment of community-acquired pneumonia in hospitalised patients and outpatients covers infection with atypical microorganisms in all types of patients [http://www.sppneumologia.pt]
Romania	Yes	Case treatment: each infectious diseases clinic receives guidelines prepared by specialists from the Regional Academic Centre.
Slovakia	Yes	Case treatment: guidance on the management of <i>M. pneumoniae</i> infection is included in guidance of management atypical pneumonia, which has been prepared by a working group of experts from the Slovakian Pneumological Society.
Slovenia	Yes	Case treatment: national treatment guidelines exist [http://www.szd.si/user_files/vsebina/Zdravniski_Vestnik/2010/marec/245-64.pdf].
Spain	Yes	Case treatment: several national guidance documents for clinicians on treatment the atypical pneumonia prepared by scientific societies such as the Spanish Society of Infectious Diseases and Clinical Microbiology and Spanish Association of Paediatric Primary Care. Institutional settings: infection control guidance for institutional care settings and nosocomial outbreaks, including respiratory tract infections.
Sweden	Yes	Case treatment: STRAMA (Swedish strategic programme against antibiotic resistance) guidance on how to treat pneumonia in outpatient care.
United Kingdom	Yes	Case treatment: guidance on the management of community-acquired pneumonia by the British Thoracic Society, which includes consideration and treatment of, <i>M. pneumoniae</i> infection [http://www.brit-thoracic.org.uk/Portals/o/Clinical%20Information/Pneumonia/Guidelines/CAPGuideline-full.pdf]. Institutional settings: the Health Protection agency has guidance on the management of outbreaks of acute respiratory infection in institutional settings.
Cyprus	Data not available	–
Poland	Data not available	–

have access to guidance on how to treat *M. pneumoniae* infections even though it is a reality that the majority of these infections remain undetected and under-diagnosed.

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Increased incidence of *Mycoplasma pneumoniae* infection in Finland, 2010–2011

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The number of cases of *Mycoplasma pneumoniae* infection detected by laboratory-based surveillance increased in Finland in late 2010. During 2011, the number of cases was four times higher than during the previous epidemic in 2005. The 2011 epidemic affected mostly school-age children. The increased number of cases was probably not due to changes in laboratory procedures, but public interest may have had an effect, since the number of Google queries followed closely the epidemic curve.

The number of cases of *Mycoplasma pneumoniae* infection in Finland started to increase in October 2010 (222 cases; 4.1 per 100,000 population) and rose further during 2011 (in October, 1,242 cases; 23.1 cases per 100,000 population). Denmark, England and Wales also saw an increased incidence of *M. pneumoniae* infections in late 2010 [1,2]. Throughout 2011, the epidemic of *M. pneumoniae* infection in Finland attracted considerable public interest and media attention.

In order to assess the extent of this ongoing epidemic, we analysed the data on *M. pneumoniae* infection from laboratory-based surveillance. We also evaluated whether changes in laboratory methods and practices as well as public interest in the epidemic during 2011 were related to the size of the epidemic.

Background

M. pneumoniae causes mainly infection of the upper respiratory tract (tracheitis, bronchitis) and, in 3–10% of cases, pneumonia. Rare neurological symptoms such as meningitis and Guillain–Barré syndrome can be observed [3]. The bacterium is spread by respiratory droplets and direct contact with an infected person. The disease occurs in all age groups but is most common among children aged 7–16 years and young adults aged 17–25 years. Presumably due to lack of

lifelong protective immunity and changes in circulating *M. pneumoniae* strains, epidemics typically occur in 3–5-year intervals [3], with seasonal peaks in autumn and winter.

National laboratory-based surveillance system

The laboratory-based surveillance system in Finland (population 5.4 million) covers 20 healthcare districts with catchment populations ranging from 68,000 to 1.4 million. Since 1995, all clinical microbiology laboratories mandatorily notify all positive findings of *M. pneumoniae* (culture, diagnostic rise in *M. pneumoniae*-specific IgG antibody titre, detection of *M. pneumoniae*-specific IgM antibodies and nucleic acid detection) to the National Infectious Disease Register, maintained by the National Institute for Health and Welfare. The following information is collected with each notification: date of birth, sex, unique national identity code, place of treatment, type of specimen and diagnostic method. Multiple notifications with the same national identity code are merged into one case, if reported within 12 months of each other. In this study, we analysed cases of *M. pneumoniae* infection notified to the National Infectious Disease Register from 1 January 1995 to 31 December 2011.

Study approach

To investigate whether there have been changes in laboratory methods or practices regarding *M. pneumoniae* diagnosis, we carried out an email survey of the five biggest laboratories in the country, located in Helsinki, Turku, Tampere and Kuopio, which notified 97.5% of all *M. pneumoniae* cases during 2010 and 2011. We asked about the total number of tests performed per month and the proportion of tests positive for *M. pneumoniae* per month in 2010 and 2011. In addition, we asked the laboratories which tests they used and whether there

had been changes in tests since the previous epidemic in 2005.

To investigate the extent of public interest in *M. pneumoniae*, we used Google Insight for Search beta and Google AdWords applications. We obtained the number of Google queries for 'mycoplasma' in Finland, during 2004 to 2011 by month.

Surveillance data

The number of cases of *M. pneumoniae* infection began to increase since October 2010 (Figure 1). The first peak was in March 2011 (n=838). The number of cases dropped between April and July 2011 and then started to increase again in September 2011 (n=667). The number of cases rose from 1,948 (36.2 per 100,000 population) in 2010 to 7,772 (145 per 100,000 population) in 2011. In 2011, the increase in the number of *M. pneumoniae* cases was detected in all healthcare districts but the incidence varied regionally (range by healthcare district: 55 per 100,000 population to 257 per 100,000 population).

During 1995 to 2011, a total of 22,835 cases were notified. Previous epidemics occurred in the winters of 2000–2002 and 2004–2006 with a peak in 2005 (1,881 cases; 36 per 100,000 population). These earlier epidemics lasted about two years, i.e. over two cold seasons.

The annual incidence during 1995 to 2011 was highest among children aged 5–14 years and lowest among elderly persons aged 65 years and older (Figure 2).

In 2011, the median age of the cases was 18 years (range: 0–85) and 4,418 (57%) were female. During

2005 to 2011, the median age of the cases was also 18 years (range: 0–104) and 13,185 (58%) were female. The difference by sex was most prominent in persons aged 15–64 years, among whom the incidence was 1.8-fold higher in females than in males both during 1995 to 2010 and in 2011.

Most of the notifications were based on testing of serum or plasma (22,486; 98.5%), a few were from bronchoalveolar lavage (63; 0.3%), pharyngeal or nasopharyngeal swabs (94; 0.4%) or cerebrospinal fluid (35; 0.2%). In 98% of the notifications, the diagnostic method was detection of *M. pneumoniae*-specific antibodies; the rest were based on nucleic acid detection by PCR.

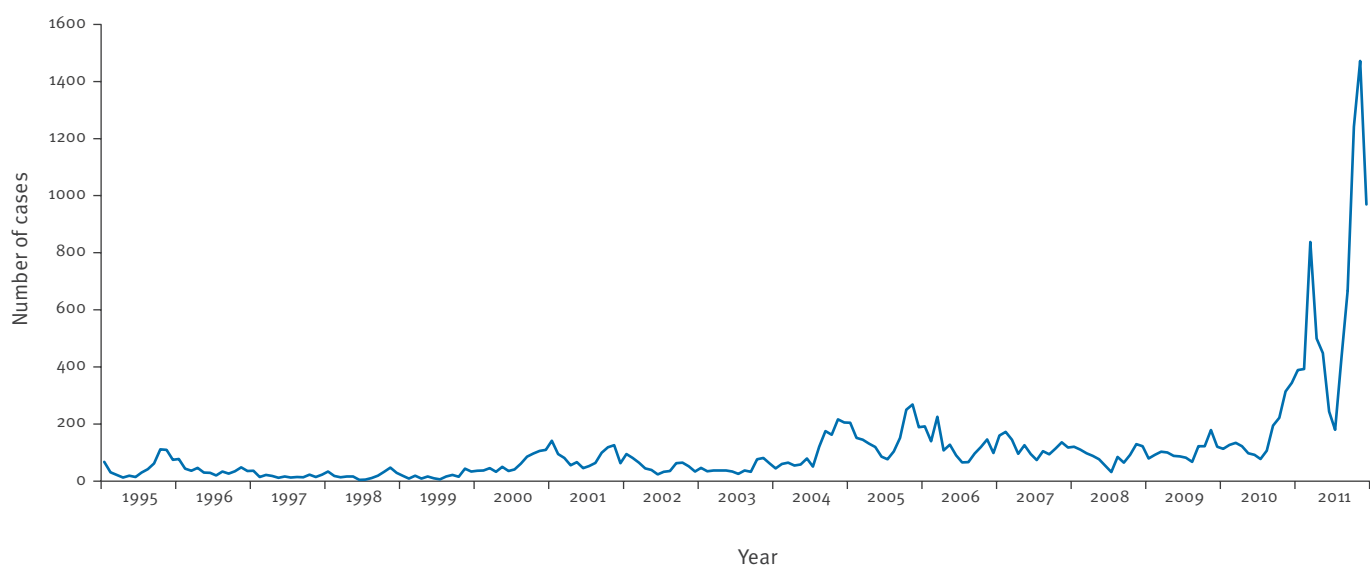
Laboratory survey

In the five laboratories taking part in the survey, detection of *M. pneumoniae* was mainly based on serological tests by enzyme immune assay (EIA). Diagnosis of infection required a diagnostic rise in *M. pneumoniae*-specific IgG antibody titre and/or detection of a *M. pneumoniae*-specific IgM. If necessary, the laboratory recommended collecting convalescent paired sera. Since the previous epidemic in 2005, there has been no change in diagnostic methods.

The number of serological tests performed for *M. pneumoniae* in the five laboratories was on average nearly four times higher in 2011 than in 2010 (range of increase by laboratory: 200–500%). The proportion of tests positive for *M. pneumoniae* during 2010 and 2011 varied between 8% and 17% in the five laboratories. There was also variation during 2010 and 2011 in four of the laboratories: in three the proportion of positive tests increased (from 8% to 9%, from 9% to 11%, from

FIGURE 1

Cases of *Mycoplasma pneumoniae* infection by month reported to the National Infectious Diseases Register, Finland, 1995–2011



Source: National Infectious Diseases Register, Finland.

11% to 17%); in one it decreased slightly (from 8.5% to 8.1%) and in one, it remained the same.

Public interest, assessed through Google queries

The first two peaks in the number of Google queries for ‘mycoplasma’ occurred during the epidemics in 2004–2005 and 2005–2006. After 2007, the number was stable. In October 2010, however, it rose again, peaking in March and November 2011 (Figure 3). As described in [4], the numbers of Google queries in Figure 3 reflect the number of searches per month for ‘mycoplasma’ relative to the total number of searches on Google between 2004 and 2011 in Finland. The data are normalised (data are divided by a common variable to cancel out the variable’s effect on the data) and

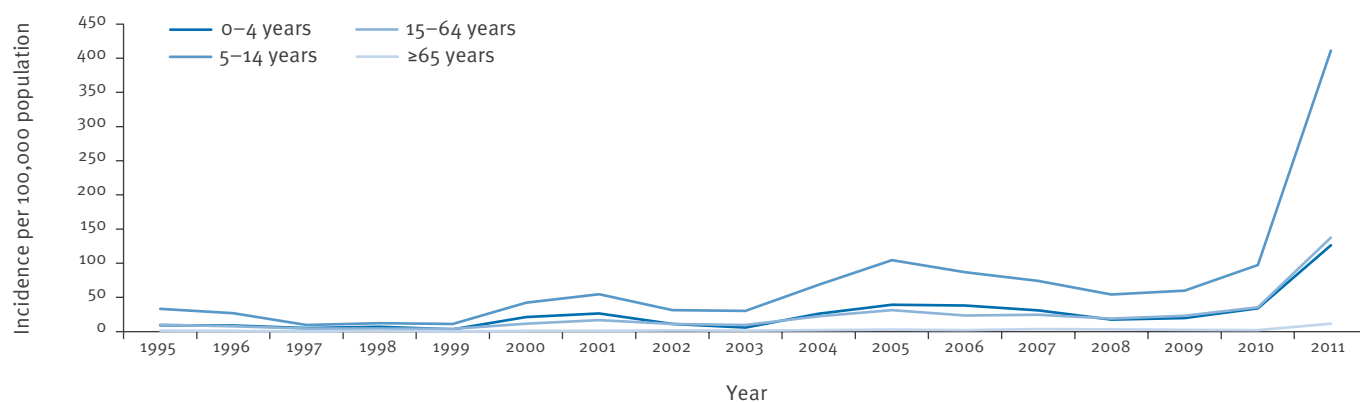
presented on a scale from 0 to 100. On the basis of data from Google AdWords, the approximate 12-month mean number of Google queries for ‘mycoplasma’ in Finland amounted to 7.3% of global searching for this term in 2011. Data on global and local searches in the previous years were not available.

Discussion

Our study based on nationwide laboratory data showed a fourfold increase in incidence and number of cases of *M. pneumoniae* infection in 2011 compared with the previous epidemic in 2005 – the highest in the history of our national surveillance. In Denmark, England and Wales, the previous epidemics were larger than their current ones (at the start of the current epidemics) [1,2]. There were no major changes in laboratory

FIGURE 2

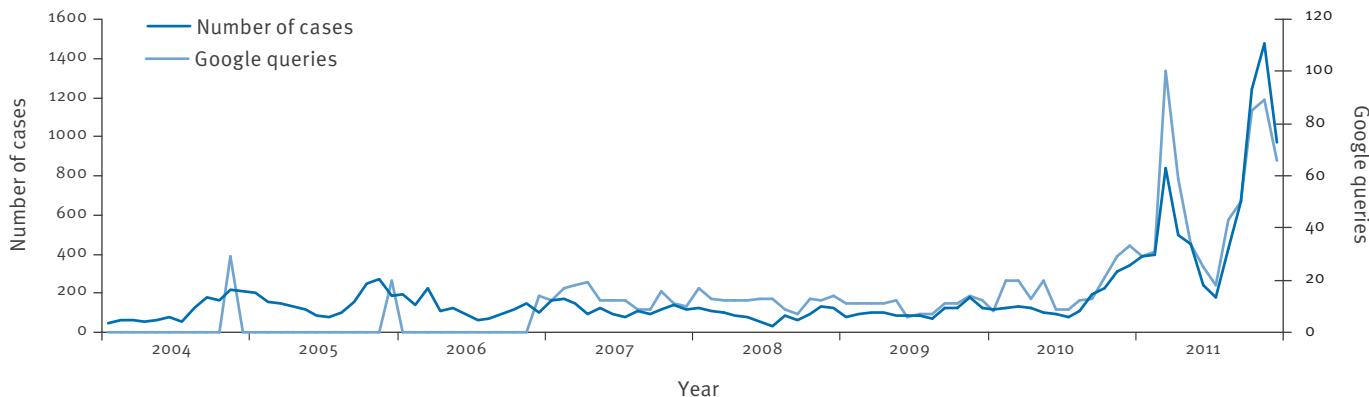
Annual incidence of *Mycoplasma pneumoniae* infection per 100,000 population by age group reported to the National Infectious Diseases Register, Finland, 1995–2011



Source: National Infectious Diseases Register, Finland.

FIGURE 3

Cases of *Mycoplasma pneumoniae* by month reported to the National Infectious Diseases Register and ‘mycoplasma’ queries in Google, Finland, 2004–2011



The Google queries shown in the graph do not represent absolute search volume numbers, because the data are normalised and scaled from 0 to 100. Normalisation means that data sets are divided by an unrelated, common Web search query. Data are scaled using the average search volume over the selected time period as a denominator.

Source: National Infectious Diseases Register, Google Insights for Search (Google data downloaded 21 December 2011).

diagnostics that could have contributed to the extent of the epidemic in Finland. However, data on the number of tests carried out from 2005 to 2006 were not available. As the number of tests performed may influence the rate of positive results, comparison of the heights of the epidemic peaks should therefore be made with caution.

Google is known to be a popular information source [5]. In Finland, Internet access is widespread: about 89% of the population aged 16–74 years used the Internet in the past three months [6]. On the basis of our results, we can assume that the high number of cases of *M. pneumoniae* infection – especially during the current epidemic – may partly reflect the intense public interest in and awareness of the disease. Patients with a prolonged cough may have been more active than in the past in seeking care and requesting testing for *Mycoplasma*, which may, in some instances, have led to unnecessary antimicrobial treatment as prolonged cough after the acute phase of infection may not benefit from such treatment.

Diagnostic testing for *M. pneumoniae* also rose around fourfold in 2011, compared with 2010. The variation in proportion of tests positive for *M. pneumoniae* between laboratories (8–17%) could be related to differences in interpreting the serological results. This finding needs further evaluation, but highlights the importance of standardisation of laboratory methodology. It may also be a sign of regional differences in diagnostic activity and case ascertainment, since the sampling was not structured for epidemiological surveillance. Laboratory diagnosis of *M. pneumoniae* infection is not easy. High levels of *M. pneumoniae*-specific IgM antibodies can persist for several weeks to up to one year after an acute infection [3,7,8]. Furthermore, *M. pneumoniae*-specific IgG antibodies may remain elevated up to four years after illness [9]. In addition, it may be difficult for clinical microbiologists to interpret borderline results, since the date of symptom onset is rarely available in the laboratories.

Our survey found that PCR was not widely used in Finland for diagnosis of *M. pneumoniae* infection. PCR has been found to be superior to serology for the diagnosis of acute *M. pneumoniae* infection and has been shown to be highly sensitive, specific and rapid [10]. However, a positive PCR may be a sign of transient asymptomatic carriage of *M. pneumoniae* or the persistence of the pathogen after infection [9]. In Denmark, where PCR-based surveillance for *M. pneumoniae* infections is established, the proportion of tests positive for *M. pneumoniae* was approximately 3% since 2007 until it rose to 15% in September 2010 when the current epidemic started [1].

We also found that culturing of *M. pneumoniae* was also scarce in Finland. It is known to be difficult, time-consuming and expensive, and therefore rarely routinely used in clinical practice [11]. Thus, information

on the molecular epidemiology of circulating *M. pneumoniae* strains is lacking, and it is also not known whether the current epidemic strains are sensitive or resistant to macrolides, the antimicrobials commonly used in treatment [3].

Since our study was based on laboratory data only, we did not have information on clinical manifestation, severity of the disease or treatment. The burden of the *M. pneumoniae* epidemic in Finland remains unknown. Although people with *M. pneumoniae* infections are mainly seen as outpatients, a register-based linkage study between laboratory-confirmed cases and hospitalisation data or a time series of pneumonia-associated hospitalisation rates could give an insight into the burden and use of macrolides could be analysed.

Physicians and the public have been informed about the symptoms and treatment of *Mycoplasma* infections, as well as the difficulties in diagnosis.

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Increased incidence of *Mycoplasma pneumoniae* infection in Norway 2011

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Epidemics of *Mycoplasma pneumoniae* have recently been reported from England and Wales and from Denmark. A similar increase in *M. pneumoniae* infections was noted in Norway late autumn 2011. The epidemic has resulted in shortage of erythromycin and the use of alternative antibiotics has been recommended.

Background

Following reports of epidemics of *Mycoplasma pneumoniae* in Denmark and England and Wales [1,2], special attention has been paid by the Norwegian Institute of Public Health to detect any similar increase in Norway. Surveillance of *M. pneumoniae* infections in Norway is solely based on a voluntary laboratory-based reporting system, and the disease is not notifiable in the Norwegian Surveillance System for communicable diseases.

Surveillance of *M. pneumoniae* infections in Norway

A voluntary laboratory-based reporting system where a selection of laboratories report to the Norwegian Institute of Public Health the number of patients testing positive for all laboratory-confirmed virus diagnoses as well as for *M. pneumoniae* each month has been in place since 1975. The number of participating laboratories has varied over the years, but there have not been any major changes in the system during the last decades. At present, 16 of 21 diagnostic microbiological laboratories in Norway participate in this surveillance system. This covers more than 80% of the Norwegian population. A total of 12 laboratories, representing all regions of the country, submit data on the number of patients testing positive by serological or molecular tests for *M. pneumoniae*. There is no common case definition for reporting a positive result, and a positive serology may include a single high titre or a rise in *M. pneumoniae*-specific IgG antibody levels. Results obtained are indicative of the *M. pneumoniae* activity in Norway as a whole. Data on the total number of tests performed or age groups among patients with positive test results is not collected in this surveillance system. Monthly reports, available at Department of

Virology, Norwegian Institute of Public Health, are submitted to all the participating laboratories, and to others who may be interested.

Since a consensus meeting of clinical microbiologists in Norway in 2003 [3], polymerase chain reaction (PCR) tests have been recommended as the most specific method of choice for laboratory diagnosis of suspected *M. pneumoniae* infection of less than four weeks duration [3]. Serology may add value to the diagnosis of long-standing infection, either by the detection of increasing antibody levels in paired serum samples, or by high antibody levels in samples drawn at least two weeks after onset of symptoms. Concurrently, the proportion of reported cases identified by PCR increased, while the proportion reported by serology decreased.

The yearly number of *M. pneumoniae*-positive tests reported to the Norwegian Institute of Public Health for the period January 1984 to December 2011 is shown in Figure 1. This figure demonstrates regular recurrent epidemics of *M. pneumoniae* in Norway, occurring with five- to seven-year intervals (2011/12, 2006, 2000, 1993 and possibly also in 1987). During the period from 2007 until August 2011 the number of reported cases remained low. From September 2011 a sharp increase in tests positive for *M. pneumoniae* was observed. PCR and serology were both used in equal measures as diagnostic methods until the epidemic was identified. Hereafter most cases were diagnosed by PCR (Figure 2).

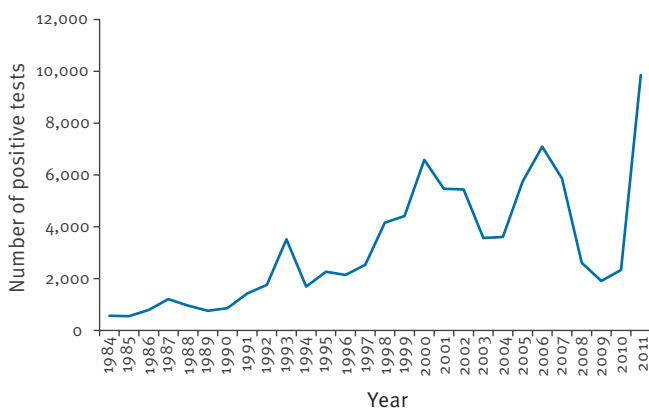
Public health response

Following the observed increase of reported positive tests for *M. pneumoniae*, respective information was published on the website of the Norwegian Institute of Public Health on 25 October 2011 [4]. This website is the main communication platform to clinicians as well as to the media and the public with regards to activity of various infectious diseases in Norway. In addition, a message was posted on a closed communication platform among laboratories in Norway. This communication platform was also used to obtain detailed

descriptions of weekly numbers and proportions of *M. pneumoniae* cases from laboratories in all regions of the country in an ad hoc manner, adding to the surveillance by monthly reporting as described above.

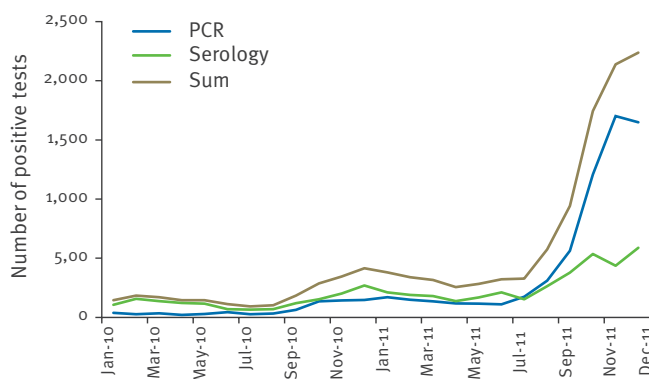
Although most general practitioners and other clinicians are familiar with *M. pneumoniae* infections, these are not considered a well known disease among the general public. Little attention had been given to the last epidemic in 2006. In a new webposting on 7 December 2011 it was emphasised that not all suspected or confirmed cases of *M. pneumoniae* infection need antibiotic treatment [5], and if such treatment was indicated clinicians should chose antibiotics according to recommendations given in the national guidelines on the use of antibiotics in primary health care [6]. In these guidelines, erythromycin and doxycyclin are recommended as the drug of choice in the treatment

FIGURE 1
Number of laboratory-reported *Mycoplasma pneumoniae* infections by year, Norway January 1984 – December 2011



Source: Norwegian Institute of Public Health

FIGURE 2
Laboratory reports of *Mycoplasma pneumoniae* infection by diagnostic methods, Norway January 2010 – December 2011



PCR: Polymerase chain reaction

Source: Norwegian Institute of Public Health

of upper or lower respiratory infections caused by *M. pneumoniae*. Azithromycin is not recommended for the treatment of respiratory tract infections in Norway due risk of resistance development.

