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## Rapid communications

# INFLUENZA A(H1N1)v VIRUS INFECTIONS IN BELGIUM, MAY-JUNE 2009

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In response to the ongoing influenza A(H1N1)v pandemic, first detected in North America in April 2009, Belgium has set up an active surveillance system for influenza-like illness among travellers returning from affected areas. This communication describes the clinical and epidemiological features of the first 43 laboratory-confirmed cases in Belgium.

### Introduction

On 25 April 2009, the World Health Organization (WHO) declared an outbreak of A(H1N1)v influenza, first reported by the United States (US) [1] and Mexico, a 'Public Health Event of International Concern' (PHEIC) under the International Health Regulations [2]. The WHO Director-General raised the pandemic alert phase to the maximum level (phase 6) on 11 June 2009 [3]. As of 14 July 2009, 30 of 31 European Union (EU) and European Free Trade Association (EFTA) countries have reported cases of influenza A(H1N1)v [4].

On 12 May 2009, the Belgian National Reference Laboratory for Influenza confirmed the first case of influenza A(H1N1)v in a person returning to Belgium from the US. A total of 130 confirmed cases have been detected in Belgium as of 14 July 2009.

An active surveillance system was implemented, following a delaying strategy. It aimed at detecting cases of A(H1N1)v influenza in travellers returning from affected areas [5] and in their contacts for the purpose of taking control measures to delay the spread of the virus.

### Methods

Table 1 shows the case definitions developed for the investigation and the case classification used.

The Interministerial Influenza Coordination Committee disseminated protocols for case and contact management regarding notification, sampling, prophylaxis, treatment and isolation. The involved physicians, mostly general practitioners (GPs), were required to contact the Community Health Inspectorate when finding a possible or suspected case. Physicians took samples and sent them on the same day to the National Reference Laboratory for Influenza. Samples were treated under biosafety level 3 (BSL3) conditions and tested by realtime reverse transcription PCR (RT-PCR) using primers directed against A and B influenza virus, and in case of a positive result for A influenza also with primers against A(H1) and A(H3); from 3 May 2009 we also used primers specific

for A(H1N1)v influenza virus, sent from the Centers for Disease Control and Prevention (CDC).

All involved public health authorities scaled up their response service to operate around the clock. A duty service with epidemiologists was available for Health Inspectorates and involved physicians through a restricted access telephone hotline in order to support them with case definitions and the organisation of sampling.

Hospitalisation was recommended for the first 25 confirmed cases for the purpose of isolation. From 2 June 2009 onwards, the recommendation was for patients to stay at home for seven days after onset of symptoms and to hospitalise severe cases only. Confirmed cases were treated with neuraminidase inhibitors.

Provincial health inspectorates performed contact tracing. Close contacts of confirmed cases should take a neuraminidase inhibitor

TABLE 1

Case definition and case classification for A(H1N1)v influenza, Belgium, May-June 2009

Case definition for investigation		
Possible case	A person with: <i>all three</i> clinical criteria <i>and</i> <i>at least one</i> epidemiological criterion during the seven days prior to onset of symptoms	<p>Clinical criteria:</p> <ul style="list-style-type: none"><li>• Fever (&gt;38°C)</li><li>• One respiratory symptom (cough, dyspnoea)</li><li>• General discomfort</li></ul> <p>Epidemiological criteria:</p> <ul style="list-style-type: none"><li>• History of travel to affected areas</li><li>• History of close contact (&lt;1 metre) with a confirmed or symptomatic probable case</li></ul>
Suspected case	A person fulfilling the epidemiological criteria for influenza A(H1N1)v infection, but not all clinical criteria for a possible case	
Case classification		
Confirmed case*	A person with laboratory-confirmed A(H1N1)v influenza	
Non-case	A person with a negative test for influenza A(H1N1)v	

\* Until 3 May 2009, real-time reverse transcription PCR (RT-PCR) for influenza A(H1N1)v virus was not available and cases were tested for influenza A and B, and subtyped for seasonal influenza A(H1) and A(H3). A person with a positive test for influenza A, untypable for seasonal strains, would have been considered as a probable case.

as prophylaxis and were requested to stay at home for seven days after the latest contact and to avoid unnecessary further contacts as a quarantine measure. Close and other contacts were advised to seek immediate medical advice if they noticed fever or respiratory symptoms.

Cases were notified to the WHO and through the Early Warning and Response System (EWRS) to the European Centre for Disease Prevention and Control (ECDC) by the Belgian Federal Public Service for Public Health (FPS).

## Results

As of 14 July, 633 people have been tested in Belgium and 130 cases of influenza A(H1N1)v have been confirmed. Two of the possible cases were not tested because they were close contacts of confirmed cases and under antiviral prophylaxis when they developed influenza symptoms.

We analysed the first 43 laboratory-confirmed cases. Infection was acquired abroad by 35 cases, of which 18 had a travel history to the US, nine had returned from the Dominican Republic and three from the United Kingdom (UK). The other imported cases had returned from Argentina (n=1), Australia (n=1), Canada (n=1), Chile (n=1) and Costa Rica (n=1). The first eight imported cases had come back from the US. Seven imported cases declared onset of symptoms prior to their return. According to information available for 26 of 28 cases, disease onset occurred up to five days after arrival (mean 1.6 days, median 2 days).

All indigenous cases (n=8) were close contacts to previously confirmed cases. Figure 1 shows the evolution of the cases by date of symptom onset and by import status.

Table 2 shows the geographic distribution of the cases by province. On 28 June 2009, eight of 11 provinces in Belgium were affected. One third of the cases were residents in the province of Antwerp. One case who had been in transit at Brussels airport was counted in the province of Flemish Brabant.

