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Outbreak of *Salmonella* Thompson in the Netherlands since July 2012

I H Friesema (ingrid.friesema@rivm.nl)[1], A E de Jong[2], I A Fitz James[2], M E Heck[1], J H van den Kerkhof[1], D W Notermans[1], W van Pelt[1], A Hofhuis[1]

1. Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands
2. Dutch Food and Consumer Product Safety Authority (NVWA), Utrecht, the Netherlands

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An ongoing outbreak of salmonellosis due to *Salmonella* Thompson is affecting the Netherlands. Between 2 August and 19 October 2012, 866 cases were confirmed. Their median age was 44 years (range: 0–95 years), 63% were female and 36% were hospitalised. A matched case–control study suggested smoked salmon as the vehicle. *Salmonella* Thompson was confirmed in four of nine batches of smoked salmon from one producer. A recall of all concerned smoked salmon products was executed starting end of September.

On 15 August 2012 (week 33), the National Institute for Public Health and the Environment (RIVM) noticed an increase in the number of *Salmonella* Thompson cases. Two weeks earlier, there had been four cases, and in week 33 another 11 cases were detected. As normally around four cases of *S.* Thompson are seen in the Netherlands per year, an outbreak investigation was started.

Epidemiological investigation

Cases were defined as persons in the Netherlands with *S.* Thompson cultured from any sample type, confirmed by the RIVM, since August 2012. A semi-structured questionnaire exploring relevant food exposures in the seven days before the onset of symptoms was administered by telephone or face to face, beginning 16 August. Data were obtained relating to food consumption and place of purchase as well as other possible exposures such as contact with pets or a person with diarrhoea. Information regarding clinical symptoms, date of onset of illness and date of hospitalisation were also gathered.

A matched case–control study was conducted by sending a similar version of the questionnaire to four controls per case, matched on year of birth, sex and municipality.

Preliminary results

Between 2 August and 19 October 2012 (week 31-42), 866 cases were confirmed with an *S.* Thompson infection (Figure 1), geographically spread throughout the country.

Women (63%) were more often affected than men (37%). The median age of the cases was 44 years (range: 0–95 years). Ten percent of the cases were between 0 and 9 years-old and another 16% were between 10 and 19 years-old. The regional public health services actively approached the first 184 cases, resulting in 111 completed questionnaires (60%). Data on hospitalisation were available for 107 cases of whom 36% were admitted to the hospital. First date of illness was known for 192 cases and ranged between 20 June and 6 October (Figure 2). Cases confirmed later than 1 October (week 39) were not contacted, as the most probable cause of the outbreak had been identified and been made public in the media. Date of onset for these cases is therefore only known when filled in on the application form for serotyping of the isolate. This has caused an artificial underrepresentation in the number of cases registered with date of onset in weeks 38, 39 and further.

As soon as new questionnaires were received, preliminary risk factor analyses were performed without a clear indication as to which food item was causing the outbreak. The latest preliminary analysis (24 September) was based on 80 case questionnaires and 175 control questionnaires. Cases had significantly more often (45%) eaten smoked fish, especially smoked salmon, than controls (28%) (adjusted odds ratio: 7.3; 95% confidence interval (CI) 2.4–22.0). Furthermore, cases were also more likely (21%) to have consumed raw salads than controls (11%) (adjusted odds ratio: 5.1; 95% CI: 1.2–21.4). Several supermarkets were reported significantly more often by cases than by controls. Most of these supermarkets turned out to do their purchases via the same organisation.

Traceback

Based on the preliminary results of the case–control study, the Dutch Food and Consumer Product Safety Authority (NVWA) performed a traceback study on smoked salmon and discovered that those

Figure 1

Cases of *Salmonella* Thompson, by week of confirmation, the Netherlands, 2 August–19 October 2012 (n=866)