Prescription of antibiotics

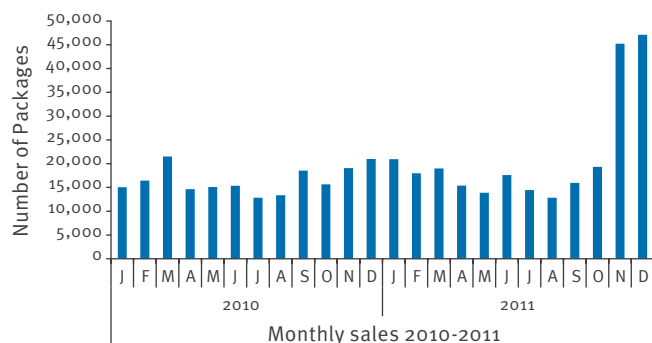
In the two months following publication, a two-fold increase in prescription of erythromycin was seen in Norway compared with the previous months and the same months in 2010. Monthly sales of erythromycin in the period from January 2010 to December 2011 are shown in Figure 3. The reason behind this increase is thought to be extensive treatment with erythromycin in respiratory tract infections suspected to be caused by *M. pneumoniae*. Awareness of the current mycoplasma epidemic might have influenced testing activity for pathogens causing respiratory tract infections, leading to an increase of positive tests.

On 4 January 2012 the Norwegian Medicines Agency reported a shortage of erythromycin in the country expected to last until March–April 2012 [7]. Clarithromycin has been recommended as an alternative to erythromycin in the treatment of respiratory tract infections.

Discussion and conclusion

An epidemic of *M. pneumoniae* has been identified in Norway since September 2011 through voluntary laboratory-based surveillance reporting. The increase in erythromycin prescriptions seen since November 2011 is probably related to extensive and in many cases unnecessary antibiotic treatment of suspected or confirmed cases of *M. pneumoniae* infections. Awareness of the epidemic might have impacted both the laboratory testing rate and the prescription of antibiotics. The regularity in temporal timing of *M. pneumoniae* outbreaks may be used to foresee new epidemics in Norway. Unfortunately, the present reporting system of *M. pneumoniae* infections in Norway is not able to provide data on the overall testing activity for *M. pneumoniae* or other respiratory infections. A better

FIGURE 3
Monthly sales of erythromycin packages in Norway January 2010 – December 2011.



Source: Norwegian Medicines Agency

laboratory-based surveillance system for identifying increase in seasonal and recurrent non-notifiable diseases infections is under consideration.

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Epidemic of *Mycoplasma pneumoniae* infection in Denmark, 2010 and 2011

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Denmark experienced two waves of *Mycoplasma pneumoniae* infection during autumn and early winter in 2010 and 2011, respectively. Both affected the whole country. The proportion of positive results was almost the same for both, indicating that the two waves were probably of equal size. High macrolide consumption during the epidemics did not seem to affect levels of macrolide resistance in *M. pneumoniae*, which remain low in Denmark (1% to 3%).

Epidemics of *Mycoplasma pneumoniae* infection are normally seen at intervals of four to seven years [1,2]. In some cases, simultaneous epidemics are seen in more than one country. In 2010, Denmark [1], England and Wales [2], Sweden [3] and Finland [4] reported more cases of *M. pneumoniae* infection than normal. In autumn 2011, reports from Norway [5], Sweden [3], the Netherlands [6] and Finland [4] indicated an epidemic of *M. pneumoniae* infection in the northern part of Europe. In Denmark, we have also seen a rise in the number of *M. pneumoniae* cases during autumn 2011.

The surveillance of *M. pneumoniae* in Denmark has been described previously [1]. The system is based on laboratory data from Statens Serum Institut (SSI). SSI receives samples (almost an equal number of blood/serum samples for serology and respiratory samples for PCR) from hospitals and general practitioners for routine diagnosis. The diagnosis and surveillance of *M. pneumoniae* infection used to be based on serology in the past, but since the beginning of the 1990s, PCR has been introduced as a routine test at SSI for rapid and early diagnosis of *M. pneumoniae*. A rise in the rate of PCR positive samples at SSI from < 5 % to 15 % or more is considered as indicative of an epidemic [1]. During the last decade, the diagnosis of *M. pneumoniae* has been moved from SSI to local hospital laboratories which have also progressively introduced PCR as a routine diagnostic test for *M. pneumoniae* over the past years. In the beginning of October 2010, SSI

saw an increase in the proportion of positive samples above the threshold (>15%) [7] (Figure 1). This tendency was confirmed by data from hospital laboratories in Denmark and in November 2010 Denmark reported a nation-wide increase in the number and proportion of *M. pneumoniae* PCR positive samples [1]. According to SSI laboratory data, the epidemic peaked in mid-December 2010, while the number decreased rapidly during the rest of December and in January 2011. The number of cases seemed to return to a normal level during spring and early summer 2011 (Figure 1). An increase was observed again in late summer and early autumn 2011 [8]. This prompted SSI to contact a selection of local laboratories all over the country, with a request to submit laboratory data on a weekly basis for *M. pneumoniae* PCR for 2011, to monitor if the rise could be confirmed and if it was nation-wide. The laboratories were selected to cover and represent most of the country, the eastern part (The Capital and Zealand) the mid-south (Funen) and the north-western part (Northern Jutland).

Macrolide resistance in *M. pneumoniae* is a growing problem especially in East Asia, but it is also seen in the United States and Europe [9]. During an epidemic of *M. pneumoniae*, the macrolide consumption is known to increase considerably [10,11]. In December 2010, Denmark saw the highest consumption in a single month (3.9 defined daily doses (DDD)/1,000 population) compared to the consumption in December during the previous nine years (2.5 DDD/1,000 population on average). According to provisional data, the consumption in November 2011 was the highest for the month of November (3.6 DDD/1,000 population) compared to the last 10 years (2.4 DDD/1,000 population on average for November months between 2001 and 2010) personal communication, Maja Laursen, the Danish Medicines Agency, January 2012.

Laboratory investigation

SSI is situated in the Capital Region of Denmark and receives samples predominantly from the Capital Region and the Region Zealand. To further investigate if the rise in the absolute number and in the proportion of positive tests was seen all over the country, the institute received and analysed weekly data from four hospital laboratories (North Denmark Region, Region of Southern Denmark and two laboratories from the Capital Region).

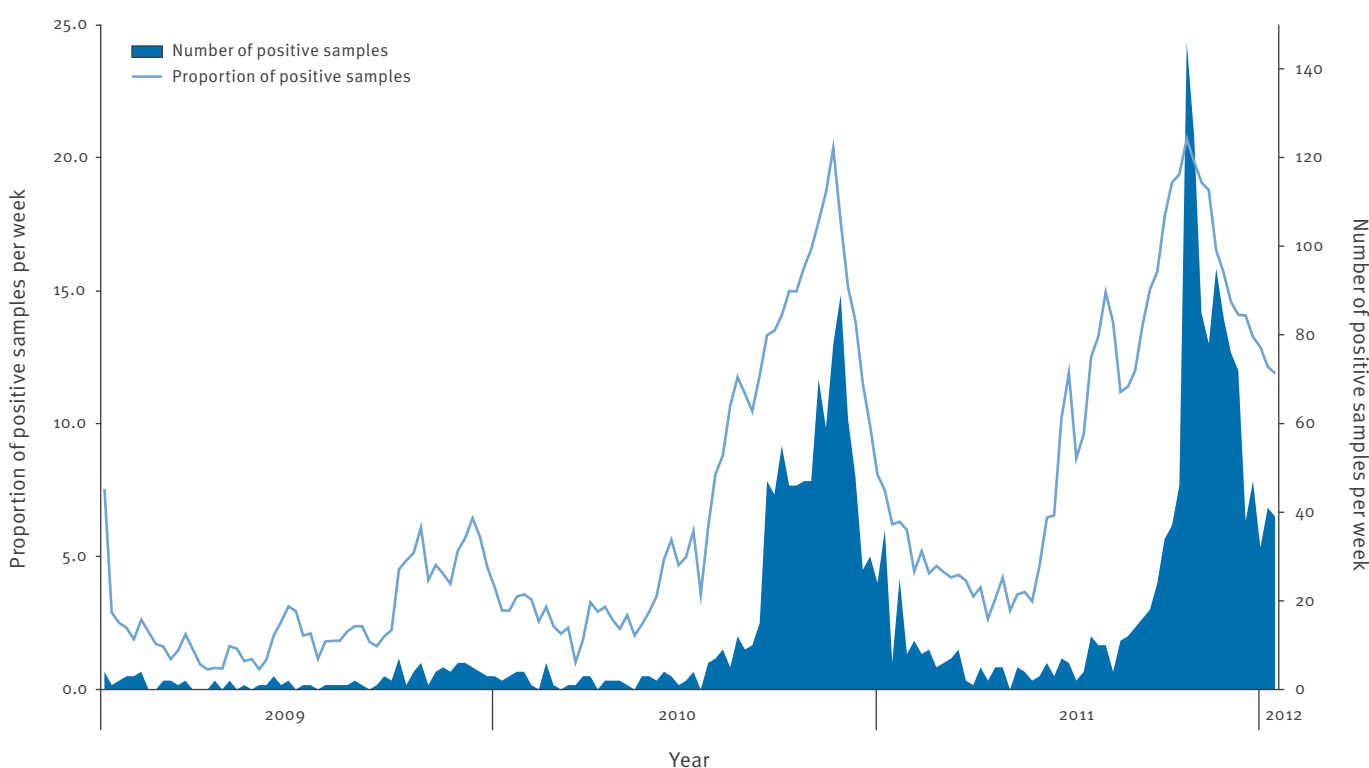
To compare the years 2009 (no epidemic) with the two epidemic years (2010 and 2011) SSI requested in January 2012 results for the period from 2009 to 2011. Data for the whole period were provided by two hospital laboratories (North and Capital 1) and by SSI. The South Denmark region laboratory provided data for 20 September 2010 (week 38) to 31 December 2011 (week 52) and Capital 2 laboratory provided data for 29 August 2011 (week 35) to 31 December 2011 (week 52). Capital 2 also provided data for the epidemic period in 2010 but only for eight weeks (25 October to 19 December 2010) and not on a weekly base but in an aggregated form (Table). The number of positive samples per week from each laboratory is presented in Figure 2. Both waves of the *M. pneumoniae* epidemic were seen in the whole country almost simultaneously (Figure 2).

To compare the two epidemic periods, data for the same period (week 43 to week 50) for the two years from the five laboratories are presented in the table. The peak periods for both epidemic waves were within the selected eight weeks. Twice the number of positive samples (1.9 times) were detected in 2011 compared with 2010, but the number of samples investigated were also almost twice (1.8 times) as high in 2011 compared with 2010. The proportion of positive samples was in general equal during both waves (in average 15%–16.3%) but for North Denmark Region, the rate was higher in 2011 (17.3%) compared with 2010 (14.5%) despite the fact that more than a double number (2.6 times) of samples were tested (Table).

In 2010, the five laboratories diagnosed approximately 70% of all cases in Denmark; assuming that this also applies for 2011, it can be estimated that more than 4,600 cases were diagnosed in Denmark (the country's population counts 5.5 million inhabitants) during the eight-week period from 24 October to 18 December 2011. This corresponds to an incidence of approximately 10 new PCR diagnosed cases per 100,000 population per week in Denmark. In the North Denmark Region, one laboratory received all samples from the region for *M. pneumoniae* PCR. The population size of the region is 580,000 and 125 samples on average were positive per week (Table) giving an estimated incidence of more than 20 new cases per 100,000 population per week. In

FIGURE 1

Positive *Mycoplasma pneumoniae* PCR samples at Statens Serum Institut, Denmark, 1 January (week 1) 2009 to 29 January (week 4) 2012



The proportion of positive tests is the floating average of four weeks.

2010, the estimated incidence for this region was only eight per 100,000 population per week. The diagnostic activity for this region was almost 1 per 100 population during the eight-week period. The diagnostic activity for the whole country can be estimated from the figures in the table. If we consider the five laboratories representing 70% of the diagnostic activity, approximately five persons per 1,000 population were investigated during the eight weeks.

At SSI, we also investigated the prevalence of macrolide resistance for both 2010 and 2011. Macrolide resistance-associated mutations in the gene for the 23 sRNA were identified with a sequencing technique developed at SSI. The technique can be performed directly on DNA purified from PCR positive samples [12]. We did a survey on 140 PCR positive samples consecutively received at SSI during late September and early October 2010 (the beginning of the first wave) and on 108 PCR positive samples consecutively received in January 2011 (the end of the first wave). During the second wave in 2011 we investigated 117 PCR positive samples received in late October and in the beginning of November, representing the beginning of the 2011 wave. In the first wave we found two (1.4%) and three (2.9%) mutations, respectively, and in the second wave we only found one sample with a mutation (0.9%). Data

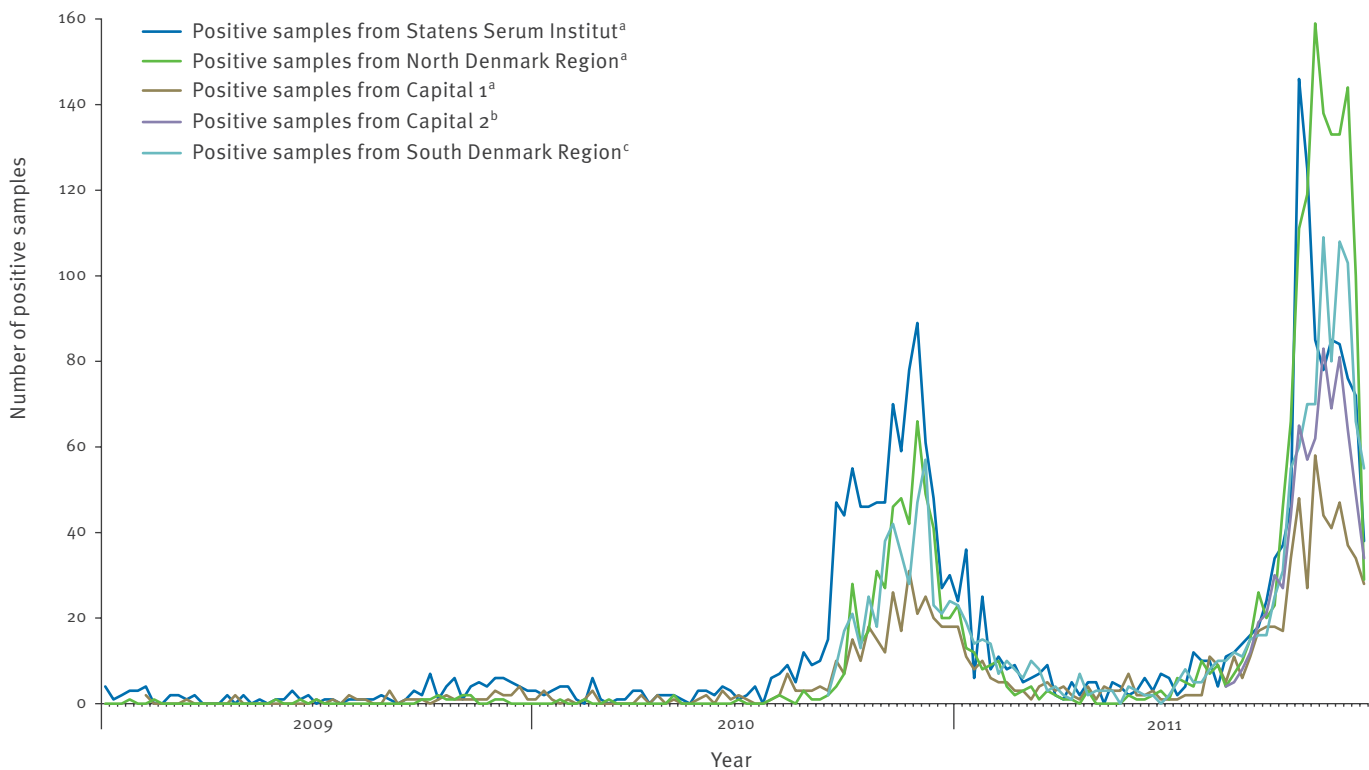
for PCR positive samples from January 2012 (the end of the second wave) are currently unavailable.

Discussion and conclusions

In two successive years, Denmark experienced a high number of *M. pneumoniae* infections during autumn and early winter. The situation can be characterised as one epidemic consisting of two waves. Epidemics spanning two autumn/winter seasons were also seen in Denmark in 1962 to 1964, in 1971 to 1973 and to some degree also in 2004 to 2006 [1]. The total number of PCR positive samples in 2011 was twice the number in 2010, but the number of investigated samples was also twice as high in 2011 compared with 2010 (Table). We are unable to determine whether this reflects a true increase in the number of cases from the 2010 wave to the 2011 wave or whether this reflects an increase in the awareness of the public and among physicians. However, as the proportion of positive samples was almost equal during the two periods, it is reasonable to assume that the two waves were of almost equal size, but the duration of the 2011/12 wave seems to be longer with a more gradual decline than the 2010 wave (Figure 1). However, it seems obvious that the 2011 wave was more extensive than the 2010 wave in the North Denmark Region, and it seems also likely that this region was more affected by the second wave than

FIGURE 2

Number of PCR positive samples from five selected laboratories in Denmark, 2009 to 2011



^a Data were provided for the whole period (2009–2011).

^b Data were provided for 25 October – 19 December 2010 and for 29 August – 31 December 2011.

^c Data were provided for 20 September 2010 – 31 December 2011.

the rest of the country. Although there are differences between the regions, both waves hit the whole country almost simultaneously (Table and Figure 2). The incidence and diagnostic activity for the other regions cannot be estimated as we do not know the population base for the other laboratories. The diagnostic activity for the whole country (5 per 1,000 population) can only be estimated under the assumption that the five laboratories represent 70% of the diagnostic activity during the epidemic. However, a diagnostic activity of approximately 1 per 100 population in North Denmark Region during the eight-week period in 2011 can be considered as high.

The estimated average incidence of PCR diagnosed cases during the epidemic in 2011 was approximately 10 new cases per 100,000 population per week; this is probably a vast underestimation of the real number of cases of *M. pneumoniae* infection during this period, as many patients with mild symptoms will not consult their general practitioner, and only a fraction of patients who visit a practitioner will have samples collected for *M. pneumoniae* PCR.

Although the consumption of macrolides is high during an epidemic of *M. pneumoniae* it does not seem to influence the prevalence of macrolide resistance in *M. pneumoniae*. This is in contrast to other respiratory pathogens, such as *Streptococcus pneumoniae*, where resistance is closely linked to increased macrolide use [13]. This link was also observed following a previous Danish *M. pneumoniae* epidemic in 1998/99 [11]. However, we still need to investigate samples collected in January 2011 before any categorical statement on *M. pneumoniae* susceptibility to macrolides. Macrolide resistance in *M. pneumoniae* may be characterised as low in Denmark, as there is still no general problem, but in specific cases, macrolide resistance can lead to relapse and prolonged disease [12].

TABLE

Number and proportion of *Mycoplasma pneumoniae* samples tested by PCR at five laboratories, Denmark, 25 October (week 43) to 19 December (week 50) 2010 and 24 October (week 43) to 18 December (week 50) 2011

Laboratory (region)	Weeks 43–50 2010		Weeks 43–50 2011	
	Number of samples	Number of positive samples (%)	Number of samples	Number of positive samples (%)
SSI ^a	3,091	497 (16.1)	4,393	725 (16.5)
Capital 1	1,109	165 (14.9)	2,412	336 (13.9)
Capital 2	2,669	349 (13.1)	3,300	519 (15.7)
North	2,253	362 (14.5)	5,787	1,003 (17.3)
Southern	1,946	290 (14.9)	3,994	655 (16.4)
Total	11,068	1,663 (15.0)	19,886	3,238 (16.3)

^a SSI: Statens Serum Institut.

We believe that it is important to have a national surveillance system for monitoring both the prevalence of the disease and the macrolide resistance in *M. pneumoniae*.

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Increased reports of *Mycoplasma pneumoniae* from laboratories in Scotland in 2010 and 2011 – impact of the epidemic in infants

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In common with reports from other European countries, we describe a substantial increase in the number of laboratory reports of *Mycoplasma pneumoniae* in Scotland in 2010 and 2011. The highest number of reports came from those aged one year and younger. However, reports from young children were more likely to come from PCR testing than serological testing.

In light of the increasing incidence of *M. pneumoniae* in other parts of the United Kingdom (UK) and Europe in 2010 and 2011, we examined the numbers of *M. pneumoniae* laboratory reports in Scotland from January 2008 to December 2011. Here we describe the temporal distribution of reports and the age groups most affected.

Background

Mycoplasma pneumoniae causes upper and lower respiratory tract infection in all age groups. However, it is a particularly important bacterial cause of community-acquired pneumonia in children [1]. *M. pneumoniae* is endemic worldwide, but epidemics are common; historically in the UK, these usually occur once every four years [2]. The most recent increase in the incidence of *M. pneumoniae* was seen in England and Wales in 2010 and 2011 [3,4]. Similar increases have also been noted in many other countries in the same period, particularly in northern Europe [5-12].

Although the main burden of infection is typically found in school-age children [4,6,10], *M. pneumoniae* has also been noted as a significant cause of respiratory tract infection in children under the age of five [13-15]. As the possibility of *M. pneumoniae* infection may be overlooked in young children, recent UK clinical guidelines emphasise that *M. pneumoniae* is not uncommon in those aged one to five years [1]. However,

the local availability of different testing methodologies for *M. pneumoniae* may determine how frequently *M. pneumoniae* is diagnosed in particular age groups.

National laboratory-based surveillance and reporting

In Scotland, some diagnostic laboratories carry out PCR testing for *M. pneumoniae* as part of a multiplex real-time PCR screening approach for respiratory viruses [16]. Therefore, young children presenting with presumed respiratory viral infection to hospitals served by these laboratories also receive concomitant testing for *M. pneumoniae*. In hospitals served by other laboratories, serology is still the mainstay of *M. pneumoniae* diagnosis. However, serology is less convenient for diagnosis in young children, since obtaining a blood specimen from an infant is more difficult than obtaining an upper respiratory tract specimen.

Reports of *M. pneumoniae* from National Health Service (NHS) laboratories in Scotland are collated centrally by the national public health body Health Protection Scotland (HPS), via the Electronic Communication Of Surveillance in Scotland (ECOSS) non-mandatory reporting system. Reports from 1 January 2008 to 31 December 2011 inclusive were analysed in this study. Denominator testing data and clinical diagnosis were not recorded via ECOSS. Data were anonymised and analysed by week of year reported, age group (year of age was available in 2010 and 2011), sex, submitting laboratory and specimen type. Estimates of incidence were based on the most recent mid-year population estimate for Scotland [17]. Reports were submitted from all NHS microbiology laboratories in Scotland which carry out *M. pneumoniae* testing. These are based in hospitals in nine locations: Aberdeen, Ayr, Dundee, Dunfermline, Edinburgh, Fife, Glasgow, Inverness and

Lanarkshire. In the case of Glasgow, results from two laboratories in the city were combined. Respiratory specimens were tested by PCR and blood specimens by serology. Laboratories used a number of different commercial and in-house PCR and serological tests. Reports of positive serology were either from a diagnostic rise in *M. pneumoniae*-specific IgG antibodies or detection of *M. pneumoniae*-specific IgM.

Analysis of laboratory reports

Temporal distribution

During the study period, there were 1,232 laboratory reports of *M. pneumoniae* in Scotland; of these, 76 (6.2%) were from 2008, 125 (10.1%) from 2009, 290 (23.5%) from 2010 and 741 (60.1%) from 2011. The highest number of reports were found in the fourth quarter of 2011 (432 reports); this was nearly three times higher than in any other quarter in the study period. The number of reports began to rise from the autumn of 2010 through the winter of 2010/11, with a second and larger rise towards the end of 2011 (Figure 1). The peak reporting frequency was 48 reports in week 47 of 2011. The estimated national incidence of *M. pneumoniae* in 2011 was 14.2 per 100,000 population.

