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Contents

Special focus: Zoonotic diseases

Surveillance and outbreak reports

Research articles

Perspectives

Rapid communications

News

Zoonoses are one of the major causes of foodborne disease in Europe. Eurosurveillance reports on several outbreaks and highlights the importance of surveillance and monitoring of potential new sources of contamination

Campylobacteriosis and other bacterial gastrointestinal diseases in Sofia, Bulgaria for the period 1987-2008

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Campylobacter **is the most commonly reported enteric bacterial pathogen in humans. We still do not have any systematic data concerning campylobacteriosis in Bulgaria. For that reason, we present data of the thermophilic *Campylobacter jejuni* and *Campylobacter* coli in the aetiology of diarrhoeal diseases in Sofia, for the period from 1987 to 2008. The study included patients from 0 to over 65 years old. A total of 51,607 faecal specimens were screened for *Campylobacter*. *C. jejuni* and *C. coli* were detected in 3.58% (1,847) of the strains, with the highest percentage in 1988 (7.5%) and the lowest in 2006 (0.3%). Campylobacteriosis occurred most frequently in the wet months of March, April, May and June, with 105, 102, 124 and 141 cases, respectively, and was rare in January with 25 cases. The most affected groups were children between 0 and 4 years of age (52%) and between five and 14 years of age (30%). *Campylobacter* infection occurred in 22% of all bacterial gastrointestinal diseases in the city of Sofia during the study period. *Salmonella* was the most frequently identified pathogen with 32%, followed by *Shigella* (30%), *Campylobacter* (22%) and diarrhoeagenic Escherichia *coli* (16%). The study shows that *Campylobacter* plays an important role as a bacterial cause of enterocolitis in Sofia, Bulgaria.**

Introduction

Campylobacteriosis is an infectious disease caused by thermophilic members of the bacterial genus *Campylobacter*. *C. jejuni* and *C. coli* are among the most important enteropathogens that cause gastroenterocolitis. The rate of *Campylobacter* infections worldwide is increasing, with the number of cases often exceeding those of salmonellosis and shigellosis [1,2]. These reported numbers of campylobacteriosis in many countries have revealed that this infection is emerging and becoming a major public health problem. According to the World Health Organization (WHO) *Campylobacter* is one of the most frequently isolated bacteria from stools of infants with diarrhoea in developing countries [3]. Despite the fact that campylobacteriosis is a notifiable disease in Bulgaria, there is no systematic data concerning this infection. In this report, we present data on the role of *C. jejuni* and *C. coli* compared to the other bacterial agents of diarrhoeal diseases in Sofia, Bulgaria.

Methods

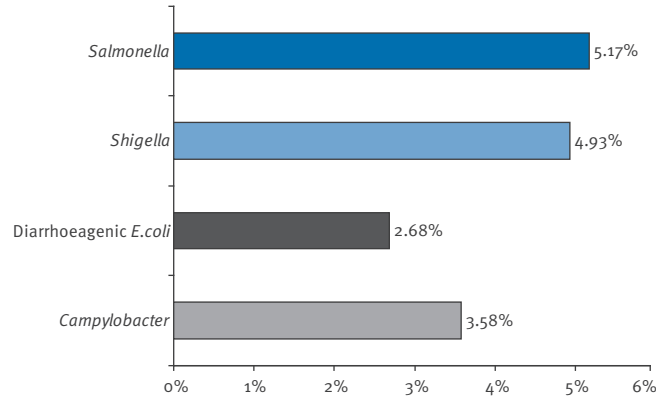
The study covered a period from 1987 till 2008 in Sofia, Bulgaria. Sofia has a population of about 1.5 million inhabitants. A total of 51,607 faecal specimens obtained from patients with enterocolitis were investigated for *Campylobacter*, *Salmonella*, *Shigella* and diarrhoeagenic *Escherichia coli*, i.e. enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC) and enterohaemorrhagic (EHEC) *E. coli*). Data were provided from the department of epidemiology at the National Centre of Infectious and Parasitic Diseases (NCIPD), Sofia, based on isolations of these bacterial pathogens by the Regional Inspectorate of Public Health Protection and Control, Sofia, and by five hospital and private laboratories in Sofia. The age of the patients ranged from 0 to over 65 years.

Culture media

The laboratory methods for *Salmonella*, *Shigella* and diarrhoeagenic *E.coli* were done according to the national standard method for diagnosis of enteric bacteria [4]. Faecal specimens for *Campylobacter* were

Figure 1

Percentage of bacterial enteropathogens isolated from 51,607 faecal samples collected in Sofia, Bulgaria from 1987 to 2008



inoculated on selective media containing 10% defibrinated sheep blood agar with *Campylobacter* selective supplement (BUL BIO-NCIPD, Bulgaria) and five antibiotics (vancomycin, trimethoprim, cefalotin, rifampicin and nystatin). The inoculated selective media were incubated for 48 hours in microaerophilic atmosphere with 10% CO2 and 5-8% O2, generated from packages Helico-Campy Pack (BUL-NCIPD, Bulgaria).

