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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%).

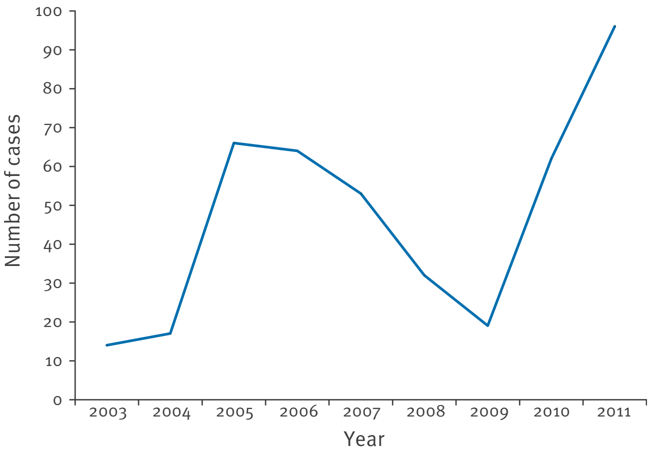
We detected a 15.1% increase in the number of respiratory samples sent to the laboratory for *M. pneumoniae* PCR from 2009 (n=819) to 2010 (n=943) and another 30.3% increase to the year 2011 (n=1,229). The main reason for this was the increased number of samples sent for testing from the paediatric emergency 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).

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)



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.

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.

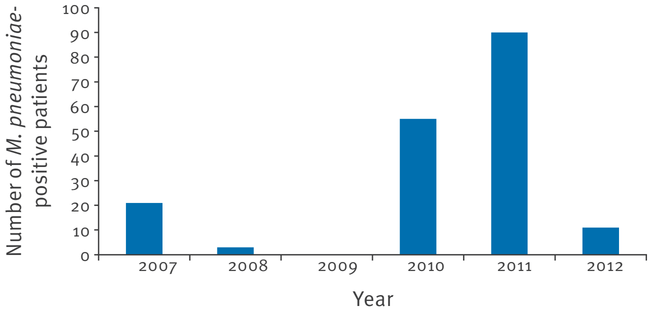
Notification of *M. pneumoniae* infection is not mandatory in Israel and currently there is no laboratory in the Central Ministry of Health Laboratories to support its diagnosis. *M. pneumoniae* diagnostics based on DNA amplification were implemented almost 10 years ago at the Hadassah-Hebrew University 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

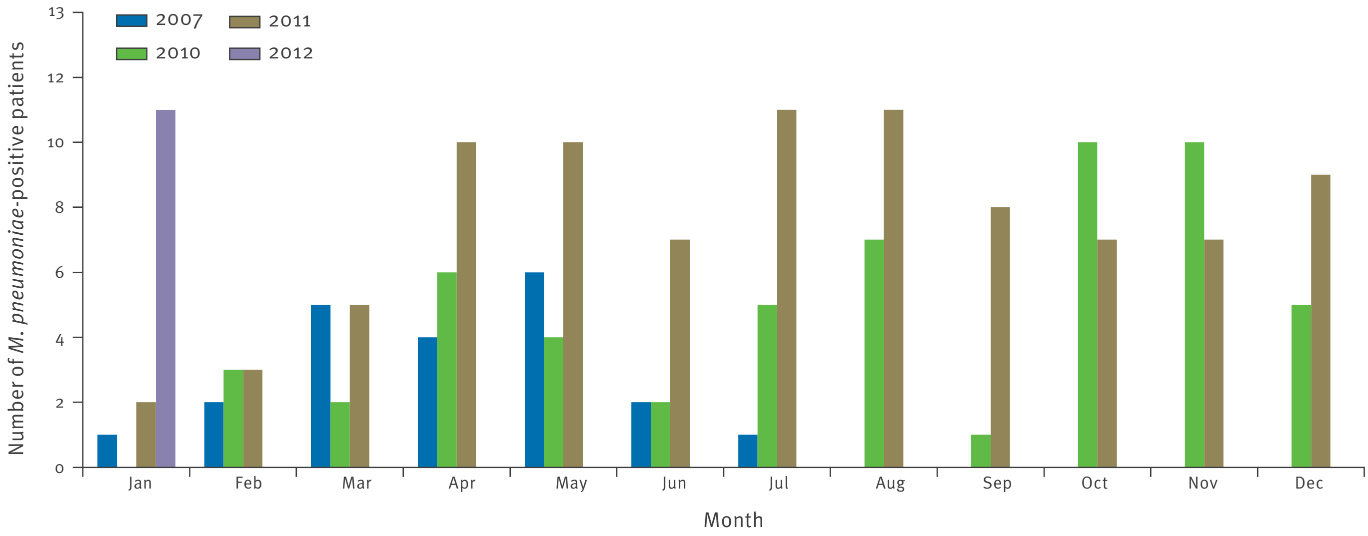
Of the major bacterial respiratory pathogens including *Haemophilus influenzae* and *Streptococcus pneumoniae*, *M. pneumoniae* is the only one for which no vaccine is available. *M. pneumoniae* is considered to cause a milder disease compared with *S. pneumoniae*, though substantial morbidity can be 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 at 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.

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) x109/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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First detection of tick-borne "*Candidatus* Neoehrlichia mikurensis" in Denmark 2011

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This is the first reporting of the tick-borne zoonotic bacterium "*Candidatus* Neoehrlichia mikurensis" in Denmark. A total of 2,625 *Ixodes ricinus* ticks from 58 locations in Denmark were collected and analysed for "*Ca.* Neoehrlichia mikurensis". A nested PCR revealed the presence of the bacterium at three geographically separate locations, which indicates that it is widely established in ticks.

Background

Since 2009, "*Candidatus* Neoehrlichia mikurensis" has been correlated with severe disease in immunocompromised people. A total of six human cases originating from Sweden [1], Germany [2], Switzerland [3] and the Czech Republic [4] have been described in the literature. In 2011, the bacterium was also isolated from a post-operative dog in Germany [5]. General clinical features in human patients have included recurrent fever, erysipelas-like rashes, arthralgias and thromboembolisms [1-4]. The infection responds well to doxycycline [1]. The pathogenic potential of "*Ca.* Neoehrlichia mikurensis" may be correlated with its putative tropism for endothelial cells [2,4].

So far little is known on the distribution, risk areas and reservoir of "*Ca.* Neoehrlichia mikurensis". If the infectious cycle resembles the other *Ehrlichia* bacteria, it has its reservoir in wild mammals and is transmitted by ticks. Accidentally humans may become infected [6]. In this study we examined *Ixodes ricinus* ticks in Denmark for the presence of "*Ca.* Neoehrlichia mikurensis" using PCR. This is the first survey for "*Ca.* Neoehrlichia mikurensis" in Denmark and in ticks in northern Europe.