Laboratory testing

Reports of *M. pneumoniae* were issued from nine laboratories, with the two laboratories serving the largest populations (Edinburgh and Glasgow) issuing 77.0% of the reports. Testing methods differed across Scotland, with five laboratories using PCR only and four using serology only. Overall, 77.4% of reports were from respiratory specimens (PCR detection), 18.0% from serology, and the specimen type was not known in 4.6% of reports. Of the respiratory specimens, 92.1% were from the upper respiratory tract.

Patient demographics

The male:female ratio was 1:0.94; there was no significant difference in the number of reports from males and females ($p=0.30$; chi-squared test). Approximately half of the reports (53%) were from children under the age of 15 years, with the age group of 0–4 year-olds accounting for 24.9% of all reports (Table). The

estimated incidence of *M. pneumoniae* in 2011 was highest in the 0–4 year-olds (67.5/100,000 population), declining to 52.2 per 100,000 in the 5–9 year-olds and 22.6 per 100,000 in the 10–14 year-olds.

Due to improvements in the quality of information provided from laboratories via ECOSS, data on individual year of age were available from 2010 onwards. The mean age of patients was 20.0 years (standard deviation (SD) +/-19.8 years; range: <1 month to 89 years), however, 16.2% of the reports from 2010 and 2011 came from patients aged one year or younger (Figure 2).

Patient age and sample type

Between 2008 and 2011, *M. pneumoniae* reports from young children were more likely to come from PCR testing than serological testing: 28.8% of reports from respiratory specimens were from 0–4 year-old children, compared to 10.4% of serology specimens ($p<0.01$ Fisher's exact test) (Table).

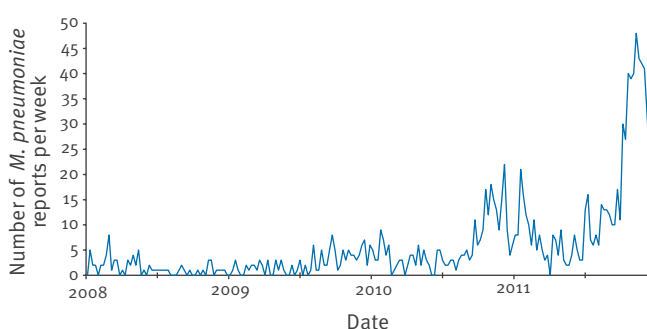
An analysis of year of age data from 2010/11 demonstrated that the mean age for PCR reports was 18.6 years (SD +/-19.4 years; range: <1 month to 89 years). In contrast, the mean age for serology reports during the same period was 27.8 years (SD +/-19.9 years; range: 1 year to 88 years).

Macrolide resistance

A full analysis of the presence of mutations in the 23S rRNA gene associated with macrolide resistance is currently underway in PCR-positive specimens.

FIGURE 1

Mycoplasma pneumoniae reports by week of year, Scotland 2008–2011 (n=1,232)



TABLE

Mycoplasma pneumoniae reports by age group and specimen type, Scotland, 2008–2011 (n=1,232)

Age group (years)	Total <i>M. pneumoniae</i> reports (%) n=1,232	<i>M. pneumoniae</i> reports from respiratory specimens (%) n=954 ^a	<i>M. pneumoniae</i> reports from serology (%) n=222 ^a
0–4	307 (24.9)	275 (28.8)	23 (10.4)
5–9	218 (17.7)	173 (18.1)	40 (18.0)
10–14	128 (10.4)	97 (10.2)	28 (12.6)
15–19	67 (5.4)	45 (4.7)	14 (6.3)
20–24	60 (4.9)	41 (4.3)	15 (6.8)
25–29	55 (4.5)	45 (4.7)	6 (2.7)
30–34	75 (6.1)	55 (5.8)	20 (9.0)
35–39	75 (6.1)	59 (6.2)	12 (5.4)
40–44	73 (5.9)	45 (4.7)	22 (9.9)
45–49	43 (3.5)	31 (3.2)	8 (3.6)
50–54	39 (3.2)	24 (2.5)	11 (5.0)
55–59	26 (2.1)	19 (2.0)	6 (2.7)
60–64	17 (1.4)	12 (1.3)	4 (1.8)
≥65	49 (4.0)	33 (3.5)	13 (5.9)

^a 56 reports were from specimens of unknown type and are therefore excluded here.

However, preliminary results indicate genotypic evidence of resistance in at least one specimen; a paediatric patient re-presenting to hospital with ongoing respiratory symptoms following first-line treatment with a macrolide for *M. pneumoniae* infection (data not shown).

Discussion

An examination of the current epidemiology of *M. pneumoniae* in Scotland was considered timely given the recent increasing incidence seen in other countries in the UK, Europe and elsewhere [3-12]. We found a substantial peak in the number of *M. pneumoniae* laboratory reports submitted to the national surveillance programme during the autumn/winter of 2011, following a smaller peak in the previous autumn/winter of 2010. The *M. pneumoniae* activity had been low from 2008 until the autumn of 2010. As expected, this picture is consistent with an increase in *M. pneumoniae* laboratory reports in England and Wales in the same period [3,4]. The estimated overall incidence of *M. pneumoniae* in Scotland in 2011 was around 10-fold lower than that reported in other northern European countries [8,10]. However, we found that the incidence was highest in the youngest age group, in contrast to a recent study in which incidence was highest in 5–14 year-olds [10]. Reporting of *M. pneumoniae* in the UK is not mandatory and reports only arise from the active microbiological investigation of patients with respiratory symptoms, mainly those presenting to hospitals. Therefore, our figures are likely to underestimate the true extent of the epidemic in Scotland, particularly in the community.

Low levels of macrolide resistance have been reported in Europe [11,18] but not from other countries in the UK [3,4]. In a preliminary analysis as part of the present study we found one genotypically resistant isolate, however, a full assessment of the level of macrolide resistance in Scotland is required and is now underway.

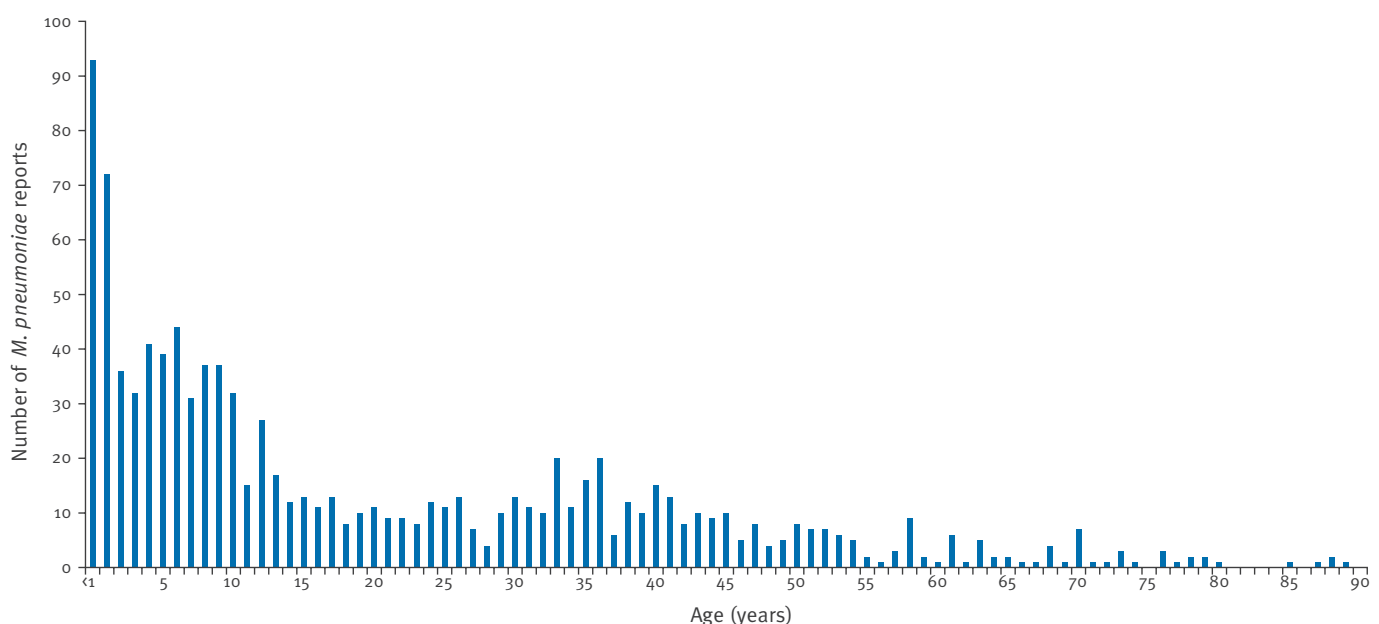
As we were able to differentiate reports into narrow age bands, it was clear that in Scotland, *M. pneumoniae* was most frequently reported in the youngest children, particularly those one year and younger. The incidence was also highest in the age group of 0–4 year-olds, with 67.5 per 100,000. A limitation of this study is that denominator testing data is not currently captured by the surveillance programme, so we are unable to determine if the proportion of *M. pneumoniae*-positive children in this age group was less than that in older age groups, as found in other studies [4,6,10]. Numerically however, we have found a significant burden in infants, which has previously been under-appreciated. A study examining the clinical course, treatment and outcomes of *M. pneumoniae* infection in infants is now underway.

We also found significantly fewer *M. pneumoniae* reports from serology compared to respiratory specimens in children aged 0–4 years. This may be due to the ease of obtaining upper respiratory tract specimens for PCR, compared to blood specimens for serology, in the youngest patients. Therefore, in hospitals where only serological testing is available, *M. pneumoniae* infections in young children may be under-diagnosed.

The majority of *M. pneumoniae* reports in Scotland originated from two large laboratories which test almost exclusively by PCR as part of in-house multiplex

FIGURE 2

Mycoplasma pneumoniae reports by year of age, Scotland, 2010–2011 (n=1,031)



real-time PCR screens for respiratory pathogens. In the future, as this molecular syndromic screening approach becomes more widespread, more infants are likely to be tested for *M. pneumoniae*, and more infections found. During *M. pneumoniae* epidemics, there may be a requirement to change empirical prescribing for community-acquired pneumonia from beta-lactam antibiotics to macrolides in the most affected age groups. However, further work is required to determine the clinical consequences of *M. pneumoniae* infection in infants and the need for antibiotic treatment.

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Increased detection of *Mycoplasma pneumoniae* infection in children, Lyon, France, 2010 to 2011

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Recent reports from several northern European countries indicate an increase in detection of *Mycoplasma pneumoniae* infection in the past two years, notably in children aged 5–15 years. Analysis of our laboratory database showed a similar pattern, with a higher proportion of respiratory samples positive for *M. pneumoniae* by real-time PCR in paediatric patients aged 5–15 years. Our data indicate that in 2010 and 2011, France experienced the first epidemic peak of *M. pneumoniae* infection since 2005.

An increased number of cases of *Mycoplasma pneumoniae* infections have recently been reported in northern Europe, including Denmark, Norway, Finland, Sweden, the Netherlands and England [1-6]. Till now, there were no available surveillance data on the current situation in France or any other country in southern Europe. The Lyon Laboratory of Virology serves the university hospitals in the metropolitan area of Lyon, with an estimated catchment area of 2.1 million people. We investigated our laboratory database in order to determine if a similar increase in the number of *M. pneumoniae* infections could be observed during the past nine years. Our study shows a striking similar pattern as that seen in Norway [3] and also confirms a current outbreak of *M. pneumoniae* infection in children.

M. pneumoniae is known to cause respiratory tract infections. It is contracted through droplets and affects primarily children aged between 5 and 15 years, with an estimated 20% of asymptomatic infections occurring in this age group [7,8]. It is the most common pathogen detected in paediatric community-acquired pneumonia [7].

Analysis of laboratory data

Laboratory diagnosis for *M. pneumoniae* has been historically based on a fourfold rise of antibody titres in a serological assay, with more sensitive methods, such

as PCR, the gold standard, being used in *Mycoplasma* diagnostics in some laboratories during recent years [9].

As infections with *M. pneumoniae* are not notifiable in France, we analysed all *M. pneumoniae*-positive reports in the Lyon Laboratory of Virology during the study period of January 2003 to December 2011. Until September 2011, we used an in-house real-time PCR based on Hardegger et al. [10], which was then replaced by the *Chlamydia pneumoniae*/*M. pneumoniae* Respiratory Multi Well System r-gene, a real-time PCR kit (bioMérieux-Argène, France).

During the study period, the *M. pneumoniae* PCR was performed on a total of 11,302 respiratory samples, with a mean of 1,280 respiratory samples per year. The samples had been mainly taken from paediatric patients, with 53.4% of the patients aged under 16 years. These paediatric samples came from the following hospital departments: paediatric emergency department (29.3%), intensive care units (14.5%) and various inpatient departments, mainly pneumology and haematology departments (56.2%). The samples from adults (aged over 15 years) were received from various inpatient departments (65.8%) and intensive care units (34.2%).

We detected a 15.1% increase in the number of respiratory samples sent to the laboratory for *M. pneumoniae* PCR from 2009 (n=819) to 2010 (n=943) and another 30.3% increase to the year 2011 (n=1,229). The main reason for this was the increased number of samples sent for testing from the paediatric emergency department, where the number of respiratory samples rose by 53.9% from the number in 2009 (n=191) to 2010 (n=294); comparison with 2009 alone showed an increase of 185.3% in 2011 (n=545). During the same time period (2009–2011), the number of samples sent

for the detection of *M. pneumoniae* from paediatric intensive care units and the adult hospital departments remained at the same level.

Coincident with the increase in the number of respiratory samples received in 2010 and 2011, we observed an increase in the number of laboratory-confirmed cases of *M. pneumoniae* infection when compared with the number in 2009 (Figure). Considering the overall pattern in the past nine years, two main epidemic periods for the detection of *M. pneumoniae* can be identified. The first occurred in 2005, followed by a slow decrease in numbers until 2009. In 2010, the number of *M. pneumoniae* started to rise again – resulting in a second epidemic period – and continued to rise until the end of the study period, December 2011 (Figure). To date, the epidemic seems to be ongoing.

When looking at the ages of patients with *M. pneumoniae* infection, we observed a general rise in the number of infections in all age groups in 2010 and 2011. The largest rise and the highest percentage of

positive samples were found in patients aged 5–15 years, with 14.8% of all samples being positive for *M. pneumoniae* in both years; in 2009, the percentage of positive samples was only 7.1%. Among patients aged 0–4 years, the percentage increased from 0.6% in 2009 to 4.0% in 2010 and 5.5% in 2011. In patients aged over 15 years, the percentage of *M. pneumoniae*-positive samples was lower, but still rose from 0.9% in 2009 to 2.8% in 2011. In the nine years, no shift in the age distribution of patients with *M. pneumoniae* infection was observed (Table).

Discussion

The proportion of *M. pneumoniae*-positive tests in our study correlates well with findings of the PCR-based study in Denmark, where approximately 3% of PCRs for *M. pneumoniae* in 2007 were positive, increasing to 15% during 2010 [11]. Surveillance data from Finland, based mainly on serology results, gave similar proportions, with 8–17% of tests positive for *M. pneumoniae* in 2010 and 2011 [2]. The detection rate of *M. pneumoniae* by PCR was highest in Sweden, at 23% in both 2006 and 2011 [6], which is as high as the percentage we observed during the peak in 2005 in the age group 5–15 years. In our study, the substantial increase in the number of samples originating from the paediatric emergency department clearly underlines the importance of *M. pneumoniae* as a community-acquired pathogen, primarily spreading in childcare facilities or schools. There was no increase in the number of samples sent for *M. pneumoniae* detection from inpatient departments. A nosocomial spread of the infection is therefore not expected.

The proportion of *M. pneumoniae*-positive PCRs among children aged 5–15 years has risen from 7.1% in 2009 to 14.8% in both 2010 and 2011. Such a high percentage has not been seen since the 2005–2007 period. A similar increase was seen, but to a lesser extent, in children aged 0–4 years (0.6% in 2009 to 4.0% and 5.5% in 2010 and 2011, respectively) and in the adult population (0.9% in 2009 to 3.3% and 2.8% in 2010 and 2011, respectively). Nevertheless, children of school age are the group mainly affected by *M. pneumoniae* infection.

FIGURE

Annual number of laboratory-confirmed cases of *Mycoplasma pneumoniae* infection, detected by real-time PCR in the Laboratory of Virology, Lyon, France, 2003–2011 (n=423)



TABLE

Annual percentage of *Mycoplasma pneumoniae*-positive samples by patient age group, detected by real-time PCR in the Laboratory of Virology, Lyon, France, 2003–2011

Patient age group in years	Percentage of positive samples (95% confidence interval)									
	2003	2004	2005	2006	2007	2008	2009	2010	2011	
0–4	1.2 (0.0–1.5)	2.0 (0.5–5.0)	6.4 (3.9–9.8)	3.5 (2.1–5.6)	3.2 (1.9–5.2)	3.8 (2.2–6.4)	0.6 (0.1–2.2)	4.0 (2.4–6.4)	5.5 (3.9–7.5)	
5–15	8.9 (4.7–15.0)	7.3 (3.2–13.8)	25.0 (18.9–32.0)	18.1 (13.5–23.7)	13.0 (8.9–18.0)	7.7 (4.6–12.1)	7.1 (4.1–11.3)	14.8 (10.8–19.5)	14.8 (11.4–18.9)	
>15	0.2 (0.0–0.5)	0.5 (0.2–1.1)	0.6 (0.2–1.6)	1.3 (0.7–2.5)	1.1 (0.5–2.3)	0.4 (0.1–1.4)	0.9 (0.1–3.0)	3.3 (1.4–6.5)	2.8 (0.9–6.3)	
Total	1.2 (0.7–2.0)	1.2 (0.7–2.0)	5.5 (4.3–6.9)	4.7 (3.7–6.0)	3.7 (2.9–4.9)	2.9 (2.0–4.0)	2.4 (1.5–3.8)	7.0 (5.5–8.9)	7.9 (6.5–9.6)	

The two epidemic periods, 2005–2007 and since 2010, correspond to the distribution of cases of *M. pneumoniae* infection in other European countries, such as Sweden, Finland and Norway [2,3,6]. Epidemic periods, occurring after a four-year interval and lasting for approximately 18 months, have also been reported from England [12].

A general surveillance system for *M. pneumoniae* as in other European countries, including typing of a single or different strains in outbreak situations [13,5], would simplify the detection of the strains responsible for the reoccurring epidemics in France.

Data on macrolide resistance of the circulating *M. pneumoniae* isolates in France are currently not available, but this issue needs to be assessed in the near future.

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Ongoing epidemic of *Mycoplasma pneumoniae* infection in Jerusalem, Israel, 2010 to 2012

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A substantial epidemic of *Mycoplasma pneumoniae* infection was reported in late 2011 in some European countries. We report here an epidemic of *M. pneumoniae* infection that began in Jerusalem during 2010 and is still ongoing. This report complements current information on what might be a worldwide epidemic of *M. pneumoniae* infection that might require substantial coordinated international public health intervention.

We describe here on an ongoing epidemic of *Mycoplasma pneumoniae* infection in Jerusalem, Israel, which started in February 2010. As of 31 January 2012, a total of 156 cases were identified among patients referred to the Hadassah-Hebrew University Medical Centers in Jerusalem.

Background

M. pneumoniae is one of the major leading respiratory bacterial pathogens, causing respiratory tract infections. It is known to cause epidemics that emerge at three-to-seven-year intervals and can last two years or more [1-3]. Until now, it was not clear whether this phenomenon was endemic to certain regions or was global in nature. Some reports have suggested that similar trends can be observed in adjacent countries [2,4-6]. Additionally, it has been suggested that most epidemics occur either in summer or autumn, without an evident explanation for this seasonal occurrence of *M. pneumoniae* outbreaks [2,7,8].

During 2006 and 2007, an increase in the number of cases of *M. pneumoniae* infection was reported in several countries including England and Norway [3,9]. A new surge was noted in a few countries in 2010, including England and Wales, Denmark and Israel [3,10,11]. In both Denmark and the United Kingdom, a decrease in the number of cases was reported in early 2011. However, a new surge of cases was noted in a few northern European countries by the end of 2011 and early 2012 [2,4-6,9,12,13] and there were also reports of an increase in the number of cases in 2011 in Japan (M. Narita, personal communication, September 2011),

which included the Emperor of Japan and his granddaughter [14].

One of the major obstacles to timely diagnosis of *M. pneumoniae* since its discovery 70 years ago has been the lack of a fast and reliable diagnostic method [15]. The past 20 years were notable for a revolution in the diagnosis of *M. pneumoniae* by direct DNA amplification methods, but only in the last few years, with the introduction of real-time PCR, has rapid diagnosis become more widely accessible.

Setting

The Hadassah-Hebrew University Medical Centers in Jerusalem provides most of the acute-care hospitalisation facilities in Jerusalem, with approximately 1,000 beds in two hospitals. It has secondary and tertiary facilities and provides, to a lesser extent, primary care consultation for some of the health maintenance organisations in Jerusalem. It currently serves a population over a million in Jerusalem and its surroundings.

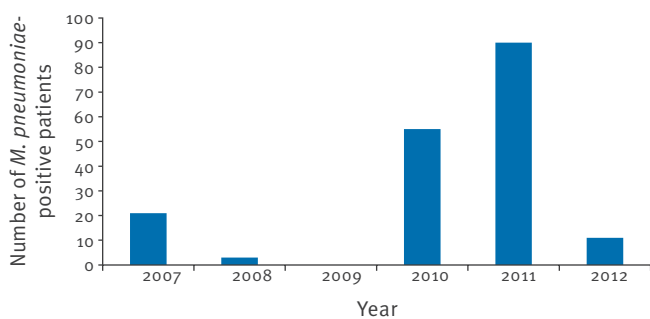
Notification of *M. pneumoniae* infection is not mandatory in Israel and currently there is no laboratory in the Central Ministry of Health Laboratories to support its diagnosis. *M. pneumoniae* diagnostics based on DNA amplification were implemented almost 10 years ago at the Hadassah-Hebrew University Medical Centers [16], but real-time PCR was introduced only in late 2006 [11], at which point serological tests were discontinued. Physicians in all admission wards, mainly paediatrics and general medicine, can submit samples, with same-day results possible five days a week.

Description of the epidemic

The past few years saw the tail of a previous epidemic in 2007 and the abrupt onset of a new epidemic in February 2010 (Figure 1). A feature of this new epidemic was a relatively high percentage (30%) of macrolide-resistant *M. pneumoniae* isolates [11], but resistance rates may be diminishing as the epidemic progresses. It is still difficult to estimate the real extent of resistance at this stage since surveillance of resistance is only

done periodically, every few months. Interestingly, no consistent seasonal or monthly influences were noted (Figure 2). The number of *M. pneumoniae*-positive samples fell from 2007, with almost no cases detected towards the end of the year, very few in 2008 and none in 2009. However, after the start of the 2010 epidemic – and unlike the phenomenon observed in Denmark [4] – there has been no notable decrease in the number of cases of *M. pneumoniae* infection, except for a temporary fall during early 2011. Since April 2011, a more or less constant number of new cases has been observed each month.

FIGURE 1
Mycoplasma pneumoniae-positive patients tested by real-time PCR referred to the Hadassah-Hebrew University Medical Centers, Jerusalem, Israel, 2007–2012^a (n=180)



The total number of patient-unique samples submitted for *M. pneumoniae* diagnosis were: 189 in 2007, 150 in 2008; 223 in 2009, 343 in 2010, 539 in 2011 and 94 in January 2012.

^a Data for 2012 include January only.

The demographic and clinical characteristics of 166 patients hospitalised at the Hadassah-Hebrew University Medical Centers during 2007 to January 2012, from whom clinical information was collected, are presented in the Table.

Since the introduction of real time-PCR, the proportion of *M. pneumoniae*-positive tests submitted to our laboratory during the epidemic years has been relatively stable: in 2007 it was 11.1%, 16.0% in 2010, 16.7% in 2011 and 11.7% in January 2012. In the non epidemic years, it was low: 2% in 2008 and 0% in 2009.