Results

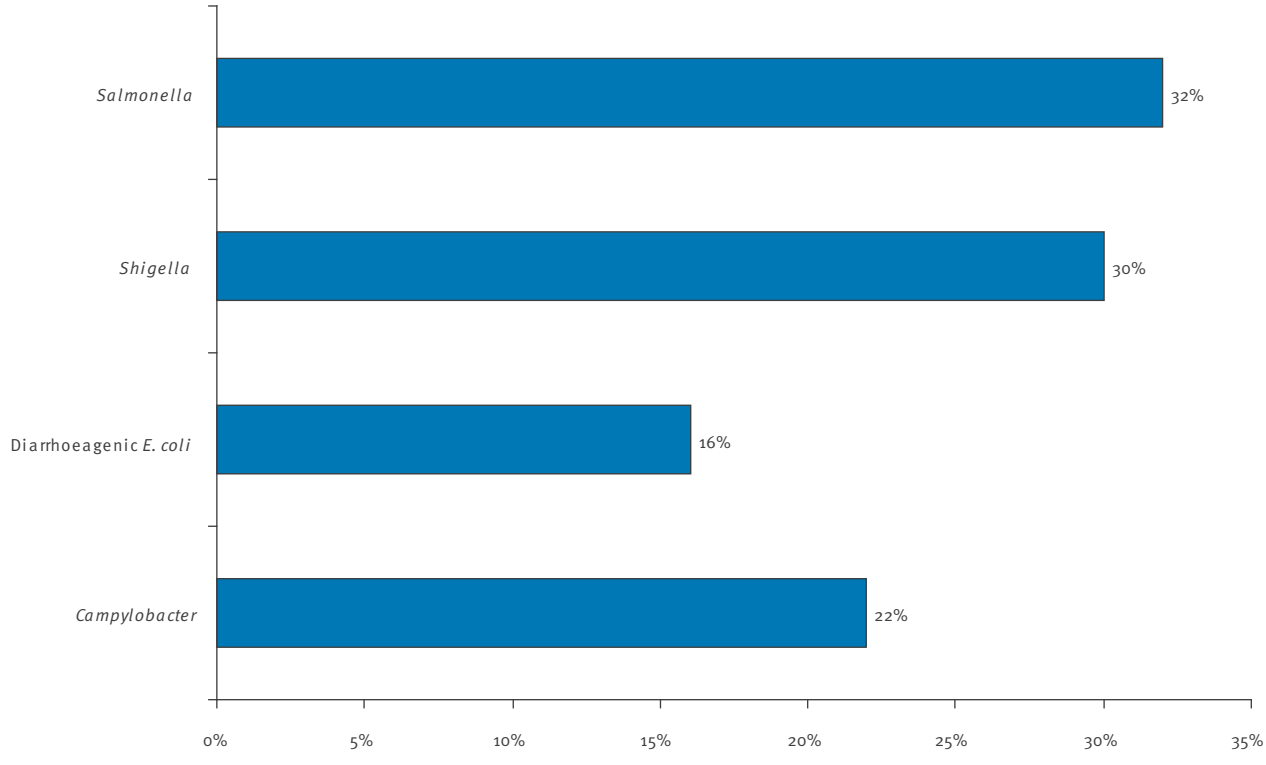
Isolates

From the 51,607 investigated stool specimens, 1,847 isolates of *Campylobacter* (3.58%) were obtained. Of these, 75% were C. jejuni, 22% were *C. coli* and 3% belonged to other species. *Salmonella* was isolated most frequently, from 5.17% of the samples, followed by *Shigella* (4.93%), *Campylobacter* (3.58%) and diarrhoeagenic *E. coli* (2.68%) (Figure 1).

For the period of the study, *Campylobacter* infection occurred in 22% of all the bacterial gastrointestinal diseases in the city of Sofia. *Salmonella* was the most frequently isolated pathogen with 32%, followed by *Shigella* (30%), *Campylobacter* (22%) and diarrhoeagenic *E.coli* (16%) (Figure 2).

Figure 2

Distribution of the pathogenic enteric bacteria isolated from faecal samples collected in Sofia, Bulgaria from 1987 to 2008 (n=8,396)



Table

Proportion of pathogenic enteric bacteria isolated from 30,033 faecal samples in Sofia, Bulgaria, 1987-1997 (n=4,235)

Year	Bacterial pathogen (%)			
	Campylobacter	*Salmonella*	*Shigella*	*Diarrhoeagenic E.coli*
1987	6.20	4.00	2.00	2.00
1988	7.51	4.95	2.66	2.17
1989	5.00	4.55	0.67	2.67
1990	5.00	3.30	2.08	1.20
1991	2.47	3.10	3.13	2.37
1992	3.30	3.10	3.30	4.40
1993	6.17	2.61	3.00	3.30
1994	1.66	2.04	11.80	2.87
1995	5.17	2.19	6.55	1.75
1996	5.54	2.33	2.37	1.98
1997	6.49	1.59	1.70	2.90
Average (%)	**4.95**	**3.07**	**3.57**	**2.51**

Although *Salmonella* was on average the predominant enteric pathogen during the study period as a whole, *Campylobacter* predominated in the years 1987 (6.42%), 1988 (7.61%), 1989 (5.00%), 1990 (5.00%), 1993 (6.17%), 1996 (5.54%), 1997 (6.49%), 1999 (4.20%), 2000 (2.70%) and 2001 (4.90%). The highest proportion of *Campylobacter* was found in 1988 (7.50%) and the lowest in 2006 (0.30%).

In our previous study of 30,033 faecal specimens from the patients with enterocolitis in the period from 1987 to 1997, *Campylobacter* ranked first (4.95%) among the bacterial causes of enterocolitis [5], followed by *Shigella* (3.57%), *Salmonella* (3.07%) and diarrhoeagenic *E. coli* (2.51%) (Table).

Seasonal distribution

The peak of *Campylobacter* infection in our study was in the wet months of spring and summer: on average 105 cases in March, 102 cases in April, 124 cases in May and 141 cases in June.

Age distribution

An analysis of the age-specific incidence (Figure 3) showed that children up to the age of four years were the age group most affected by campylobacteriosis in Sofia (52%), followed by the group of 5-14-year-olds (30%), the group above the age of 65 years (6%), the 15-24-year-olds (5%), the 45-64-year-olds (4%) and the 25-44-year-olds (3%). In our study, *C. jejuni* and *C. coli* were most frequently isolated in the children up to the age of 14 years, totalling 82%.

Discussion

Diarrhoeal diseases are a major problem for many countries in the world. The determination of the aetiological agent is an important step in the prophylaxis and the prompt treatment of enterocolitic infections.

In our study of 51,607 stool specimens, *Salmonella* was isolated most frequently, which correlates with reports of increasing incidence of human salmonellosis in Europe and the United States (US) in recent years [1,5]. The distribution of the different enteropathogenic bacteria among the positive faecal samples in our study was also similar to that observed in the US, where *Campylobacter* was isolated from 4.4%, *Salmonella* in 2.3% and *Shigella* in 0.9% of faecal samples in the same time period [7]. Campylobacteriosis was the leading cause of bacterial gastroenteritis reported in Belgium, Canada, Finland, Sweden, Central and South America, and southern states of Australia [8-10] during the time of our study.

In our study, *C. jejuni* and *C. coli* were most frequently isolated in the children up to the age of 14 years, totalling 82%. These data correlate with findings of other authors [1,10].

