Outbreaks of monophasic *Salmonella enterica* serovar 4,[5],12:i:- in Luxembourg, 2006

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A monophasic *Salmonella enterica* serovar 4,[5],12:i:- phage type DT193 emerged as the dominant serovar in Luxembourg in 2006, when it caused two major outbreaks involving 133 laboratory-confirmed human cases, 24 hospitalisations, and one death. The outbreak strain had an uncommon pulsed-field gel electrophoresis pattern STYMXB.0031 and antibiotic resistance profile ASSuT. A high proportion of cases were clustered in institutions for the elderly and in day-care centers. Strains identical to the outbreak strain were recovered from two control meals, a nappy changing table, retail sausages and caecal porcine samples at an abattoir. Locally produced pork meat is strongly suspected to have been the vehicle for the outbreaks, although the precise mechanisms remain unclear.

Introduction

Salmonella enterica is one of the most common causes of foodborne gastroenteritis. Reflecting the trend in Europe as a whole, most human cases in Luxembourg have recently been due to serovars *S.* Enteritidis (typically associated with eggs or chicken) or *S.* Typhimurium (typically associated with pork) [1]. Between 2000 and 2004, an annual average of 360 laboratory-confirmed *Salmonella* isolates were referred to the National Health Laboratory in Luxembourg. Of those, 66% were *S.* Enteritidis and 20% were *S.* Typhimurium.

In recent years, the emergence of a *Salmonella enterica* monophasic serovar 4,[5],12:i:- has been described that was responsible for human cases in New York [2], Spain [3], Brazil [4], Thailand [5] and Taiwan [6]. The strains are called monophasic because they lack the second-phase flagellar antigen, represented by the '-' after the second colon in the antigenic formula 4,[5],12:i:-. Genotypic, biochemical and phenotypic characterisations indicate that such strains usually represent monophasic variants of the serovar *S.* Typhimurium [7]. Between 2000 and 2005, human cases with this monophasic serovar 4,[5],12:i:- were rare in Luxembourg, with on average two to three cases reported annually. However, *Salmonella* serovar 4,[5],12:i:- was responsible for the two large outbreaks in 2006 that are described in this report and is now the dominant human serovar in Luxembourg.

Methods

The microbiology unit of the National Health Laboratory in Luxembourg is the reference laboratory for human salmonellosis and member of the European Enter-net surveillance network [8]. Human *Salmonella* isolates from all private and hospital laboratories as well as veterinary isolates from food safety, animal feed control and animal pathology laboratories in Luxembourg are characterised by serotyping, antibiotic resistance typing (disk diffusion method) and, since 2003, also by pulsed-field gel electrophoresis (PFGE) using the Pulsenet protocol [9]. In 2005, the cooperative research project EPIFOOD was initiated between all public institutions involved in food safety in Luxembourg. It systematically conducts enhanced sampling of different levels of the food chain and compares bacterial pathogens in the food chain with human isolates using molecular typing methods. In particular, routine sampling of bovine and porcine caecal contents was started in all three abattoirs in Luxembourg with the aim of isolating *Salmonella*. On those farms where the routine programme detected *Salmonella*, additional samples were taken.

During the investigation of the 2006 outbreaks, the patients were contacted and sent a detailed questionnaire on medical symptoms and food consumption prior to illness. In supermarkets and catering facilities of institutions in which patients were staying, samples were taken from food items that were considered at risk.

Results

An unexpected increase in gastrointestinal disease was noted in the *Salmonella* reference laboratory during a three-week period

Figure 1

Weekly number of human monophasic *Salmonella* cases in Luxembourg in 2006, based on date of stool sample collection

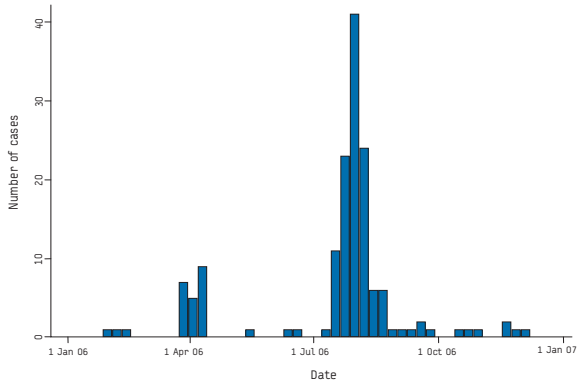
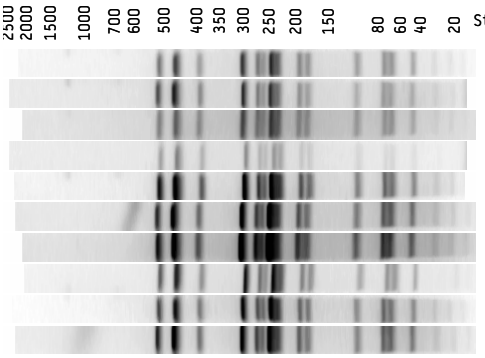


Figure 2

Pulsed-field gel electrophoresis profiles of human, food and veterinary strains implicated in the outbreaks in Luxembourg, 2006



Strain ID	Date	Strain origin
CO60533	April 06	Human feces
CO61153	July 06	Human feces
CO61233	July 06	Cooked ham, institution for elderly
CO61272	July 06	Control meal with ham, day care centre
CO61273	July 06	Control meal, day care centre
CO61314	August 05	Nappy changing table, day care center
EO60804	August 06	Mixed barbecue meat, butcher shop
EO50287	December 05	Porcine feces, abattoir
EO60510	June 06	Porcine feces, abattoir
CO61323	August 06	Porcine feces, farm

in spring 2006. Initially, *S. enterica* monophasic serovar 4,[5],12: i:- was confirmed in 21 human cases by the National *Salmonella* Reference Laboratory (Figure 1). Almost all strains isolated from patients had the pulsed-field gel electrophoresis (PFGE) profile STYMXB.0031 (Figure 2) and antibiotic resistance type (R-type) ASSuT, i.e. were resistant to ampicillin (A), streptomycin (S), sulfonamides (Su) and tetracycline (T) [10-12]. The PFGE and antibiotic resistance profiles of this outbreak strain were identical to strains isolated during a monitoring programme in late December 2005 from caecal contents of swine slaughtered at one of three abattoirs in Luxembourg (Abattoir A).