Sampling methods and analysis

The analysed ticks originated from two different sampling procedures (Table): ticks collected by flagging (n=1,552) and a tick DNA/RNA archive (n=1,073).

Flagging was performed during September 2011 at four distinct localities known for an abundance of ticks and a recent history of human cases of tick-borne encephalitis (TBE). A white flannel flag was dragged over the vegetation and 1,552 ticks collected into plastic containers. These were frozen a few hours after collection and stored at -20°C for up to one month before DNA extraction. The flagging for ticks was carried out as part of a project investigating TBE virus. However, with the emergence of "*Ca.* Neoehrlichia mikurensis" as a public concern in our neighbouring countries, the DNA was additionally screened for the presence of this potentially emerging pathogen in December 2011 and January 2012.

Furthermore, a tick archive was investigated for the presence of "*Ca.* Neoehrlichia mikurensis". During 2010 and 2011, the Veterinary Institute's National Center for Wildlife Health collected 1,073 ticks from roe deer submitted for diagnosis and routine surveillance from 53 locations in Denmark. A sample of 40 ticks from a domestic sheep flock was additionally included in the archive. After removal, ticks were stored in ethanol for up to 1.5 years. DNA and RNA were extracted and stored as a tick archive of genetic material.

Before laboratory analysis, ticks from sites with large sample sizes were distributed into smaller pools (Table). Ticks were crushed and homogenised in 1 ml

Table

Ticks collected by two different sampling procedures in Denmark in 2010 and 2011 (n=2,625)

Sampling procedure	Number of locations	Number of pools	Mean number of ticks per pool (range)	Nymphs	Adult males	Adult females	**Total ticks**
Flagging	4	21	74 (3–106)	1,444	52	56	**1,552**
Tick DNA/RNA archive	54	58	19 (1–62)	30	399	644	**1,073**
Total	**58**	**79**		**1,474**	**451**	**700**	**2,625**

phosphate buffered saline (PBS). The homogenate was centrifuged and supernatant collected and stored at -80°C until DNA was extracted from 200 µL homogenate in a MagNA Pure 96 robot using MagNa Pure 96 DNA and Viral Nucleic Acid Small Volume Kit version 4.0 (Roche) according to the manufacturer's instructions.

The 16S rRNA gene was amplified with the universal bacterial primers 519F (5'-CCA GCA GCC GCG GTA ATA C-3') and 1054R (5'-ACG AGC TGA CGA CRR CCA TG-3') [7]. This was followed by a nested PCR with newly designed 16 S rRNA gene primers specific for "*Ca.* Neoehrlichia mikurensis": micurensis729F (5'-GGC GAC TAT CTG GCT CAG-3') and micurensis1016R (5'-GCC AAA CTG ACT CTT CCG-3'). The positive PCR amplicons were sequenced on an ABI PRISM 373 DNA Sequencer (PE Biosystems, Foster City, United States) and aligned with published 16S rRNA gene sequences using SEQMATCH in the Ribosomal Database Project (http://rdp.cme.msu.edu).

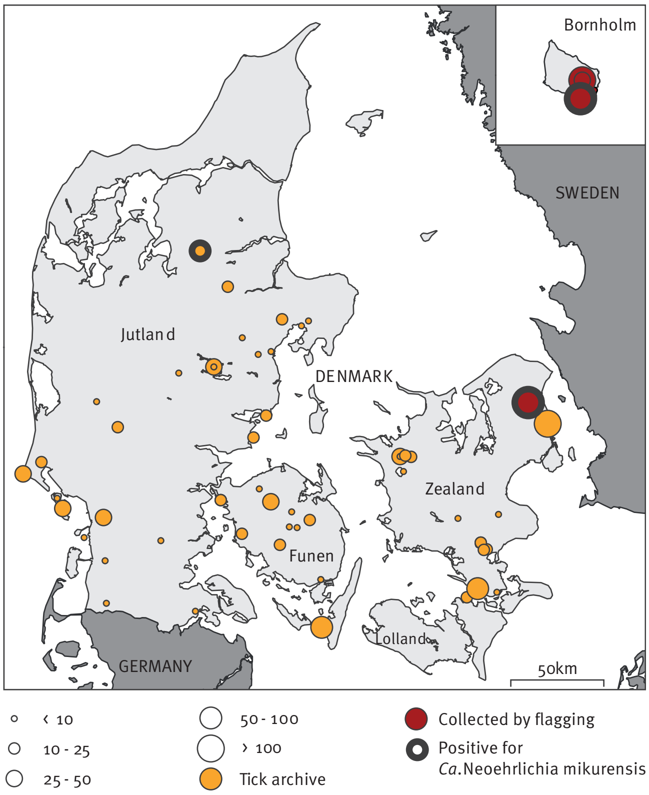
Results

Three of the 79 pools contained ticks positive for "*Ca.* Neoehrlichia mikurensis"; they originated from three locations separated from each other by the sea (Figure).

The first positive sample came from flagging in Øster Sømarken on the island of Bornholm. From this location six pools with a total of 467 ticks were collected. One pool, containing 100 nymphs, was found positive for "*Ca.* Neoehrlichia mikurensis". The second positive pool was found after flagging in the forest of Tokkekøb Hegn in Northern Zealand in the same one-hectare area where emerging TBE in both *I. ricinus* and humans were reported in 2009 [8]. At this location eight pools with a total of 736 ticks were collected, of which one, containing 100 nymphs, was found positive. The third positive sample originated from a pool of 12 male and 28 female ticks collected for the tick archive from domestic free-grazing sheep in the area of Viborg on the Danish mainland. All three isolates were verified to be 100% similar to 16S rRNA gene sequences from "*Ca.* Neoehrlichia mikurensis".

Figure

Location of tick collection, Denmark, 2010 and 2011 (n=1,552)



Discussion

"*Ca.* Neoehrlichia mikurensis" belongs to the family *Anaplasmataceae* [9] which comprises a variety of emerging tick-borne human pathogenic bacteria [10]. Former studies have suggested a potential widespread occurrence of "*Ca.* Neoehrlichia mikurensis" in the wild fauna of Asia and Europe, including our neighbouring countries [9,11-14], but it has never been reported in Denmark. In this study we examined 2,625 *I. ricinus* ticks divided in 79 pools and identified the presence of "*Ca.* Neoehrlichia mikurensis" at three distinct locations, indicating that the bacterium is widely distributed in the Danish tick population.