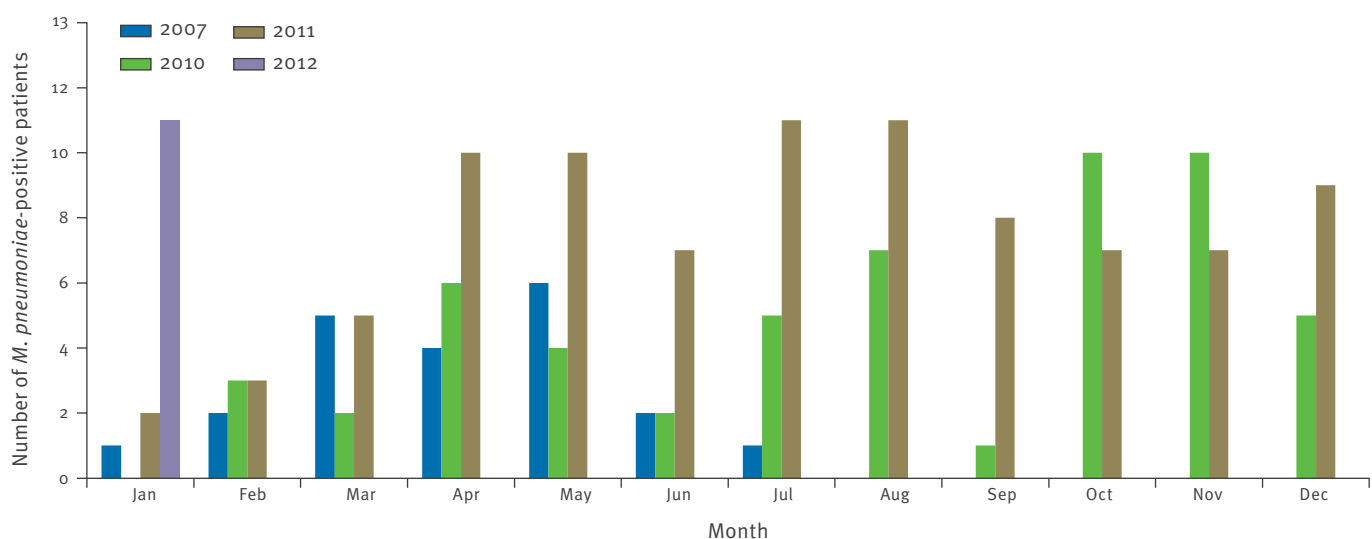
Discussion

Of the major bacterial respiratory pathogens including *Haemophilus influenzae* and *Streptococcus pneumoniae*, *M. pneumoniae* is the only one for which no vaccine is available. *M. pneumoniae* is considered to cause a milder disease compared with *S. pneumoniae*, though substantial morbidity can be observed [17]. Indeed the median duration of admission in our cohort was four days.

In many laboratories, serology is still being used [2,6]. The resulting delay in diagnosis poses a problem for clinicians [2], who need to ensure prompt treatment of patients with *M. pneumoniae* infection. Problems in diagnosis have led to under-investigation in the past and have also impeded our ability to understand the epidemiology of the local outbreak setting as well as the nationwide or worldwide spread of this pathogen.

A study from Germany suggested that no single clone was responsible for nationwide *M. pneumoniae* infections [18]. Indeed, Chalker et al. suggested from multi-locus variable number tandem repeat analysis (MLVA)

FIGURE 2
Mycoplasma pneumoniae-positive patients tested by real-time PCR, by month and year of referral to the Hadassah-Hebrew University Medical Centers, Jerusalem, Israel, during the epidemic years 2007, 2010–2012^a (n=177)



^a Data for 2012 include January only.

typing of a small sample in the United Kingdom that epidemics are multiclonal in nature [13]. In contrast, Pereyre et al. have evidence that a small outbreak in Bordeaux, France, might be related to a single clone [19]. In influenza, the epidemics generally involve a single or very few clones of influenza virus that spread worldwide at the same time. Interestingly, it seems that *M. pneumoniae* epidemics do occur worldwide and are a global phenomenon affecting countries both adjacent and distant. This is demonstrated by the fact that in 2007, epidemics were noted in several countries, some of which are not adjacent to each other [3,9], including Israel. Similar observations were made in 2010 and 2011 [2,4-6,9,12-14]. It seems that for unknown reasons some countries are spared from such epidemics [2]. For example countries in the south of western Europe are not affected by the current epidemic [2,6]. Additionally, the specific epidemiological pattern within each country seems to differ: in some countries the epidemic is abrupt and subsides relatively quickly [4], while, as in our case, the epidemic has so far being maintained for more than two years.

Our study has a few limitations. Being a single-institution study, selection bias in the population referred to our hospitals may have resulted in the inclusion of more severe cases, possibly with more underlying conditions or co-morbidities. In addition, since currently there is no nationwide surveillance programme for *M. pneumoniae* in Israel and no published data are available from other Israeli medical institutions, we do

not know the extent of the infection in the rest of the country.

Our report is in line with recent observations published in *Eurosurveillance* [4-6,9,12,13] and emphasises the need to understand the epidemiology and pathogenesis of epidemics of *M. pneumoniae* infection better. To this end, it would be appropriate for countries to establish sentinel institutions equipped with up-to-date dedicated diagnostics for *M. pneumoniae*. A network of such facilities, working in a coordinated fashion, would provide invaluable information for epidemic and inter-epidemic periods.

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TABLE

Characteristics of *Mycoplasma pneumoniae*-positive patients tested by real-time PCR referred to the Hadassah-Hebrew University Medical Centers, Jerusalem, Israel, during the epidemic years, 2007-2012^a (n=166)^b

Characteristic	Details
Age	Median: 12 years (range: 0.3-77)
Sex	98 (59.0%) male, 68 (41.0%) female
Ethnicity ^c	41 (24.7%) Arabs, 123 (74.1%) Jews
Having underlying disease	47 (28.3%)
Did not improve on antibiotics prescribed in the community	73 (44.0%)
Body temperature on admission	Median: 37.5 °C (range: 35.8-40.1)
Chest X-ray performed	149 (89.8%)
Infiltration compatible with pneumonia on chest X-ray	92/149 (61.7%)
Bilateral pneumonia on chest X-ray	21/149 (14.1%)
White blood cell count on admission	Median: 9.8 (range: 1.2-37.4) x10 ⁹ /L
Length of hospital stay	Median: 4 days (range: 0-50)

^a Data for 2012 include January only.

^b Patients for whom data were available.

^c Two patients were not from Israel.

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

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

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

Background

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

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

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

Methods

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

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

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

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

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

The number of *M. pneumoniae*-positive laboratory reports from regional laboratories and hospitals submitted to the HPA during the study period is shown in Figure 1: one report is received per patient and four-weekly moving averages are plotted. From week 40

of 2010 to week 3 of 2011, there were a total of 322 reports, the highest number since the previous peak of *M. pneumoniae* infections seen during the same sampling period in 2005 to 2006 (n=455). The mean number of reports received from 2006 to 2009 (from week 40 of one year to week 3 of next) was 234.

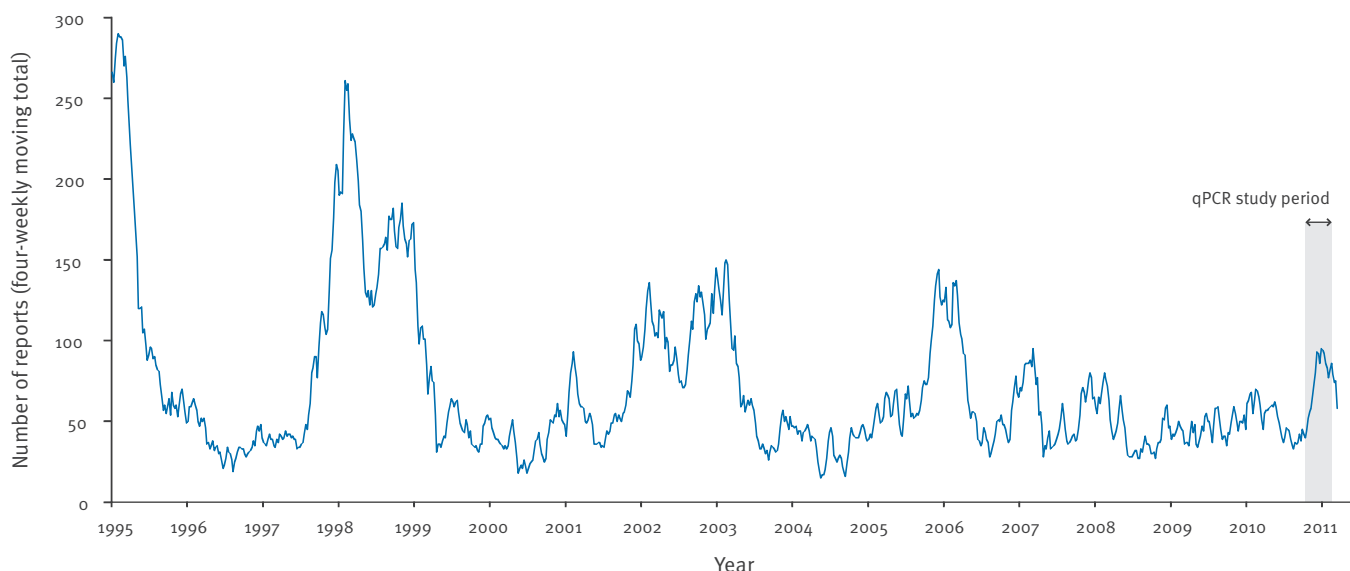
A total of 21 of 1,221 (1.7%; 95% CI: 1.1 to 2.6) community surveillance samples from week 40 of 2010 to week 3 of 2011 were *M. pneumoniae*-positive by qPCR. The percentage of positive cases per week is shown from 2005 to 2011 in the Table, showing an increase from October to December (weeks 40–49)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

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

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

FIGURE 1

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



qPCR: quantitative real-time polymerase chain reaction.

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

Source: [9].

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

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

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

Discussion

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

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

TABLE

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

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

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

^a Determined by quantitative real-time PCR.

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

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

It is not known whether increases in incidence of *M. pneumoniae* infections are due to an increased incidence of an individual strain or a concurrent increased incidence of several strains. Speculation that a shift in P1 adhesin type may be the cause of epidemics has been disputed [7,10]. Evidence from our study does not support the hypothesis that a single strain type of *M. pneumoniae* was responsible for the observed increase in infection in England and Wales. Rather, a decline in immunity or increase

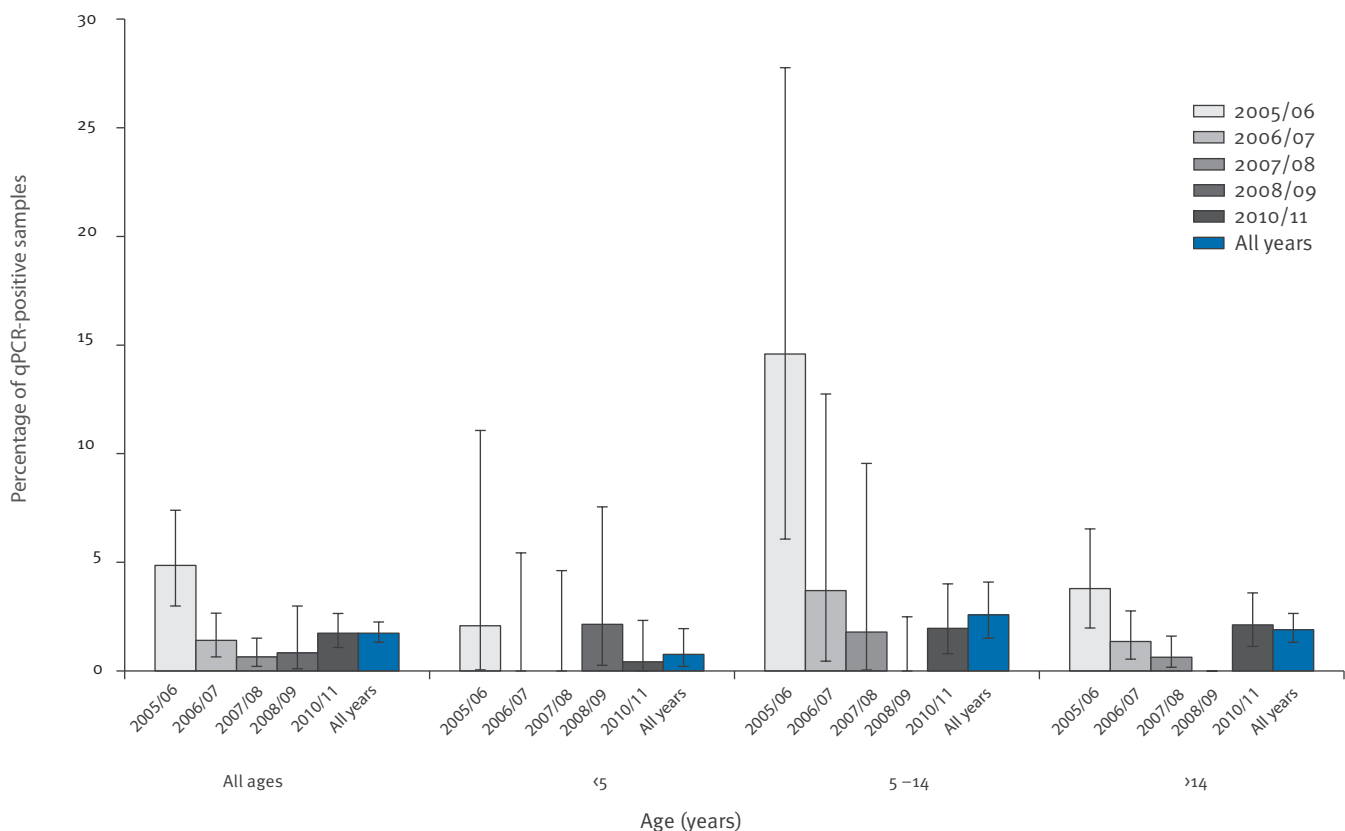
of the immunologically naive population may have triggered the four-year cycle of epidemic periods. It would be interesting to type *M. pneumoniae* strains from Denmark and other countries during the same period to determine how strains differ geographically during periods of increased infection.

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

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

FIGURE 2

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



qPCR: quantitative real-time polymerase chain reaction.

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

^a Determined by qPCR.

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

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Increased incidence of *Mycoplasma pneumoniae* infections detected by laboratory-based surveillance in Denmark in 2010

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In Denmark recurrent epidemics of *Mycoplasma pneumoniae* infections have been described since the 1950s at intervals of approximately four to six years. The latest epidemic occurred in 2004/05 followed by two years of high incidence and more than three years of low incidence. Due to a recent increase in diagnosed cases since late summer 2010, we conducted a survey of positive *M. pneumoniae* PCR tests performed by clinical microbiology departments in Denmark, which indicated that a new epidemic may be underway.

Introduction

Mycoplasma pneumoniae is a common cause of upper and especially lower respiratory tract infections such as bronchitis and pneumonia. In addition, *M. pneumoniae* causes neurological symptoms and sequelae in a high proportion of cases [1,2]. The highest prevalence is seen in children and younger adults. Cases occur throughout the year, but the incidence is highest during autumn and winter. In Denmark, regular epidemics have been described since 1949/50. With the exception of a nine-year endemic period from 1978 to 1987 [3], these epidemics usually begin during summer, culminate in late autumn/early winter and fade out during winter. In some instances the epidemics span two winters: this was seen in 1962 to 1964 and 1971 to 1973 [3]. The latest epidemic in 2004/05 [4,5] was followed by two years of high incidence, but since 2007 the incidence has been very low judging by the low rate of on average approximately 3% positive samples seen in this period (Figure 1).

From 1946 until the late 1990s the central national laboratory at Statens Serum Institut (SSI) received samples from the whole country for the diagnosis of *M. pneumoniae* infections [3]. In the last decades

the local clinical microbiology departments have taken over a large part of the laboratory tests for *M. pneumoniae*. The diagnosis had previously been based on serology but since the beginning of the 1990s PCR has been introduced as a routine diagnostic test at SSI for rapid and early diagnosis of *M. pneumoniae* infection [6], and in more recent years, most of the local departments have also adopted PCR. The countrywide use of PCR for diagnosis and surveillance of *M. pneumoniae* infections is probably unique for Denmark.

Although SSI is now predominantly receiving samples from the eastern part of the country only, the institute is the one laboratory in Denmark performing most tests for *M. pneumoniae* overall, and thus results obtained at SSI may be seen as indicative of the *M. pneumoniae* activity in Denmark as a whole. Each week the rate of positive samples is calculated, and a rise from approximately 5% to 15% or more positive samples within approximately six weeks are considered as indicative of an *M. pneumoniae* epidemic [4].

At SSI we saw an increase in the number of positive samples above the threshold in the beginning of October 2010. This prompted us to investigate whether this was the beginning of an epidemic of *M. pneumoniae* infections in Denmark in the autumn of 2010.

Methods

Because PCR is found superior to serology for the diagnosis of *M. pneumoniae* infection during the early phases of infection [7], we included in our investigation only those records that were diagnosed by a PCR-based method. The departments use a range of different PCR

assays, of which some are published [6,8,9] or commercial kits, but most are unpublished but validated *in-house* assays.

A survey was conducted collecting data from all clinical microbiology departments in Denmark performing PCR testing for *M. pneumoniae* for general practitioners and hospitals. In addition to SSI, there are 12 such departments in the country that perform this analysis and we received data from 11 of them. They represented all five regions in Denmark (Figure 2): Capital Region of Denmark (data from three of four departments), Region Zealand (data from the sole department), Region of Southern Denmark (data from three of three departments), Central Denmark Region (data from two of two departments) and North Denmark Region (data from the sole department).

From the local departments we obtained data on the total number of PCR analyses performed and the number of analyses positive for *M. pneumoniae* for week 1 in 2009 to week 41 in 2010. Only data for weeks 34 to 41 in 2009 and 2010 are compared in the analysis presented here. From SSI we obtained data from week 1 in 2004 to week 41 in 2010 (October 16). We present the number of positive tests and the weekly proportion of positive tests among all tests performed. Since the catchment areas of the departments are not well defined, i.e. the general practitioner can send the specimen to any department, it was not possible to calculate the regional incidences. However, the total population of Denmark is 5.5 million and we used this to calculate an estimated incidence of PCR-diagnosed *M. pneumoniae*.

Results

Figure 1 shows the *M. pneumoniae* tests performed at SSI from week 1 in 2004 to week 41 in 2010. From 2007

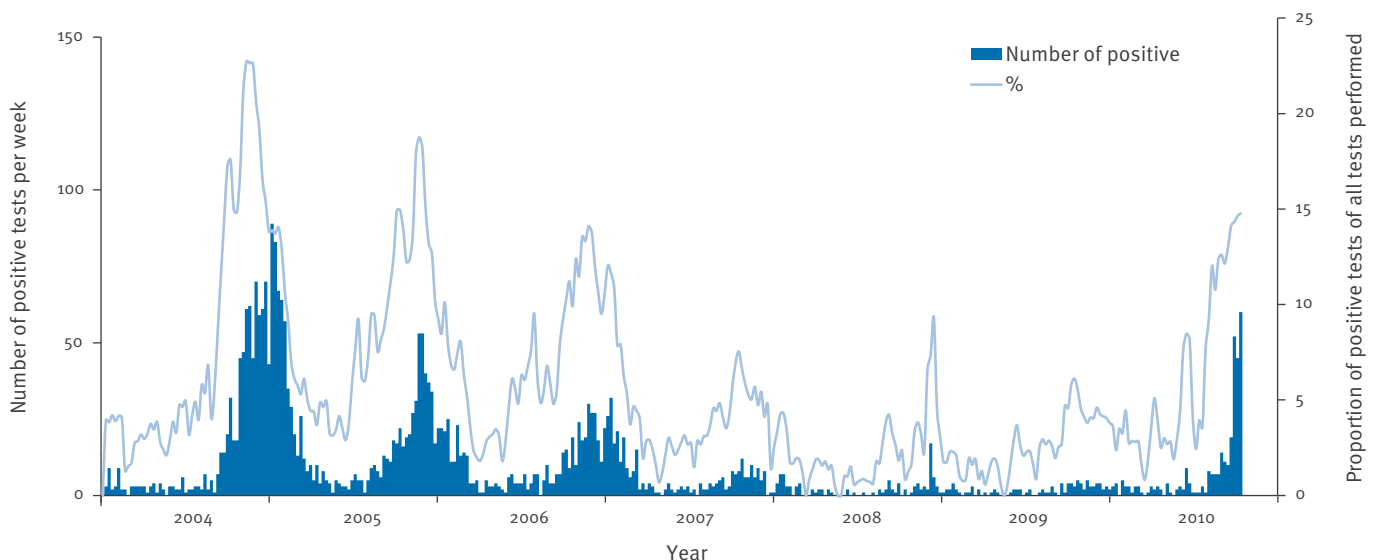
to 2010 the average positivity rate of *M. pneumoniae* infection in Denmark remained very low, at approximately 3% positive samples (Figure 1). Apart from a short peak in the number of positive tests observed in week 50 in 2008, the first increase in the positivity rate since 2007 was observed in late August 2010 (weeks 33–35) when it rose to approximately 10%. The rate increased further in the following weeks and reached approximately 15% in late September/early October (weeks 39–40) despite a three- to fourfold increase in the number of samples received for PCR in this period (Table). This increase in the rate of positive *M. pneumoniae* tests occurred in all regions, but was seen a little later in the regions than at SSI (Table). The estimated national incidence of PCR-diagnosed *M. pneumoniae* infections in 2010 rose from 0.4 per 100.000 in week 34 to 3 per 100.000 in week 41.

Discussion and conclusion

Recurrent epidemics of *M. pneumoniae* infection are also well known in other countries [10,11] and a few reports indicate simultaneous epidemics in more than one country [12,13]. *M. pneumoniae* epidemics have a high impact on the community, and a laboratory-based system for the surveillance of this disease is recommendable. According to our knowledge Denmark is the only country with a PCR-based surveillance system for *M. pneumoniae*. A rapid increase in macrolide-resistant *M. pneumoniae* has been reported from Asia in the recent years, but macrolide resistance it is also seen in Europe and in the United States [14]. In Denmark SSI did a survey after the epidemic in 2004 and found 1-2% of macrolide resistance. This is in accordance with a recent German study [15] indicating a limited but not negligible level of resistance in Europe. If an epidemic is recognised it is possible to guide the hospitals and general practitioners in the diagnosis and antibiotic treatment of the disease. Only a focused use of

FIGURE 1

Mycoplasma pneumoniae PCR tests done at Statens Serum Institut, Denmark, week 1 2004 – week 41, 2010*



The percentages are the floating average of three weeks.

FIGURE 2

The five administrative regions of Denmark and population numbers



A: Capital Region of Denmark; B: Region Zealand; C: North Denmark Region; D: Central Denmark Region; E: Region of Southern Denmark

Population 1 July 2010. Source Statistics Denmark (<http://www.dst.dk/HomeUK.aspx>).

TABLE

Number and proportion of positive tests for *Mycoplasma pneumoniae* performed by Statens Serum Institut and the clinical microbiology departments in the regions, Denmark, 2009 and 2010

Region and year	Number of positive test and number of all tests performed (%)							
	Week 34	Week 35	Week 36	Week 37	Week 38	Week 39	Week 40	Week 41
SSI^a								
2009	1 of 55 (1.8)	3 of 69 (4.3)	1 of 70 (1.4)	0 of 66 (0)	4 of 61 (6.6)	1 of 60 (1.7)	4 of 71 (5.6)	4 of 66 (6.1)
2010	7 of 68 (10.3)	14 of 91 (15.4)	11 of 96 (11.5)	10 of 101 (9.9)	20 of 112 (17.9)	52 of 362 (14.4)	45 of 338 (13.3)	60 of 374 (16.0)
Capital								
2009	0 of 30 (0)	1 of 34 (2.9)	5 of 29 (17.2)	0 of 37 (0)	1 of 37 (2.7)	1 of 49 (2.0)	1 of 47 (2.1)	0 of 39 (0)
2010	6 of 53 (11.3)	5 of 74 (6.8)	4 of 84 (4.8)	6 of 59 (10.2)	3 of 75 (4.0)	16 of 233 (6.9)	25 of 218 (11.5)	24 of 224 (10.7)
Zealand								
2009	0 of 5 (0)	0 of 12 (0)	0 of 14 (0)	0 of 15 (0)	1 of 17 (5.9)	0 of 13 (0)	0 of 11 (0)	0 of 11 (0)
2010	2 of 10 (20.0)	1 of 11 (9.1)	3 of 13 (23.1)	2 of 30 (6.7)	3 of 20 (15.0)	15 of 86 (17.4)	7 of 61 (11.5)	20 of 85 (23.5)
Southern Denmark								
2009	2 of 45 (4.4)	1 of 37 (2.7)	1 of 51 (2.0)	0 of 68 (0)	1 of 60 (1.7)	1 of 57 (1.8)	1 of 62 (1.6)	1 of 61 (1.6)
2010	2 of 41 (4.9)	1 of 40 (2.5)	3 of 43 (7.0)	2 of 82 (2.4)	2 of 81 (2.5)	10 of 137 (7.3)	20 of 165 (12.1)	22 of 189 (11.6)
Central Denmark								
2009	0 of 8 (0)	0 of 16 (0)	1 of 17 (5.9)	0 of 25 (0)	0 of 16 (0)	0 of 13 (0)	1 of 16 (6.3)	1 of 26 (3.8)
2010	4 of 25 (16.0)	1 of 18 (5.6)	2 of 23 (8.7)	4 of 23 (17.4)	1 of 22 (4.5)	7 of 53 (13.2)	11 of 60 (18.3)	10 of 65 (15.4)
North Denmark								
2009	0 of 6 (0)	0 of 3 (0)	0 of 8 (0)	0 of 10 (0)	0 of 15 (0)	0 of 11 (0)	1 of 12 (8.3)	1 of 8 (12.5)
2010	0 of 17 (0)	3 of 17 (17.6)	1 of 15 (6.7)	1 of 14 (7.1)	2 of 19 (10.5)	4 of 44 (9.1)	7 of 55 (12.7)	28 of 165 (17.0)
Total								
2009	3 of 149 (2.0)	5 of 171 (2.9)	8 of 189 (4.2)	0 of 221 (0)	7 of 206 (3.4)	3 of 203 (1.5)	8 of 219 (3.7)	7 of 211 (3.3)
2010	21 of 214 (9.8)	25 of 251 (10.0)	24 of 274 (8.8)	25 of 309 (8.1)	31 of 329 (9.4)	104 of 915 (11.4)	115 of 897 (12.8)	164 of 1102 (14.9)

^a Statens Serums Institut (SSI) receives samples not only from the capital region but also from the rest of the country and is therefore presented separately.

macrolide antibiotics in diagnosed cases can diminish the risk of spreading resistant bacteria.