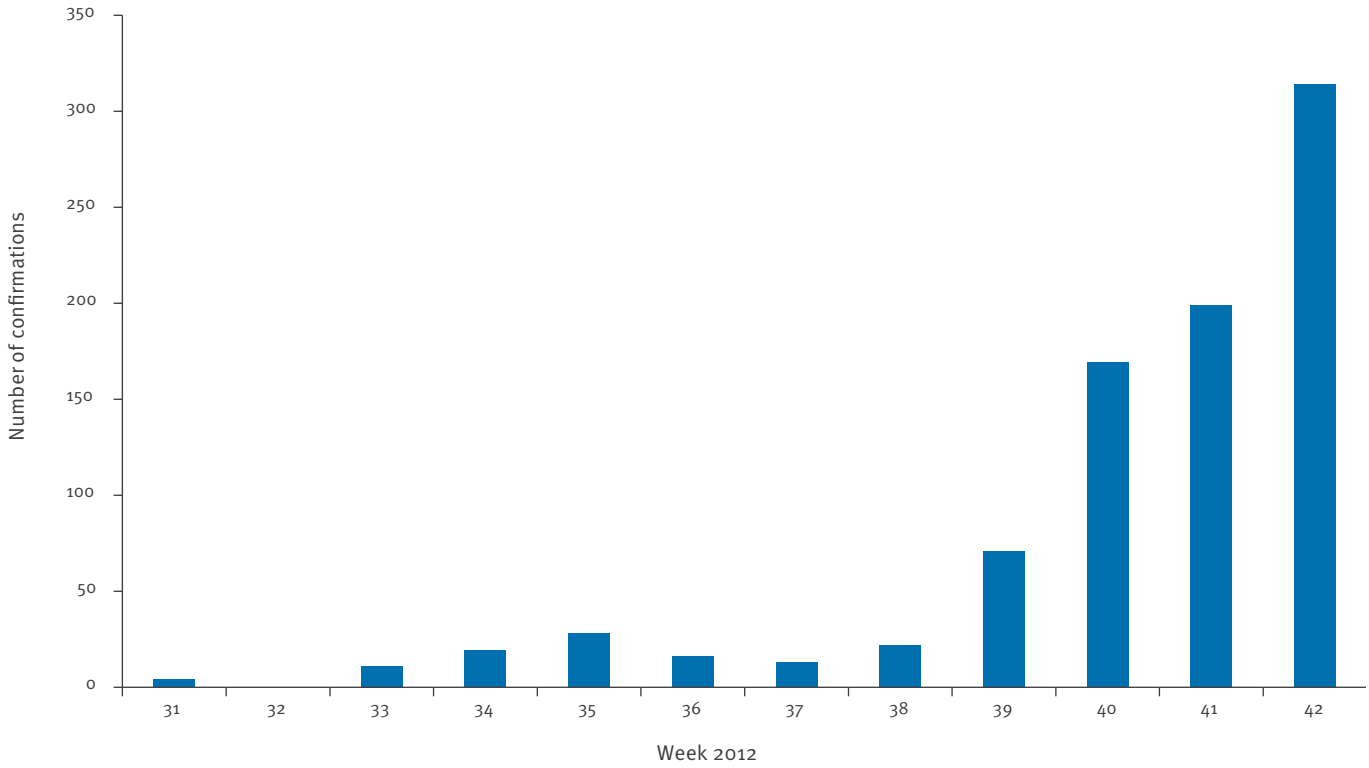
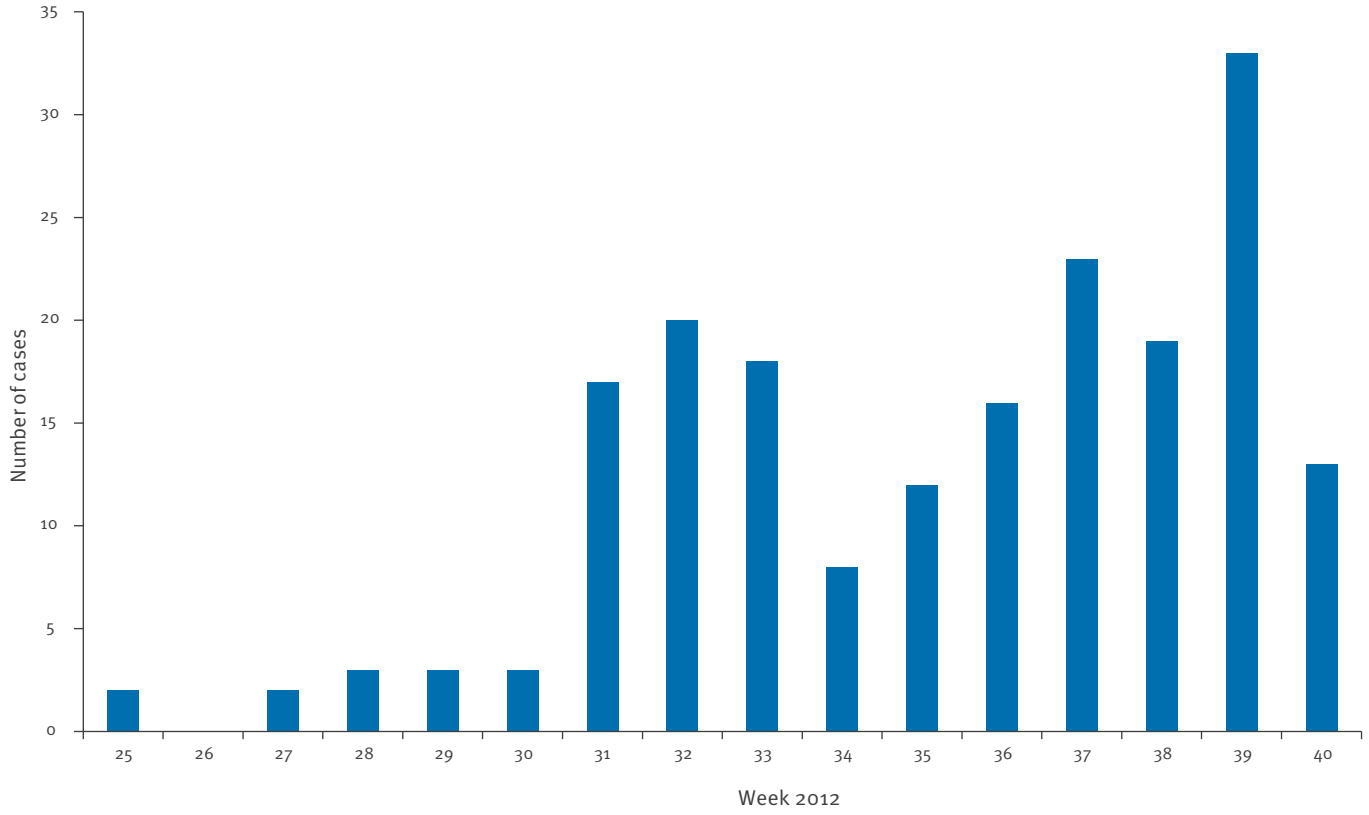


Figure 2

Cases of *Salmonella* Thompson, by week of onset, the Netherlands, 20 June–6 October 2012 (n=192 with known onset date)



supermarkets using the same purchasing organisation, as well as another often mentioned supermarket, purchased all or part of their smoked salmon from the same fish producer. On 26 September, the NVWA held an inspection at this fish production site and took samples from different batches of smoked salmon products. *S.* Thompson was detected in four of nine sampled batches of smoked salmon. Subsequently, all smoked salmon from this producer was recalled, starting Friday, 28 September (week 39). During the following week, other products containing salmon, such as salads, were recalled. How the contamination happened is still being investigated by the producer, supervised by the NVWA.

Microbiological investigation

Isolates of *Salmonella enterica* subsp. *enterica* Thompson from patients and sampled smoked salmon were subjected to molecular typing analysis by means of pulsed-field gel electrophoresis (PFGE) according to the PulsNet international protocol [1]. The enzyme used for digestion in the PFGE was *Xba*I. PFGE patterns of strains from the patients and the smoked salmon were indistinguishable using BioNumerics 6.6 (Applied Maths, Sint-Martens-Laten, België) with tolerance and optimisation both set on 1%. Historical strains from patients and food products that did not belong to this outbreak showed different fingerprints. Further analysis of these strains is ongoing to confirm these findings.

International investigations

An urgent inquiry was sent out on 23 August 2012 to European Union (EU) Member States via the Epidemic Intelligence Information System (EPIS, managed by the European Centre for Disease Prevention and Control). Members were asked to report any increase in the number of cases of *S.* Thompson in their countries. Eighteen EU countries replied and reported no increase. In the United States (US), a cluster of *S.* Thompson infections with a PFGE pattern indistinguishable from the current outbreak strain is being investigated (personal communication, Dr. Laura Gieraltowski and Dr. Peter Gerner-Smidt, Centers for Disease Control and Prevention, US). That ongoing investigation has not identified a connection between the US cluster and the current Dutch outbreak or a connection with the consumption of fish, nor with any other particular exposure.