The recorded minimum prevalence of three of 2,625 was, however, substantially lower than that found in studies from the Netherlands in 2006, the Baltic regions of Russia in 1997–98 and a recent central European study, which all estimated 6–7% of the ticks to carry the bacterium [12,14,15]. The latter study investigated ticks from the Czech Republic, France, Germany, Poland and Portugal and found a prevalence ranging from 0% to 10% that was highest in the Czech Republic and Germany [14]. In southern Sweden, "*Ca.* Neoehrlichia mikurensis" was in 2008 found to be widespread in the wild rodents of this region with a prevalence ranging from 0% to 12.5% in the investigated locations [11].

An increase and spread of other tick-borne infections such as Lyme borreliosis and TBE, has been reported in Denmark and neighbouring countries. This trend has been attributed to increased awareness, climate change and increasing tick populations [16,17]. In this study the tick-borne pathogen was found at a known TBE site on Bornholm and at an emerging TBE site in Tokkekøb Hegn forest [8]. The recent appearance of several human clinical cases of "*Ca.* Neoehrlichia mikurensis" infection in Europe, as well as the findings in the wild fauna, indicate that this is an emerging tick-borne pathogen. However, lack of knowledge and a diagnostic test combined with a low pathogenic potential may have hindered previous detection in Denmark. Whether or not the newly reported cases are the result of previous misdiagnosis or a true emerging risk, it is important that medical doctors in the affected areas are aware of the risk for immunocompromised patients. The State Serum Institute will now establish a diagnostic assay. Finding the pathogen on production animals suggests there may be veterinary risk as well.

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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.

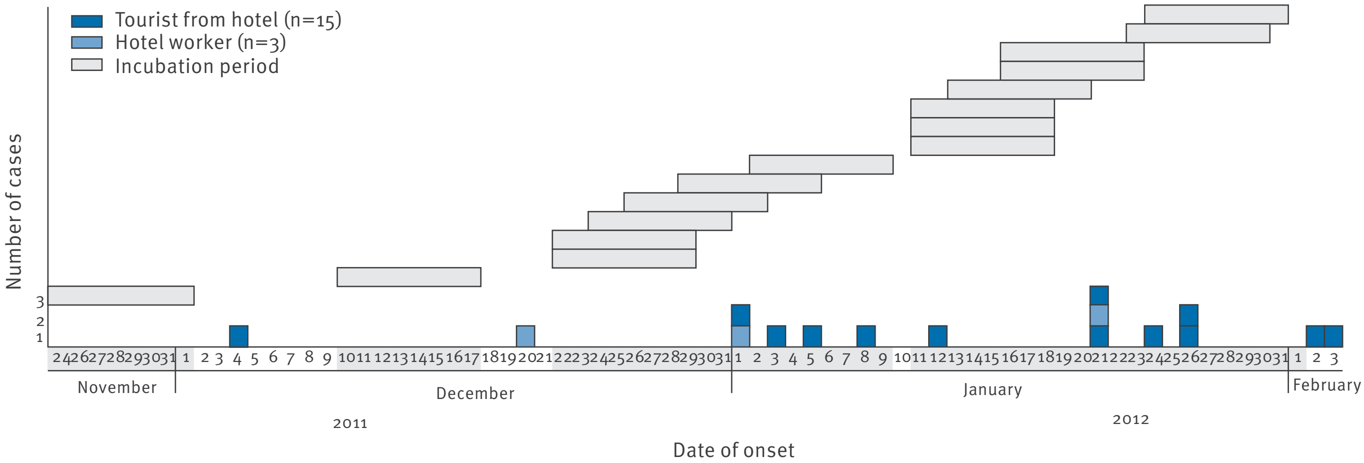
When the second case of Legionnaires' disease, a hotel employee, was reported, a new on-site investigation was immediately launched. In addition to the previously inspected documents, we obtained the analytical results, dated from 29 November 2011, of routine water samples from the hot-water deposits, 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 *Legionella*
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 hyperchloration 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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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, is 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-ketogluterate (BCYE-α) and (i) the antibiotics polymyxin B, cefazolin, and pimaricin; and (ii) the antibiotics polymyxin B, anisomysin, 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 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 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)**

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 microenvironment 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 *Legionella* spp.					
	Positive for *Legionella* spp.				Negative for *Legionella* spp.	**Total**
	L. pneumophila n (%)	non-*pneumophila Legionella* spp. n (%)	*L. pneumophila* and non-*pneumophila Legionella* spp. n (%)	Total positive n (%)	Total negative n (%)	**Total 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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Surveillance of acute infectious gastroenteritis (1992–2009) and food-borne disease outbreaks (1996–2009) in Italy, with a focus on the Piedmont and Lombardy regions

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We describe trends in the occurrence of acute infectious gastroenteritis (1992 to 2009) and food-borne disease outbreaks (1996 to 2009) in Italy. In 2002, the Piedmont region implemented a surveillance system for early detection and control of food-borne disease outbreaks; in 2004, the Lombardy region implemented a system for surveillance of all notifiable human infectious diseases. Both systems are internet based. We compared the regional figures with the national mean using official notification data provided by the National Infectious Diseases Notification System (SIMI) and the National Institute of Statistics (ISTAT), in order to provide additional information about the epidemiology of these diseases in Italy. When compared with the national mean, data from the two regional systems showed a significant increase in notification rates of non-typhoid salmonellosis and infectious diarrhoea other than non-typhoid salmonellosis, but for food-borne disease outbreaks, the increase was not statistically significant. Although the two regional systems have different objectives and structures, they showed improved sensitivity regarding notification of cases of acute infectious gastroenteritis and, to a lesser extent, food-borne disease outbreaks, and thus provide a more complete picture of the epidemiology of these diseases in Italy.

Introduction

Acute gastroenteritis of infectious aetiology is a public health problem worldwide [1]. Although cases in industrialised countries are usually characterised by low mortality, the economic impact on health services (direct costs) and on the general public (indirect costs) can be considerable [2]. Any initiative aimed at controlling acute infectious gastroenteritis in a population should be based on the extent of the problem. However, the true incidence of the disease in the population, based on data from national surveillance systems, is usually underestimated [e.g. 3]. In Italy and other countries, this problem can be attributed to several factors: (i) most cases have mild, self-limiting symptoms, which do not motivate patients to seek medical attention; (ii) stool examination is not always recommended by the attending physician and an aetiological diagnosis is rarely made; (iii) diagnostic capabilities and protocols differ greatly among laboratories; and (iv) under-reporting, as it is known that physicians rarely report cases.