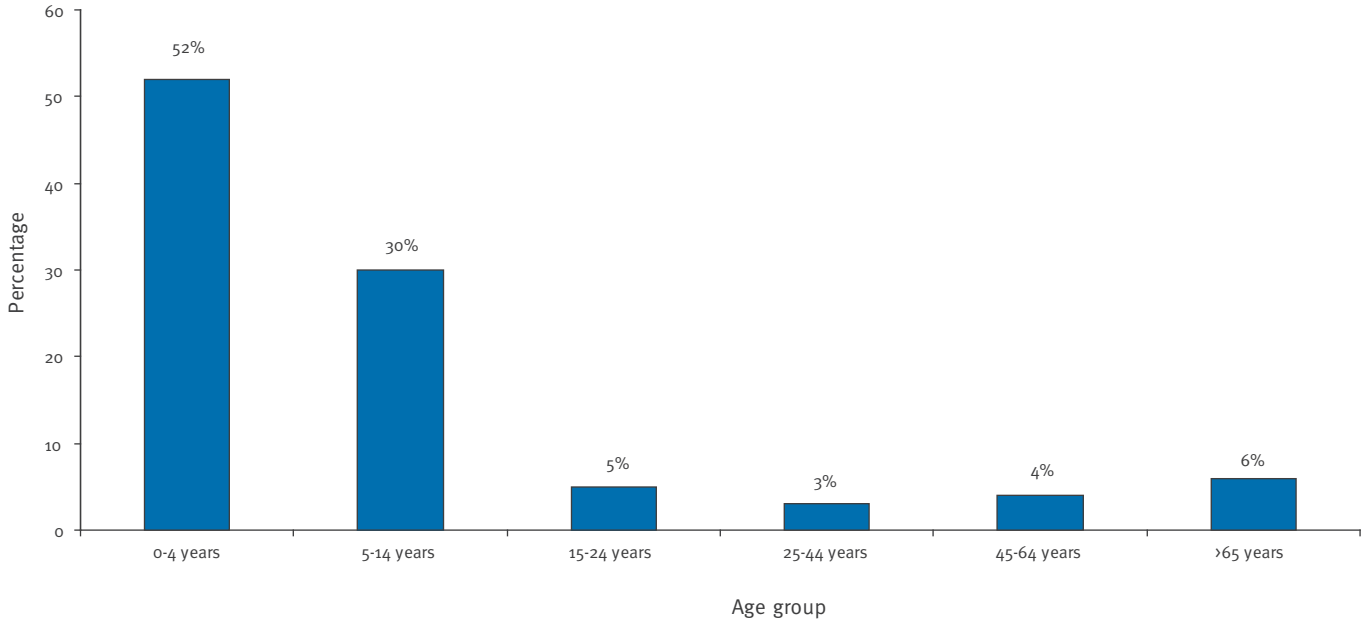
The diagnosis of *Campylobacter* in Sofia for the second decade in the study period, 1998-2008, was limited due to a shortage of data from hospital and private laboratories where the investigations are episodic.

Campylobacter enteritis has no seasonal preference in developing countries. In contrast, epidemics occur in summer and autumn in developed countries [1,11]. According to other authors in countries with moderate climate such as Bulgaria, *Campylobacter* is isolated most frequently in May, June and July [1,5], while the peak of *Campylobacter* infection in our study was in the wet months of spring and summer.

Our study provides data only for one region of Bulgaria, Sofia, although campylobacteriosis is notifiable disease in Bulgaria. The study showed the importance of thermophilic *Campylobacter* as a food-borne pathogen

Figure 3

Age distribution of patients with campylobacteriosis in Sofia, Bulgaria, 1987-2008



and underlines the need to strengthen surveillance of *Campylobacter* in Bulgaria. A lot of effort is needed to improve surveillance of campylobacteriosis in our country. Only a small number of laboratories are currently reporting *Campylobacter* cases. The main reason of the underreporting of campylobacteriosis in Bulgaria is the limited laboratory capacity for *Campylobacter* detection. The National Centre of Infectious and Parasitic Diseases in Sofia provides training in practical and theoretical courses on the diagnosis, treatment and epidemiology of campylobacteriosis. *Campylobacter* should be included in the set of enteric pathogens (*Salmonella*, Shigella, diarrhoegenic *E.coli*, *Yersinia*) tested for in cases of diarrhoea.

In conclusion, the results of our investigation for the period of 1987–2008 show that *Campylobacter* plays an important role as a bacterial pathogen that causes enterocolitis in Sofia, Bulgaria. The most affected group were 0-14-year-old children. Despite the fact that campylobacteriosis is a notifiable disease, the investigations are episodic and there is no systematic data for our country. For that reason we consider it an urgent need to introduce systematic surveillance of this infection in Bulgaria.

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Nationwide outbreak of Salmonella serotype Kedougou associated with infant formula, Spain, 2008

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On 5 August 2008, the National Centre of Microbiology in Madrid, Spain, notified an increase in *Salmonella* Kedougou isolations compared to 2007, with 21 cases including 19 children under one year of age. Active case finding and a matched case-control study were carried out to confirm this increase, identify source, transmission mode and risk factors in order to implement control measures. Cases were defined as any child under one year of age with *S.* Kedougou isolated since 1 January 2008, and were matched for age, sex, medical practitioner and diagnosis week with controls who were selected among patients of the cases' medical practitioners. An *ad hoc* questionnaire was completed for cases and controls and a univariate analysis was conducted to identify risk factors. We found 42 isolates from 11 of the 19 Spanish Regions. Completed questionnaires were available for 39 of 42 patients identified; 31 were children under one year of age and fulfilled the case definition. The median age of the 31 cases was 4.3 months and 13 were male. Main symptoms were diarrhoea (n=31) and fever (n=13). Ten cases required hospitalisation. All 31 cases had consumed infant formula milk of Brand A which was associated with illness in the univariate analysis (exact matched odds ratio: 74.92; 95% confidence interval: 12.89-∞). All patient isolates showed indistinguishable pulsed-field gel electrophoresis and antimicrobial susceptibility patterns. Five milk samples from three cases' households were negative for *Salmonella*. Our results suggest that Brand A was the transmission vehicle of *S.* Kedougou in the outbreak that occurred in Spain between January and August 2008. Food safety authorities recalled five batches of Brand A milk on 26 August 2008. No further cases have been detected as of 15 September 2009.