Discussion

The epidemiological, molecular and traceback evidence gathered for the currently ongoing outbreak of salmonellosis due to *S.* Thompson in the Netherlands indicates that the food involved is contaminated smoked salmon. Previous outbreaks due to *S.* Thompson were related to contaminated fresh coriander [3], rucola lettuce [4] and pet treats [5,6]. However, *S.* Thompson has been found in a wide range of products and animals, such as poultry, pigs, cattle, birds and reptiles [7-10]. In the United States, 12,000 seafood samples were tested for salmonellae over a nine-year period, and 7% were found positive [11]. *S.* Thompson was the seventh most frequently isolated serovar with 22 of 830 positive samples. Among the smoked fish and seafood samples, 3% were positive for *Salmonella*. The current outbreak could have been caused by one batch of contaminated smoked salmon, as the production size of the identified producer is very large. Insufficient cleaning and disinfection of equipment may have resulted in an increase and persistence of the contamination of the production line, especially since *S.* Thompson has been reported to easily form a persistent biofilm [12].

As of 19 October, the number of isolates sent for confirmation remained very high (150 to 200 isolates per week). These high numbers of cases per week after recall of the incriminated food could be related to a lagging effect of about 20 days between date of disease onset and laboratory confirmation. Furthermore, people who consumed smoked salmon just before the recall and became ill want to be tested. Moreover, laboratories normally not participating in the surveillance now send *Salmonella* group C isolates for confirmation, increasing the number of confirmed cases that would otherwise have been missed. In addition, in the week before the recall, the identified smoked salmon was offered at a special discount at one of the largest supermarkets and people may therefore have bought larger quantities for storage in the freezer.

At the time of writing this report which includes data until 19 October, the outbreak seemed to be ongoing. Preliminary numbers as of 25 October indicate that the outbreak may have come to an end. The situation is and will be followed actively until the number of cases is back to normal.

Acknowledgments

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An invasive mosquito species *Aedes albopictus* found in the Czech Republic, 2012

O Šebesta[1,2], I Rudolf[1], L Betášová[1], J Peško[1], Z Hubálek (zhubalek@brno.cas.cz)[1]
1. Institute of Vertebrate Biology, v.v.i., Academy of Sciences of the Czech Republic, Brno, Czech Republic
2. Regional Public Health Authority of South Moravian Region, Brno, Czech Republic

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Between July and September 2012, seventeen larvae of the invasive mosquito species *Aedes (Stegomyia) albopictus* (Skuse) were discovered using 60 ovitraps at four study sites alongside two main road exits in South Moravia, Czech Republic. This is the first report of imported *Ae. albopictus* in the Czech Republic. The findings highlight the need for a regular surveillance programme to monitor this invasive species throughout western and central Europe.

Background

Of the invasive mosquitoes discovered in Europe recently, the Asian tiger mosquito *Aedes albopictus* (Skuse) represents the major threat to public health. Historically, this species originated in South-East Asia, but it has spread to the Americas, parts of Africa, northern Australia, and 19 European countries (Albania, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, France, Germany, Greece, Italy, Malta, Monaco, Montenegro, the Netherlands, San Marino, Serbia, Slovenia, Spain, Switzerland, Vatican City State) during the last decades. The species is now widely established and reportedly a nuisance mosquito in Italy, parts of France and Spain [1]. *Ae. albopictus* is globally an important vector of human pathogens such as chikungunya and dengue viruses as well as filarial nematodes represented by *Dirofilaria* spp., and an experimentally proven vector of eastern equine encephalitis, Venezuelan equine encephalitis, La Crosse encephalitis, Japanese encephalitis, West Nile and several other viruses [2,3].

Its eggs are frequently transported via used tire trade or by importation of lucky bamboo [2]. However, the most important mode of long-distance dispersal of *Ae. albopictus* in Europe in the last decade seems to be transportation by ground vehicles (i.e. lorries, cars, caravans) from southern Europe [4,5].

While two frequently used main roads connecting the Czech Republic with southern European countries cross the border in South Moravia, no systematic surveillance of invasive mosquito species has been conducted until present. This led us to periodically monitor invasive mosquito species at this so-called 'Moravian entrance gate' using ovitrap installations.

Trapping of mosquitoes

To monitor the presence of *Ae. albopictus* we used traditional ovitraps [6]. These were constructed from a dark blue 800 ml plastic cup and supplemented with 500 ml of dechlorinated tap water and a floating wooden tongue depressor paddle wrapped into rough cotton fabric that was in contact with the water line to ensure *Ae. albopictus* oviposition. Ovitraps were placed on shrubs, columns or public lighting in close proximity to parking spaces about 50 cm above the ground. Wooden paddles and water were periodically replaced (every 7 days) and transported in closed containers to the laboratory. The paddles were incubated at 25°C in humid atmosphere for three days and then kept immersed below the water surface at 25°C for another 12 days. Additionally, water from the ovitrap containers was incubated in the laboratory at 25°C for one week. Both components were daily examined for the presence of hatching eggs or larvae. Larvae and adults reared from larvae were morphologically identified according to recent entomological keys [6,7].