In Italy, surveillance of acute infectious gastroenteritis and food-borne disease outbreaks is part of the activities of the Italian National Surveillance System of Infectious Diseases (SIMI), which has been in place since 1990 [4]. Notification data of cases of acute infectious gastroenteritis and food-borne disease outbreaks are also shared with the National Institute of Statistics (ISTAT), which produces official statistics on economic, social and health matters in Italy. The Piedmont and Lombardy regions, in the north of the country, have implemented two different Internet-based surveillance systems since 2002 and 2004, respectively. The Piedmont system is dedicated to surveillance of food-borne diseases, with an emphasis on outbreaks (including but not limited to acute infectious gastroenteritis, as this can frequently be caused by food-borne pathogens), whereas the Lombardy system is aimed at improving the surveillance and reporting of all notifiable human infectious diseases, including acute infectious gastroenteritis and food-borne diseases. Both systems notify to the national surveillance system. As the two regions together account for about a quarter of the Italian population (in 2009: Piedmont: 4,432,571 inhabitants; Lombardy: 9,742,676; national: 60,045,068 [5]) estimates of disease incidence from these regional surveillance systems can be considered relevant for comparisons at the national level.

At present, the national surveillance system does not collect notifications of acute infectious gastroenteritis as one syndrome; instead, laboratory-confirmed cases of diarrhoeal disease are generally notified in two categories: non-typhoid salmonellosis (hereafter referred to as salmonellosis) and infectious diarrhoea other than salmonellosis (hereafter referred to as infectious diarrhoea). These two categories therefore include diarrhoeal diseases caused by all identified enteric pathogens. For the purposes of this article, the official notifications of salmonellosis and infectious diarrhoea were used as proxies for acute infectious gastroenteritis, but we analysed the data separately due to the large difference in the number of cases in the two categories.

Cases of salmonellosis and infectious diarrhoea are notified to the national surveillance system according to its criteria, which, for these diseases, are based on laboratory results [4]. Food-borne disease outbreaks are generally notified to the system as the occurrence of the same disease in two or more people belonging to the same community (family, school, etc.) or exposed to a common source of infection.

The aim of our analysis was to describe the epidemiology of acute infectious gastroenteritis and food-borne disease outbreaks in Italy using official notification data collected in 1992–2009 and 1996–2009, respectively. We have also taken into account the contribution of the notification data from Piedmont and Lombardy and speculated on the impact that the notifications from the two regions could have at the national level. Our findings may help decision-makers in developing novel approaches aimed at improving the surveillance of acute infectious gastroenteritis and food-borne disease outbreaks in the general population.

Methods

Data collection

Notification data were obtained from the SIMI online databases from 1996 to 2009 (for salmonellosis, infectious diarrhoea and food-borne disease outbreaks) [6] and the ISTAT from 1992 to 1995 (for salmonellosis and infectious diarrhoea) [7]. Data are available on request.

The SIMI started publishing data in 1996, while data of the previous four years were made available by the ISTAT only. There were no available data on food-borne disease outbreaks before 1996. Data on salmonellosis and infectious diarrhoea were collected per year, region, age group (0–14 years, 15–24 years, 25–64 years, 65 years and older) and sex, while those on food-borne disease outbreaks were only available per year and region. Population data per year, region, age group and sex were also collected from the ISTAT.

In order to obtain information on the two regional surveillance systems, we developed a questionnaire according to guidelines provided by the United States Centers for Disease Control and Prevention [8]. The questionnaire is available on request. It was completed by the heads of the two systems.

Data analysis

Annual notification rates (annual number of notified episodes per 100,000 inhabitants) of salmonellosis and infectious diarrhoea (from 1992 to 2009) were calculated per region, age group and sex, while those of food-borne disease outbreaks (from 1996 to 2009) were calculated per region only. Age- and sex-standardised annual notification rates of salmonellosis and infectious diarrhoea were then calculated per region using 2001 population data. Rates were calculated for the Piedmont and Lombardy regions and for the country as a whole (calculated as the mean of the 20 Italian regions).

Temporal trends in annual notification rates of salmonellosis, infectious diarrhoea and outbreaks of food-borne diseases were assessed using the Cuzick test [9]. Annual rates of salmonellosis and infectious diarrhoea were compared between the sexes using the Mann–Whitney test and among age groups using the Kruskal–Wallis test. Post hoc paired comparisons after the Kruskal–Wallis test were tested using the Mann–Whitney test on each pair of age group and p-value adjustment according to Bonferroni's method [10].

To evaluate any difference in notification rates in Piedmont and Lombardy, compared with the national mean, the standardised annual notification rates of salmonellosis, infectious diarrhoea and food-borne disease outbreaks in both regions were centred on (i.e. subtracted from) the corresponding national mean and then intra-regionally compared between the periods before (Piedmont: 1992 or 1996 to 2001; Lombardy: 1992 or 1996 to 2003) and after the implementation of their respective systems (Piedmont: 2002–2009; Lombardy: 2004–2009), using the Mann–Whitney test.

Statistical analysis was performed with STATA 10.1 and Excel. Statistical significance was set at a p value of 0.05.

Regional surveillance systems

All regions other than Lombardy notify cases according to the SIMI criteria [4]. Cases notified to SIMI are not divided into possible, probable or confirmed cases, as in the European Union (EU) case definition [11]. The cases notified to the SIMI are later reported to the EU by the Ministry of Health through the European Surveillance System (TESSy). In contrast, Lombardy, uses the EU case definition, but the cases are then reported to the national surveillance system according to SIMI criteria.

Piedmont

The surveillance system of Piedmont is structurally independent of the SIMI. It collects data on all food-borne diseases – including episodes due to food-poisoning (e.g. those involving mushrooms, marine biotoxins and histamine) that are not notified to the SIMI. Basically, it is a passive system focused on the early detection of food-borne disease outbreaks, with the aim of improving the rapid alert and investigation of the outbreaks to prevent further cases.

Data generated from the system are also used for: (i) monitoring of spatio-temporal trends in food-borne diseases, including identification of pathogens, food items involved, related risk factors and the at-risk population; (ii) driving the development and evaluation of control programmes (for prioritising resource allocation); (iii) detecting changes in the impact of acute gastroenteritis in response to public health actions; and (iv) providing a basis for epidemiological research.