Background

Salmonella Kedougou belongs to serogroup G *Salmonella* and is one of the nearly 2,000 *Salmonella* serotypes that can cause illness in humans, but it is a rare serotype identified in Spain. The National Centre of Microbiology (NCM) in Madrid isolated a mean of three *S.* Kedougou strains from humans per year between 2002 and 2007 (unpublished data). We only found two outbreaks involving this serotype in the literature: one in Norway in 2006 linked to consumption of Salami [1] and one in the United Kingdom in 1992 linked to cooked meat [2].

On 5 August 2008, the NCM notified an increase in number of *S.* Kedougou isolates during the first half of 2008: 21 isolates from seven Spanish regions, compared to six isolates in 2007 and two in 2006. Nineteen of these 21 isolates were from children under one year of age. The widespread distribution and the cases' age suggested a commercial infant food product as the likely vehicle of transmission in this *Salmonella* outbreak.

On 6 August 2008, the National Centre of Epidemiology (NCE) in collaboration with NCM, regional epidemiologists and microbiologists of the Spanish epidemiological surveillance network began an epidemiological study and sent an alert to the Spanish Food Safety and Nutrition Agency (SFSNA) and to the Ministry of Health. The alert was also sent to the European Food and Waterborne Diseases Network, asking for *S.* Kedougou increases during 2008. Eleven member countries answered but did not report any increase in *S.* Kedougou isolates.

The objectives of our study were to confirm the increase in number of cases and to identify the source of infection, the transmission mode and associated risk factors in order to implement appropriate control measures.

Materials and methods

Epidemiological investigation

An active case finding and a matched case-control study were conducted by the NCE in collaboration with NCM and regional and local epidemiologists to test the hypothesis that consumption of commercial infant food product was associated with the illness.

Active Case Finding

An outbreak case was defined as any person with an isolate of *S.* Kedougou identified during 2008. NCE sent a request to all regions in Spain through the Spanish Epidemiological Surveillance Network, to notify any case from whom *Salmonella* Group G was isolated in 2008.

We collected information on the cases with confirmed *S.* Kedougou infection using a structured questionnaire. They were filled in by regional and local epidemiologists in interviews with the cases or their parents.

We asked for demographic information (age, sex, place of residence), clinical information (date of onset, main symptoms, severity, hospitalisation) microbiological information, human and/or animal contact, food consumed in the 72 hours before the onset of symptoms (including brands and batch numbers of infant food consumed), and information about the way of preparation and disinfection as well as the time from preparation to consumption.

The epidemiological data and food history of the first identified cases (see below) raised the hypothesis that consumption of infant formula could be the cause of infection and we started an analytical study.

Analytical study

A case was defined as any child under one year of age with *S.* Kedougou isolated between 1 January 2008

Figure 1

Cases of *Salmonella* Kedougou by region, Spain 2008 (N=42)

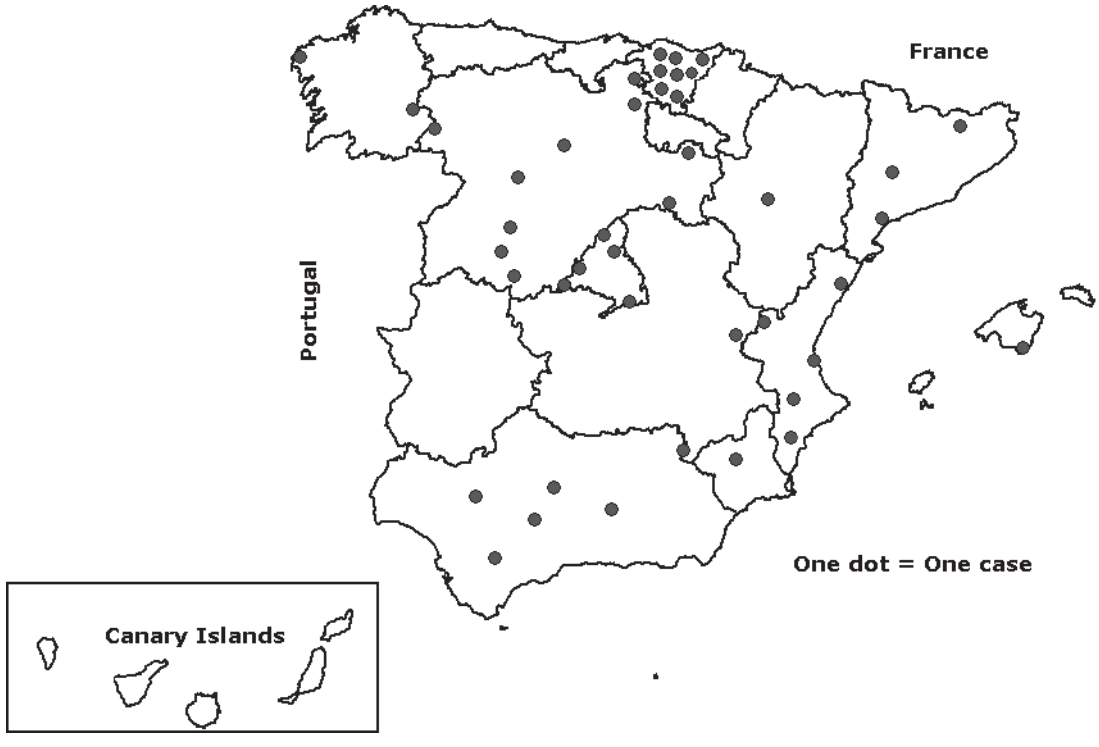
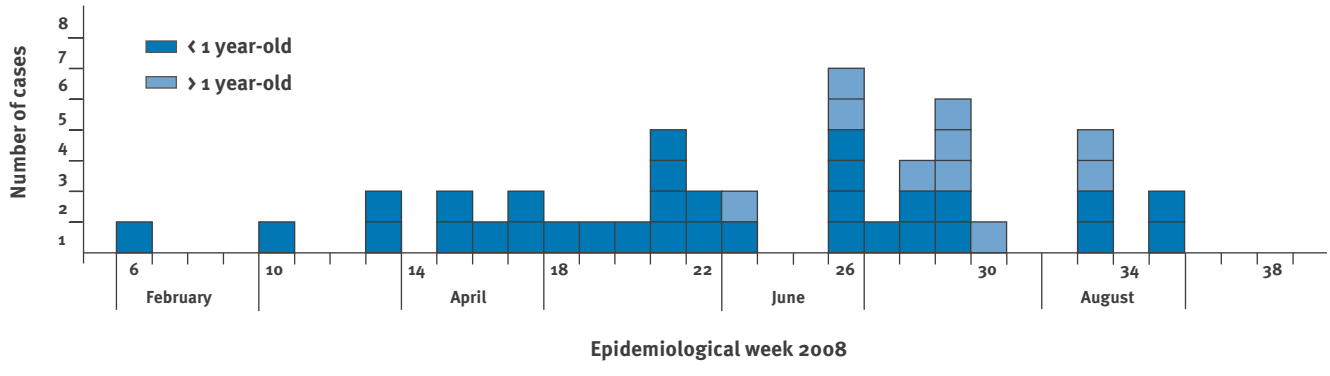