The female to male ratio was 1.05 (22 women and 21 men). The age range was from eight months to 51 years (median: 28 years, mean: 29 years). Seven cases were younger than 20 years. The most affected age group were the 20-29 year-olds with 16 cases.

Information about symptoms was available for 42 cases. The most commonly reported symptoms were cough in 40 cases

followed by general discomfort in 38 cases and fever or history of fever in 36 cases. Dyspnoea was reported by 12 cases and diarrhoea by five cases; nausea was reported by two cases and vomiting, sore throat and headache were reported by one case each.

No complications have been detected so far. One confirmed case, already under treatment with oseltamivir, was hospitalised because influenza symptoms persisted and the patient had asthma as underlying condition. Respiratory samples from this patient are currently being cultured and tested for resistance against oseltamivir. One pregnant woman was confirmed to be infected with influenza A(H1N1)v. Information on underlying factors for the other 41 patients was not available.

## Discussion

When Belgium detected the first confirmed case of influenza A(H1N1)v, many neighbouring countries had already notified cases. We assume that the number of Belgian travellers to Mexico is small compared to that of more populated European countries and that the number of Belgian travellers returning from the US is larger than the amount of those returning from Mexico. This may explain why Belgium started detecting imported cases when sustained community transmission happened in the US.

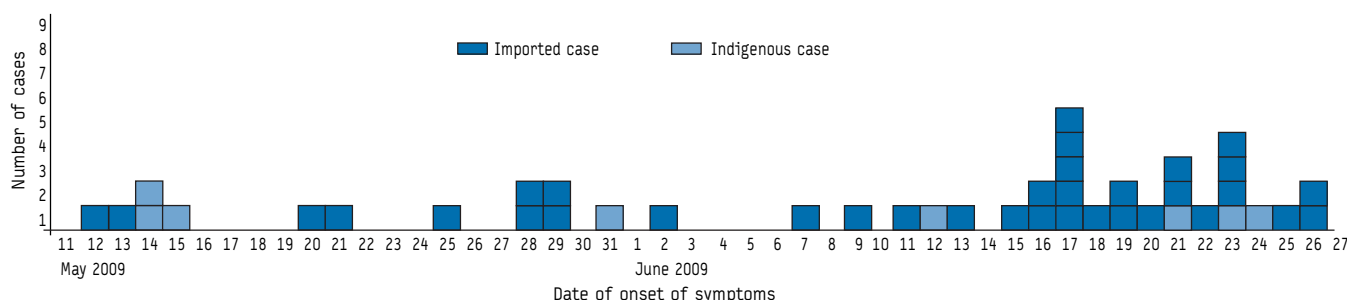
Continuous monitoring of affected areas worldwide and consequent updating of the case definition allowed the detection of cases returning from countries with a low number of cases but with evidence of community transmission like Costa Rica or the Dominican Republic.

The age distribution of the cases may reflect the age of the people that travel and is not representative of the Belgian population. Children of school age were only sporadically affected until 11 July 2009, and this may have played an important role in the disease not spreading in the community. Secondary cases occurred in the same age groups as imported ones, reflecting the importance of contact patterns. However, an outbreak in a summer language school that has affected 14 people between 10 and 18 years-old, is currently under investigation.

This preliminary analysis of the 43 first confirmed cases of influenza A(H1N1)v in Belgium suggests that the clinical manifestation resembles that of seasonal influenza. This is consistent with an analysis by the ECDC on aggregated data of European cases of influenza A(H1N1)v [6].

FIGURE 1

Distribution of laboratory-confirmed cases of influenza A(H1N1)v by date of onset and by import status, Belgium, 12 May-28 June 2009 (n=43)



Currently, mitigation strategies are being adopted by countries in the southern hemisphere that are facing the influenza season, such as Chile, New Zealand and Australia, but also by European countries where sustained community transmission has been declared, like the UK [7]. In Belgium, the Interministerial Influenza Coordination Committee announced the switch to a mitigation strategy on 13 July 2009. This will require appropriate surveillance of influenza-like illness. A GP-based sentinel surveillance network for seasonal influenza is being reinforced in Belgium and from 14 July 2009 onwards has taken over the enhanced system put in place from the beginning of the pandemic. This network aims at characterising the circulating influenza viruses, seasonal or pandemic strain, as well as estimating the burden of disease at community level. The Belgian system for the monitoring of mortality will contribute to observing the situation.

## Conclusions

The introduction of influenza A(H1N1)v virus in Belgium happened in the same way as in other EU/EFTA countries, causing a small but increasing number of cases. Given the uncertainty of the evolution of the current influenza A(H1N1) pandemic, and the emergence of complications in a small proportion of the cases, the Belgian health authorities continue to closely monitor the severity and the spread of the disease in order to provide an adequate response during the coming months.

## Working group:

The Belgian working group on influenza A(H1N1)v is formed by the Flemish Community, the French Community, the Brussels Region, the Hospital Saint-Pierre in Brussels, the Federal Public Service for Public Health and the Belgian Scientific Institute of Public Health, under the coordination of the Interministerial Influenza Coordination Committee. The corresponding author is S. Quoilin, IPH (s.quoilin@iph.fgov.be).