In conclusion, we have seen an increase in the number of positive tests and also in the positivity rate of submitted samples since late summer 2010, indicating increased transmission of *M. pneumoniae*. The findings suggest that Denmark may be in the early phase of an epidemic. Other European countries, if data are available, should assess if they are in a similar situation.

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***Authors' correction:**

On request of the authors, Figure 1 was exchanged on 18 November 2010.

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Preliminary report: Outbreak of Legionnaires' disease in a hotel in Calp, Spain, update on 22 February 2012

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Research is ongoing on eighteen cases of Legionellosis, including four deaths, identified among tourists and employees in a hotel in Calp, Spain. Cases occurred during a period of two months, indicating the possibility of a point-source transmission at the hotel. An environmental investigation identified several positive samples in the hotel, which as a precautionary measure, was closed until requested improvements were made. Surveillance measures currently remain active.

Outbreak description and epidemiological investigation

On 14 December 2011 a Spanish tourist, who had stayed at a hotel in Calp, on the east coast of Spain, between 27 and 29 November was confirmed as a case of *Legionella* pneumonia. Thirteen days later, on 27 December 2011, a Spanish employee at the same hotel was identified as a second case. This prompted an epidemiological investigation to confirm or rule out an outbreak.

On 11 January 2012 another case was reported via the European Legionnaires' Disease Surveillance Network (ELDSNet) and involved an English tourist who had also stayed at the hotel. Following this, on 17 January 2012, three additional cases related to the hotel were reported, all British citizens.

The European case definition [1] was adapted for this outbreak, and a confirmed case was defined as a patient with clinical diagnosis of pneumonia, who had stayed or worked at the hotel between two and ten days before the onset of symptoms, with laboratory findings indicative of *Legionella* infection, including a positive urine test for *Legionella pneumophila* antigen, or a positive culture or isolation from respiratory secretions.

Currently, the outbreak is restricted to 18 cases. All cases were confirmed by positive urine antigen. Seven samples are pending sequencing by the Genomics and

Health Joint Unit, Centro Superior de Investigación en Salud Pública (CSISP) -University of Valencia, Spain. There have been four deaths, all involving male travel-related cases, over 70 years of age. Two of the cases who died had not sought prior medical care, while the other two cases died in the hospital 12 and 39 days after onset of symptoms.

All cases had stayed or worked at the same hotel in Calp during the incubation period of their illness. There were a total of 11 men and seven women with a mean age of 70 years (range: 44–88 years). Partial information is available on predisposing factors of cases: smoking in 3/9, heart disease in 2/13 and chronic respiratory disease in 1/13.

Fifteen of the eighteen cases were travel-associated (one Spanish, twelve English and two French) and three were members of the hotel staff. The three cases who were part of the hotel staff had an average age of 58 years (range: 47–74 years). For all of the 18 cases but two, symptoms began between 4 December 2011 and 2 February 2012. The date of onset of symptoms is unknown for two of the four cases who died (Figure).

Travel-associated cases occupied different rooms in the hotel, except for three couples, who respectively shared a room. Only two cases used the hotel's spa facilities.

Environmental investigation

When the first case appeared, on 4 December, the registered documentation on the Facilities Management Program Risk of the hotel was reviewed. We verified that certificates of cleaning and disinfection of water deposits, as well as of the network of cold water for human consumption and hot water were compliant with the Spanish *Legionella* surveillance legislation [2]. The documents certifying compliance were dated from 31 January 2011.

When the second case of Legionnaires' disease, a hotel employee, was reported, a new on-site investigation was immediately launched. In addition to the previously inspected documents, we obtained the analytical results, dated from 29 November 2011, of routine water samples from the hot-water deposits, jacuzzi, cold-water tank and rooms. All of the seven water samples that had been analysed had been negative for *Legionella*.

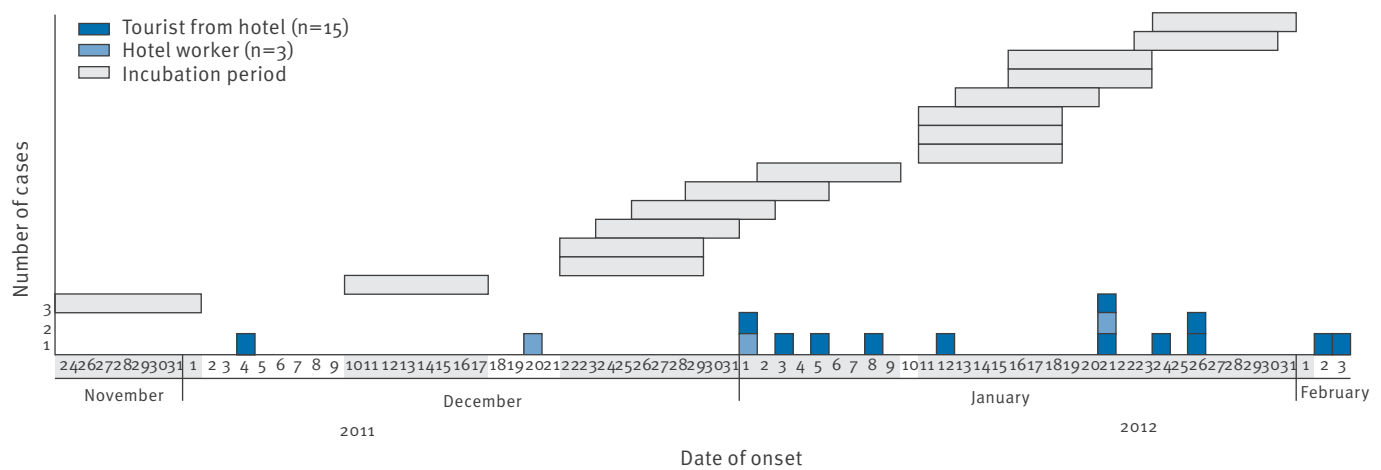
On 11 January, via ELDSNet a third, travel-associated case was reported. As a result, a thorough inspection of the hotel premises was performed. Chlorine levels and temperatures were checked in each column of the drinking water pipes. Deficiencies in the hot-water

temperature and other structural points were detected, as hot water stagnated in the feedback circuit. Twelve new water samples that were taken, yielded negative results a few days later. Nevertheless, all the hotel facilities were cleaned by hyperchlorination [2]. Two days later, additional water and biofilm samples were collected to check the efficiency of the cleaning procedures. All the samples tested negative.

On 31 January, new water and biofilm samples were taken from the network of cold water for human consumption and the hot water. Fourteen biofilm samples tested positive and the hotel was immediately closed on 2 February.

FIGURE

Cases of Legionnaires' disease, by date of symptom onset, ongoing outbreak in Calp, Spain, 24 November 2011–22 February 2012 (n=18)



Dates of symptom onset for two of 18 cases who died are not known. These two cases are not shown on the figure.

TABLE

Environmental investigation, outbreak of Legionnaires' disease in Calp, Spain, 24 November 2011–22 February 2012

Date of action	Action	Result
04 December 2011	Review of the registered documentation on the Facilities Management Program Risk of the hotel	Certificates, dating from 31 January 2011, of cleaning and disinfection of water deposits, the network of cold water for human consumption and hot water were obtained
03 January 2012	On-site inspection	A whirlpool cleaning and disinfection certificate dated from 02 November 2011 was obtained The certificate dating from 29 November 2011 showed that analytical results of seven routine water samples had been negative for <i>Legionella</i>
12 January 2012	On-site investigation Water chlorine levels and temperatures were checked Seven new water samples were taken	All water samples were negatives
16–17 January 2012	All the hotel facilities were cleaned by hyperchlorination	One water and 12 biofilm samples were taken on 19 January 2012 to check the result of the cleaning procedure
19 January 2012	New on-site investigation One water and 12 biofilm samples were taken	Deficiencies in the hot-water temperature and other structural points All the samples tested negative
31 January 2012	32 water and 24 biofilm samples were taken	14 biofilm samples were positive on 2 February 2012
2 February 2012	14 biofilm samples were positive	Precautionary closure of the hotel
8–9 February 2012	Cleaning and hyperchlorination after correction of deficiencies in the water distribution network was conducted between 8 and 9 February	The hotel reopens to the public on 10 February

Environmental intervention requested from the hotel

As a result of the environmental investigations, the hotel had to make changes in the hot water system to prevent the growth of *Legionella*. The changes had to ensure that the hot water temperature would be higher than 50°C in all endpoints. Improvements in the water disinfection system were also requested and the use of well-water for irrigation and toilets' cisterns was prohibited.

Discussion

From 1999 to 2009, 26% of Legionnaires' disease outbreaks in Spain have been travel-associated, and have affected 435 people [3]. However, in recent years there has been a decrease in the number of cases and outbreaks affecting travellers [4,5]. Interestingly, travel-associated Legionnaires' disease mortality in non-Spanish citizens is 2.6 times higher than in Spanish citizens travelling in their own country [3].

Here we report on the ongoing investigations into an outbreak in a single hotel in Calp, affecting 18 individuals and causing four fatalities. In the last 10 years, the incidence of travel-associated *Legionella* clusters in Calp has been very low. In 2006, an outbreak in the same hotel involved six cases. During 2011, a cluster of two travel-associated cases was reported in a different hotel of the same city.

Unlike other point-source transmission outbreaks, the onset of the one reported here was insidious with 13 days between the notifications of the two first cases. In addition, the second case was a hotel worker. These circumstances have made the early stages of the investigation quite difficult [6-8].

In this outbreak, the majority of hotel guests were from the European Union (EU), especially from the United Kingdom, France, Italy and Belgium. There were also Spanish guests and some from other countries outside the EU (United States, Russia, Kazakhstan, Brazil, New Zealand, Australia). Identified cases were from three EU countries. For the surveillance of Legionnaires' disease and especially for the detection of travel-associated clusters, collaboration among European countries through ELDSNet is very important and facilitates a rapid risk assessment [9-11]. Nevertheless, it would be interesting to have more detailed information about the patients involved in travel-associated clusters to improve research and control of outbreaks.

Guests and tour operators have been informed about the outbreak and strict control and cleaning measures, including the closure of the hotel, were implemented. The hotel resumed normal operation once the structural deficiencies and additional cleaning procedures were performed. Surveillance measures will remain active until further notice.

The results of genomics analyses of human and environmental samples are still awaited. The final report on the outbreak will be delivered once it is considered closed and we have all the results related to the investigation.

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Increase of cases of legionellosis in Latvia, 2011

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An increased number of legionellosis cases in 2011 has been reported in Latvia, compared to the ten previous years. A total of 30 legionellosis cases (1.35 per 100,000 inhabitants), including 19 females, have been confirmed until the end of September 2011. The majority of cases (n=23) were inhabitants of the capital city Riga. The reason for the increase in legionellosis is unclear. Twenty-six of the 30 cases are not travel-related.

In 2011, increased numbers of legionellosis case notifications have been noted in Latvia, compared with previous years. From 2001 to 2010, a total of 22 cases were notified to the State Agency "Infectology Center of Latvia" (LIC). In 2011, there were at least two cases per month from March onwards, contributing to a total of 26 autochthonous cases until September 2011.

Legionellosis or Legionnaires' disease is a mild to severe pneumonia caused by bacteria of the genus *Legionella*. *Legionella* bacteria are found in environmental fresh waters, and have a potential to proliferate in great quantities in badly maintained human-made water systems, such as spas, baths, cooling towers, hot and cold water systems. Legionellosis can occur when *Legionella*-contaminated water aerosols created by for example showers and taps are inhaled [1-3]. In most cases, legionellosis is caused by the *Legionella pneumophila* serogroup 1 [4-6].

Notification of legionellosis in Latvia

In Latvia, legionellosis was included in the list of mandatorily notifiable diseases in 1999. Healthcare practitioners are legally responsible for notifying infectious diseases and each legionellosis case or professionally well-founded suspicion of legionellosis have to be notified to the regional epidemiologists within 24 hours by phone, and by sending a special urgent notification form. Notification is required for suspected *Legionella* cases and then additional notifications are required if a diagnosis is changed or discarded, as well as for the final diagnosis and outcome of disease and laboratory confirmation of the diagnosis. Since 2009, cases of legionellosis have to be notified by microbiological laboratories as well. The case classification as

probable or confirmed is based on the European Union (EU) case definition [7].

Regional epidemiologists of the LIC State Agency after receipt of the information from healthcare practitioners or laboratories collect, store and analyse the epidemiological data. They can also perform an investigation of the cases, and take environmental samples for laboratory testing, including water from suspected *Legionella*-contaminated water systems. The LIC is also responsible for organising and advising on preventive and control measures.

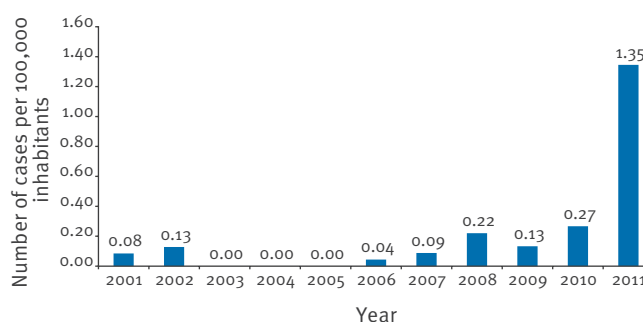
Legionellosis in Latvia from 2001 to 2011

The first autochthonous legionellosis cases in Latvia were registered in 2001 and 2002. Subsequently no cases were reported during the three following years. The average number of cases per year in the period from 2001 to 2009 was 2.2 (range: 1–5), which corresponds to a mean incidence of 0.09 per 100,000 inhabitants (Figure 1).

The number of cases reached six (0.27 per 100,000 inhabitants) in 2010. Among cases, two were likely to have been infected abroad, while for the rest, the source of infection remains unconfirmed. None of the water samples taken at the patients' dwellings revealed *Legionella* prevalence.

FIGURE 1

Legionellosis incidence rate in Latvia, 1 January 2001–30 September 2011 (n=52)



Epidemiological situation in 2011

In 2011, a total of 30 legionellosis cases (1.35 per 100,000 inhabitants) were registered until the end of September (Figure 2) and an epidemiological investigation of all cases was performed. Of the 30 registered cases, 17 were confirmed serologically by demonstration of a specific antibody response to *Legionella pneumophila* by single high titre, while 11 were confirmed by detection of specific *Legionella* antigen in urine, and two were confirmed by both of the mentioned methods.

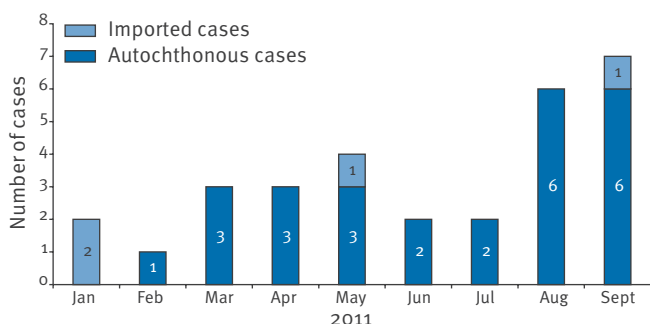
Of the legionellosis cases, 17 were treated in the only specially designated hospital for infectious diseases in the country, which is at the LIC in Riga. The rest were admitted to six other different hospitals/rehabilitation centres. Two of the cases in the age group 45–55 years were fatal and consisted of a woman and a man, who was a heavy smoker. Neither fatal case had any documented underlying diseases.

A standard questionnaire was used during the epidemiological investigation in order to interview patients with legionellosis. The questionnaire included travel history and other possible risk factors/exposures. There were only four cases likely to have been infected abroad in 2011, either in Germany, Czech Republic, India or Mexico, where they had travelled/worked during their incubation period (two of them mentioned that they could have been infected during a stay in a hotel). The 26 remaining patients reported no travel abroad. Among them, 23 were inhabitants of the capital city Riga, with their residences scattered at either side of the Daugava river which divides the city (Figure 3). The other three were from other cities in the western and central part of the country.

In 2011, 19 legionellosis patients were females while only 11 were male. For female patients, the highest incidence occurred in the 18–29 and older than 60 year age groups, while most male patients were between 40 and 59 years old (Figure 4).

FIGURE 2

Legionellosis cases by month of registration, Latvia, 2011 (n=30)



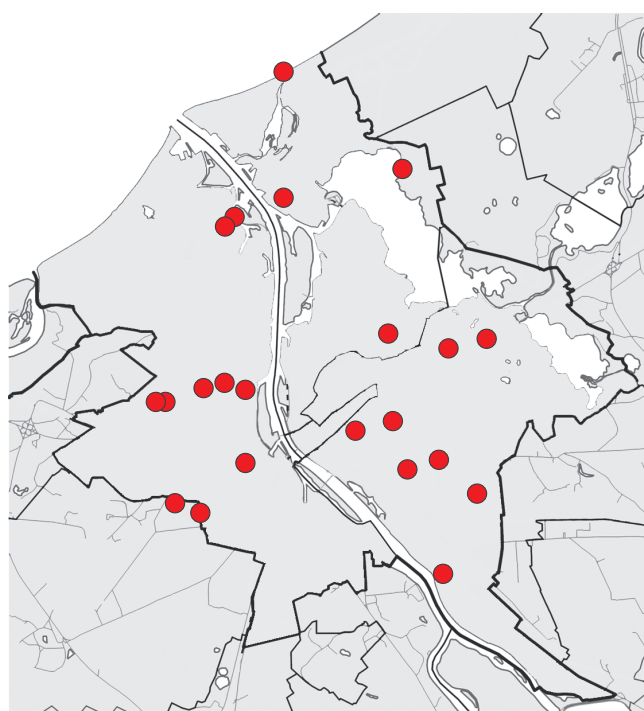
Environmental investigation

During the epidemiological investigation of cases, a total of 52 households were visited and 114 water samples were collected and tested for the prevalence of *Legionella* spp. (Table 1).

For 12 legionellosis cases, *Legionella* spp. were found in the water-supply system of the patients' households, including the heating units of the apartment house. In the majority of samples, bacteria were found in the hot water (55% in the house heating units, and 24% in the

FIGURE 3

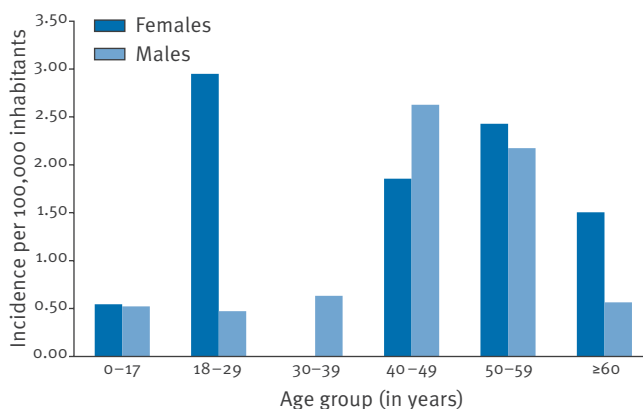
Distribution of cases of legionellosis in Riga, Latvia, 1 January–30 September 2011 (n=23)



Only 22 locations are indicated on the map for the 23 cases, because two cases occurred from the same location.

FIGURE 4

Legionellosis incidence rate by age group and sex, Latvia, 2011 (n=30)



flats), while in cold water samples - only 15% and 8% accordingly. *Legionella pneumophila* serogroup 1 was found in seven of 26 positive samples (27±8.87%), while other serogroups (2-14) were found in 19 samples (73±8.87%).

Control measures

As soon as an increase of legionellosis cases in Latvia was detected, the LIC prepared and provided information for practitioners and clinicians of all hospitals, including case definitions and diagnostic methods. As a response to the emerging situation, a notable information campaign was undertaken, to involve and educate institutions responsible for water system maintenance, such as city councils, house management offices, city heating suppliers, city water suppliers, as well other competent bodies such as the Ministry of Health, Health Inspection, Association of the Family doctors, hospitals and society via mass media.

Discussion

The reasons for the increased legionellosis case numbers in Latvia in 2011 are unclear. Apart from the four cases who travelled abroad, no common risk factor or exposure could be identified. There were, moreover, no changes in the availability of diagnostic tests in Riga, compared with previous years, which could have accounted for differences in the number of confirmed cases in 2011. Among possible factors that could have contributed to the increase, the enhanced awareness of healthcare practitioners could have played a role, as it would have resulted in a reduction of underdiagnosed cases of pneumonia. It has been reported that the main reason for not diagnosing legionellosis in patients is a lack of clinical awareness [5]. Another explanation for the increase of legionellosis cases could be the unfavourable economical situation, which compels the population to spare water and energy. In this case, inhabitants request heating regulators to decrease the temperature of hot water systems leading an increased contamination of these systems. *Legionella* can multiply between 25°C and 42°C, and the optimal proliferation temperature of the bacteria is 35°C [5]. Also, it cannot be excluded that two exceptionally hot summers in 2011 influenced the drinking water contamination load with *Legionella*.

In our study, the male/female ratio of cases was also inverse to the usual trend, where males dominate [6,8].

This could be due to chance and the small numbers did not really allow reliable statistical analysis, but could be also partially explained by the male/female ratio in the Latvian population which is 0.86 (1,029,391 males/1,200,250 females) [9]. Some unstated activities at households, more specific to women could influence the situation as well. .

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TABLE 1

Investigation of environmental samples for *Legionella* spp. prevalence, Latvia, 1 January–30 September 2011 (n=114)

Sample collection site	Sample type	Number of samples tested	<i>Legionella</i> positive n (% ± Standard deviation)
Apartment house (heating units)	Hot water	20	11 (55±11)
	Cold water	13	2 (15±10)
Flats (taps or showers)	Hot water	42	10 (23±7)
	Cold water	39	3 (15±10)

Cluster of travel-associated Legionnaires' disease in Lazise, Italy, July to August 2011

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Since 18 August 2011, 17 cases of travel-associated Legionnaires' disease have been reported. They were tourists from five European countries who had stayed in five accommodation sites in Lazise, Italy. The dates of symptom onset ranged from 18 July to 25 August 2011. Control measures were implemented and no further cases associated with stays at the sites have been reported after disinfection. Timely notification of any further cases potentially associated with stay in Lazise is recommended.

Cluster description

A total of 17 cases of travel-associated Legionnaires' disease have been reported since 18 August 2011 that were associated with a stay in several accommodation sites in Lazise, Italy. All cases – seven from the Netherlands, six from Germany, two from Italy, one from Austria and one from Denmark – stayed at five different accommodation sites (two campsites and three hotels) in Lazise between the beginning of July and end of August 2011. Dates of symptom onset ranged from 18 July 2011 to 25 August 2011 (Figure). The ages of the cases ranged from 42 to 78 years (mean: 57; standard deviation: 11.9) and the male to female ratio was 3.3 to 1.