Figure 2

Cases of *Salmonella* Kedougou by week of isolation and age, Spain, 2008 (N=42)



and 31 August 2008. For each case, four controls were selected and matched for age (±one month), sex, same medical practitioner and week of diagnosis (±one week), without gastrointestinal symptoms and non-exclusive breastfeeding. The same questionnaire was applied to cases and controls, asking for information on their food intake during the three days previous to the onset of symptoms of the case.

Statistical analysis

Odds ratios (OR) and their 95% confidence intervals (CI) for the association between risk factors and disease were estimated using exact conditional logistic regression [3]. Maximum likelihood estimates (MLE) were applied when possible, and median unbiased estimates (MUE) when MLE could not be calculated. All analyses were carried out using STATA 10.0 [4-5].

Microbiological Investigation

Strains isolated from cases at regional hospital laboratories were sent to NCM for serotyping, and comparison of pulsed-field electrophoresis (PFGE) profiles and susceptibility patterns. The strains were tested for susceptibility to ampicillin, cefalotin, cefotaxime, amoxicillin/clavulanic acid, chloramphenicol, gentamicin, kanamycin, nalidixic acid, ciprofloxacin, tetracycline and trimethoprim/sulfamethoxazole.

Samples of infant foods (opened or unopened) provided by cases' households were also collected and sent to the laboratory of the SFSNA and to the regional laboratories for *Salmonella* testing.

Results

A total of 42 isolates from 42 patients were identified from January to August 2008. Sixteen patients were male and 32 were children under one year of age. Ten children under one year of age and a pregnant woman required hospitalisation. Patients were from 11 of the 19 regions in Spain (Figure 1).

The first isolate of *S.* Kedougou was identified on 4 February 2008 and the last one on 29 August 2008 (Figure 2).

Questionnaires for the children were answered by the parents. Completed questionnaires were available for 39 of 42 patients identified. For further analysis, we only considered cases under the age of one year. These were 31 of the 39 respondents, with a median age of 4.3 months, and 13 were male. Main symptoms were diarrhoea (n=31), fever (n=13) and vomiting (n=7). Blood was present in the stools of 20 of the patients. Ten children were hospitalised; none of them had a history of immunosuppression.

Five children had a mixed diet (breast feeding and infant formula) and 26 used infant formula exclusively. All the infants had consumed the same milk, Brand A, in the 72 hours before the onset of symptoms. Table 1 shows products consumed by the patients in the 72 hours before onset of symptoms, as well as other exposures.

The eight patients over one year of age, for whom a completed questionnaire was available, had a median age of 28 years (range: 1-84 years of age). Two patients were parents of cases under one year of age. Three patients had consumed powder infant formula of Brand A in the 72 hours before the onset of symptoms.

We included 22 cases and 70 controls in the matched case-control study (12 cases were matched with four controls, two cases with three controls and eight cases with two controls).

All cases included in this analytical study consumed infant formula of Brand A in the 72 hours before onset of symptoms compared with seven (10%) of the controls. Because all cases consumed an infant formula of Brand A, the maximum likelihood estimation for the OR of association with illness was infinite. The median unbiased estimate for the Mantel-Haenszel OR (OR_{M-H}) and the lower limit of the CI were thus calculated (exact OR_{M-H}: 74.92; 95% CI: 12,89–∞). Other food products, food preparation, or preservation and disinfection habits were not associated with the disease (Table 2).

Antimicrobial susceptibility tests and PFGE were done on all the strains. All PFGE pattern were indistinguishable (SAL-XBA-KDG-1) and all strains had the same sensitivity profile to all antibiotics tested.

We tested five samples of milk consumed before the occurrence of symptoms and provided by three cases' families. *Salmonella* was not detected in any of them.

Table 1

Distribution of exposures during the 72 hours before onset of symptoms in children under one year of age with isolation of *Salmonella* Kedougou, Spain, 2008 (N=31)

Product	N
Breast feeding	5
Milk of Brand A	31
Water	
Tap water	5
Bottled water	24
Baby cereal	11
Baby puree	
Fruit (homemade)	7
Fruit (commercial food)	5
Vegetables and chicken (homemade)	8
Baby bottle disinfection	
Boiling bottles	10
Using steriliser	11
Consumption of formula milk, cereal or puree immediately after preparation	28
Animal contact	9

No further cases' families were able to provide the batch number of the product consumed.

The infant formula Brand A distributed in Spain up to the day of the study had been produced in a local production plant. The company had closed this production plant in March 2008, five months before the outbreak alert. The results of factory quality control tests provided by the producers from raw materials and incriminated end products were negative for *Salmonella*, but positive for *Enterobacteriaceae* in some batches of end product.

On 26 August, the Spanish food safety authorities recalled five batches of infant formula of Brand A. This product was distributed only in Spain. A press release was issued informing people to avoid the use of these batches of milk Brand A and a contact telephone help line was set up to provide information to consumers.

Discussion

Our results suggest that the consumption of an infant formula of Brand A was associated with *S.* Kedougou infection. In our analytical study, 100% of the cases had consumed this milk compared with only 10% of the controls.

Outbreaks associated with infant powder formula are not uncommon because this is not a sterile product. This type of feeding is now usual because of many reasons such as the increased survival of premature babies and newborns with low birth weight, maternal illnesses in which breastfeeding is not recommended, early return of women to work after giving birth, or difficulties in breastfeeding [6].