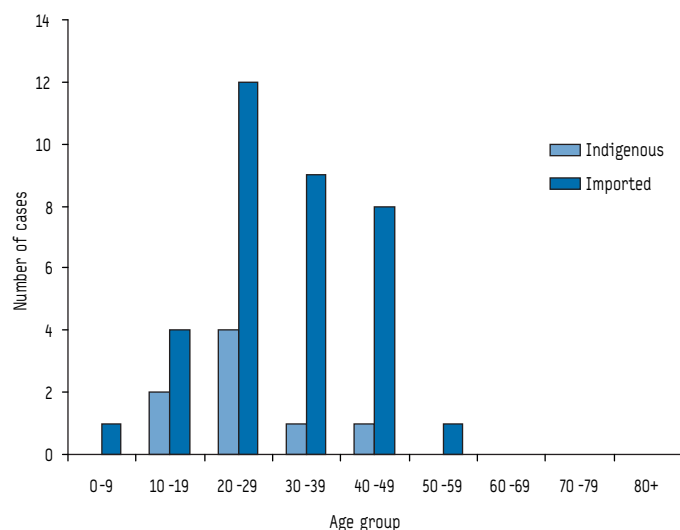
TABLE 2

### Distribution of laboratory-confirmed cases of influenza A(H1N1)v by province of residence, Belgium, 12 May-28 June 2009 (n=43)

Province	Frequency	Percentage
Antwerp	14	32.6
Brussels	4	9.3
East Flanders	7	16.3
Flemish Brabant	10	23.3
Hainaut	2	4.7
Liege	0	0
Limburg	2	4.7
Luxembourg	0	0
Namur	2	4.7
Walloon Brabant	2	4.7
West Flanders	0	0
Total	43	100

FIGURE 2

### Distribution of cases of influenza A (H1N1)v by age group and import status, Belgium, 12 May-28 June 2009 (n=43)



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## Rapid communications

# AN OUTBREAK OF *SHIGELLA DYSENTERIAE* IN SWEDEN, MAY–JUNE 2009, WITH SUGAR SNAPS AS THE SUSPECTED SOURCE

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We report an outbreak of *Shigella dysenteriae* type 2 infections during May–June 2009 in Sweden, involving 47 suspected cases of whom 35 were laboratory-confirmed. The epidemiological investigation based on interviews with the patients pointed at sugar snaps from Kenya as the source. *Shigella* was not detected in samples of sugar snaps. However, *Escherichia coli* was confirmed in three of four samples indicating contamination by faecal material. During April to May 2009 outbreaks with *Shigella* connected to sugar snaps from Kenya were reported from Norway and Denmark. In the three countries trace back of the indicated sugar snaps revealed a complex system with several involved import companies and distributors. In Sweden one wholesale company was identified and connections were seen to the Danish trace back. These three outbreaks question whether the existing international certification and quality standards that are in place to prevent products from contamination by faecal pathogens are strict enough.

### Introduction

Shigellosis is a notifiable disease in Sweden. Annually approximately 500 cases are notified to the Swedish Institute for Infectious Disease Control (Smittskyddsinstitutet, SMI) and about 20% are domestic cases. The majority of the *Shigella* strains are sent to SMI for verification and further typing. Most of the cases are caused by *Shigella sonnei*. Cases with *Shigella dysenteriae* are rare in Sweden. In average five cases are reported each year, including domestic cases and cases infected abroad.

On 10 June 2009 the laboratory at SMI detected six domestic cases of *Shigella dysenteriae* from four different counties and informed the department of epidemiology. Minutes later the county medical officer of another Swedish county (not one of the four mentioned above) telephoned SMI and reported that 25 persons who had visited a restaurant on 31 May were ill with gastrointestinal symptoms. This first information also revealed that a number of them (at the time it was unclear how many) were diagnosed with *S. dysenteriae*. The restaurant was visited by 320 guests that day as it was a holiday (Mother's Day in Sweden).

Six cases of *S. dysenteriae* in a short period of time, although geographically spread, clearly indicated an outbreak. The coinciding report from another county pointed to the possibility of a large outbreak. An outbreak team including investigators from the involved county medical offices, the SMI and the National Food Administration (Livsmedelsverket, SLV) was formed. As this was a national outbreak, the outbreak investigation was coordinated from the SMI.

Routine typing of the isolates from the six domestic cases revealed *S. dysenteriae* type 2. This is a rare type of *S. dysenteriae* with only four cases reported last year and all of them acquired abroad.

All five counties with cases were contacted. In one of the counties a birthday party with 60 guests took place on 30 May. Five persons were ill and one of them was diagnosed with *S. dysenteriae*.

In cooperation with the National Food Administration an investigation was started to try to identify any common food product consumed by known cases.

### Methods

#### Epidemiological investigation

In the county where 25 persons got ill after visit to a restaurant, a list of food items that had been delivered to the restaurant was produced. The persons affected were asked about food items they had consumed at the dinner according to the delivery list. Due to summer vacations and shortage of staff it was unfortunately not possible to perform a cohort study for the restaurant.

In the county where a case of *S. dysenteriae* was linked to a birthday party, the person responsible for purchasing food for the party was asked to list the products served and where they were bought. People who became ill after the party were asked what they had consumed.

In the remaining three counties the infected persons were either interviewed according to a general questionnaire for gastrointestinal diseases or asked by phone what they had consumed. The interviews were performed at the county medical offices and the results were gathered at the SMI for analysis and discussion with the National Food Administration.

#### Microbiological investigation

PFGE was performed on 12 clinical isolates with *S. dysenteriae* type 2 at SMI using the enzyme XbaI.

After sugar snaps had been suspected as the possible source of infection, four samples of sugar snaps were sent to the section for water and environmental microbiology at the SMI for analysis of *Shigella*. Three samples had been collected from supermarkets in two counties and one from a private person in a third county. The sample from this private person was of the same batch as a sample

from one of the supermarkets. Coliforms and *Escherichia coli* were also analysed as indicators for possible faecal contamination.