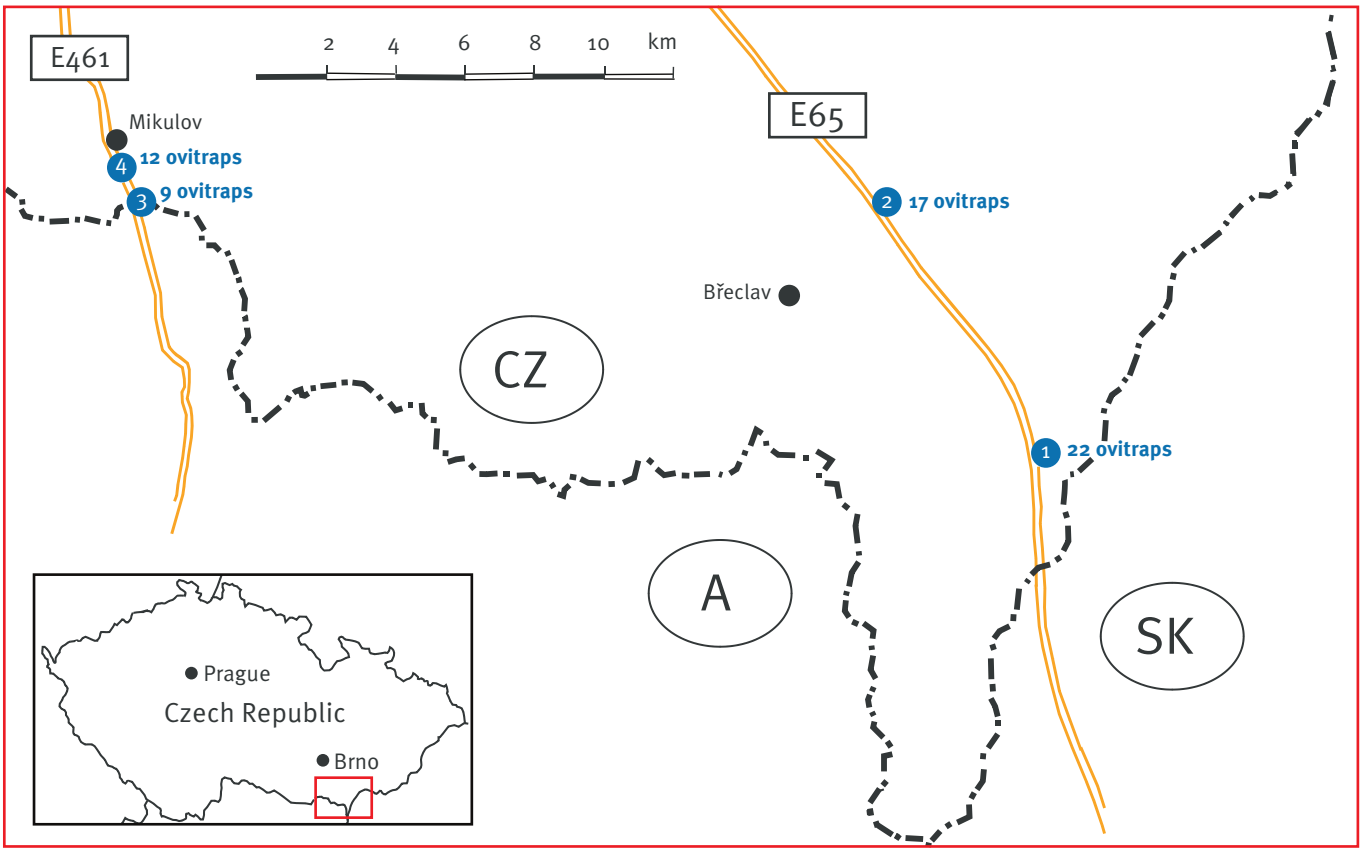
Study sites and findings

Several ovitraps were placed at four study sites (parking lots) in close proximity to exits of two main roads respectively connecting Austria and Slovakia with the Czech Republic (Figure). A total of 60 ovitraps were installed between the beginning of July and the end of September 2012.

The first two ovitrap sites (study sites 1 and 2) were situated near the main road E65, a transit route for goods to the Czech Republic from Slovakia and Hungary as well as from Balkan countries (Romania, Bulgaria, Croatia, Serbia, Greece). Individual and collective transport between western (e.g. Germany, Belgium, the Netherlands), central and southern Europe also operates through this main road. Study site 1 (22 ovitraps) was in Lanžhot (N 48°43,554´, E016°59,041´, 155 m above sea level (a.s.l.)), at a one km distance from Slovakia. The location is used for refreshment and

Figure

Locations (n=4) of ovitraps (n=60) for invasive mosquito monitoring, South Moravia, Czech Republic, July–September 2012



Location where ovitraps were placed, the number in the circle indicates the site number for the purpose of the study.

A: Austria; CZ: Czech Republic; SK: Slovakia.

Mosquito traps (ovitraps) were placed at four locations near the two main roads E461 and E65 which are respectively shown on the map in yellow.

refueling, with a parking capacity of about 100 spaces. Study site 2 (17 ovitraps) was at Ladná (N 48°48,669´, E016°53,600‘, 177 m a.s.l.) and situated approximately 16 km north of the first study site alongside the same main road. The site serves mainly as a refueling and rest area with a parking capacity of about 40 spaces. Two additional ovitrap sites (study sites 3 and 4) were chosen beside main road E461, where this road enters the Czech Republic from Austria. The main road E461 is frequently used for transit of goods from southern Europe (Italy, Slovenia, Croatia, Serbia, Montenegro, Macedonia, Albania) to the Czech Republic. Study site 3 (9 ovitraps) was Mikulov II (N 48°47,424´, E016°38,154´, 198 m a.s.l.), a former customs' house now solely intended for refreshment. It is located on the Czech–Austrian border and has a parking capacity of about 10 spaces. Study site 4 (12 ovitraps) was Mikulov I (N 48°47,845´, E016°37,970´, 207 m a.s.l.), at the periphery of the town of Mikulov about 1.2 km north of study site 3. It serves a rest and refueling purpose and has a parking capacity of about 20 spaces.

From study site 4, we found 16 larvae of *Ae. albopictus*. Eight larvae in stage IV were euthanised for identification while the remaining eight were left to rear to adult stage (five females and three males) and also subsequently identified. Interestingly, all mosquito larvae developed from ovitraps set up within two subsequent intervals (20 August and 27 August 2012). Furthermore, one larva of *Ae. albopictus* developed from an ovitrap situated at the study site 3, on 10 September 2012, while no deposited eggs were detected in the study sites 1 and 2.