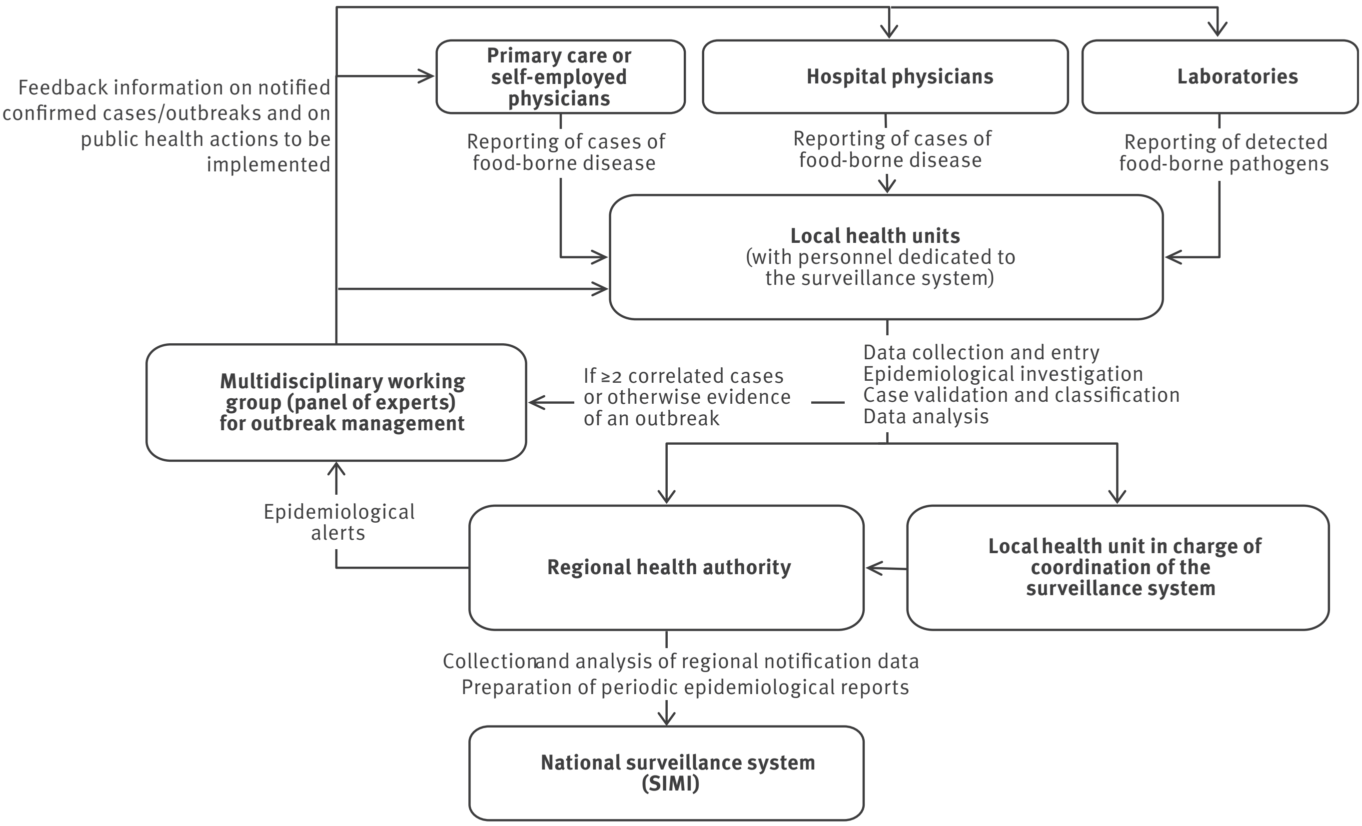
The system collects information on food-borne disease outbreaks and laboratory-confirmed individual cases of food-borne diseases, thus including salmonellosis and other diarrhoeal pathogens, which are frequently transmitted by contaminated food (Figure 1). Reporting of food-borne diseases is managed separately from other diseases. Each local health unit in the region has dedicated staff who manually enter the received data (usually by fax, email or telephone) into an Internet-based database shared by local health units and the regional health authority. Entry of all validated data is performed on a weekly basis. One person in each local health unit is in charge of validating the data, ensuring that the data are entered and coordinating a multidisciplinary panel of experts to investigate every outbreak of food-borne diseases detected by the system. In the local health unit in the city of Turin, there is a regional coordinator who is in charge of coordinating all other local health units and report to the regional health authority.

Lombardy

The surveillance system of Lombardy represents an Internet-based improvement of the SIMI and it is fully integrated with it. The system has primarily been implemented to improve aetiological diagnosis and data quality for individual cases. Its main objective is to provide data for real-time analyses on spatio-temporal trends aimed at preventing secondary cases by means of prompt public health actions.

Figure 1

Surveillance system of Piedmont region, Italy



SIMI: National Infectious Diseases Notification System.

The structure of the Lombardy system (Figure 2) is basically the same as that of the SIMI, which has a pyramidal structure from the bottom (physicians) to the top (regional health authorities) and finally to the Ministry of Health, which hosts the SIMI, but compared with the SIMI, the procedure for physicians reporting to local health units was modified by: (i) reducing the information requested to a minimum (additional information requested by the SIMI for completing the notification is provided by the local health units later on); (ii) shortening the deadline for reporting (e.g. for acute infectious gastroenteritis, notification of cases should be immediate instead of within 48 hours, as required by Italian law) [4]; and (iii) defining different levels of detail required for cases detected at hospitals and for those detected by primary care or self-employed physicians. Data of the notified cases received by each local health unit are manually entered into an Internet-based database and automatically matched with the corresponding patient information stored in the regional health registry. Further epidemiological investigations are carried out when necessary. Cases are automatically validated and classified as notifiable to the SIMI or not notifiable. The database is shared among all local health units and the Lombardy regional health authority, which is in charge of the final data cleaning and analysis.

In both systems, access to the database is restricted to authorised staff of the local health units and regional health authority. All data are managed according to Italian legislation on privacy.