Sugar snaps were treated as environmental samples where extraction was performed by washing an appropriate amount of sugar snaps in PBS+Tween80. Extracts were then used for analysis of coliforms and *E. coli* by using Colilert-18 as well as an enrichment procedure for the analysis of *S. dysenteriae* where enriched broth was used both for plating on DC-agar and PCR.

## Results

A case was defined as having a domestic laboratory-confirmed *S. dysenteriae*. The case definition was not more specific than that since the infection is so rare and there were no cases to exclude at the time. Of the 47 persons reported to have been affected by the outbreak, 35 were laboratory-confirmed, including three secondary cases (Figure, excluding the three secondary cases). One of the cases with *S. dysenteriae* type 2 was identified in a sixth county more than two weeks after the earliest reported date of onset (Figure). This case was included in the outbreak as it fit the above case definition and had also consumed sugar snaps.

The cases were reported from six counties, all but one situated in the southern or middle part of Sweden. The cases were between 1 and 82 years old and 50% were women. 20 confirmed cases were reported from the restaurant, seven from the birthday party and eight from the remaining four counties. The cases from the birthday party were single cases in five different families. In all, seven persons were infected after the party since the parents of a child who was ill became secondary cases.

Date of onset for all cases in the outbreak was between May 24 and June 15 with the majority of cases reporting onset of symptoms on June 1 to 3 (Figure). One single case with date of onset on June 15 had kept sugar snaps in the refrigerator and consumed them continuously. This person still had sugar snaps left during the time of investigation and they were sent to SMI for analysis.

*Shigella* was not detected in any of the four samples of sugar snaps sent to SMI. However, *E. coli* was confirmed in three samples.

11 of the 12 isolates analysed by PFGE were identical. These isolates were collected from five counties.

The investigation pointed at sugar snaps from Kenya as the source of infection since the majority of the cases from the restaurant, the birthday party and the other counties had consumed sugar snaps. As a result, the local health authority in one municipality decided to impose sales restrictions on sugar snaps, something that was not done in any other municipality. In Sweden this can be decided on local level and does not require decision by the National Food Administration.

At the time of the outbreak sugar snaps from Kenya as well as from other African countries were sold all over Sweden. At least four large wholesale companies and an unknown number of smaller companies import sugar snaps to Sweden. Information from the restaurant and the cases indicated however that the implicated sugar snaps had been distributed by the same wholesaler. Interestingly enough this company is in liaison with the wholesaler that distributed the sugar snaps suspected to have caused the outbreak of *S. sonnei* earlier this year in Denmark.

No more domestic cases with *S. dysenteriae* were reported after the case with the latest date of onset, 15 June.

## Discussion

It was difficult to find samples consumed by cases representative of the suspected food batch. One package from the time of the outbreak was found in one of the case's home. *Shigella* was not isolated from this sample or from samples of sugar snaps from the other two counties. However, it is known that isolating *Shigella* from food specimens can be difficult. *E. coli*, on the other hand, was confirmed in three of the samples and since both bacterial species represent intestinal microorganisms the finding of *E. coli* could still be a good indication that the analysed sugar snaps were contaminated by faecal material.

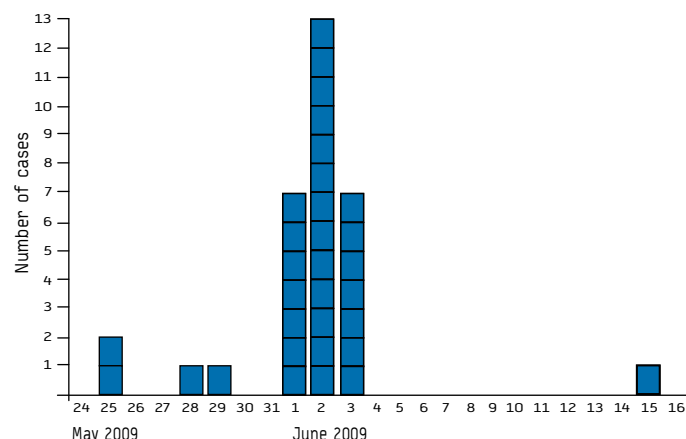
No cohort or case control study was performed in this outbreak as these studies are time consuming and the outbreak coincided with vacations. Personnel at the county medical offices in the involved counties interviewed the persons who were ill and sugar snaps were the only common denominator. Our conclusion is therefore that the most probable source of infection in this outbreak was sugar snaps.

During April to May 2009 outbreaks with *Shigella* connected to sugar snaps from Kenya were reported from two other northern countries; Norway and Denmark [1,2]. Strains of *S. sonnei* were isolated from patients and in Norway a sample of sugar peas was tested positive for *S. sonnei* by PCR. It was probably not a coincidence that *Shigella* outbreaks were connected to sugar snaps from Kenya in three Scandinavian countries within such a short time period.

The investigation performed by the Swedish National Food Administration showed that the trade routes from Kenya are many and diversified. The wholesale companies in Sweden usually have more than one local supplier in Kenya and each supplier in turn packs products from up to 200 local farmers. Trace-back to the farm of origin thus becomes very difficult. The wholesale companies require that each local producer is certified according to GlobalGap which is the golden international quality standard for produce. The question then arises whether this programme is strict enough to

## FIGURE

Confirmed cases of *Shigella dysenteriae* type 2 in an outbreak in Sweden in May-June 2009, by date of onset of symptoms (n=32)





prevent products from being contaminated by faecal pathogens or whether these regulations have not been followed adequately. According to available information, the period of growth this year in Kenya was dry and that normal production volumes could not be reached. Maybe the dry conditions led local producers to use contaminated water for irrigation.

The number of cases included in this outbreak is probably an underestimation of the actual number of persons affected as is the case in food-borne outbreaks in general. The county medical officer in the county with the restaurant outbreak was convinced that a number of people who had visited the restaurant and fallen ill afterwards did not seek healthcare and were not sampled. We may suppose that this was probably the case also in other counties.