Conclusion

South Moravia is owing to its mild climate the most favourable habitat for breeding of mosquitoes within the Czech Republic [8]. Massive broods of mosquitoes (predominantly *Aedes* spp.) periodically occur here along the rivers Dyje and Morava. This area has been known for a long time as a natural focus of several mosquito-borne viruses: mainly Ťahyňa virus, the etiologic agent of Valtice fever, and since 1997 also

West Nile virus lineage 3 – Rabensburg [9,10]. Many mosquito species occurring in the Czech Republic were only recorded in this region, e.g. *Anopheles atroparvus, An. hyrcanus, An. labranchiae, Aedes nigrinus, Uranotaenia unguiculata, Culex martinii* [11,12]. We should take this region into consideration when searching for a suitable habitat for possible introduction and subsequent establishment of invasive mosquito species in central Europe. Our findings suggest that *Ae. albopictus* may be able to complete its developmental cycle in this region, and in case of a mild winter might also survive in the stadium of eggs [13]. Our results also indicate that ovitraps are a suitable tool for monitoring invasive mosquitoes on parking lots alongside main roads where alternative egg depositing water is likely less available.

In conclusion, we provide the first evidence of import of *Ae. albopictus* in the Czech Republic. Interestingly, *Ae. albopictus* has not yet been reported from the neighbouring central-European countries Austria, Slovakia, Hungary or Poland.

Acknowledgments

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Sequence-based typing of *Legionella pneumophila* serogroup 1 clinical isolates from Belgium between 2000 and 2010

E Vekens (labomicro@uzbrussel.be)[1], O Soetens[1], R De Mendonça[2], F Echahidi[1], S Roisin[2], A Deplano[2], L Eeckhout[1], W Achtergael[1], D Piérard[1], O Denis[2], I Wybo[1]

1. National Reference Centre for Legionella pneumophila, Department of Microbiology and Infection Control, Universitair Ziekenhuis Brussel, Vrije Universiteit Brussel (VUB), Brussels, Belgium
2. National Reference Centre for Legionella pneumophila, Department of Microbiology, Erasme Hospital, Université Libre de Bruxelles, Brussels, Belgium

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Sequence-based typing (SBT) is a discriminatory method widely used to genotype *Legionella pneumophila* strains. A total of 86 clinical *L. pneumophila* serogroup 1 (sg1) isolates, collected between January 2000 and December 2010 in the two Belgian National Reference Centres for *Legionella pneumophila*, were genotyped using the internationally standardised SBT protocol of the European Working Group for *Legionella* Infections (EWGLI). The isolates could be classified into 31 different sequence types (ST, index of diversity: 0.879). The obtained STs were submitted to the EWGLI SBT-database for *L. pneumophila*. In our study, ST47 (27.9%) and ST1 (19.8%) were the most frequently detected STs. The detected profiles were a combination of both frequently isolated and unique STs, and of both worldwide distributed and more local strains. Two STs, ST880 and ST881, were new to the EWGLI database. In conclusion, we characterised *L. pneumophila* sg1 isolates with the SBT method, and created a Belgian profile database that will be useful for future epidemiological studies.

Introduction

Legionella spp. are rod-shaped, gram-negative bacteria, which are ubiquitously spread in aqueous environments [1] where they survive as intracellular parasites of protozoa [2]. *Legionella* is transmitted to humans by inhalation of contaminated aerosols. Common sources are air conditioning systems, cooling towers, dental devices and showerheads [3]. Legionellosis can appear in two distinct clinical presentations, Legionnaires' disease (LD), a mild to fatal pneumonia with an approximate case fatality rate of 6.6% [4], and Pontiac fever, an acute self-limited influenza-like illness [5]. One species of *Legionella*, *L. pneumophila*, is the aetiological agent of approximately 90% of legionellosis cases, and serogroup 1 (sg 1) accounts for about 84% of these cases [6]. Notification of legionellosis to the health authority is mandatory in Belgium. Most reported cases are single infections, but outbreaks do occur [7].

The characterisation of clinical isolates by molecular typing methods is essential for epidemiological investigations of sporadic cases and outbreaks. *L. pneumophila* sg1 isolates can be genotyped by sequence-based typing (SBT) using the seven loci (*flaA, pilE, asd, mip, mompS, proA* and *neuA*) proposed by the European Working Group on *Legionella* Infections (EWGLI, renamed to European Study Group for *Legionella* Infections, ESGLI, since September 2012) [8,9]. We determined the SBT of clinical *L. pneumophila* sg1 isolates recovered over a 10-year period in Belgium, and compared these results to available data in other countries.

Methods

Legionella pneumophila isolates

Clinical isolates of *L. pneumophila* sg1 collected between January 2000 and December 2010 in the laboratories of Microbiology of UZ Brussel and ULB-Erasme, the two Belgian National Reference Centres for *L. pneumophila*, were retrospectively analysed. Clinical laboratories of both general and university hospitals may refer clinical samples for culture and PCR or strains for molecular typing to the reference centres. This service is free of charge for all clinical laboratories and supported by the health authorities. During the 10-year study period, we gathered 106 *L. pneumophila* sg 1 isolates from 29 hospitals, of which 91 were available for further SBT analysis. All isolates were unduplicated and collected from different patients with LD (diagnosed with pneumonia according to the EU case definition [10]). Epidemiological data for each isolate included the patient's age and sex, hospital from which specimens were submitted, and if available, the patient's place of residence and probable origin

of infection. A case of LD was considered nosocomial if the patient had been hospitalised during the entire incubation period (10 days), or travel-associated if the patient had spent at least one night away from home, either in Belgium or abroad, 10 days before onset of the symptoms. For the other cases, the patient's place of residence, if available, was used as a proxy for the place of infection. Clinical cases were subdivided into related or single cases. Isolates were considered related to each other if they were recovered, within the same year, from patients with a probable or confirmed common source of contamination.

Identification methods

The *L. pneumophila* isolates were cultured on buffered charcoal yeast extract agar (*Legionella* CYE, Oxoid, UK) supplemented with ACES buffer, alpha-ketoglutarate, ferric pyrophosphate and L-cysteine and with and without the antibiotics cefamandole, polymyxin B and anisomycin (*Legionella* BMPA-alpha selective supplement SR0111B, Oxoid, UK). Isolates were identified as *L. pneumophila* by determination of cellular fatty acid composition by gas-chromatography [11,12]. Identification to serogroup level was performed by latex agglutination using Microgen *Legionella* latex kit (Microgen Bioproducts Ltd., UK) or Oxoid *Legionella* latex test (Oxoid, UK). Isolates were stored at -80 °C in nutrient broth supplemented with 15% glycerol until analysis.