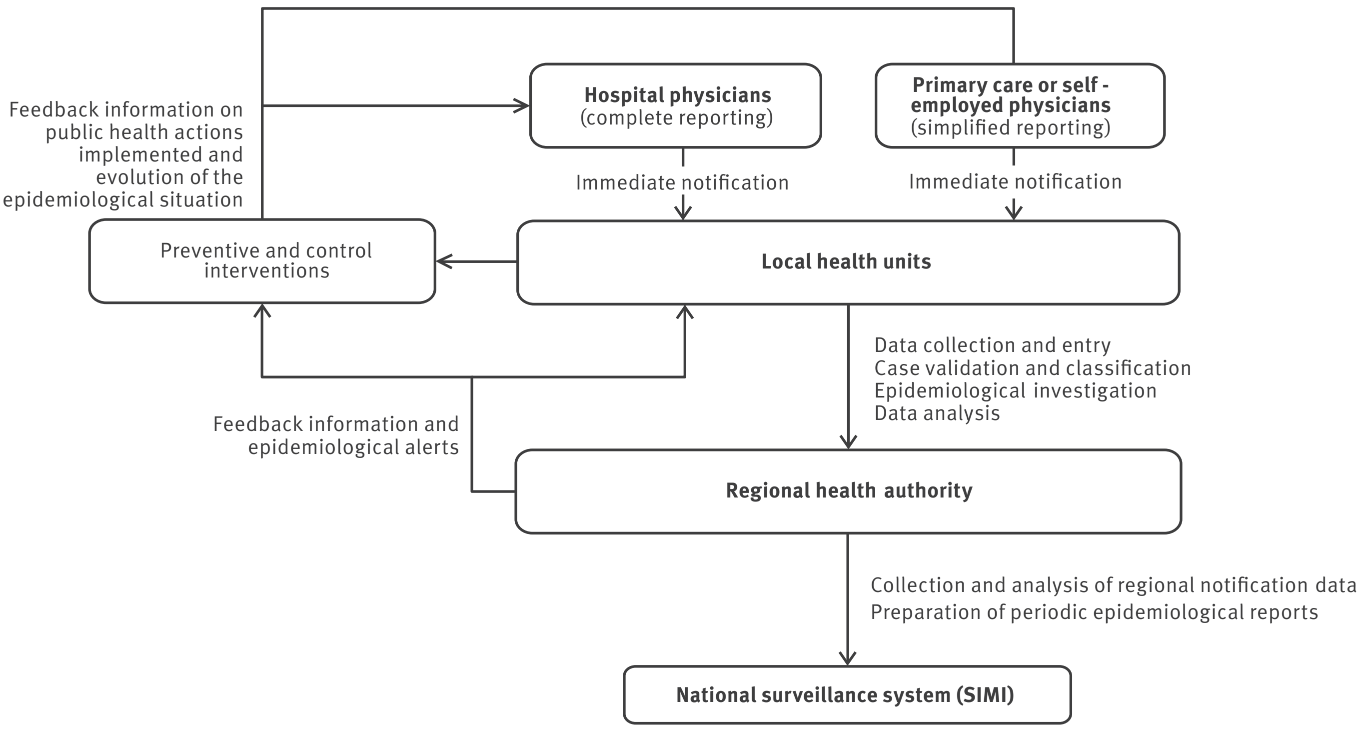
Both systems regularly notify to the SIMI only those cases (divided into salmonellosis and infectious diarrhoea) and food-borne outbreaks that meet the SIMI notification criteria (the set of information that must be collected in order to notify the case to the system is described in the legislation [4]).

Epidemiology of acute infectious gastroenteritis and food-borne disease outbreaks in Italy

During the period analysed (1992–2009 for salmonellosis and infectious diarrhoea and 1996–2009 for food-borne disease outbreaks), a total of 222,277 cases of salmonellosis, 46,903 cases of infectious diarrhoea and 7,937 food-borne disease outbreaks were notified in Italy. Piedmont notified 16,431 cases of salmonellosis (7.4% of the total), 4,012 cases of infectious diarrhoea (8.6%), and 570 food-borne disease outbreaks (7.2%), while Lombardy notified 43,040 cases of salmonellosis (19.4%), 14,797 cases of infectious diarrhoea (31.5%), and 1,663 food-borne disease outbreaks (21.0%). Annual notification rates of salmonellosis, infectious diarrhoea and food-borne disease outbreaks in Piedmont and Lombardy, together with the national mean, are shown in Figure 3.

Figure 2

Surveillance system of Lombardy region, Italy



SIMI: National Infectious Diseases Notification System.

Salmonellosis notifications

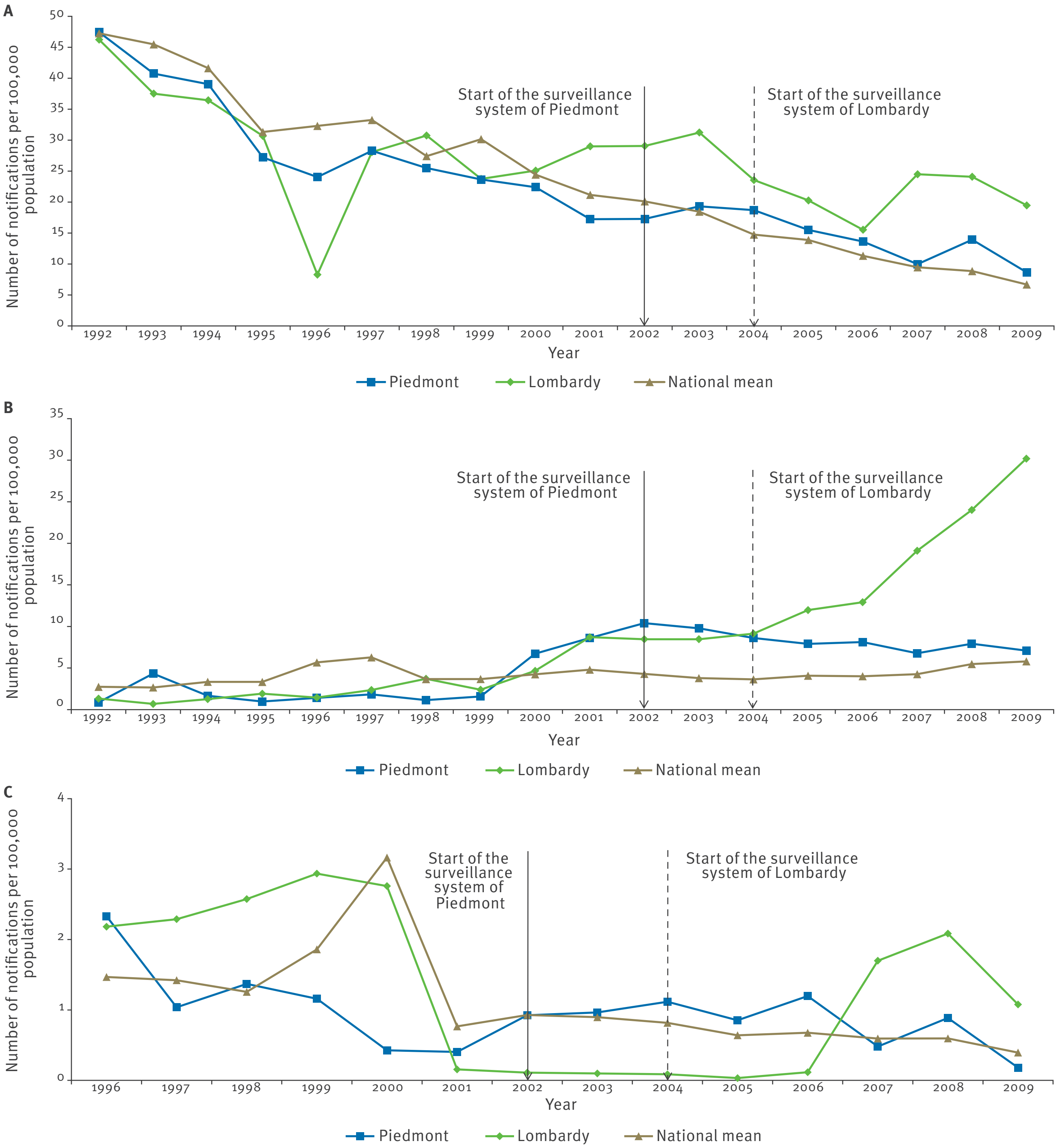
At the national level, salmonellosis notification rates significantly decreased from 47.3 per 100,000 population in 1992 to 6.7 per 100,000 population in 2009 (a decrease of 86%). Statistically significant decreasing trends were also observed in Lombardy (–58%, from 46.2 per 100,000 population in 1992 to 19.5 per 100,000 population in 2009) and Piedmont (–82%, from 47.4 per 100,000 population in 1992 to 8.6 per 100,000 population in 2009).