Outbreaks with *Shigella* sp. are uncommon in Sweden but in 2008 there was a large outbreak in Stockholm with 140 cases infected with a very rare type of *S. sonnei* (mannitol negative). This was the largest outbreak of shigellosis in Sweden during the last 30 years. The cases had visited the same lunch restaurant. A cohort study pointed at grated carrots of Swedish origin as the suspected vehicle in the outbreak but this was not laboratory-confirmed [3].

The recent *Shigella* outbreaks in Denmark, Norway and Sweden, most likely associated with imported sugar peas from Africa, revealed a complex import system for sugar peas involving various wholesalers and distributors and numerous growers. The dimension of the system raises concern whether the existing international certification and quality standards that prevent products from being contaminated by faecal pathogens are strict enough.

As sugar peas are sold as a ready-to-eat product, consumers should be aware of the risk of possible contamination by faecal bacteria that can cause gastroenteritis. It is advisable to wash the vegetables or even better heat them up quickly. During the outbreak information on correct handling of vegetables to avoid infection was published on SMI and SLV websites. However, it will be discussed whether this kind of information should be disseminated more widely to prevent similar outbreaks in the future.

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# Surveillance and outbreak reports

## A FOODBORNE OUTBREAK DUE TO *CRYPTOSPORIDIUM PARVUM* IN HELSINKI, NOVEMBER 2008

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We report the first foodborne outbreak caused by *Cryptosporidium parvum* in Finland. The outbreak occurred among personnel of the Public Works Department in Helsinki, who had eaten in the same canteen. 72 persons fell ill with diarrhoea, none was hospitalised. Four faecal samples obtained from 12 ill persons were positive for *Cryptosporidium* by an antigen identification assay and microscopy. The vehicle of infection could not be identified with certainty but a salad mixture was suspected.

### Introduction

*Cryptosporidium* infection is transmitted by the faecal-oral route and results from the ingestion of *Cryptosporidium* oocysts through the faecally contaminated water or food or through direct person-to-person or animal-to-person contact [1]. The infectious dose is low, 10-30 oocysts [2,3]. The reported foodborne outbreaks are not as common as those caused by swimming in water. In the United States, *Cryptosporidium* is the leading cause of reported recreational water-associated outbreaks [1,4,5].

On 12 November 2008, the Food Control Unit and the Epidemiology Unit of the Helsinki City Health Department were alerted of a gastroenteritis outbreak among the clients of the canteen of Public Works Department. Tens of people had fallen ill within the two weeks since 31 October. The main symptoms were watery diarrhoea, which lasted approximately one week, abdominal pain, fatigue and nausea. All persons affected had eaten at the canteen of the Public Works Department.

### Materials and methods

The canteen of Public Works Department belongs to a large chain of catering services. The daily lunch includes three dishes of warm food, salad buffet and bread. Approximately 100-150 persons of the total personnel of 400 use daily the services of the canteen.

According to the standard procedures, the Food Control Unit listed the foods served between 22 and 31 October. The number of different foods and drinks served in the canteen was about 30 per day. A retrospective cohort study was carried out among the personnel by the Food Control Unit. A detailed questionnaire on symptoms and consumption of canteen food during the period of 22 to 31 October was e-mailed to all 400 staff members on

18 November. Completed questionnaires were obtained from 127 persons (response rate 32%). A case was defined as a person with diarrhoea (at least four loose stools a day) or laboratory-confirmed *Cryptosporidium* infection during the period from 31 October to 14 November. Associations between food items and illness were assessed by univariate analysis using the chi-squared test.

In late November, the health inspector examined the consignment records of the canteen and found that some salads had not been included in the questionnaires. These included a mixture of lettuce packed of red and green colour by a Swedish company. The salad mixture had been served during two or three days on the week before the beginning of the outbreak. A separate case-control study was carried out on 19 December. In order to find out about the consumption of the salad mixture, 30 cases and 30 controls randomly identified from the cohort study were interviewed by phone. Of the cases 29, and of the controls 30 replied.

The canteen was inspected on 14 November and 19 samples of foods and spices used between 27 and 31 October were taken. The food samples of the previous week had already been disposed. The food samples were analysed for *Escherichia coli*, enterococci, *Staphylococcus aureus*, *Campylobacter*, yeasts and molds, and later for *Cryptosporidium*. Some specimens were analysed also for total aerobic bacteria count, *Bacillus cereus* and *Enterobacteriaceae*. Two drinking water samples were taken on 11 November and analysed for total number of aerobic microbes, faecal coliforms, *Escherichia coli* and free and total chlorine, and estimated for colour, taste, odour and appearance as part of the internal quality control. No irregularities in the kitchen conditions, functions of the staff or in complying with internal quality control were found.

Stool samples were taken on 12 to 14 November from 10 ill guests of the canteen and from two ill members of the kitchen staff. The samples were initially tested for *Campylobacter*, *Salmonella*, *Shigella* and *Yersinia* spp. as well as norovirus. On 17 November, the investigating team requested stool samples to be analysed for *Cryptosporidium*. The samples were analysed by using Remel's (Lenexa, US) ProSpecTRGiardia/Cryptosporidium and ProSpecTR. Presence of *Cryptosporidium* was further verified from all positive



samples by modified Ziehl-Nielsen staining. Faecal DNA samples of three patients were available for PCR analysis [6].