We are not aware of any outbreak of *S.* Kedougou associated with infant formula. However, other serotypes of *Salmonella* had been associated with infant formula as a vehicle of transmission in many outbreaks in the world, such as in France (*S.* Agona, 2005), Korea (*S.* London, 2000), Spain (*S.* Virchow, 1994), Canada and the United States (*S.* Tennessee, 1993) [7-16]. One of the latest outbreaks occurred in France at the time of our study (*S.* Give, 2008) [17].

Outbreaks associated with commercial products like infant formula tend to have a low epidemic profile (small number of cases spread over long periods of time) because of the low bacterial load usually contained in the product [11]. However, continuous exposure to the factor for several months increases the probability of infection. The current Codex Alimentarius specification for *Salmonella* considers food products as fit for consumption when 60 samples of 25 gr are free from microorganisms [18]. Data provided by the infant food industry and inspection authorities indicate that *Salmonella* is rarely detected in powder infant products; nevertheless the microorganism can survive in powdered formula milk for up to 15 months and the method of detection can fail [19].

The increase in *S.* Kedougou isolations in Spain in 2008 was detected by the NCM because of the low expected frequency of this serotype in our country. This highlights the crucial role of the microbiology laboratories detecting outbreaks involving rare serotypes of microorganisms. Laboratory networks with a role in early detection of alert signals can complement surveillance systems in detecting uncommon microorganisms that otherwise might go unnoticed.

The higher attack rate in children under the age of one year, the identical PFGE pattern, the wide geographical distribution in Spain and the consumption of a particular brand of infant formula by the first cases identified lead to the hypothesis that the milk could be the vehicle of infection. Moreover, almost all cases older than one year could be explained by consumption of Brand A milk or by epidemiological link with younger cases.

In our study, most adult cases were probably secondary cases. Those cases for whom no contact with children under the age of one year could be established had consumed Brand A formula milk. By restricting our study to cases under one year of age we increased the specificity of the case definition because only primary cases were included. In case-control studies recall bias usually differs between cases and controls; parent cases tend to recall better than parent controls. In our study, we minimised this bias by restricting the

Table 2

Matched univariate analysis between *Salmonella* Kedougou infection and different exposures, Spain, 2008

Variable	Matched OR	95% CI
Tap water	2.11	0.43–10.22
Fruit baby food	0.8	0.08–7.51
Baby puree (vegetables and chicken)	0.34	0.01–26.07
Infant formula milk Brand A	**74.92[a]**	**12.89–∞**
Boiled water for baby bottle	5.77	0.95–35.02
Disinfection: water and detergent	0.48	0.12–1.92
Disinfection: boiled water	2.07	0.54–7.85
Animal contact	0.98	0.3–3.12

CI: confidence interval; OR: odds ratio.

[a] Median unbiased estimates; maximum likelihood estimation= infinite.

analysis to cases under the age of one year, for whom food consumption patterns are usually constant and thus less prone to recall bias. However, some bias could be present given the delay between the interview and the onset of symptoms (mean: 108 days, range: 9-222 days).

The matched case-control study design chosen [20] included matching for medical practitioner as a way to facilitate the control search and selection. This could have lead to overexposure among controls because doctors could tend to recommend the same infant formula to their patients. Nevertheless this assumption was not confirmed by our data, as only few controls consumed the involved milk brand compared with 100% of the cases.

In the case-control questionnaires, up to five varieties of Brand A milk were reported, but the small number of exposed controls did not allow further analysis to identify a specific variety associated with the outbreak. Except in two cases, it was not possible to obtain details on the batch of formula milk consumed by the patients.

The number of cases confirmed at the laboratory (42 cases) might be an underrepresentation of the real number of infections since only a small proportion of people with gastroenteritis seeks medical assistance and provide a sample for laboratory testing. This might be also applicable for the age group at risk (under one year) because even when parents seek medical assistance for their children, gastrointestinal illness might be frequently misdiagnosed as milk/food intolerance as initially happened in two of the cases identified.

This is the first outbreak of *S.* Kedougou associated with the consumption of infant formula in Spain. The results of the investigation involving epidemiological services of all Spanish regions and the NCE support the hypothesis that the Brand A formula milk was the vehicle of the *S.* Kedougou gastroenteritis outbreak, occurring between February and August 2008.

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Multiresistant Salmonella enterica serovar 4,[5],12:i:- in Europe: a new pandemic strain?

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A marked increase in the prevalence of *S. enterica* serovar 4,[5],12:i:- with resistance to ampicillin, streptomycin, sulphonamides and tetracyclines (R-type ASSuT) has been noted in food-borne infections and in pigs/pig meat in several European countries in the last ten years. One hundred and sixteen strains of *S. enterica* serovar 4,[5],12:i:- from humans, pigs and pig meat isolated in England and Wales, France, Germany, Italy, Poland, Spain and the Netherlands were further subtyped by phage typing, pulsed-field gel electrophoresis and multilocus variable number tandem repeat analysis to investigate the genetic relationship among strains. PCR was performed to identify the *fljB* flagellar gene and the genes encoding resistance to ampicillin, streptomycin, sulphonamides and tetracyclines. Class 1 and 2 integrase genes were also sought. Results indicate that genetically related serovar 4,[5],12:i:- strains of definitive phage types DT193 and DT120 with ampicillin, streptomycin, sulphonamide and tetracycline resistance encoded by bla_{TEM}, *strA-strB*, *sul2* and *tet*(B) have emerged in several European countries, with pigs the likely reservoir of infection. Control measures are urgently needed to reduce spread of infection to humans via the food chain and thereby prevent the possible pandemic spread of serovar 4,[5],12:i:- of R-type ASSuT as occurred with *S.* Typhimurium DT104 during the 1990s.

Introduction

Infections with *Salmonella enterica* account for the second largest burden of bacterial gastrointestinal disease in the European Union (EU) [1]. The majority of *Salmonella* infections result in mild, self-limited illness and may not require treatment with antimicrobials. Nevertheless treatment with an appropriate antimicrobial can be life-saving in immunocompromised patients and in invasive disease, such as *Salmonella* bacteraemia and meningitis.