Molecular typing

The first recovered isolate from each group of related isolates and all unrelated isolates were included and genotyped using the standard protocol from the EWGLI SBT scheme [8,9,13]. DNA was prepared directly from colonies by incubating the bacterial suspensions in 250µL Tris-EDTA buffer for molecular biology (pH 8, Sigma Aldrich, Bornem, Belgium) at 100 °C for 10 minutes. Polymerase chain reaction (PCR) was performed using primers targeting the gene loci *flaA* [13], *pilE* [8], *asd* [8], *mip* [8], *mompS* [8,13], *proA* [8] and *neuA* [9] on an iCycler (Bio-Rad). Purification of PCR products and sequencing with the amplification primers, except for the reverse sequencing primer of *mompS* [8], was done by VIB Genetic Service Facility (University of Antwerp, Wilrijk, Belgium). Sequence alignment, trimming and allele designation were performed using the online EWGLI Sequence Quality Tool. The obtained STs were submitted to the EWGLI SBT-database (http://www.hpa-bioinformatics.org.uk/legionella/legionella_sbt/php/sbt_homepage.php). The data from our study were compared to literature and to data submitted to the EWGLI database until 25 January 2012.

Statistical analysis

The index of diversity (IOD) was calculated by using Hunter and Gaston's modification of Simpson's index of diversity as previously described [14]. The Mann-Whitney U-test was used to assess whether there was a significant change in frequency of ST isolation over years. Statistics were performed with Analyse-it for Microsoft Excel (version 2.21 Analyse-it Software Ltd, UK). P values ‹0.050 were considered as statistically significant.

Results

Clinical isolates

From 2000 to 2010, 212 cases of LD were confirmed by the Belgian National Reference Laboratories. Of these, 122 (57.5%) were diagnosed by culture and isolation of *Legionella* spp. *L. pneumophila* sg1 was the causative agent in the majority of culture-confirmed cases (n=106, 86.9%). Of the 91 *L. pneumophila* serogroup 1 clinical isolates available for SBT analysis, 82 were from clinically single cases. Over the study period, four outbreaks occurred (involving nine cases in total) and all of them were linked to a hospital facility. After inclusion of the first isolate for each outbreak (no discrepancy was observed between isolates from the same outbreak), 86 isolates were included in our study and typed by SBT analysis.

Epidemiological data

The median age of included patients was 58 years (range: 19–92 years; excluding one patient whose age was unknown) and 62 patients (73%) were men. There were 15 travel-associated cases (17.4%), eight nosocomial cases (9.3%), 11 community-acquired cases (12.8%) and 52 cases for which the source could not be identified (60.5%) (Table).

Legionella pneumophila sequence-based typing

SBT analysis assigned the 86 unrelated clinical isolates to 31 distinct STs (IOD: 0.879). As shown in the Table, 47.7% of the isolates belonged to two main STs with 24 and 17 isolates, respectively. Eight STs consisted of groups containing between two and five isolates and the remaining STs (n=21) accounted for only one single isolate each.

The ST with the largest number of isolates (n=24) was ST47, which represented 27.9% of all clinical isolates. The ST47 profile was found ubiquitously across Belgium as shown in Figure 1. Two ST47 isolates were associated with travelling to France and Italy, respectively. A suspected source could be identified for a further three cases: a decorative fountain, a visit to a garden centre and the maintenance of an outdoor swimming pool.

Genotype ST1 was found in 17 isolates (19.8%). The geographic distribution of ST1 in Belgium was mainly restricted to Brussels (Figure 1). Six isolates were associated with nosocomial infections. Four of these isolates were found between 2000 and 2005 in one hospital, two of which were related to outbreaks involving two cases each. One ST1 isolate was travel-associated; the infection was acquired in Spain.

Other major STs were ST6 (n=5), ST23 (n=5), ST42 (n=3) and ST62 (n=3). One of the ST6 isolates was associated with a nosocomial outbreak involving three cases.

Table

Distribution of *Legionella pneumophila* serogroup 1 isolates by sequence type and origin of acquisition, Belgium, January 2000–December 2010 (n=86)

ST	Allelic profile[a]	N	%	Community-acquired	Travel-associated	Nosocomial	Undetermined origin
ST47	5, 10, 22, 15, 6, 2, 6	24	27.9	6	2	0	16
ST1	1, 4, 3, 1, 1, 1, 1	17	19.8	1	1	6	9
ST6	1, 4, 3, 1, 1, 1, 15	5	5.8	0	0	1	4
ST23	2, 3, 9, 10, 2, 1, 6	5	5.8	0	1	0	4
ST42	4, 7, 11, 3, 11, 12, 9	3	3.5	1	2	0	0
ST62	8, 10 3, 15, 18, 1, 6	3	3.5	0	1	0	2
ST9	3, 10, 1, 3, 14, 9, 11	2	2.3	0	0	0	2
ST48	5, 2, 22, 27, 6, 10, 12	2	2.,3	0	0	0	2
ST110	2, 10, 3, 3, 9, 4, 9	2	2.3	1	0	1	0
ST664	3, 13, 1, 3, 14, 9, 9	2	2.3	0	1	0	1
ST109	5, 1, 22, 15, 6, 10, 6	1	1.2	0	0	0	1
ST146	2, 10, 18, 10, 2, 1, 6	1	1.2	0	0	0	1
ST16	2, 10, 18, 10, 2, 1, 9	1	1.2	0	0	0	1
ST196	3, 10, 1, 28, 14, 9, 11	1	1.2	0	0	0	1
ST20	2, 3, 18, 15, 2, 1, 6	1	1.2	0	1	0	0
ST22	2, 3, 6, 10, 2, 1, 6	1	1.2	0	0	0	1
ST301	2, 10, 3, 12, 9, 4, 9	1	1.2	1	0	0	0
ST345	6, 10, 19, 3, 19, 4, 11	1	1.2	0	0	0	1
ST37	3, 4, 1, 1, 14, 9, 11	1	1.2	0	0	0	1
ST438	3, 10, 1, 1, 14, 9, 15	1	1.2	0	0	0	1
ST479	5, 1, 22, 10, 6, 10, 12	1	1.2	1	0	0	0
ST487	3, 6, 1, 28, 14, 11, 11	1	1.2	0	1	0	0
ST579	3, 13, 1, 3, 14, 9, 11	1	1.2	0	1	0	0
ST744	2, 3, 5, 5, 18, 1, 10	1	1.2	0	0	0	1
ST751	8, 10, 3, 15, 9, 14, 6	1	1.2	0	1	0	0
ST77	6, 10, 14, 10, 2, 3, 6	1	1.2	0	1	0	0
ST862	4, 17, 11, 15, 29, 12, 20	1	1.2	0	1	0	0
ST880	6, 10, 19, 3, 2, 4, 11	1	1.2	0	0	0	1
ST881	7, 7, 17, 3, 13, 11, 9	1	1.2	0	1	0	0
ST94	12, 8, 11, 5, 20, 12, 2	1	1.2	0	0	0	1
ST99	4, 8, 11, 5, 29, 12, 10	1	1.2	0	0	0	1
Sum		**86**		**11**	**15**	**8**	**52**
				(12.8%)	**(17.4%)**	**(9.3%)**	**(60.5%)**

ST: sequence type

[a] Sequence of genes *flaA, pilE, asd, mip, mompS, proA, neuA*.