## Results

Seventy-two persons (41 women, 31 men) met the case definition. The mean age was 48 years. The outbreak peaked on 3 to 4 November when 38 cases fell ill (Figure). Two members of the kitchen staff reported diarrhoea with the onset on 3 November. Watery diarrhoea (100%), fatigue (85%), abdominal pain (76%), nausea (69%) and headache (61%) were the most common symptoms. Fever (31%) and vomiting (21%) were reported less often and some patients reported arthralgia or myalgia. The epigastric pain was often described as very severe. Two persons had to visit hospital emergency services, but none was hospitalised.

Four stool samples of 12 persons were found positive for *Cryptosporidium*. None of them belonged to kitchen staff. No other pathogens were found. Control samples taken from the infected persons approximately two weeks later were found negative for *Cryptosporidium*. In one sample, the amplification of *Cryptosporidium*-specific PCR product was successful and the sequence had 100% similarity with the sequence of *C. parvum*.

Food samples were negative for *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella*, *Campylobacter* and enterococci. Methods to analyse *Cryptosporidium* from food samples have not been built up and validated in Finland, but the salads were examined by using the same method as for stool samples. These results were negative, too. Analyses of drinking water suggested no faecal contamination. The total number of aerobic bacteria was 1 and 0 cfu/ml.

The analysis of the cohort study did not show significant association between any of the foods served and the illness. In the case-control study, the odds ratio for consumption of the salad mixture was 22.5 (95% CI 3.5–177.9).

The imported lot of the salad mixture weighted 486 kg and consisted of two batches. The batches contained salads from Denmark, France, Spain, Italy and Sweden. According to the records, the lot was divided and sold in small quantities to 130 premises in various municipalities all around Finland. The Building

Department canteen received 1.5 kg of the salad mixture possibly originating from both of the batches. Thus, the exact tracing was not possible.

## Discussion

More than 70 guests of a canteen of the Public Works Department of Helsinki fell ill with gastroenteritis in October 2008. The symptoms were compatible with cryptosporidiosis and *Cryptosporidium* spp. was detected in stool specimens of four patients. Genotyping of one isolate showed that the causative agent was *C. parvum*. A case-control study suggested that mixed salad was the source of the outbreak. This was the first time that *Cryptosporidium* was found to cause an outbreak in Finland.

All workers of the Public Works Department, personnel of occupational health authorities and the National Public Health Institute were immediately informed about the outbreak. Persons having diarrhoea were instructed about their personal hygiene and were forbidden to use public swimming pools until the end of November. On 21 November, a press release about the outbreak was issued by the local authorities.

Vegetables, and especially salads, have been shown to be an important source of foodborne outbreaks recently [7,8,9]. Specifically, *Cryptosporidium* was linked to consumption of vegetables in Nordic countries [10,11]. In addition, *Cryptosporidium* was found in samples from fresh produce [12,13]. In our outbreak, the vehicle transmitting *Cryptosporidium* was unfortunately not found. Information about the outbreak came so late to the municipal authorities that relevant food samples were no longer available. Food Control Department of Helsinki recommends that in institutional kitchens, frozen samples of 200 g from all served foods should be stored for two weeks to enable microbiological investigations after possible outbreaks. Operators of either the producer or the importer of the suspected salad did not comply with the legislation of the European Union. The Article 18 of the Regulation 178/2002 of the European Parliament and of the Council states that the traceability of food or any substance intended to be, or expected to be, incorporated into a food product shall be established at all stages of production, processing and distribution. Food business operators should be able to identify the operators from whom they have been acquiring food and also the ones where food has been delivered to. In addition, the salad finally suspected to be the vehicle, was not included in the initial questionnaire due to an error of the kitchen personnel.

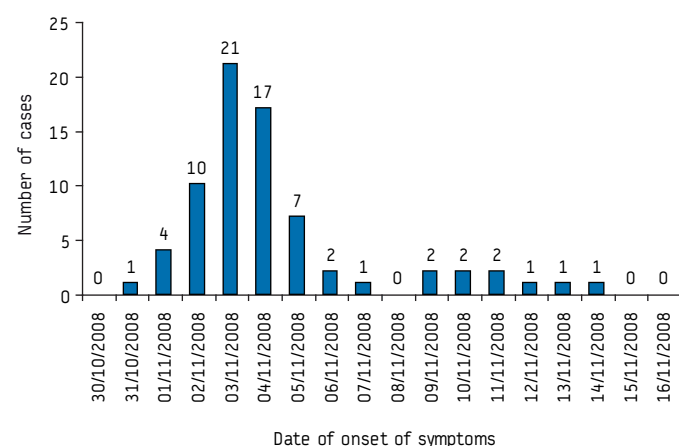
The outbreak described here shows that the public health authorities should be aware of the possibility of foodborne infections caused by protozoa, not only by bacteria and viruses. Testing for *Cryptosporidium* should be included in the panel of tests performed in gastrointestinal illness and appropriate methods to detect *Cryptosporidium* in food samples should be developed. It is also imperative that food handlers are aware that proper handling of vegetables is an important method to prevent transmission.

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## FIGURE

Date of onset of symptoms in persons affected by an outbreak of cryptosporidiosis, November 2008, Helsinki, Finland (n=72)



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## Research articles

# SEQUENCE-BASED AND MONOCLONAL ANTIBODY TYPING OF *LEGIONELLA PNEUMOPHILA* ISOLATED FROM PATIENTS IN PORTUGAL DURING 1987-2008

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The monoclonal antibodies and the sequence-based typing (SBT) are two methodologies widely used to characterise *Legionella pneumophila* strains serogroup 1 (sg1). In this study, we analysed the clinical strains received in two Portuguese laboratories since 1987, including the strains isolated in Portugal during the four years of the surveillance scheme for Legionnaires’ disease implemented in 2004. In total, 63 clinical isolates of *L. pneumophila* sg1 were differentiated by SBT into 19 different sequence types. Ten of them were new in the SBT database of the European Working Group for Legionella Infections (EWGLI). As a result of the combination of the two methodologies, these strains were discriminated into 25 different profiles. This study enabled, for the first time in Portugal, not only to characterise the *L. pneumophila* sg1 clinical isolates, but also to create a database of Portuguese profiles for use in epidemiological surveillance efforts.