Serotyping according to the Kauffmann-White scheme is a widely used method for the initial characterisation of *Salmonella* isolates and is based on the antigenic variability of the somatic (O) and flagellar (H) antigens present in the cell wall of the organism [2]. Despite identification of more than 2,500 different serovars, the majority of cases of human infection are caused by a limited number of serovars. Most serovars are biphasic and express two distinct flagellar antigens encoded by *fliC* (phase-1 flagellin) and *fljB* (phase-2 flagellin). However, some serovars fail to express either the phase-1 or phase-2 flagellar antigen, therefore are classed as monophasic.

S. enterica serovar 4,[5],12:i:- is considered a monophasic variant of serovar Typhimurium (4,[5],12:i:1,2) due to antigenic and genotypic similarities between the two serovars [3,4]. Serovar Typhimurium is the second most common serovar associated with human cases of *Salmonella* infection in the EU [1]. In contrast isolates of serovar 4,[5],12:i:- were rarely identified before the mid-1990s but are now among the top 10 most common serovars isolated from humans in several countries [3-8]. According to Enter-net data this serovar was the fourth most common serovar in confirmed cases of human salmonellosis in the EU in 2006 [1]. Cases of infection with serovar 4,[5],12:i:- have reportedly been severe, with a 70% hospitalisation rate during an outbreak in New York City in 1998 [9], although a much lower rate of 21% was observed during an outbreak in Luxembourg in 2006 [6]. Infections have also been particularly associated with cases of septicaemia in Thailand and Brazil [7,10]. Overall, cases of infection have been linked to a number of sources, including

poultry and cattle, but particularly pigs and pork products [4,6,10-13]. Serovar 4,[5],12:i:- was among the top 10 most common serovars isolated from both pigs and pig meat in the EU in 2006 [1].

A marked increase in prevalence of *S. enterica* serovar 4,[5],12:i:- with resistance to ampicillin, streptomycin, sulphonamides and tetracyclines (R-type ASSuT) has been noted both in food-borne infections and in pigs/pig meat in several European countries over the last ten years [6,8,14,15]. In the baseline study from fattening pigs (Commission Decision 2006/668/EC), Spanish strains of *S. enterica* serovar 4,[5],12:i:- represented 14.3% of the isolates, 52.5% of which were of R-type ASSuT (VISAVET *Salmonella* database, unpublished data). In England and Wales cases of serovar 4,[5],12:i:- infection have risen from 47 in 2005 to 151 in 2009 (a 321% increase) against a backdrop of an overall decrease in the number of salmonellosis cases, with R-type ASSuT accounting for approximately 30% of these strains (Health Protection Agency (HPA) *Salmonella* database, unpublished data). In France isolations of serovar 4,[5],12:i:- increased from 99 to 410 between 2005 and 2008 to become the third most common serovar isolated from humans, with 62% of strains in 2007 being of R-type ASSuT [16]. In Italy cases of serovar 4,[5],12:i:- infection have risen from 59 in 2003 to 641 in 2009, with 75% of monophasic strains isolated in 2009 belonging to R-type ASSuT (with or without additional resistances) (Istituto Superiore di Sanità *Salmonella* database, unpublished data). A recent study described emergence of a clonal group of serovar Typhimurium and 4,[5],12:i:- R-type ASSuT strains in Italy, Denmark and the United Kingdom (UK) [17]. Resistance genes bla_{TEM-1}, *strA-strB*, *sul2* and *tet*(B) encoding resistance to ampicillin, streptomycin, sulphonamides and tetracyclines were localised on the bacterial chromosome. On the basis of resistance gene content and the lack of class 1 integrons these observations have suggested the existence of a new resistance island that differs from the *Salmonella* Genomic Island-1 [17].

In response to the rapid increase in the frequency of *S. enterica* serovar 4,[5],12:i:-, R-type ASSuT strains, isolates from England and Wales, Germany, France, Italy, Poland, Spain and the Netherlands were compared using phage typing, resistance gene characterisation, pulsed-field gel electrophoresis (PFGE) and multilocus variable number tandem repeat (MLVA) analysis to evaluate the possibility of clonal spread of this emerging multidrug-resistant (MDR) strain.

Methods and Materials

Isolate collection

The eight participating laboratories (the HPA Centre for Infections, London and the Veterinary Laboratories Agency, Weybridge in the UK, the Agence Française de Sécurité Sanitaire des Aliments in Maisons-Alfort, France, the Federal Institute for Risk Assessment in Berlin, Germany, the Istituto Superiore di Sanità in Rome, Italy, the National Institute of Public Health in Warsaw, Poland, the Health Surveillance Centre (VISAVET), University Complutense in Madrid, Spain and the Central Veterinary Institute of Wageningen in Lelystad, the Netherlands) were asked to submit a maximum of 10 isolates of *S. enterica* serovar 4,[5],12:i:- exhibiting resistance (according to local protocols) to ampicillin, streptomycin, sulphonamides and tetracyclines, and isolated from humans, pigs or pig meat between 2006-2008. In addition, laboratories were invited to send a maximum of 10 isolates of serovar 4,[5],12:i:- exhibiting other resistance phenotypes. All isolates were sent to the HPA.

Strain characterisation

The *Salmonella* serotype was confirmed on the basis of the Kauffmann-White scheme and phage typing performed in accordance with HPA protocols [2,18]. In addition, isolates were screened using a duplex PCR targeting regions specific to serovar Typhimurium and to definitive phage type (DT) 104 and related strains of phage type (PT) U302 [19]. PCRs targeting the variable regions of the *fljB* genes encoding the phase-2 flagellar antigens H:1,2, H:1,5, H:1,6, H:l,7, H:e,n,x, H:e,n,z15 and H:1,w, were performed as previously described [20].

Susceptibility to a panel of 18 antimicrobials was determined by a breakpoint method in Isosensitest agar (Oxoid, Basingstoke, UK). The final plate concentrations (µg/mL) used routinely by the HPA on the basis of long-term studies were: ampicillin (A; 8), chloramphenicol (C; 8), gentamicin (G; 4), kanamycin (K; 16), neomycin (Ne; 8), streptomycin (S; 16), sulphonamides (Su; 64), tetracycline (T; 8), trimethoprim (Tm; 2), furazolidone (Fu; 8), nalidixic acid (Nx; 16), ciprofloxacin (low-level (Cpl) 0.125; high-level (Cp) 1.0), amikacin (Ak; 4), cephalexin (Cx; 16), cephradine (Cr; 16), cefuroxime (Cf; 16), ceftriaxone (Cn; 1) and cefotaxime (Ct; 1). Resistance genes bla_{TEM}, *strA-strB*, *sul2* and *tet*(B), and classes 1 and 2 integrase genes were sought by PCR using previous described primers [21,22].