Infectious diarrhoea notifications

National notification rates of infectious diarrhoea increased significantly from 2.7 per 100,000 population in 1992 to 5.8 in 2009 (an increase of 53%). From 1992 to 2009, the annual notification rates in Piedmont

Figure 3

Trends of annual notification rates of (A) non-typhoid salmonellosis (1992–2009), (B) infectious diarrhoea other than non-typhoid salmonellosis (1992–2009) and (C) food-borne disease outbreaks (1996–2009) in Piedmont and Lombardy regions and the Italian national mean



increased significantly from 0.9 per 100,000 population to 7.1 per 100,000 population (+87%) and from 1.3 per 100,000 population to 30.2 per 100,000 population in Lombardy (+96%). Figure 3 shows that in both regions, notification rates of infectious diarrhoea were above the national mean from 2000 onwards.

Food-borne disease outbreaks notifications

The mean national notification rates of food-borne disease outbreaks significantly decreased from 1.5 per 100,000 population in 1996 to 0.4 per 100,000 population in 2009 (–73%). No statistically significant trends were detected in Lombardy (–50%, from 2.2 per 100,000 population in 1996 to 1.1 per 100,000 population in 2009), where notification rates were below the national mean from 2000 to 2006. From 1996 to 2009, there was no statistically significant trend in Piedmont, although the notification rate decreased from 2.3 per 100,000 population in 1996 to 0.2 per 100,000 population in 2009 (–91%). As shown in Figure 3, notification rates were above the national mean from 2003 to 2006, and then again in 2008, but were below the national mean in 2007 and 2009.

Significant differences in notification rates of salmonellosis and infectious diarrhoea by age group were observed in Piedmont, Lombardy and the country as a whole (Table 1). The highest notification rates were observed in children aged 0–14 years, in both regions and nationally. Apart from the 0–14year-olds, the only significant difference was observed in elderly patients (≥65 years) in Lombardy for infectious diarrhoea; in this age group the notification rates was 14.10 cases per 100,000 population in Lombardy, while in Italy and in Piedmont the rates were lower (2.84 and 4.36 per 100,000 population, respectively). No statistically significant differences were detected between male and female cases for either salmonellosis or infectious diarrhoea.

Impact of the regional surveillance systems on acute infectious gastroenteritis notification rates

Differences in notification rates from the two regions of salmonellosis, infectious diarrhoea and food-borne disease outbreaks with those of the whole of the country (national mean) before and after the implementation of the regional systems is described in Table 2. In Piedmont, after implementation of its system, there was a significant increase in notification rates of both salmonellosis (an increase of 1.6 cases per 100,000 population per year) and infectious diarrhoea (an increase of 3.9 per 100,000 population per year) compared with the national mean. In Lombardy, the increase after the implementation of its system was significant for both salmonellosis (an annual increase of 10.3 cases per 100,000 population) and infectious diarrhoea (an annual increase of 13.3 per 100,000 population). The observed increases in the notification rate of food-borne disease outbreaks after the implementation of the two regional systems (annual increases of 0.1 and 0.2 per 100,000 population in Piedmont and Lombardy, respectively) were not statistically significant.

Discussion and conclusions

Analysis of the notifications of salmonellosis, infectious diarrhoea and food-borne disease outbreaks showed important differences between the figures provided by the regional surveillance systems of Piedmont and Lombardy and those of the national surveillance system. When we compared the regional figures with the national mean, we found significantly higher notification rates of salmonellosis and infectious diarrhoea in the two regions after the implementation of

Table 1

Mean annual notification rates by age group and sex of non-typhoid salmonellosis and infectious diarrhoea other than non-typhoid salmonellosis, Piedmont and Lombardy regions and Italian national mean, 1992–2009

Disease, by region or nationwide	Mean annual notification rate[a]					
	Age group[b]				Sex[c]	
	0–14 years	15–24 years	25–64 years	≥65 years	Male	Female
Non-typhoid salmonellosis						
Piedmont	99.73±6.09§	24.06±8.00†	21.73±9.04†	14.92±2.12†	41.98±6.50	38.24±6.12
Lombardy	127.58±5.9§	19.49±6.13†	19.11±7.35†	18.11±1.71†	48.03±7.10	44.12±6.63
National mean	98.20±6.89§	32.65±12.41†	24.72±9.93†	17.33±2.60†	44.45±7.26	42.00±7.21
Infectious diarrhoea other than non-typhoid salmonellosis						
Piedmont	25.80±3.15§	1.36±0.18†	1.49±0.30†	4.36±0.67†	8.83±1.76	7.68±1.54
Lombardy	32.43±4.14§	2.85±0.39†	1.97±0.30†	14.10±3.77‡	14.02±2.62	11.66±2.26
National mean	19.80±1.04§	1.97±0.06†	1.22±0.05†	2.84±0.51†	7.04±1.09	5.88±0.89

[a] Mean number of cases per 100,000 population±standard error.