### Introduction

*Legionella pneumophila* is a Gram-negative facultative intracellular pathogen, which is responsible for Legionnaires’ Disease. This microorganism has increasingly been recognised as an important cause of pneumonia since its first description in 1977 [1]. The characterisation of clinical isolates of *L. pneumophila* enables us to learn about its epidemiology in a certain geographic region, as well as to create a database of circulating profiles [2-7].

The combination of a genotypic method with monoclonal antibody (MAb) typing has been described as a useful approach for epidemiological typing of *L. pneumophila* isolates [7-10]. MABs of the Dresden panel allow subdividing the serogroup 1 of *L. pneumophila* as having, or not having, the epitope recognised by the MAB 3/1. According to epidemiological studies, this epitope appears to be associated with virulence [11]. Sequence-based typing (SBT) is one of the genotypic methods that can be applied for this purpose. It was adopted as an international standard and is widely used by the members of the European Working Group for Legionella Infections (EWGLI), since it is a simple, rapid and discriminatory typing method. Furthermore, it also allows the exchange of data between laboratories [7,8].

In 1999, the Portuguese public health authorities implemented a surveillance scheme for Legionnaires’ Disease based on clinical reports. Later, in 2004, a surveillance scheme based on

laboratory notifications was added. The Legionella laboratory in the microbiology department of the Faculty of Medical Sciences in Lisbon and the National Institute of Health Dr Ricardo Jorge (INSA) are the two laboratories involved in this surveillance scheme.

The aim of this study was to investigate the distribution of sequence types (ST) and monoclonal antibody subtypes among clinical isolates of *L. pneumophila* in Portugal.

### Material and methods

As far as the present study is concerned, the SBT methodology, using seven genes (*flaA*, *pilE*, *asd*, *mip*, *mompS*, *proA* and *neuA*), was applied to 63 clinical isolates of *L. pneumophila* serogroup 1 (sg1), and four from non-sg1 (one isolate was sg 10, another was sg 12 and the two remaining reacted with “*Legionella pneumophila* serogroups 2-14 Latex Test Reagent” (Oxoid), but the serogroup could not be determined using our MABs protocol) (see Table). The *L. pneumophila* strains were typed with MABs of the Dresden panel, by using an indirect immunofluorescence test [10,11].

We analysed the clinical strains received since 1987 by the laboratories of Santa Cruz Hospital and the Faculty of Medical Sciences, including the 19 strains isolated during the four years of the surveillance scheme for Legionnaires’ disease. In total, 67 strains were sent for typing by 17 Portuguese hospitals. Thirty of them were isolated from patients with nosocomial infections and 20 from patients with community-acquired infections; the remaining 17 had an undetermined origin.

The genomic DNA used for the SBT method was extracted with the InstaGene Matrix kit (Bio-Rad), and the PCR amplification was performed by using puRe Taq Ready-to-Go beads (Amersham Biosciences). The primers and the PCR conditions were the same as those used by Gaia *et al.* and Ratzow *et al.* [7,8,12]. After purification with the Qiaquick PCR purification Kit (Qiagen), both strands of the amplicons were sequenced by StabVida on a 3700 ABI DNA sequencer (Applied Biosystems) using the Big Dye terminator DNA sequencing kit. The nucleotide sequences obtained were compared to those in EWGLI-SBT database [13]. All putative new sequences were confirmed before being sent to the curators of the database.



## Results

In this study, all but three of the strains included were typable by SBT using the seven genes (see Table). The *neuA* primers failed to type these three strains, all of which were non-sg1 (one sg 10 and the other two could not be identified with MABs of Dresden panel), suggesting that *neuA* primers described by Ratzow *et al.* [12] are not always suitable for serogroups other than sg1. Other teams have also reported amplification problems with the *neuA* primers [14].

Applying SBT, the sample was discriminated into 7, 7, 11, 8, 12, 7 and 7 types, based on the sequences of *flaA*, *pilE*, *asd*, *mip*, *mompS*, *proA* and *neuA*, respectively. As a consequence,

the 67 isolates were divided into 23 STs in total. The distribution was as follows: the 63 *L. pneumophila* sg1 isolates were included into 19 ST, and the four *L. pneumophila* non-sg1 isolates into the remaining four ST.

Ten of the 19 STs from *L. pneumophila* sg1 and the four STs from *L. pneumophila* non-sg1 were different from the ones that already existed in the EWGLI-SBT database. In addition, six new allele numbers (22 and 29 for the *mip* gene, and 24, 20, 34 and 23 for the *pilE*, *asd*, *mompS* and *proA* genes, respectively) were assigned by the curators after our data were submitted to the database. It is interesting to notice that five of these new allele numbers were detected only in *L. pneumophila* non-sg1 strains

TABLE

Twenty-three SBT profiles of 67 Portuguese *L. pneumophila* clinical isolates, 1987-2008