Background

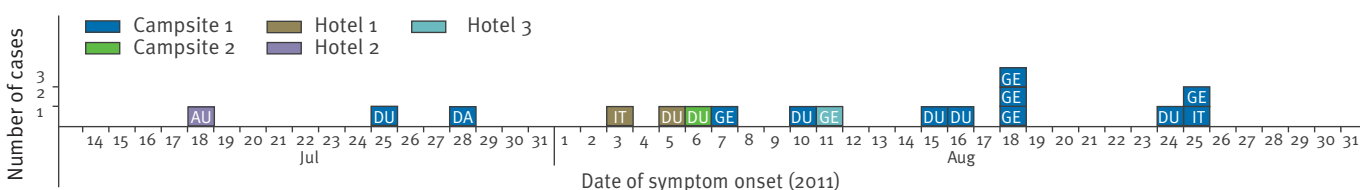
Legionnaires' disease is a lung infection caused by *Legionella* bacteria. The bacteria live in water or wet soil and must be inhaled to cause infection. *Legionella* can cause a severe form of pneumonia (Legionnaires' disease), which in Europe can be fatal for about 5–15% of people with the disease, but it can also cause a mild influenza-like infection without pneumonia, called Pontiac fever [1].

Over the last 10 years, the number of cases of Legionnaires' disease in Italy has been steadily increasing, from 325 cases in 2001 to 1,200 cases in 2009, with an incidence in 2009 of 2 per 100,000 population [2,3]. The number of cases of travel-associated Legionnaires' disease has also been increasing: every year, several clusters associated with accommodation sites, involving tourists from Italy and elsewhere in Europe, are reported [4,6]. Most of this increase has been attributed to improved diagnostic tools, in particular the urinary antigen detection test [7].

The European Legionnaires' Disease Surveillance Network (ELDSNet), coordinated by the European Centre for Disease Prevention and Control (ECDC) since

FIGURE

Cases of travel-associated Legionnaires' disease by date of symptom onset and nationality, Lazise, Italy, notified by ELDSNet as of 21 September 2011 (n=17)



AU: Austrian; DA: Danish; DU: Dutch; GE: German; IT: Italian.
ELDSNet: European Legionnaires' Disease Surveillance Network.

April 2010, carries out surveillance of Legionnaires' disease, involving all European Union Member States, Iceland and Norway. It aims to identify relevant public health risks, enhance disease prevention and monitor epidemiological trends. In this context, surveillance of travel-associated disease is carried out on a day-to-day basis to inform urgent public health action, with the aim of preventing subsequent cases. Each travel-associated case of Legionnaires' disease diagnosed in a participating European country is reported by national ELDSNet collaborators to ELDSNet as quickly as possible. If other cases are found to have been associated with a particular accommodation site within a two-year period, a cluster is identified. A rapid risk assessment of the accommodation site associated with the cluster is undertaken by the country in which the site is located: the results are reported to ECDC and shared with all countries in the network [8,9].

Testing isolates and data collection

Of the 17 reported cases reported in Lazise, 16 were confirmed by a urinary antigen test and one case remained probable because diagnosis was on the basis of a single high *Legionella*-specific antibody titre. *Legionella pneumophila* serogroup 1 was isolated from two patients: one had stayed at Campsite 1 and one at Campsite 2. There were no deaths.

Lazise is a small town located about 20 km north-west of Verona, by Lake Garda (the largest lake in the country). It has 7,000 inhabitants and there are an estimated 60,000 visitors during the summer holiday period. Legionnaires' disease was not reported in Lazise inhabitants in July and August 2011. The disease has been reported in tourists staying in neighbouring villages in the Lake Garda area, as expected based on the previous years' notifications (unpublished data).

Patients were contacted by ELDSNet national collaborators in their country of residence. Information about potential exposure in the 10 days preceding the onset of symptoms (incubation period for Legionnaires' disease is 2–10 days) was obtained using a standardised questionnaire: national ELDSNet collaborators of the countries where cases were reported recorded the details in an ad hoc restricted-access web-based database set up by ELDSNet. Analysis of the data revealed common accommodation sites but no other common exposure.

Ongoing investigations

Epidemiological and environmental investigations, which started immediately after notification of the cluster by ELDSNet on 19 August 2011, are ongoing. The Istituto Superiore di Sanità is supporting the local health authorities in Lazise.

Of the 17 reported cases, 12 had stayed in Campsite 1 (accommodating about 3,500 people), two had stayed in Hotel 1 (with about 40 rooms), two in two different hotels (Hotels 2 and 3 with about 50 rooms each)

and one in Campsite 2 (accommodating about 1,800 people).

Three of the five accommodation sites (Campsite 1, Campsite 2 and Hotel 1) were found to be within approximately 500 metres of each other. The water sources for the five accommodation sites are different: the two campsites are supplied by private wells while the three hotels are supplied by the same public service. Local rapid risk assessment was promptly carried out [10] and several water samples were collected for testing by the regional and the national reference laboratories according to procedures indicated for the control and prevention of legionellosis [11]. In the first round of sampling, 56 samples of cold and hot water were collected from water tanks, taps, shower heads, swimming pools, water sprinklers, decorative fountains and jacuzzis at the five accommodation sites. Two samples from Campsite 1 were found positive for *L. pneumophila* serogroup 1, with a concentration of 900 and 4,100 colony forming units per liter (CFU/L). These two samples had been collected from distal water outlets in one of the seven washing and toilet facilities. In Hotel 2, three samples were found positive for *L. pneumophila* serogroup 1, at concentrations ranging from 2,000 to 12,000 CFU/L. *L. pneumophila* serogroup 2-14 was isolated from other water points in all five accommodation sites.

No cooling towers were found in Lazise and its outskirts. To date, no installations have been identified as a potential source of *Legionella*.

Typing of *Legionella* isolates

The two *L. pneumophila* serogroup 1 clinical isolates were characterised by sequence-based typing [12]: both were sequence type (ST) 23, as were the two *L. pneumophila* serogroup 1 isolates from the environmental samples. Further molecular investigations are ongoing.

Control measures

A rapid risk assessment conducted promptly in all five accommodation sites allowed us to implement control measures. Disinfection of the water systems in all five accommodation sites involved was carried out as a control measure and all devices generating aerosols (e.g. spa pools, lawn sprinklers and decorative fountains) were immediately deactivated. Hospitals and general practitioners (GPs) in the area were alerted in order to enhance clinical surveillance of the disease. People staying at Campsite 1 (which reported the greatest number of cases) and for whom email addresses were available were informed by email of the ongoing cluster of the disease and were encouraged to contact their GPs if they developed symptoms. Managers of all the accommodation sites, spas and other recreational sites in the municipality were also informed through a note issued by the Mayor of Lazise and were made aware of the importance of adopting adequate measures to prevent legionellosis.

Environmental sampling, repeated after disinfection of the water systems, was negative for *Legionella* and no further cases have been notified after the risk management measures were adopted.

Conclusion

As a common source of infection in Lazise has not yet been identified, there may be an ongoing risk of exposure to *Legionella* for persons visiting or residing in the town. For this reason, we encourage timely notification of further cases potentially associated with stay in Lazise.

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Wellness centres: an important but overlooked source of Legionnaires' disease. Eight years of source investigation in the Netherlands, 1 August 2002 to 1 August 2010

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Visiting wellness centres is considered safe and relaxing and might provide health benefits for visitors with certain cardiovascular, dermatological or respiratory diseases. On the other hand, wellness centres could pose health risks, especially with respect to Legionnaires' disease. We investigated the role of wellness centres in the occurrence of Legionnaires' disease by analysing the data of eight years (2002–2010) of source investigation in the Netherlands. There were 15 wellness centres identified as potential sources of infection for a total of 35 Legionnaires' disease patients. Twelve of these centres were positive for *Legionella* spp.: six for *Legionella pneumophila*, six for non-*pneumophila* *Legionella* spp.. Of the 65 positive environmental samples found during the wellness centre investigations, 41 were derived from shower heads. For two centres, the *Legionella pneumophila* strains in the collected samples had a genotype that was indistinguishable from the patient isolates. These results show that wellness centres are potential sources of Legionnaires' disease.

Introduction

Apart from massages and beauty care most wellness centres offer a mix of saunas, swimming pools, whirlpools, and other bathing facilities to the general public. Visiting these wellness centres is considered safe and relaxing and might even provide health benefits for visitors with certain cardiovascular, dermatological or respiratory diseases [1,2]. On the other hand, it has been shown that facilities with whirlpools or saunas could comprise health risks, for example with respect to Legionnaires' disease [3-5]. This acute pneumonia is caused by *Legionella* spp., which are thought to be responsible for two to 15% of all community-acquired pneumonias [6-8]. *Legionella* spp. live in aquatic environments and are particularly prevalent in man-made habitats [9]. The major route of transmission for Legionnaires' disease is inhalation of the

bacterium that is spread into the air as an aerosol from either natural or man-made sources [10]. Modern use of devices that aerosolise water or settings with such devices (e.g. air conditioners, showers, cooling towers, fountains, wellness centres), largely contribute to the emergence of Legionnaires' disease as an important waterborne disease.

Previous reports showed that in several cases of Legionnaires' disease, wellness centres (with saunas and/or whirlpools) were indeed identified as the source of infection [3-5]. However, further clarification of the role of these centres in *Legionella* infections warrants a systematic identification and investigation of potential sources of Legionnaires' disease. In 2002, based on the observation that outbreaks of Legionnaires' disease are often preceded and followed by small clusters of cases [11], the Netherlands established the *Legionella* Source Identification Unit (LSIU) as part of a National *Legionella* Outbreak Detection Programme (NLODP) [12]. The aim of this programme was to improve source identification, thereby preventing or controlling outbreaks of Legionnaires' disease by swift elimination of the source.

In this study we aimed to assess the importance of wellness centres in the occurrence of Legionnaires' disease by analysing the data of eight years (2002–2010) of systematic source investigation within the NLODP in the Netherlands.

Methods

National *Legionella* Outbreak Detection Programme

As part of the NLODP, a LSIU was available to all Municipal Health Services for sampling of potential sources of *Legionella* infection in reported cases of Legionnaires' disease. Between 2002 and 2006, all identified potential sources of infection were

investigated. From 2006 onwards, the LSIU has only investigated potential sources if at least one of the following four sampling-criteria was met: (i) A patient isolate of *Legionella* spp. from respiratory secretions or lung tissue is available; (ii) one of the potential sources of infection identified by a Legionnaires' disease patient was previously identified as a potential source of a different Legionnaires' disease patient; (iii) the residence of a reported Legionnaires' disease patient is situated within a range of less than one kilometre from the residences of at least two other Legionnaires' disease patients who were reported in the last six months; (iv) the patient stayed in a hospital during the incubation period.

Patients

Legionnaires' disease has been notifiable in the Netherlands since 1987. Treating physicians are required to report cases of Legionnaires' disease to a public health physician at one of the 29 Municipal Health Services within 24 hours of diagnosis. The public health physicians are then required to report all confirmed and probable cases of Legionnaires' disease to the Ministry of Health and, since 2006, to the Centre for Infectious Disease Control, within 24 hours. A confirmed case of Legionnaires' disease is defined as a patient suffering from symptoms compatible with pneumonia, with radiological signs of infiltration, and with laboratory evidence of *Legionella* spp. infection (including isolation of *Legionella* spp. from respiratory secretions or lung tissue, detection of *L. pneumophila* antigen in urine, seroconversion or a four-fold or higher rise in antibody titres to *Legionella* spp. in paired acute- and convalescent-phase sera). A probable case of Legionnaires' disease is defined as a patient suffering from symptoms compatible with pneumonia, with radiological signs of infiltration, and with laboratory findings suggestive of *Legionella* spp. infections (including a high antibody titre to *Legionella* spp. in a single serum, direct fluorescent antibody staining of the organism or detection of *Legionella* species DNA by polymerase chain reaction (PCR) in respiratory secretions or lung tissue). All 62 microbiological laboratories in the Netherlands involved in the diagnosis and treatment of patients with pneumonia are requested to send the available isolates of *Legionella* spp. from respiratory secretions or lung tissue of patients to the LSIU.

Given the purpose of the programme to identify Dutch sources of infection, patients who had stayed abroad for five days or more during their incubation period of two to 10 days were not considered for source identification.

Source identification and sampling procedure

Potential sources of infection were identified by public health physicians and nurses from the Municipal Health Service who interviewed the patient and/or a relative. The interview focused on tracking each patient's exposure to potential sources of infection during the two

weeks before their first symptoms occurred. If at least one of the four sampling criteria was met, trained laboratory staff from the LSIU took water and swab samples from the identified potential sources. For each location, sampling points were selected by the LSIU staff in cooperation with the technical team of a facility (when available) to obtain a comprehensive collection of water and swab samples for further analysis. The sampling procedure was in accordance with national guidelines [13,14]. It is noteworthy that the LSIU sampling method differs slightly from the European guidelines, which recommend samples of one litre in volume to be collected immediately after the opening of the water outlet [15], while the LSIU samples 500 ml in volume.

Laboratory investigations

The water samples were concentrated by filtration and filtered residues were resuspended in 1 ml sterile water. Of this suspension, 100 µl samples were cultured without dilution and after 10-fold dilution on two media at 35°C, with increased humidity. The two media used were buffered charcoal yeast extract supplemented with α-ketoglutarate (BCYE-α) and (i) the antibiotics polymyxin B, cefazolin, and pimaricin; and (ii) the antibiotics polymyxin B, anisomycin, and vanomycin. In cases of bacterial overgrowth, cultures were repeated after pre-treatment by heating 30 minutes at 50°C. Swab samples were dispersed by immersion in 1 ml sterile water and cultured as described above. Both patient and environmental *Legionella* isolates were serogrouped by using commercially available kits containing antisera against *L. pneumophila* serogroups 1-14, *L. longbeachae* 1 and 2, *L. bozemanii* 1 and 2, *L. dumoffii*, *L. gormanii*, *L. jordanis*, *L. micdadei*, and *L. anisa* (*Legionella* latex test, Oxoid Limited, Hampshire, England; *Legionella* antisera "Seiken," Denka Seiken Co. Ltd., Tokyo, Japan). All *Legionella pneumophila* serogroup 1 strains that were found in patient isolates or in the collected samples were subsequently genotyped by amplified fragment length polymorphism (AFLP) analysis, and by sequence based typing (SBT), as recommended by the European Working Group for Legionella Infections (EWGLI) [16-18]. Patient isolates were then compared with environmental strains that were found in the samples of potential sources that were investigated.

Control measures

Whenever a wellness centre was found positive for *Legionella* spp. after sampling, the responsible government agency (usually the Inspectorate of the Ministry of Housing, Spatial Planning and the Environment (VROM Inspectorate)) was informed by the Municipal Health Services. They assessed how codes of practice and legal regulations concerning the prevention of Legionnaires' disease had been followed, and recommended or enforced control measures such as thermal or chemical disinfection and adaptation of the plumbing system to prevent new cases of Legionnaires' disease.

Results

Patients

From 1 August 2002 until 1 August 2010, 2,076 confirmed or probable cases of Legionnaires' disease were notified to the Centre for Infectious Disease Control. The 619 (30%) patients who had stayed abroad for five days or more during their incubation period (2–10 days) were excluded from the analyses. The remaining 1,457 patients were investigated by the Municipal Health Services and the LSIU. Patient characteristics are shown in Table 1. Patients had a median age of 59.5 (interquartile range (IQR): 50.7–70.0) years, and 29% were female.

The 2,343 potential sources of infection that were mentioned by the patients during the interviews with the Municipal Health Service are shown in Table 2. Patient homes were mentioned by the majority of patients, followed by garden centres, workplaces, hospitals, cooling towers, and sports facilities. Wellness centres ranked 11th on the list of most often mentioned potential sources.

Source investigation

Source investigation resulted in the sampling of 1,317 of the 2,343 potential sources by the LSIU that were related to one or more of the 1,457 patients. Some of the potential sources were more frequently associated with *Legionella* findings than others, which is reflected in the proportion of investigations where *Legionella* was found in the investigated source. The sampling results are shown in Table 3, where the sources are ranked by the percentage of positive source investigations (from high to low). It should be noted that an individual source was sometimes investigated more than once (some sources were repeatedly identified by new patients during the study period). The proportion of potential source investigations that were positive for *Legionella* spp. was highest for wellness centres (28 of 33 source investigations), followed by cooling towers, hospitals, hotels, swimming pools, sports facilities, holiday parks, and home residences (Table 3).

When the different species of *Legionella* are considered, the data show that in 21 of the 33 wellness centre investigations *Legionella pneumophila* was found in one or more of the investigated samples, ranking

TABLE 1

Probable or confirmed cases of Legionnaires' disease, by age group, the Netherlands, 1 August 2002–1 August 2010 (n=1,457)

Age group (years)	Female n (%)	Male n (%)
0–25	7 (1.7)	8 (0.8)
26–50	87 (20.7)	238 (23.0)
51–75	244 (58.1)	640 (61.7)
>75	82 (19.5)	151 (14.6)
Total	420 (100.0)	1,037 (100.0)

wellness centres first before cooling towers, hospitals, hotels, swimming pools, sport facilities, and holiday parks (Table 3). The majority of the 65 positive samples found during the wellness centre investigations were derived from shower heads (n=41). Other positive sample locations within the wellness centres were: taps (n=12) and whirlpools (n=3).

The 33 investigations of wellness centres were performed at 15 unique sites. Twelve of these centres were positive for *Legionella* spp. (six centres for *Legionella pneumophila*, and six centres for non-*pneumophila* *Legionella* spp.). The number of investigations on individual wellness centres testing positive for *Legionella* spp. ranged from one to seven. The 15 investigated wellness centres were identified by 35 patients, of whom 25 were part of different clusters associated with seven large and small wellness centres all positive for *Legionella*. There was one wellness centre with seven clustered patients, two centres with four patients, two centres with three patients, and two centres with two patients.

Genotype comparison

For 129 of the 333 positive source investigations that were performed between 2002 and 2010, there was a patient isolate available for genotyping which allowed comparison with the genotypes of the environmental strains found in the samples. In 33 cases the available patient isolate had an indistinguishable genotype from those of the environmental strains reflecting a success rate of 25 % (33/129). The majority of these 'matches' were made with strains from investigated hospitals (13 matches of 13 positive investigation with an available patient isolate), home residences (nine matches of 47), hotels (two matches of two), swimming pools (two matches of seven), and wellness centres (two matches of 13).

TABLE 2

Potential sources of infection (n=2,343) reported by Legionnaires' disease cases (n=1,457), the Netherlands, 1 August 2002–1 August 2010

Reported potential source of infection	n (%)
Home residence	1,149 (49.0)
Garden centre	146 (6.2)
Workplace	138 (5.9)
Hospital	115 (4.9)
Cooling tower	89 (3.8)
Sports facility	68 (2.9)
Swimming pool	59 (2.5)
Holiday park	48 (2.0)
Hotel	47 (2.0)
Car wash installation	47 (2.0)
Wellness centre	44 (1.9)
Campsite	39 (1.7)
Fountain	38 (1.6)
Other	316 (13.5)

Discussion

Given the low ranking of potential sources mentioned by Legionnaires' disease patients, wellness centres do not seem to contribute much to Legionnaires' disease transmission. However, our data show that in 85% (28 of 33) of all investigations wellness centres were positive for *Legionella* spp. This rate is remarkably higher compared to other types of potential sources like cooling towers (18 of 33 (55%)), hospitals (34 of 68 (50%)), homes (139 of 693 (20%)) and garden centres (eight of 63 (13%)) that were identified, investigated and sampled under identical conditions. Moreover, typing results indicate that in more than 60% (six of 33) of all wellness centre investigations, *Legionella pneumophila*, which is thought to be the etiologic agent in over 90% of all Legionnaires' disease patients [19], was found in at least one of the samples. Compared to the other potential sources that were investigated, wellness centres account for the highest percentage of *Legionella pneumophila* positive source investigations, which further indicates the relatively high potential of wellness centres as sources of Legionnaires' disease.

There are several possible explanations for our findings. One of them is that the circumstances in wellness centres contribute to a *Legionella*-friendly environment. The abundant presence of showers, whirlpools, swimming pools and even air-perfused footbaths can clearly form a *Legionella*-friendly habitat and lead to

free *Legionella* in the air. Additionally, the complexity of water piping systems due to subsequent enlargements of wellness centres could lead to standing or slow-flowing water and thereby create a stable micro-environment for growth of *Legionella*.

Another possibility is that the visitors of wellness centres may be more at risk for Legionnaires' disease compared to individuals who do not visit these centres. Underlying chronic diseases and smoking status are known risk factors for Legionnaires' disease [20]. If an overrepresentation of individuals who are at higher risk for Legionnaires' disease among wellness centres visitors is confirmed, a possible public health intervention would be to inform this group on the risks of wellness recreation. We were unfortunately not able to study this possibility in the current study setting. However, considering the remarkable source investigation results we do think that there is a role awaiting for public health education aimed at wellness centre visitors who are at increased risk for Legionnaires' disease.

It is difficult to compare our results with previous European studies on surveillance of Legionnaires' disease because of the absence of a systematic source identification and investigation programme in other countries. Although several outbreak reports have acknowledged wellness centres as an important

TABLE 3

Results of investigations (n=1,317) of potential sources of infection reported by Legionnaires' disease cases (n=1,457), the Netherlands, 1 August 2002–1 August 2010

Source type (n) ^a	Number of investigations ^b for <i>Legionella</i> spp.					Total n (%)
	Positive for <i>Legionella</i> spp.			Negative for <i>Legionella</i> spp.	Total n (%)	
	<i>L. pneumophila</i> n (%)	non- <i>pneumophila</i> <i>Legionella</i> spp. n (%)	<i>L. pneumophila</i> and non- <i>pneumophila</i> <i>Legionella</i> spp. n (%)	Total positive n (%)		Total negative n (%)
Wellness centre (n=15)	15 (45)	7 (21)	6 (18)	28 (85)	5 (15)	33 (100)
Cooling tower (n=30)	15 (45)	2 (6)	1 (3)	18 (55)	15 (45)	33 (100)
Hospital (n=48)	14 (21)	15 (22)	5 (7)	34 (50)	34 (50)	68 (100)
Hotel (n=14)	3 (20)	2 (13)	1 (7)	6 (40)	9 (60)	15 (100)
Swimming pool (n=32)	5 (15)	5 (15)	2 (6)	12 (35)	22 (65)	34 (100)
Sports facility (n=26)	4 (15)	3 (12)	1 (4)	8 (31)	18 (69)	26 (100)
Holiday park (n=19)	3 (14)	3 (14)	0(0)	6 (27)	16 (73)	22 (100)
Other (n=199)	19 (9)	31 (15)	3 (1)	53 (26)	150 (74)	203 (100)
Home residence (n=693)	39 (6)	93 (13)	7 (1)	139 (20)	554 (80)	693 (100)
Workplace (n=78)	6 (7)	8 (10)	2 (2)	16 (20)	66 (80)	82 (100)
Car wash installation (n=11)	0(0)	2 (18)	0(0)	2 (18)	9 (82)	11 (100)
Garden centre (n=51)	2 (3)	6 (10)	0(0)	8 (13)	55 (87)	63 (100)
Fountain (n=11)	0(0)	1 (9)	0(0)	1 (9)	10 (91)	11 (100)
Campsite (n=23)	1 (4)	1 (4)	0(0)	2 (9)	21 (91)	23 (100)
Total (n=1,250)	126 (10)	179 (14)	28 (2)	333 (25)	984 (75)	1,317 (100)

L. pneumophila: *Legionella pneumophila*.

^a This number represents the number of unique sources.

^b A unique source could be the subject of more than one investigation if it was repeatedly identified by Legionnaires' disease cases over the eight year period covered by this study.

source of exposure in Legionnaires' disease outbreaks [4,5], most European surveillance programmes do not include these specific potential sources in their surveillance data [21,22]. The installation of a European surveillance programme in which systematic environmental investigations are incorporated could elucidate the role of different potential sources in Legionnaires' disease cases.

The strengths of this study are the nationwide detection and registration of new Legionnaires' disease cases and additional source identification within the NLODP, which resulted in a systematic and uniform collection of data. Together with the systematic sampling procedure of potential sources and the advanced serotyping and genotyping (AFLP and SBT) techniques, this enabled us to further clarify the role of wellness centres in *Legionella* infections in eight years of Legionnaires' disease source identification efforts in the Netherlands.