[b] Post hoc paired comparisons of mean annual notification rates between age groups were tested by the Mann–Whitney test. Symbols (§, † and ‡) indicate the results of the pairwise comparisons: in the same row, age groups marked with different symbols are statistically different when compared (Bonferroni-adjusted p<0.05), while the same symbol in the same row indicates no difference between the age groups.

[c] No statistically significant differences between rates in male and female groups were observed (Mann–Whitney test p>0.05).

their systems. In addition to these increased rates, the absence in these two regions of the significantly decreasing trend in food-borne disease outbreaks observed at the national level can be considered a positive performance of the systems.

The better performance of the two regional systems could be related to increased motivation of those involved (e.g. physicians, epidemiologists, public health professionals and laboratory staff) to report cases of acute infectious gastroenteritis, increased awareness of the disease and better coordination between laboratory and local health unit teams. In both regional systems, the web-based management and sharing of notification data have facilitated the reporting process and improved the completeness of the information collected. Web-based surveillance systems have become increasingly widespread and it is known that they can improve sensitivity [12-14]. Nonetheless, both Italian regional systems have major weaknesses, in particular: (i) limitations in events covered (the Piedmont system is focussed on food-borne diseases only); (ii) limitations in automatic outbreak detection (spatio-temporal clusters); and (iii) data entry is carried out far from the source. Points ii and iii, in particular, are consequences of the lack of real-time data collection and analysis and of the labour-intensive activity required by both systems. These two constraints could considerably be balanced out by full electronic reporting and management of notification data.

Concerning the epidemiology of acute infectious gastroenteritis in Italy, we identified a significantly decreasing trend of salmonellosis over the period analysed, which has also been observed in other industrialised countries, possibly resulting from improved *Salmonella* control measures in the food chain [15,16]. Although the national trend is decreasing, salmonellosis rates in Lombardy and Piedmont showed a rise from 2006 and 2007 onwards, respectively. In 2009, data provided to the European Food Safety Authority (EFSA) showed an increase in the number of *Salmonella* isolates from human cases in Italy of 22.2%, compared with those in 2008 (from 3,232 to 4,156 isolates) [16]. This increase was detected one or two years in advance by the surveillance systems of Piedmont and Lombardy (in 2008 and 2007, respectively), but not by the national surveillance system. The difference between our data and those provided to EFSA can be explained by the different sources: our data are the official notification data, while the data provided to EFSA are from Enter-net, a laboratory-based surveillance network for enteric pathogens [17].

In Lombardy, and to a lesser extent in Piedmont, the trend of salmonellosis observed during 2006 to 2009 seems related to the trend seen for food-borne disease outbreaks in the same period. Taking into account that in the EU most of the acute infectious gastroenteritis outbreaks in humans are caused by *Salmonella* [15,16], we can hypothesise that, at least in Lombardy, improved outbreak detection could have contributed to the increase of salmonellosis cases notified to the system.

The observed trends of infectious diarrhoea notification rates suggest an increasingly prominent role of pathogens other than *Salmonella* – in particular *Campylobacter jejuni* – which is the most frequent cause of acute infectious gastroenteritis in the EU [15,16]. The increasing trend of infectious diarrhoea was particularly evident in Lombardy, but was also seen in Piedmont, and could be related to the improved routine laboratory capacity for the detection and notification of pathogens other than *Salmonella*. In both regions, improvement in laboratory capacity (particularly in Lombardy) was implemented at the same time the surveillance systems were introduced. This enabled the regional diagnostic and microbiology laboratories

Table 2

Differences in annual notification rates of non-typhoid salmonellosis, infectious diarrhoea other than non-typhoid salmonellosis, and food-borne disease outbreaks, Piedmont and Lombardy regions with the Italian national mean, before and after implementation of regional surveillance systems

Disease	Differences in annual notification rate[a,b]					
	Piedmont			Lombardy		
	Before implementation (1992/1996–2001)[c]	After implementation (2002–2009)	P value	Before implementation (1992/1996–2003)[c]	After implementation (2004–2009)	P value
Non-typhoid salmonellosis	−4.05±0.79	+1.58±0.83	<0.01	−1.54±2.79	+10.27±1.87	<0.05
Infectious diarrhoea other than non-typhoid salmonellosis	−1.12±0.89	+3.90±0.61	<0.01	−0.25±0.87	+13.34±2.95	<0.01
Food-borne disease outbreaks[d]	−0.53±0.49	+0.13±0.08	>0.05	+0.16±0.32	+0.22±0.40	>0.05

[a] Mean number of cases per 100,000 population±standard error.
[b] Reference value (national mean) = 0.
[c] From 1992 for salmonellosis and infectious diarrhoea and from 1996 for food-borne disease outbreaks.
[d] In Piedmont, includes also outbreaks due to food poisoning.

to extend the range of assays routinely performed and pathogens searched for, and to improve the timeliness of diagnosis and their communication with the staff of the local and regional health authorities involved in the system.

Acute infectious gastroenteritis notification rates by age group confirmed the higher incidence of both salmonellosis and infectious diarrhoea in children (0–14 years), in line with what has been observed in the United States [18] and in other European countries [e.g. 19].

Concerning the trend of food-borne disease outbreaks, Lombardy showed a very low notification rate between 2001 and 2006. This is probably related to the changes in the notification procedure of such outbreaks to the SIMI (but not the notification of single cases) that Lombardy made in 2001, during the period considered for the analyses. After 2006, however, the reporting of these outbreaks was redefined, in agreement with the SIMI definitions.

In Lombardy, we observed that the implementation of the system improved notification rates of acute infectious gastroenteritis and food-borne disease outbreaks, with a reduction of the under-reporting, and consequently gave a better estimate of the impact of acute infectious gastroenteritis on the population. The Piedmont surveillance system, which is dedicated to acute infectious gastroenteritis, allows broader collection of information that is not easy to obtain in other ways, in particular concerning food-poisoning outbreaks.

With regard to the extension of the surveillance systems of Piedmont and/or Lombardy to the other Italian regions, and even to other countries, decisions should be made on the basis of cost–benefit analyses that take into account the expected improvements in terms of efficacy of the surveillance and the resources needed to achieve them, as well as the long-term sustainability of the systems.

In conclusion, improving the surveillance of acute infectious gastroenteritis at the Italian national level requires additional efforts, which can be defined by looking at the experience at the regional level, such as that of Lombardy and Piedmont. Such efforts should be focused on the integration and harmonisation of different surveillance activities and sources of information, as well as evaluation of such activities, to obtain the best achievable impact on the burden of acute infectious gastroenteritis in the population.

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