ST	Allelic profile <sup>a</sup>	No. of strains	Dresden panel			Epidemiological relatedness
			Serogroup	MAB subgroup	No. of strains	
100 <sup>b</sup>	3,8,1,10,14,12,2	32	1	Allentown/France	18	Related
				Philadelphia	14	
1	1,4,3,1,1,1,1	3	1	Philadelphia	2	Unrelated
				Olda	1	
23	2,3,9,10,2,1,6	3	1	Philadelphia	2	Unrelated
				Knoxville	1	
62	8,10,3,15,18,1,6	3	1	Allentown/France	2	Unrelated
				Philadelphia	1	
103 <sup>b</sup>	1,4,3,22 <sup>b</sup> ,1,1,1	3	1	Philadelphia	3	Unrelated
20	2,3,18,15,2,1,6	2	1	Knoxville	2	Unrelated
42	4,7,11,3,11,12,9	2	1	Knoxville	1	Unrelated
				Benidorm	1	
44	4,8,11,10,10,12,2	2	1	Allentown/France	1	Unrelated
				Philadelphia	1	
99 <sup>b</sup>	4,8,11,5,29,12,10	2	1	Knoxville	2	Unrelated
101 <sup>b</sup>	6,10,15,15,21,4,6	2	1	Philadelphia	1	Unrelated
				Knoxville	1	
16	2,10,18,10,2,1,9	1	1	Knoxville	1	
22	2,3,6,10,2,1,6	1	1	Philadelphia	1	
94	12,8,11,5,20,12,2	1	1	Knoxville	1	
98 <sup>b</sup>	8,10,3,10,2,5,6	1	1	Philadelphia	1	
102 <sup>b</sup>	8,19,5,15,18,5,10	1	1	Philadelphia	1	
146	2,10,18,10,2,1,6	1	1	Philadelphia	1	
172 <sup>b</sup>	1,4,3,1,1,1,2	1	1	Philadelphia	1	
173 <sup>b</sup>	6,10,14,15,21,4,6	1	1	Knoxville	1	
174 <sup>b</sup>	4,8,11,5,10,12,15	1	1	Allentown/France	1	
153 <sup>b</sup>	2,10,3,28,9,14,3	1	12	---		
c,b	6,10,21,28,4,14,0	1	10	---		
c,b	2,24,20,29,34,23,0	1	d	---		
c,b	3,24,1,29,34,23,0	1	d	---		

SBT: sequence-based typing; ST: sequence type.

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

b New profiles and allele numbers are in bold.

c Problems in amplifying the *neuA* gene.

d Strain reactive with "Legionella pneumophila serogroups 2-14 Latex Test Reagent" (Oxoid). Serogroup could not be determined using MABs of Dresden panel.

that were non-typable with MABs from the Dresden panel (see Table). The ST100 (3,8,1,10,14,12,2) was the most frequent allele (32/67). This is a new profile and all of the ST100 strains had been isolated in patients of the same hospital over a period of several years. Twenty-four of the 32 strains with this profile came from nosocomial infections and the remaining eight from undetermined origin. These eight patients had subacute diseases and needed hospital care frequently, suggesting that some or even all of these sporadic cases could be hospital-acquired, too. The STs 1, 20, 23, 42, 44, 62, 99, 101 and 103 were found in more than one strain. The 22 strains belonging to these nine STs were unrelated according to their source origin. In this study, ST1 (1,4,3,1,1,1,1), the most frequent profile reported in the world, was found only in three isolates (see Table).

The 19 strains sent by the surveillance scheme during the past four years, showed high profile diversity. Eleven distinct STs were obtained, five of them for the first time in Portugal. These strains were isolated in 11 different hospitals, five, four and two, respectively, from the north, the centre and the south of Portugal. The majority of the isolates came from community-acquired infections (12/19).

Using the Dresden panel of MABs, the 63 *L. pneumophila* sg1 strains had previously been divided into five different subgroups (unpublished data). All strains but one possessed the virulence-associated epitope recognised by MAB 3/1 [11], and the Philadelphia subgroup was the most frequent with 28 of the 63 strains (see Table). As a result of the combination of the two methodologies, MABs and SBT, these strains were now differentiated into 26 different profiles. The results showed that the Philadelphia subgroup was the most heterogeneous as it was divided into 12 different STs. On the other hand, identical STs were found among strains reactive with different MABs (see Table). These two facts support the idea that it is valuable to add genotyping methods to MAB typing when defining profiles within a phenotypic subgroup [7,9].

## Discussion

As far as our experience is concerned, the SBT scheme is technically simple for a laboratory with basic molecular expertise and equipment, provided that there is access to a sequencing laboratory. Although this method proved to be a good genotypic method for epidemiological investigations, showing unambiguous results that are easy to interpret [4,6-8], one of the limitations of the epidemiological studies is the fact that most diagnoses are made by urinary antigen test, without strain isolation. The EWGLI 2008 database showed that culture was the methodology used in only 62 of the 866 reported cases in the 35 countries participating in EWGLINET [15]. In Portugal, the data were similar: in the past four years, the strain was isolated for only 19 of 237 *Legionella* notifications (unpublished data). Thus, *Legionella* isolates are not available for the majority of cases and therefore the results of this study may not entirely reflect the distribution of the *Legionella* strains responsible for the disease in Portugal. However, our collection contains the majority of the clinical isolates collected in Portugal since 1987; so it is possible that this sampling is representative of the profiles circulating in Portugal.

The significant profile diversity we observed is in accordance with reports from the other countries [4,14,16,17]. Due to the relatively low number of isolates in each ST, with the majority

(13/23) of the STs being detected only once, it is not possible to establish a correlation between the ST and the infection origin.

To summarise, this study enabled us, for the first time in Portugal, to characterise the *L. pneumophila* clinical isolates with SBT methodology and MABs, as well as to create a database of Portuguese *L. pneumophila* profiles for use in epidemiological surveillance efforts. It was also a contribution to the EWGLI-SBT database and to the knowledge of the European *L. pneumophila* diversity, owing to the high rate of new STs obtained.

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