Nevertheless, it should be kept in mind that the investigated wellness centres were not a random selection of all available centres in the Netherlands. Sampling of wellness centres was only performed according to the protocol of the NLODP. Furthermore, the ranking of the potential sources of infection that were mentioned by the patients is influenced by the overall presence of particular sources (there are clearly more home residences than wellness centres or car wash installations present in the environment). Random sampling of centres that are not directly linked to Legionnaires' disease patients, for presence of *Legionella* could further elucidate the contribution of these centres to Legionnaires' disease in the Netherlands. It should also be noted that despite the large number of positive source investigations in wellness centres, only two matches in genotype were found during the eight years of this study period. Although this is partly a reflection of the limited number of clinical isolates that were available for genotype comparison in case of a positive source investigation, a larger number of genotype matches that actually linked cases to wellness centres would have strengthened the evidence for the role of wellness centres in Legionnaires' disease.

In conclusion, wellness centres are not merely the health promoting facilities they are often seen as, but also potential sources for Legionnaires' disease. Despite control measures that are taken after identification of a first patient, some individual centres have been related to an accumulating number of Legionnaires' disease patients over time. This questionable role of wellness centres requires increased attention from wellness centre owners, the VROM Inspectorate, water companies, and Municipal Public Health Services. Furthermore, as many sources remain unknown at the moment this could increase the number of identified sources of Legionnaires' disease.

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Cluster of Legionnaires' disease in a newly built block of flats, Denmark, December 2008 – January 2009

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During December 2008 to January 2009, two persons contracted Legionnaires' disease in a newly built block of flats in a suburb of Copenhagen in Denmark. Polymerase chain reaction and culture was used to diagnose Legionnaires' disease in this cluster. Isolates from both patients tested positive for *Legionella pneumophila* serogroup 1 subgroup Philadelphia sequence type 1 and the same strain was detected in hot water samples taken from the residential area indicating that the hot water supply system was the most likely source of infection. *Legionella* was not detected in the cold water. Two interventions were conducted to limit the *Legionella* colonisation of the piping and storage tanks and the effect was monitored by investigating water samples from various sites in the block of flats. Only the second intervention had a sufficient effect on the *Legionella* colonisation. The cluster described here points to several risk factors regarding growth of *Legionella* in hot water systems: (i) stagnancy of water from when the building is constructed and piping installed and until residents move in, (ii) stagnancy and low temperature (from room temperature to approximately 38 °C) of water in shower hoses and (iii) failure in operation of and control measures for the hot water system.

Introduction

Legionnaires' disease (LD) is a severe pneumonia with high mortality caused by the inhalation of aerosolised *Legionella* bacteria. *Legionella* occurs naturally in water sources but the bacteria multiply to high numbers at temperatures between 20 °C and 45 °C [1]. The multiplication of *Legionella* is, associated with several other factors apart from water temperature such as water being stagnant, type of pipe material used, the presence of a biofilm (a micro-environment between surface and water) and amoebae [1,2]. These factors are the reasons why man-made water systems, often harbour *Legionella* in high numbers. Outbreaks of LD are often associated with aerosols from cooling towers [3,4], spas [1,5,6], and hot and cold water systems at hospitals [7,8] and hotels [9]. Studies have shown that domestic hot water systems are often colonised with

Legionella [10,11] but outbreaks are rarely associated with potable water distribution systems.

Each year 100-130 cases of LD are notified in Denmark (approximately 20 per million) which is a rather high incidence compared to other European countries [12]. Most of the cases are sporadic and only few outbreaks have been identified. This study was conducted to investigate factors associated with risk of *Legionella* colonisation in new buildings and to monitor and investigate the effect of control measures.

Cluster description

A cluster of two culture-confirmed LD cases was identified during December 2008 to January 2009 in a suburb of Copenhagen in Denmark. Neither case had any recent history of travel. On 11 November 2008, the first case (Case 1), a man in his early forties with an underlying condition, was hospitalised 250 km away from the building that was later found to be the source of infection. Case 1 was linked to the block of flats only after the second case (Case 2) was diagnosed, since he had only spent a few days in the newly built block of flats, in an apartment which had not been used before. On 30 December the second patient (Case 2), a man in his mid-sixties who had been treated for an underlying condition, was hospitalised and on 5 January 2009 he was diagnosed with LD. He died 20 days after admission. Case 2 lived in the building later found to be the source of infection. His family had earlier complained about the low temperature of the hot water in the apartment and the hot water of this apartment was therefore the first to be investigated.

Both cases were positive for *L. pneumophila* by polymerase chain reaction (PCR) on samples from tracheal secretions and *L. pneumophila* was subsequently isolated by culture by standard techniques. Isolates were identified by agglutination test (*Legionella* latex test DRo800M, Oxoid); and sero- and subgrouping were performed with monoclonal antibodies [13]. Extracted DNA was analysed by sequence-based typing (SBT) according to the European Working Group

for *Legionella* Infections (EWGLI) standard procedure [14]. Both cases were found to be infected with *L. pneumophila* serogroup 1 subgroup Philadelphia sequence type (ST) 1.

All residents of the block of flats, as well as visitors, were informed about the outbreak and advised to go and see their general practitioner and take blood samples if showing symptoms of LD and 16 of these chose to do so. These 16 samples were tested for *Legionella* antibodies. Three of them were also tested for *Legionella* urinary antigen but none of the samples were positive. None had pneumonia but some may have had Pontiac fever based on clinical symptoms (influenza-like illness caused by *Legionella* infection), although this was not confirmed by laboratory tests.

Methods

The water system

The building identified as the most probable source of contamination had 225 apartments distributed in six blocks. Of these 225 apartments, 210 were inhabited at the time the cluster was detected. The hot water system had two boilers in use and a circulation pump in place to circulate the hot water. In each stairway, proportional thermostatic adjusting valves set at 50 °C regulated the flow of hot water. The water supply in the area was based on ground water, which was

not disinfected and distributed without disinfection residuals.

Sampling and analysis

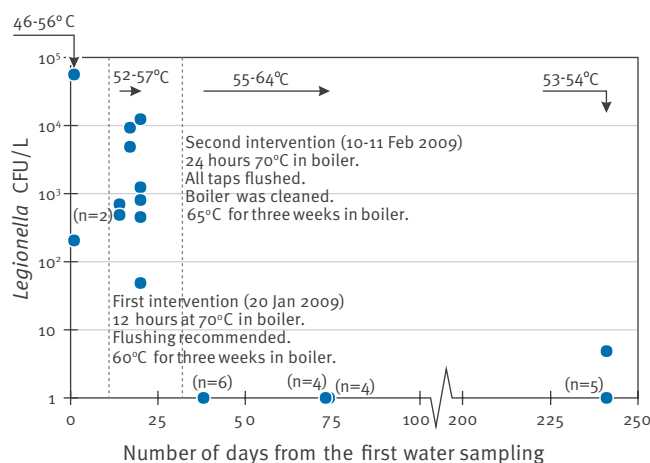
Water was collected from the building on eight occasions from 9 January to 7 September 2009. Two types of water samples were collected each time from kitchen and bathroom taps as well as from shower hoses: A-samples – the first litre of water (first flush) – and B-samples – one litre collected after flushing until constant water temperature (warm or cold) was reached. Apartments located as distant from and as close to the boiler as possible and apartments with no, low or normal levels of water consumption, as well as apartments associated with the cases, were sampled. B-samples from shower hoses all had a temperature of 38 °C due to the thermostatic mixing valves installed on shower fixtures in all apartments.

The temperature ranges in the samples in the different apartments are given at the top of the figure. The first water samples were collected on 9 January 2009. Unless otherwise indicated, each dot represents one sample. The dotted lines indicate the two interventions.

The first water samples were collected on 9 January 2009. Unless otherwise indicated, each dot represents one sample. The dotted lines indicate the two interventions.

FIGURE 1

Concentration of *Legionella* spp. in the hot water system (B-samples^a), data collected from seven different apartments, Copenhagen, Denmark, January – September 2009



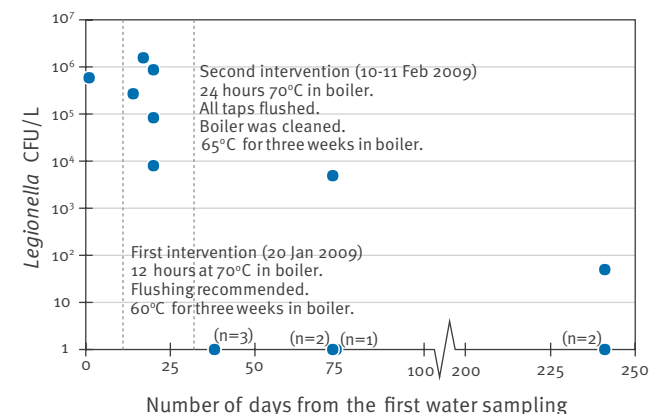
CFU: colony forming unit.

^a One litre samples collected after flushing until constant water temperature (warm or cold) was reached.

Concentration of *Legionella* spp over time in the hot circulation water in the block of flats. The water samples were collected after flushing until constant temperature (B-samples), and the temperature interval in the samples in the different apartments are given at the top of the figure. Each dot represents one sample but there are two samples with 800 CFU/L on day 20, six samples with no *Legionella* detected on day 38, four samples with no *Legionella* detected on day 73, four samples with no *Legionella* detected on day 74 and five samples with no *Legionella* detected on day 241. The dotted lines indicate the first and the second intervention, respectively. The first water samples were collected on 9 January 2009.

FIGURE 2

Concentration of *Legionella* spp. in the first litre of water sampled (A-samples^a), data collected from seven different apartments, Copenhagen, Denmark, January – September 2009



CFU: colony forming unit.

^a A-samples are the first litre of water from the shower hose (first flush).

Concentration of *Legionella* spp over time in the first litre of water sampled (A-samples) from different shower hoses. There are 3 samples on day 38, where no *Legionella* could be detected. Each dot represents one sample but there are two samples on day 73 and one sample on day 74 where no *Legionella* could be detected. At day 241, there are two samples with no *Legionella* detected. The dotted lines indicate the first and the second intervention, respectively. The first water samples were collected 9 January 2009.

Testing of water samples for the detection of environmental *Legionella* by cultivation was done according to the ISO standard 11731:1998 [26]. One-litre water samples were concentrated 10-fold and 100-fold respectively by 0.2 µm membrane filtration and subsequent centrifugation. Modified Wadowsky Yee and Glycin, Vacomycin, Polymyxin, Cycloheximide agar plates were both seeded with 0.5 ml water directly from the sample before concentration, 0.1 ml after filtration and 0.1 ml after centrifugation. Plates were incubated at 37 °C for seven to 10 days before colonies were counted. The highest colony count from any of the three steps was considered the result and expressed as colony-forming units (CFU) per litre.

Isolates from water were analysed as isolates from clinical samples, but only two environmental isolates of *Legionella pneumophila* serogroup 1 subgroup Philadelphia from two different samples were selected for further DNA typing as described above. Isolates (>10) identified as *Legionella* species by OXOID were identified to species level by sequencing of the *Legionella mip* gene [15].

Control measures

To control the *Legionella* contamination of the hot water system, two interventions were conducted. The first was initiated on 16 January 2009 (11 days after the diagnosis of Case 2) when the temperature and the flow of the water system were increased. On 20 January, water in the boiler was heat-treated at 70°C for 12 hours together, after which all residents were requested to flush their taps for five minutes. Subsequently, the water in the boilers was completely replaced with fresh water and the temperature was reduced to 60 °C for three weeks. Circulation pumps were set at maximum flow.

The second intervention was performed on 10–11 February 2009. For 24 hours the water in the boilers was heated to approximately 70°C and all taps were flushed for five minutes. The hot water temperature in the taps was kept at a minimum of 65 °C. The boilers were hyperchlorinated and the temperature was set at 65°C. All shower hoses in all apartments were replaced with new ones and over the next month the boiler temperature was regulated to ensure the water in the most distant taps was kept at 50°C. To monitor how the second heat treatment affected the *Legionella* level in the long term, samples were collected one week, six weeks and seven months after the intervention.

The design, dimensions and regulation of the hot water system, including boilers, pumps, valves and control procedures were evaluated by consulting engineers.

Information to residents

The residents were informed about the *Legionella* colonisation of the water system by letters delivered to each apartment on 15, 20 and 21 January 2009. In addition, posters were displayed on the entrance doors of

the building, and an information meeting for residents and visitors was organised on 4 February 2009 by the administration. Residents and visitors to the block of flats who had symptoms compatible with *Legionella* infection (influenza-like symptoms and/or respiratory symptoms) were asked to contact their general practitioner for consultation and collection of samples for laboratory testing.

Results

Water samples

The sample collected after flushing (B-samples) from the tap in the apartment of Case 2 revealed a hot water temperature below 50 °C (46 °C after 15 minutes of flushing) and 5.5×10^4 *Legionella* CFU/L by culture (Table 1) whereas the temperature should be above 50 °C as a minimum.

The apartment of Case 2 was situated far from the boilers (only two apartments were placed further away in that direction). The first flush samples (A-samples) collected from shower hose in the apartment of Case 2 showed more than 6×10^5 CFU/L. This shower hose had rarely been in use, so water had been stagnant for several days. *L. pneumophila* serogroup 1 subgroup Philadelphia was found in both the A-sample and in the B-sample tapped after 15 minutes. One B-sample was collected from a tap in an apartment very close to the boilers; the temperature measured 56 °C and only 2×10^2 CFU/L were detected in that sample. Only *L. pneumophila* serogroup 3 was found in the sample. The subgroup Philadelphia isolated from the patient's apartment was also found to be ST 1.

The water system

During the investigation to reveal the cause of the low hot water temperature in the apartment of Case 2, operational problems were detected. These problems were caused by a combination of low flow in the hot water system and inadequate temperature in the boilers. The circulation pump was adjusted to low capacity, which made the circulation slow. The slow circulation was also caused by small pipe dimensions. In fact the resistance in the pipes was so high that the water was prevented from circulating at the required flow. The slow circulation caused heat loss, and despite the thermostatic adjusting valves being opened, a circulation speed high enough to compensate for the heat loss could not be obtained. Thus, the temperature of the water leaving the boilers was not high enough to compensate for the low flow and the heat reduction throughout the water system. The monitoring arrangements were problematic as well, since a thermometer installed to manually control the water temperature of the hot water return, was found not to function as it showed too high a temperature.

The high concentration of *Legionella* in some parts of the hot water system resulted in the first intervention which unfortunately failed to eradicate *Legionella* from the hot water system. The concentration of *Legionella*

TABLE 1

Effect of heat treatments on the number and species, serogroups, strains of *Legionella*, data collected from seven different apartments, Copenhagen, Denmark, January – September 2009

Timing of the sampling	Sampling site	Type of sample ^a	Number of samples	Number of positive samples	Temperature of water tested (°C)	<i>Legionella</i> concentration CFU/litre CFU/L Median	Type of <i>Legionella</i> identified
Before the first intervention (9/1/09)	Shower hose	A	1	1	not measured	> 6 *10 ⁵	<i>L. pneumophila</i> serogroup 1 subgroup Philadelphia. Sg 3
	Tap	A	1	1	not measured	1,4 *10 ⁵	<i>L. pneumophila</i> serogroup 1 subgroup Philadelphia. Sg 3
	Tap (hot water)	B	1	1	46	5.5 *10 ⁴	<i>L. pneumophila</i> serogroup 1 subgroup Philadelphia
	Kitchen tap (hot water)	B	1	1	56	2,0 *10 ²	Sg 3
After the first intervention	Shower hose	A	5	5	not measured	8,0 *10 ² – 1,6 *10 ⁶	Sg 1. <i>L. pneumophila</i> serogroup 1 subgroup Philadelphia. Sg 3. Sg 2-14. Sg 4 subgroup Portland.
	Shower hose 38 °C ^b	B	4	4		2,0 *10 ² – 1,2 *10 ⁴	Sg 1. <i>L. pneumophila</i> serogroup 1 subgroup Philadelphia. Sg 3. Sg 2-14
	Bathroom tap (hot water)	A	5	5	not measured	5,0 *10 ³ – 1,2 *10 ⁵	Sg 1. <i>L. pneumophila</i> serogroup 1 subgroup Philadelphia. Sg 3. Sg 2-14
	Bathroom tap (hot water)	B	5	5	51.5–56	4,5 *10 ¹⁺² – 1,2 *10 ⁴	<i>L. pneumophila</i> serogroup 1 subgroup Philadelphia. Sg 3. Sg 2-14.
	Kitchen tap (hot water)	A	5	5	not measured	7 *10 ²⁻³ *10 ⁵	<i>L. pneumophila</i> serogroup 1 subgroup Philadelphia. Sg 3. Sg 2-14
	Kitchen tap (hot water)	B	5	5	52–57	5 *10 ¹⁻⁵ *10 ³	Sg 3. Sg 2-14
	Bathroom tap (cold water)	B	4	0	8.5–16	BD	–
	Shower hose	A	7	1	not measured	BD – 5 *10 ³	spp. <i>L. anisa</i>
	Shower hose 38 °C ^b	B	7	3		BD – 1 *10 ²	<i>L. anisa</i>
	Bathroom tap (hot water)	A	7	0	not measured	BD	–
After the second intervention	Bathroom tap (hot water)	B	7	0	55.3–64	BD	–
	Kitchen tap (hot water)	A	7	3	not measured	BD – 1 *10 ³	spp. <i>L. anisa</i>
	Kitchen tap (hot water)	B	7	0	56.7–64	BD	–
	Bathroom tap (cold water)	B	3	0	7.3–16.7	BD	–
	Shower hose	A	3	1	not measured	BD – 5 *10 ¹	Sg 3. Sg 2-14
	Shower hose 38 °C ^b	B	3	2		BD – 1 *10 ²	spp.
	Bathroom tap (hot water)	A	3	2	not measured	BD – 2 *10 ¹	<i>L. pneumophila</i> serogroup 1 subgroup Philadelphia. Sg 3. Sg 2-14
	Bathroom tap (hot water)	B	3	0	53–54	BD	–
	Kitchen tap (hot water)	A	3	1	not measured	BD – 5 *10 ¹	Sg 3. Sg 2-14
	Kitchen tap (hot water)	B	3	1	54	BD – 5	Sg 3

BD: below detection by culture; CFU: colony-forming unit; Sg: serogroup.

^a A-samples are the first litre of water from the tap or shower hose (first flush); B-samples are one-litre samples collected after flushing to reach constant water temperature (warm or cold) was reached.

^b Samples of taps are collected after flushing until constant temperature. B-samples from shower hoses were collected when the thermostats were mixing cold and warm water to 38 °C. If B-samples are not referred to in the text as being from shower hoses, B-samples are from samples collected at constant temperature from taps.

TABLE 2

Distribution of the different *Legionella* species serogroups and strains before and after the two interventions, data collected from seven different apartments, Copenhagen, Denmark, January – September 2009

Type of <i>Legionella</i> identified	Tap samples						Shower samples ^a									
	Before the first intervention		After the first intervention		After the second intervention		Seven months after the second intervention		Before the first intervention		After the first intervention		After the second intervention		Seven months after the second intervention	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
<i>L. pneumophila</i> serogroup 1 subgroup Philadelphia	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Sg 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sg 3	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+
Sg 4 portland	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sg 2-14	-	-	+	+	-	-	+	+	-	-	-	-	-	-	-	-
<i>L. anisa</i>	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Any <i>Legionella</i> species	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+

^a Includes shower samples 38 °C ('B' samples), as in Table 1

+: detection by cultivation, agglutination test, monoclonal antibodies and DNA typing.
-: not detected.

decreased in the hot water B-samples (taps), but they remained present, with an average of more than 3×10^3 CFU/L (Figure 1). In the A-samples (first flush) from shower hoses, the number of *Legionella* was high: 8.0×10^3 to 8.8×10^6 CFU/L (Figure 2).

Since *Legionella* remained present in the water system after the first intervention, a second intervention was conducted. Samples were collected one week (day 38) and six weeks after the second heat treatment (day 73 and 74) and revealed none or only very few *Legionella* in samples collected from the taps after flushing to constant temperature (B-samples). This indicated that the increased temperature suppressed *Legionella* growth in the circulating water. However, *Legionella* remained present in some A-samples although in low numbers. Of seven samples from shower hoses, only one contained *Legionella* (5×10^3 CFU/L) (Table 1). The detection of *Legionella* in A-samples but not in B-samples, from the same tap or shower hose, indicated local growth. Local growth can be established when the most distant parts of the pipework (shower hose or tap) have not been effectively included in an intervention or if this habitat is particularly favoured for rapid regrowth. *Legionella* was not detected in the cold water system probably because water temperatures (less than 20 °C) were outside the optimal growth temperature for *Legionella* (Table 1).

Seven months after the second heat treatment, only a few *Legionella* (five *Legionella* CFU/L in one sample) were detected by culture of B-samples from taps. Only 50 CFU/L were detected in one of three A-samples from shower hoses. The second heat treatment and the continuing flow and temperature regulation seemed to control the number of *Legionella* in this newly built block of flats.

The composition of *Legionella* species and serogroups changed during the course of the treatments (Table 2).

Before and after the first heat treatment only *L. pneumophila* was detected (dominated by serogroup 3 and serogroup 1 subgroup Philadelphia) in both A- and B-samples but after the second heat treatment only *L. anisa* was detected, and mainly in A-samples. Seven months after the second heat treatment, *L. pneumophila* serogroup 3 and serogroup 1 subgroup Philadelphia were found again, but serogroup 1 Philadelphia was only detected in one A-sample, at a very low (5 CFU/L) concentration.

Discussion and conclusion

The hot water in the building was not kept at temperatures outside the range within which *Legionella* can multiply. Two interventions were conducted to eradicate the *Legionella* contamination but only the second intervention (water in the boilers heated to 70 °C for 24 hours followed by 65 °C for three weeks) followed by a generally increased temperature of the whole warm water system compared to before any of

the interventions was effective with only very limited regrowth after seven months. Other studies [16-18] have investigated different kinds of heat treatments but none of them have proven to be effective over a longer period of time. These studies showed that an important factor common to all treatments was that the normal day-to-day operation of the water systems was not adjusted after the different interventions. In this case permanent changes were made in the functioning of the water system after the second heat treatment (higher circulation speed and flushing in unoccupied apartments).

Bacterial biofilms are important for the survival of *Legionella* and may limit the effectiveness of any intermittent systemic disinfection regime [18]. If not totally erased they constitute a serious factor for potential regrowth. The change we found in the composition of *Legionella* species before and after the different heat treatments indicated a higher heat tolerance for *L. anisa* than for *L. pneumophila* as only *L. anisa* was cultured immediately after the second heat treatment. Another explanation for this change could be a faster colonisation of *L. anisa* than other *Legionella* species. When investigating the composition of species it was also shown that *L. pneumophila* either had survived in the biofilm or had been supplied with the water from the waterworks since this species was detected seven months after the second heat treatment when the temperature was lowered. The finding of *L. pneumophila* serogroup 1 subgroup Philadelphia emphasises the importance of keeping the water system under strict temperature control. *L. pneumophila* serogroup 1 subgroup Philadelphia was not found in water with temperatures above 55 °C.

L. anisa, which was detected right after the second heat treatment, is common in Danish residential water systems [19], but has only very rarely been associated with infections in humans [20]. To get a real picture of the risk of a given water system, it is important to be able to discriminate between different species and serogroups. In this specific cluster, we would probably not have seen any LD cases had *L. pneumophila* serogroup 1 subgroup Philadelphia not been part of the *Legionella* flora in the residential area. The subgroup Philadelphia belongs to a virulent subgroup of *L. pneumophila* serogroup 1 (called Pontiac or MAb 3/1 positive) [21], which is seldom cultured from hot water systems (< 5% in Denmark) [20].

L. pneumophila serogroup 1 subgroup Philadelphia ST 1 is uncommon in hot water systems. The finding of this particular strain in both patients and in the water system of the new block of flats where both cases had lived or spent time during the incubation period, clearly points to the water as the infectious route.

Legionella is often found in private houses and apartments [11,23,24]. In old buildings with old water installations, the risk of *Legionella* contamination is normally

considered to be larger compared with newer buildings with newly established water systems [10,11,25]. However, this cluster demonstrated that newly built blocks of flats can present a risk of *Legionella* infection. From when a building is finished and water is let into the system until all apartments are inhabited, water may be stagnant in the pipes at ambient temperature, and a biofilm with *Legionella* can be established in the system. This was probably the situation in the apartment of Case 1. In order to prevent high levels of *Legionella* in the water pipe systems in new buildings, standard procedures to clean the systems should be applied before occupation. Treatment with biocides could be a solution. In a newly built residential area with many unoccupied apartments, it should also be taken into consideration that the water consumption (both cold and hot water) is lower than the consumption the system is designed for. Hence the water system should be designed to accommodate varying levels of water consumption.