The outbreak investigation did not reveal any common sources of exposure or other risk factors. No unexpected increase was reported in other countries through the European Enter-net surveillance network, although a few concurrent human cases of this monophasic serovar with the same antibiotic resistance pattern were reported in Germany, Hungary (N Nogrady, personal communication), Scotland (D Brown, personal communication) and Switzerland (H Hächler, personal communication). Strains with the same PFGE profile and R-type were also found in pork food samples of German origin in Germany and Denmark (M Torpdahl, personal communication). The initial spring outbreak in Luxembourg eventually stopped in mid-April (Figure 1). No additional public health measures were taken. No common source or food vehicles were identified, although the local and international circumstantial evidence suggest that the cause was probably a pork product.

A much larger outbreak of *S. enterica* started in mid-July, involving 112 cases over a period of six weeks (Figure 1). Again, almost all strains had phage type DT 193, PFGE profile STYMXB.0131 [10-12] and the antibiotic resistance profile ASSuT. The outbreak investigation revealed that approximately half of the cases were clustered in institutions for the elderly or handicapped, and in a day-care centre for young children. 24 patients (21%) were hospitalised and one person aged 64 years died of bacteraemia. The hospitalisation rate of 21% is similar to those reported for all *Salmonella* serovars in Denmark (mean 18%) [13], and for multidrug-resistant *Salmonella* outbreaks in the United States (median: 26%) [14]. Following the identification of the outbreak, 145 food samples were obtained either from the kitchens of institutions that were linked to patients (one hotel, three institutions for the elderly, one restaurant and one day-care centre for children), or directly from retail outlets (five supermarkets and one wholesale store linked to the institutions mentioned above). Strains identical to those isolated from the patients could be recovered from two control meals: the first at an institution for the elderly, the second at a day-care centre in which the same strains were also recovered from a nappy changing table (Figure 2). The day-care centre was closed temporarily for professional disinfection, and hygiene procedures were reinforced through staff education programmes. Most *Salmonella*-positive food samples could be linked to meat from Abattoir A .

An identical *S. enterica* strain had again been recovered, three weeks prior to the July outbreak, from porcine caecal contents at Abattoir A as part of the monitoring programme (Figure 2). During the veterinary inspection, Abattoir A reported problems with hygiene procedures during the summer due to a combination of unusually hot weather and temporary staff during the holiday period.

In addition, on two occasions in August the outbreak strain was recovered at a farm from porcine faeces that had been found positive at the abattoir. No *Salmonella* could be isolated from animal feed used on this farm. Following the main outbreak wave in July/August, a further 22 sporadic human cases were reported in the nine month period between 1 September 2006 to 31 May 2007. This suggests that the outbreak is still ongoing due to continued presence of the *Salmonella* serovar 4,[5],12:i:- in the food chain, albeit at lower levels than during the summer of 2006.

Discussion

To our knowledge, this is the first report of a human outbreak of multidrug-resistant *Salmonella* monophasic serovar 4,[5],12: i:- phage type DT193 with resistance pattern ASSuT. Phage type DT193 with the antibiotic resistance pattern ASSuT has previously been reported only in swine in Spain and the United States [15]. It represents the largest *Salmonella* outbreak recorded in Luxembourg in the last 20 years. As a result, the monophasic serovar 4,[5],12: i:- has become the dominant serovar in 2006, surpassing both *S.* Enteritidis and *S.* Typhimurium in frequency.

The reasons behind this large outbreak in summer were probably multi-factorial and included a high prevalence of the strain on pig farms prior to the outbreaks and poor compliance with hygiene procedures at an abattoir during the holiday period and by catering staff of the institutions involved in the human outbreaks. However, the precise details of what went wrong in the abattoir or further

down along the food chain, and how it could be prevented in the future, remain unclear. Equally unclear are the reasons behind the prevalence of this *Salmonella* strain on pig farms in Luxembourg. July 2006 was an exceptionally warm month in Luxembourg, and hot weather has been linked to an elevated incidence of *Salmonella* infections before, even in the absence of particular outbreak situations [16].

Our outbreak investigation was clearly helped by genotyping methods. The outbreak strain had an uncommon antibiotic resistance and PFGE profile. Moreover, we were able to detect the outbreak strain at an abattoir several months prior to the major outbreak, which facilitated the identification of a likely vehicle of the outbreak. One practical implication for laboratories in the human, food safety and veterinary field is that testing the second phase of the flagellar antigen is essential in order to identify this serovar correctly and to distinguish it from *S.* Typhimurium.

The emergence of this monophasic serovar also has implications with regards to public health reporting, nomenclature and food safety regulations. Genetically, phenotypically and in terms of pathogenicity, the monophasic serovar should be considered a variant of *S.* Typhimurium [3]. Regarding it as distinct from serovar *S.* Typhimurium could imply that it is a rare and unusual serovar, and its public health importance could easily be underrated.

We believe that routine comparison of food chain and human *Salmonella* isolates using molecular typing tools is a powerful tool for monitoring food safety and protecting public health. However, close cooperation between all veterinary, food safety and human public health sectors is key to quick detection and successful control of both well-known and newly emerging foodborne pathogens.

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