ST110 was recovered twice among our isolates: in one nosocomial and one community-acquired infection. These two clinical isolates could be matched with environmental isolates recovered from shower water in a hospital and from water in a service flat, respectively. The nosocomial isolate was associated with a hospital-outbreak involving two cases.

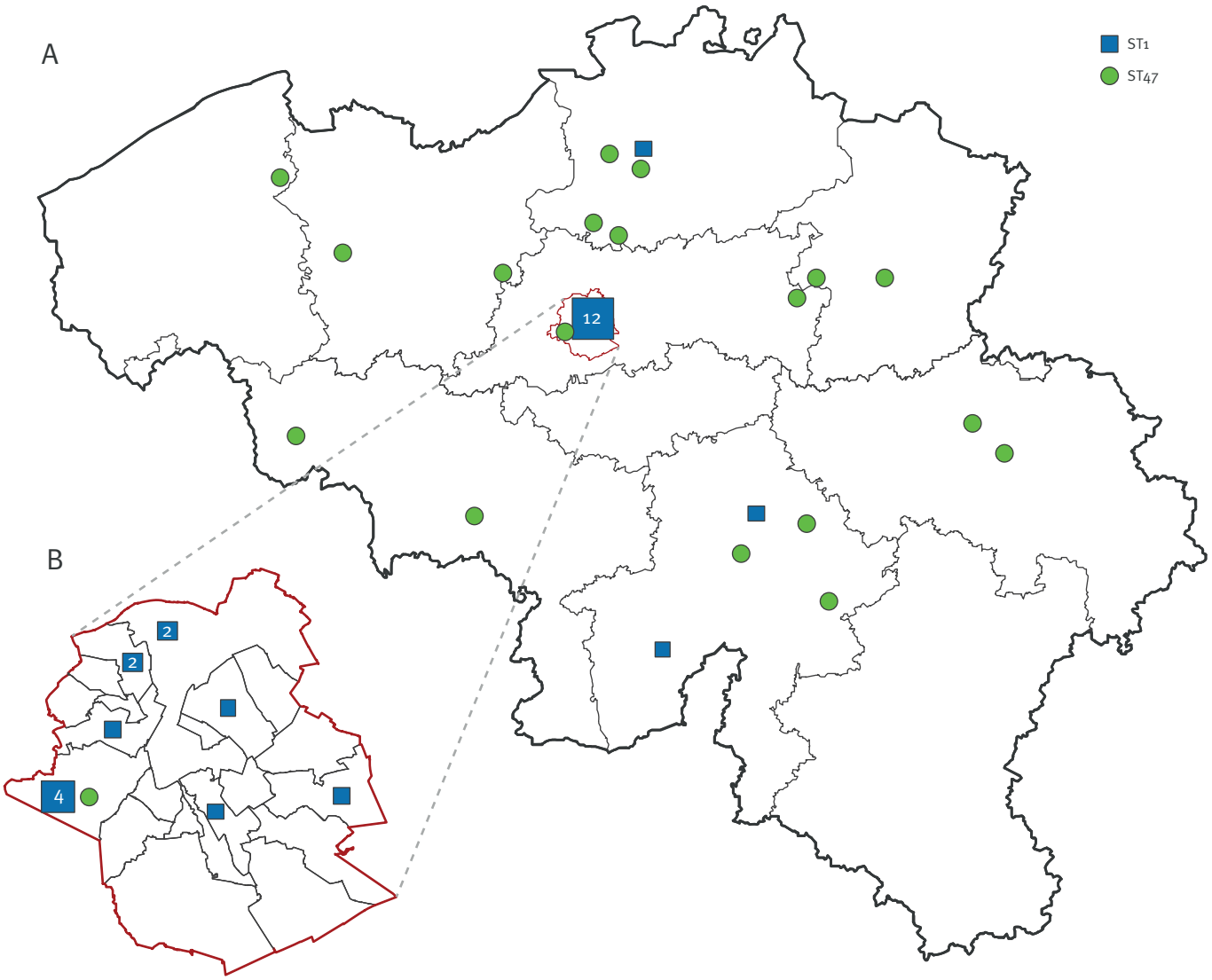
Two STs were new to the EWGLI database: ST881 was detected in an isolate from a patient probably infected in Spain, and ST880 was detected in a clinical isolate of unknown origin.

Sequence type distribution by year of isolation

The profile distribution of *L. pneumophila* sg1 clinical isolates by year of isolation was heterogeneous (Figure 2). From 2000 through 2005, ST1 was regularly isolated and accounted yearly for 14.3% to 60% of clinical isolates. The incidence of ST1 was significantly lower from 2006 through 2010 with an average recovery in 8.2% of clinical isolates (p=0.009). Although isolation of ST47

Figure 1

Geographic distribution of *Legionella pneumophila* serogroup 1 isolates with sequence type ST1 and ST47 in Belgium (A) and in Brussels (B), January 2000–December 2010 (n=33)



The number of cases is shown for locations with more than one case. Travel-associated cases (n=3) and cases with no available geographic information (n=5) are not shown.

peaked in 2007 (80% of clinical isolates), there was no significant difference in the frequency of ST47 isolation between 2000–05 and 2006–10 (p=0.662).