Shower hoses were found to be important risk factors in this study since we found a high number of *Legionella* in them. This may be due to the material of the hose, temperature and flow of the water. If not regularly flushed with hot water, the low temperature and stagnancy of water in them could pose a risk for infection.

Another risk factor – obvious but nevertheless often overlooked, as in this newly built block of flats – is the control and regulation of the water system. Thermostatic heating systems should be properly controlled and correctly sized, including adequate boilers and pumps to run the system optimally. Circulation pumps should have the capacity to keep the water circulating sufficiently also during periods of low water consumption, when the circulation pumps provide the main force in circulating the water. Water should leave the boiler at a temperature hot enough to maintain the temperature above 50 °C even at the most distant tap and in the return water. Pipe dimensions should be scaled according to the size of the building and flow should be adjustable according to the water consumption.

In the building described in this study, some of the pipes have been changed in order to reduce the resistance. The water system has now two separate re-circulation systems each with a pump, and taps in the apartments that are not occupied are flushed once a week.

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Contamination of the cold water distribution system of health care facilities by *Legionella pneumophila*: Do we know the true dimension?

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German water guidelines do not recommend routine assessment of cold water for *Legionella* in healthcare facilities, except if the water temperature at distal sites exceeds 25 °C. This study evaluates *Legionella* contamination in cold and warm water supplies of healthcare facilities in Hesse, Germany, and analyses the relationship between cold water temperature and *Legionella* contamination. Samples were collected from four facilities, with cases of healthcare-associated Legionnaires' disease or notable contamination of their water supply. Fifty-nine samples were from central lines and 625 from distal sites, comprising 316 cold and 309 warm water samples. *Legionella* was isolated from central lines in two facilities and from distal sites in four facilities. 17% of all central and 32% of all distal samples were contaminated. At distal sites, cold water samples were more frequently contaminated with *Legionella* (40% vs 23%, $p < 0.001$) and with higher concentrations of *Legionella* ($\geq 1,000$ colony-forming unit/100 ml) (16% vs 6%, $p < 0.001$) than warm water samples. There was no clear correlation between the cold water temperature at sampling time and the contamination rate. 35% of cold water samples under 20 °C at collection were contaminated. Our data highlight the importance of assessing the cold water supply of healthcare facilities for *Legionella* in the context of an intensified analysis.

Introduction

Legionnaires' disease (LD) is an important cause of hospital-acquired pneumonia [1]. Potable water was recognised as the major environmental source of healthcare-associated LD (hca-LD) in the early 1980s [1]. After this discovery, almost all cases of hca-LD have been linked to potable water [2-5]. For example, in the United Kingdom, 19 of 20 hospital LD outbreaks from 1980 to 1992 could be attributed to the water distribution system (WDS) [6]. Microaspiration is the major mode of transmission of hca-LD [7]. Because the clinical manifestations are non-specific, and specialised labo-

ratory testing is required, LD is easily underdiagnosed [1,8].

Routine testing for *Legionella* of environmental water samples by culture has emerged as an effective strategy for prevention of hca-LD. Guidelines mandating routine monitoring of *Legionella* contamination of the WDS in hospitals and other healthcare facilities have been implemented in many European countries, including Spain, France, the United Kingdom, and Germany [1,9]. In contrast, the Centers for Disease Control and Prevention (CDC) recommends environmental cultures only when cases of hca-LD are discovered [10], an approach which remains controversial, taking into account that a specific diagnostic for LD is not routinely performed in many laboratories. For example, in the United States of America (USA) only 19% of the hospitals that participated in the CDC National Nosocomial Surveillance System did routinely provide *Legionella* testing of patients at high risk for developing hca-LD [11]. In Germany, the Federal Environment Agency (Umweltbundesamt) and the German National Public Health Institute (Robert Koch Institute) recommend periodical analysis of the WDS of hospitals, nursing homes and other healthcare facilities [12]. If a moderate to high level contamination is detected, i.e. at *Legionella* concentration of $\geq 1,000$ colony-forming unit (cfu)/100 ml, an intensified analysis with additional sampling points according to the guidelines of the German Technical and Scientific Association for Gas and Water (DVGW) is recommended [12,13].

Legionella can grow and amplify at temperatures between 25 °C and 45 °C with an optimum between 32 °C and 42 °C. *Legionella pneumophila* is able to withstand temperatures of 50 °C for several hours, but does not multiply at temperatures below 20 °C [9]. Therefore, keeping water temperature outside the range for *Legionella*, i.e. ≥ 55 °C and < 20 °C is an effective prevention and control measure for both warm and cold water systems. In Germany, which has

a temperate climate, the temperature of cold water at entry to a building is usually below 20 °C. The German guidelines do not recommend routine assessment of cold water for *Legionella* contamination. In the context of intensified analysis, assessment of cold water is recommended if the water temperature at the distal site exceeds 25 °C [12].

The Hesse State Health Office (HSHO) is a federal institution in charge of surveillance, prevention, and control of LD in Hesse, a state with six million inhabitants located in west-central Germany. The diagnostic laboratories of HSHO offer a broad spectrum of chemical and microbiological analysis for water samples. Our institution is usually consulted by the communal health authorities when cases of hca-LD are detected in a healthcare facility or if routine environmental cultures reveal a notable contamination by *Legionella* species. We here present the results of the evaluation of the WDS of four healthcare facilities, which had contacted us for assistance to control and prevent *Legionella* contamination of their WDS. Two cases of hca-LD had been diagnosed in one facility, an acute care hospital with a solid organ transplantation unit, whereas a moderate to high *Legionella* contamination had been detected upon routine assessment in the other facilities, which included a rehabilitation centre and two nursing homes. A multidisciplinary team was sent to each facility in order to determine the extent of contamination of the WDS, to assess the contamination of cold and warm WDS independently and to investigate a possible correlation between the water temperature at sampling time and the extent of *Legionella* contamination.

Methods

Healthcare facilities

The healthcare facilities included in this study consisted of an acute care hospital specialised in thoracic surgery and solid organ transplantation (260 beds), a rehabilitation centre with cardiologic, orthopaedic and psychosomatic departments (183 beds), a nursing home for physically disabled individuals (47 beds), and a nursing home for elderly people (220 beds). These facilities had been requested by the Communal Health Office to conduct intensified *Legionella* monitoring because high *Legionella* concentrations had been detected during periodical assessment and/or cases of hca-LP had been reported. Each facility was visited by a team of specialists of the Communal Health Office and the HSHO several times (four to six times) between March 2009 and August 2010. The results presented in this study are derived from the analysis of samples that were obtained at the first visit of our team to the facilities between March 2009 and February 2010.

Sampling procedure

Sampling points were selected by the team of specialists in cooperation with the technical teams of the facilities to obtain a comprehensive sample of cold and warm water for intensified analysis, in accordance with the recommendations of DVGW [13]. Fifty-nine

samples were obtained from central lines (cold and hot-water tanks, return lines) of all facilities, including facility A (one warm sample), facility B (four cold samples), facility C (24 warm, 25 cold samples), and facility D (three warm, two cold samples). Six hundred and twenty-five samples were obtained from distal sites (467 showerheads, 155 taps, one pond and two spring fountains) of the facilities, comprising facility A (10 warm, 12 cold samples), facility B (15 warm, 16 cold samples), facility C (252 warm, 256 cold samples), and facility D (32 warm, 32 cold samples). Cold and warm water were generally sampled in parallel at distal sites. The temperature was documented and samples of approximately 200 ml were collected at central sites after discarding 3 L of cold or 3 L of warm water, and at distal sites after discarding 3 L of cold or 5 L of warm water, according to recommendations of the Federal Environment Agency [12]. It is noteworthy that the latter sampling method differs slightly from the European guidelines, which recommend samples of one litre in volume to be collected immediately after the opening of the water outlet [14].

Laboratory investigation

Legionella culture was performed on GVPC agar (Oxoid) according to recommendations of the Federal Environment Agency [15]. Two aliquots of 0.5 ml water were inoculated directly to GVPC agar and 100 ml was filtered through a 0.45 µm cellulose-nitrate membrane. The filter was overlaid with 20 ml 0.2 M HCl-KCl [pH 2.2] and incubated for 4–5 min. The buffer was discarded, the filter was rinsed with 10 ml sterile water and placed on GVPC agar. The cultures were incubated at 37 °C in a humidified atmosphere and examined after three, five, seven and 10 days. The detection limit of our method was one cfu/100 ml.

Identification was conducted by performing subcultures of at least three colonies per sample on BCYE agar (Oxoid) and sheep-blood agar. *Legionella* isolates grew on BCYE agar but not on sheep-blood agar. Serotyping was performed with a latex agglutination kit (*Legionella* Latex Test, Oxoid), which allows the identification of *Legionella pneumophila* serogroup 1, *L. pneumophila* serogroups 2–14, and non-pneumophila *Legionella* species.

Statistical analysis

Statistical analysis was performed with Stata, Version 11.1, 2009 (StataCorp LP, Texas, USA). Chi square test or Fisher exact test were used for analyzing qualitative data. Results were considered statistically significant when the P value was <0.05.

Results

Contamination rate in cold and warm water

Fifty-nine samples were collected at central lines, including 28 warm (temperature range: 46–75 °C) and 31 cold (temperature range: 7–14 °C) water samples. A total of 10 of 59 central samples were contaminated, comprising nine of 28 warm and one of 31 cold water

samples (Table 1). Hence, among the central samples, warm water was more frequently contaminated with *Legionella* than cold water ($p < 0.001$).

Six hundred and twenty-five distal samples were analysed, including 309 warm (temperature range: 32–70 °C) and 316 cold (temperature range: 7–29 °C) water samples. A total of 197 of 625 (32%) distal samples were contaminated. *Legionella* was detected in 125 of 316 (40%) cold water samples and 72 of 309 (23%) warm water samples (Table 1). Thus, among the distal samples, cold water was more frequently contaminated with *Legionella* than warm water ($p < 0.001$).

We next evaluated the results at the level of individual facilities. The temperature of cold and warm water differed slightly between the facilities. At distal sites, cold water temperatures of 8–25 °C (facility A), 9–24 °C (facility B), 7–28 °C (facility C), and 13–29 °C (facility D) and warm water temperatures of 40–64 °C (facility A), 36–65 °C (facility B), 32–70 °C (facility C), and 50–66 °C (facility D) were measured at sampling time. *Legionella* contamination was detected in distal cold and warm water of all facilities. The overall positivity rate was nine of 22 (41%), 25 of 31 (81%), 146 of 508 (29%), and 17 of 64 (27%) in distal water of the facilities A, B, C, and D, respectively. Remarkably, contamination was more frequently detected in cold water than in warm water in three facilities (Figure 1). The contamination rate of cold and warm water in the facilities A, B, C, and D were 25% versus 60%, 88% versus 73%, 39 versus 19%, and 28 versus 25%, respectively (Table 2).

Legionella species and serogroups detected

Serological differentiation of the *Legionella* isolates from the WDS revealed *L. pneumophila* serogroup 1 in facility A, C, and D, *L. pneumophila* serogroup 2-14 in facility B, and non-pneumophila *Legionella* spp. in facility A and C. *L. pneumophila* serogroup 1 was also isolated from the bronchoalveolar lavage fluid of the index patient with hca-LD in facility C. The *L. pneumophila* isolates obtained from the patient and the water supply displayed the same geno- and serotype, as determined by multilocus sequence typing (MLST) and monoclonal antibody serotyping, which were performed at the *Legionella* Reference Laboratory, University of Dresden, Germany.

Legionella concentration in cold and warm water

Of 316 distal cold water samples analysed, 60% were tested negative for *Legionella*, 4% revealed minimal contamination (colony count 1–99 cfu/100 ml), 20% moderate contamination (100–999 cfu/100 ml) and 16% high contamination ($\geq 1,000$ cfu/100 ml). Of 309 distal warm water samples analysed, 77% were negative, 6% displayed minimal contamination, 11% moderate contamination, and 6% high contamination (Table 3). In detail, a total of 69 samples comprising 49 cold and 20 warm water samples revealed a high *Legionella* concentration ($\geq 1,000$ cfu/100 ml). Thirty three of 49 (67%) highly contaminated cold water samples displayed a temperature of < 20 °C at collection time, whereas three of 20 (15%) highly contaminated warm water samples displayed a temperature of ≥ 55 °C at sampling time. Together, cold water samples were more frequently contaminated with higher *Legionella* concentrations compared to warm water samples. The difference between cold and warm water was significant in all categories except for minimal contamination (Table 3).

We next evaluated the prevalence of high *Legionella* concentrations, i.e. $\geq 1,000$ cfu/100 ml, in cold and warm water of different facilities. As shown in Table 2, a high grade contamination was detected in three of four facilities. Cold water samples were more frequently contaminated with high *Legionella* concentrations than warm water samples in three of four facilities (Table 2).

Relationship between temperature and Legionella contamination

We next examined the relationship between the temperature of distal water at sampling time and *Legionella* contamination. Cold and warm water samples were assigned to four groups, cold water < 20 °C, cold water ≥ 20 °C, warm water < 55 °C, and warm water ≥ 55 °C and the contamination rate was calculated for each group. The positivity rate was 94 of 265 (35%), 31 of 51 (61%), 45 of 52 (87%), and 27 of 257 (11%) in the latter groups, respectively (Figure 2). It is noteworthy that 35% of cold water samples that displayed an optimal temperature in terms of *Legionella* prevention at sampling time, that is < 20 °C, were contaminated. In contrast, only 11% of warm water samples that displayed an optimal

TABLE 1

Legionella contamination rate in cold and warm water samples obtained from four healthcare facilities, Hesse, Germany, March 2009–February 2010 (n=684)

Sample collection site	Sample type	<i>Legionella</i> positive n (%)	<i>Legionella</i> negative n (%)	Total n
Central line	All	10 (17)	49 (83)	59
	Cold water	1 (3)	30 (97)	31
	Warm water	9 (32)	19 (68)	28
Distal	All	197 (32)	428 (68)	625
	Cold water	125 (40)	191 (60)	316
	Warm water	72 (23)	237 (77)	309

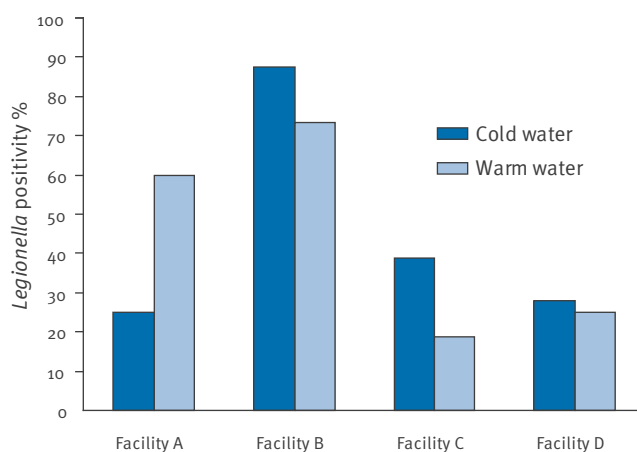
temperature in terms of *Legionella* prevention, that is ≥ 55 °C, were contaminated. Outside the temperature range of *Legionella* growth, there was significantly less contamination in warm water than contamination in cold water ($p < 0.001$).

We further examined whether we may find a threshold temperature that would allow a reliable discrimination between contaminated and non-contaminated distal water. The threshold temperatures of 15 °C, 20 °C and

25 °C were tested for cold water, and 50 °C, 55 °C, and 60 °C for warm water. The contamination rate of samples beyond the selected temperature was calculated separately. As shown in Figure 3, 43 of 156 (28%) of water samples that were below 15 °C at sampling time, which is below the lower limit (20 °C) of the range of *Legionella* growth, were contaminated by *Legionella*. This suggests that measuring cold water temperature at sampling does not allow the defining of a reliable temperature threshold, below which cold water would be considered free from *Legionella* contamination.

FIGURE 1

Legionella contamination in cold and warm water collected at distal sampling sites in four healthcare facilities, Hesse, Germany, between March 2009 and February 2010 (n= 625)



Discussion

We here present the results of assessment of the water supplies of four healthcare facilities in Germany. The investigation was initiated because cases of hca-LD were diagnosed in one facility (Facility C) or because periodical analysis had suggested a severe contamination of the WDS with *Legionella* (facilities A, B, and D). The contamination rate of distal water samples was 41%, 81%, 29% and 27% in the four facilities examined. The very high rate in some cases (81%) was not entirely unexpected in light of the circumstances that had led to the enrolment of the facilities in this study.

We found higher contamination rates and higher *Legionella* concentrations in cold water samples than in warm water samples collected from distal sites in three facilities (Figure 1, Table 2). Legionellosis has been traditionally associated with inadequately heated warm water [1]. There is a common belief that only the

TABLE 2

Legionella contamination in distal cold and warm water samples collected in four healthcare facilities, Hesse, Germany, March 2009–February 2010 (n=625)

Healthcare facility	Cold water			Warm water		
	Total	<i>Legionella</i> positive	<i>Legionella</i> $\geq 1,000$ cfu/100 ml	Total	<i>Legionella</i> positive	<i>Legionella</i> $\geq 1,000$ cfu/100 ml
Facility A (n=22)	12	3	0	10	6	0
Facility B (n=31)	16	14	8	15	11	5
Facility C (n=508)	256	99	37	252	47	15
Facility D (n=64)	32	9	4	32	8	0

TABLE 3

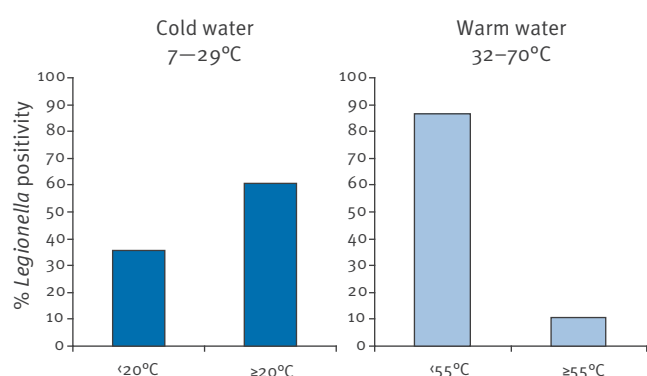
Legionella concentration and temperature range of cold and warm water collected at distal sites in four healthcare facilities, Hesse, Germany, March 2009–February 2010 (n= 625)

<i>Legionella</i> concentration (cfu/100 ml)	Cold water			Warm water			P value ^a
	Temperature range (°C)	n	%	Temperature range (°C)	n	%	
<1	7–28	191	60	38–70	237	77	<0.001
1–99	8–25	13	4	39–65	18	6	0.361
100–999	11–27	63	20	37–64	34	11	0.003
$\geq 1,000$	11–29	49	16	32–62	20	6	<0.001
Total	7–29	316	100	32–70	309	100	

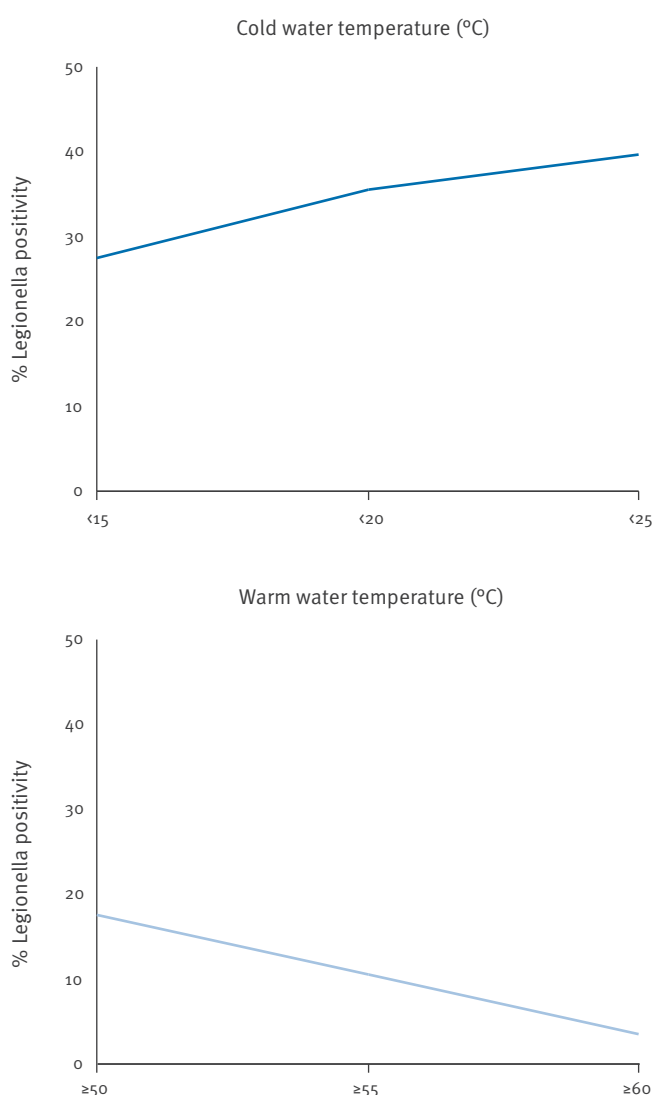
^a The P values were calculated by comparing the proportion of cold water samples displaying a distinct *Legionella* concentration among all cold water samples with the proportion of warm water samples with the similar *Legionella* concentration among all warm water samples.

FIGURE 2

Relationship between the temperature of distal water at sampling time and *Legionella* contamination, Hesse, Germany, March 2009–February 2010 (n= 625)

**FIGURE 3**

Relationship between contamination rate of distal water and the threshold temperature for cold and warm water, Hesse, Germany, March 2009–February 2010 (n= 625)



warm water supply may serve as a source of infection. Nonetheless, previous studies have shown that the cold water supply of healthcare facilities may be heavily contaminated with *Legionella* species [16]. Other investigators have reported cases of hca-LD that were attributed to contamination of the cold water supply. Hoebe et al. [17] reported two cases of fatal LD in a rehabilitation centre linked to the cold water supply. Johansson et al. [18] described a case of hca-LD in Sweden that was clearly linked to the cold WDS. Graman et al. [19] reported a case of hca-LD that was traced back to a contaminated ice machine. Our data show that the cold water supply of healthcare facilities may be even more heavily contaminated by *Legionella* species than the warm water supply. We found *Legionella* concentrations of up to 10,000 cfu/100 ml in distal cold water samples (data not shown). Different factors may have contributed to this interesting phenomenon. It is possible that a thermal disinfection of warm WDS was performed shortly prior to our visit to the facility. This could have resulted in a temporal suppression of *Legionella* in the warm water supply. Another possible explanation is a “warming-up” of cold water, which may occur after long intervals of stasis or when the cold and warm water pipes are closely fitted in the same shaft and run together over a long distance without appropriate insulation. The warming-up effect may not be detectable at the time of sampling, which is usually during daytime on a weekday. In the latter case, hot water flushing of warm water tubes may even have a paradoxical effect on contamination of the cold WDS by aggravating the warming-up effect.

Analysis of the temperature of distal samples revealed that only 16 of 316 (5%) cold water samples displayed a temperature of 25 °C or more at sampling time, which is the threshold temperature recommended by the German water guidelines for assessment of cold water [12]. We therefore tested other threshold temperatures. We found that 94 of 265 (35%) and 43 of 156 (28%) of the distal cold water samples that displayed a temperature of <20 °C and <15 °C at sampling time were contaminated (Figure 3). Taken together, our data show that high *Legionella* concentrations may be found in cold water samples displaying a temperature of as low as 11 °C at sampling time, whereas no or very low *Legionella* concentrations may be associated with cold water temperatures of up to 28 °C at sampling time (Table 3). Hence, our data suggest that there is no reliable correlation between the temperature of cold water at sampling time and the extent of *Legionella* contamination. A possible explanation for this incoherence is that the temperature at sampling time, which is usually a busy time on a working day, is not representative of the temperatures that the sampled water has undergone prior to sampling.

After release of the results of our investigation, the infection control precautions were reassessed in all facilities and additional decontamination measures and prevention strategies were initiated for the warm

and cold WDS. The results of the intervention activities were controlled by follow-up investigation.

In conclusion, our data suggest that the cold water supply of healthcare facilities may be heavily contaminated with *Legionella* species. We did not find a reliable correlation between cold water temperature at sampling time and *Legionella* contamination rate or concentration. If we had restricted our analysis to cold water samples that displayed at least 25 °C at sampling time, we would have missed many cases of severe contamination. Our results highlight the importance of assessment of cold water in the context of intensified analysis of the water supply of healthcare facilities.

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