Discussion

This report represents the first SBT analysis of *L. pneumophila* serogroup 1 clinical isolates in Belgium from 2000 to 2010. Over this period, 212 cases of LD were confirmed by the Belgian National Reference Laboratories, of which 122 (57.5%) were diagnosed by the isolation of *Legionella*. In line with other reports, the majority of culture-confirmed LD cases in Belgium was caused by *L. pneumophila* sg1. The incidence of other serogroups (13 clinical isolates: four sg6, three sg4, one sg10, one sg3, three sg2-15 and one undefined) and non-pneumophila species of *Legionella* (two *L. bozemanii* and one *L. longbeachae*) (data not shown) was similar to the distribution in the rest of Europe [4,15].

Diagnosis of LD in Belgium is based on culture, PCR, serology and urinary antigen detection. Since many clinical laboratories in Belgium use the urinary antigen test as a primary diagnosis tool, culture and strain isolation of *L. pneumophila* from respiratory samples is, in our experience, seldom undertaken in less severe cases. In the future, clinicians should be encouraged to refer respiratory samples for *Legionella* culture in case of confirmed LD. During the 10-year study period, we gathered 106 *L. pneumophila* serogroup 1 isolates, of which 91 were available for further SBT analysis. The incidence of Legionnaires' disease in Belgium between 2003 and 2010 was about 138 cases per year [4,16,17,18,19]. Therefore, our study may not represent

all LD cases, but it gives a good representation of the circulating isolates. A comparison of epidemiological characteristics of included cases and non-culture verified cases showed no statistically significant difference in age, sex and distribution into nosocomial and travel-associated cases (data not shown). Although the cases for which urine samples were referred to our laboratories could be biased, this finding suggests that the included cases might be a good representation of all cases of *L. pneumophila* sg1 infection in Belgium.

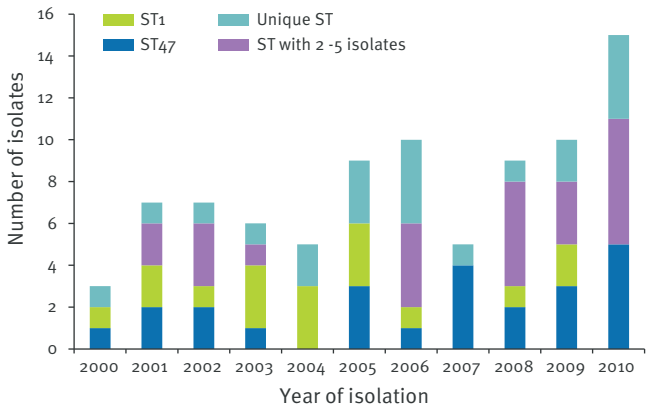
In our study, 31 distinct allelic STs were detected among 86 clinical *L. pneumophila* sg1 isolates which reflects a high profile diversity. The index of diversity (0.879) in our investigation was lower than that described previously in Japan (0.979) [20], the United States (0.946) [21], Canada (0.964) [22] and England and Wales (0.901) [23]. This could be explained by the smaller geographic area of Belgium as compared to the other countries. The combination of SBT analysis with monoclonal antibody subgrouping [24] would allow a further characterisation of the clinical isolates. This subgrouping can have relevance for source investigation especially for common STs, like ST1 [22,25].

The detected STs in our study are a combination of both frequently isolated and unique STs, and of both worldwide distributed and more local STs. The most common ST in Belgium was ST47 (27.9%). This observation is in accordance with published data; ST47 is also the major ST found in France, the Netherlands, and England and Wales [23,26,27]. The most frequent ST reported in the world, ST1, was found in 17 isolates (19.8%). ST6 was recovered from five isolates and is an important but more local strain since isolation of this genotype is restricted to the region of Germany, France and Belgium according to the data submitted to the EWGLI database. Strains occurring worldwide that were responsible for three to five cases in Belgium included ST23 and ST42. The ST23 genotype has repeatedly been isolated from clinical cases in Europe, mostly in France (n=291) and the Netherlands (n=27), according to the EWGLI database. Interestingly, one of our cases was associated with travelling to France. Apart from the frequent isolation in Europe [25,28], this ST was also found to be responsible for two large outbreaks in Japan associated with a bath facility [20]. The ST42 profile is also widely distributed and most frequently isolated in the Netherlands (n=30), France (n=17) and England (n=16) according to the EWGLI database. Data from England and Wales showed that this profile was often associated with travelling outside the UK. This is in accordance with our data since two of our three ST42 strains were associated with travel to Turkey and Italy. Only one strain with ST37 profile was detected among our isolates. In contrast, this profile accounted for 11.4% of clinical isolates in England and Wales [23] and was detected in 21 clinical isolates in Canada [29].

During this 10-year surveillance period, only four small nosocomial outbreaks were detected. This is in contrast

Figure 2

Distribution of *Legionella pneumophila* serogroup 1 sequence types by year of isolation, Belgium, January 2000–December 2010 (n=86)



ST: sequence type.

with the large ST36 outbreak of 1999 at Kappellen in Belgium, where more than 90 cases occurred during a fair [7]. Of notice is that the ST36 from the 1999 outbreak was not detected in the present study. Of the four outbreaks recorded in Belgium during the study period, two were associated with ST1, while the two other outbreaks were associated with ST6 or ST110, respectively. Four of the ST1 nosocomial infections occurred within the same hospital. This institution was probably colonised by ST1, which caused two outbreaks and two sporadic cases over several years. In France and Canada, this sequence type was found to be responsible for sporadic as well as outbreak cases [22,28,29].

In the 10-year study period, differences could be observed in profile distribution by year of isolation. The decreased incidence of ST1 over the years is in accordance with observations made in Canada and Japan [20,29]. Irrespective of the temporal differences between the ST47 and ST1 strains, the pattern of geographical distribution varies between both strains. The ST47 profile showed a dispersed distribution in Belgium whereas detection of the ST1 profile was generally restricted to Brussels.

As demonstrated in our study and in previous publications, Legionnaires' disease occurs both in sporadic and epidemic forms. Prompt recognition of LD cases and outbreaks is necessary to control epidemics quickly and to treat patients effectively. As a result of this study a Belgian national database of *L. pneumophila* SBT profiles was created, which is a useful tool for the investigation and management of local outbreaks. Our data were uploaded to the EWGLI SBT-database which allows comparison between countries and is valuable in epidemiological investigations, since cases might be dispersed over different regions and countries.

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