Molecular subtyping

PFGE was performed after digestion of genomic DNA with XbaI according to a standardised protocol [23]. The patterns were analysed using the Bionumerics software package (version 5.10; Applied Maths, Sint-Martens-Latem, Belgium) and resulting band profiles were submitted to the PulseNet Europe database for assigning profile names. Dendrograms were constructed using the Dice similarity coefficient and the unweighted pair group method with arithmetic averages (UPGMA) with optimisation and position tolerance set at 1.5%. Multilocus variable number tandem repeat (MLVA) analysis was performed according to a previously described protocol [24]. MLVA profiles were assigned based on the fragment size amplified from each locus, with 'NA' used to denote a locus not present [25].

Results

Some 122 serovar 4,[5],12:i:- isolates were sent to the HPA Laboratory of Gastrointestinal Pathogens, of which 116 were confirmed as serovar 4,[5],12:i:-. These comprised 41 from England and Wales (20 from pigs and 21 from humans, including three from patients with a history of recent travel to Thailand, Greece and an undisclosed destination), 10 isolates from France (isolated from pig meat), 19 from Germany (12 from pigs, six from pig meat and one from a human), 23 from Italy (from humans), five from Poland (from humans), eight from Spain (from pigs) and 10 from the Netherlands (seven from human cases of infection; three from pigs). The H:1,2 phase-2 flagellar antigen could be serologically detected in the remaining six isolates.

Phage typing using the Typhimurium typing phages identified 16 different PTs (Table 1). The most commonly identified PTs were DT193 (51 isolates), DT120 (27 isolates) and RDNC (reacts but does not conform; 11 isolates). DT193 was the most common PT identified

Table 1

Phage type distribution among serovar 4,[5],12:i:- isolates from seven European countries, 2006-2008 (n=116)

Country	Phage type (number of isolates)
England and Wales	21 var (1), 120 (13[a]), 191 (1), 193 (21[b]), 208 (1), RDNC (2), U302 (2)
France	68 var (1), 120 (2), 193 (5), U311 (1), UT (1)
Germany	193 (13), 208 (1), 104b (2), RDNC (3)
Italy	7 var (3), 18 var (2), 120 (6), 193 (3), RDNC (5), U311 (3), UT (1)
Poland	120 (4), 104 (1)
Spain	18 (1), 193 (4), RDNC (1), U302 (1), U311 (1)
The Netherlands	12 (2), 120 (2), 193 (6)

RDNC: isolates that react with the typing phages, but do not conform to a recognised pattern; UT: isolates that do not react with any of the typing phages; var: variant.

Phage type as determined by the scheme of Anderson *et al.* [18].

[a] Including two strains associated with foreign travel.

[b] Includes one strain associated with foreign travel.

Table 2

Comparison of phage type and R-type with PFGE profile of serovar 4,[5],12:i:- isolates from seven European countries, 2006-2008 (n=116)

	PFGE profiles (STYMXB.)																	
	ASSuT						ASSuT and other resistances[b]						Other resistance patterns (not ASSuT)[c]					
PT	0131	0083	0079	0010	0022	Other[a]	0131	0083	0079	0010	0022	Other	0131	0083	0079	0010	0022	Other
193	24	1		1	2	7	2				1	3	4		1		1	4
120	1	4	1	6	1	1		4	1			1		2	3	1		1
RDNC	1		2	1		2				1		1						2
U311			1		1							3						
U302			1									1						1
7 var			1															2
12																		2
208																		2
18 var									1					1				
UT						1						1						
104B low														2				
104 low												1						
18																		1
191																		1
21 var						1												
68 var																		1
Total	**26**	**5**	**6**	**8**	**4**	**14**	**2**	**4**	**2**	**1**	**1**	**11**	**4**	**5**	**4**	**1**	**1**	**17**

PFGE: pulsed-field gel electrophoresis.

[a] Includes two untypable strains.

[b] Includes resistance patterns ACGNeKSSuTTmNxCpl (1 strain), ACKSSuT (1), ACSSuSpTTm (2), AGKNeSSuTTm (n=1), AGSSpSuT (n=1), AGSSuTTm (n=1), AKSSuT (n=1), ASSpSuTNxCpl (n=1), ASSuTNxCpl (n=2), ASSuTNxCpl (n=1) and ASSuTTm (n=9).

[c] Includes fully sensitive strains (n=6), AGST (n=1), AGSuT (n=1), AGT (n=1), ASSu (n=1), ASuT (n=1), SSuTm (n=1), SSuTTm (n=6), SuT (n=1), SuTTm (n=1) and T (n=9).

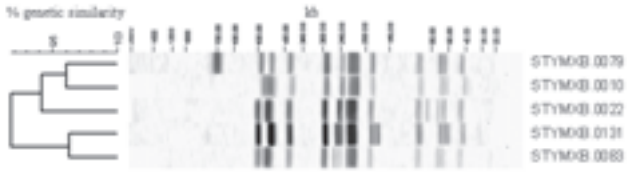
in England and Wales, France, Germany, Spain and the Netherlands, while DT120 predominated in Italy and Poland. All 116 isolates were PCR-positive for the Typhimurium-specific fragment of the malic acid dehydrogenase gene but only four isolates (one belonging to DT104, two to PT U302 and one untypable) gave a product with primers targeting the 16S to 23S spacer region specific to DT104 and the related PT U302 [19].

Overall, 94 of 116 isolates were PCR-negative for all variants of the *fljB* gene coding for the phase-2 flagellar antigen, including 48 of 51 DT193 and 17 of 27 DT120 isolates. H:1,2-specific amplicons were detected in the remaining 22 isolates.

Eighty-four isolates (72%) expressed resistance to ampicillin, streptomycin, sulphonamides and tetracyclines (R-type ASSuT), with or without additional

Figure

Comparison of the five most common PFGE profiles identified in serovar 4,[5],12:i:- isolates from seven European countries, 2006-2008



PFGE: pulsed-field gel electrophoresis.

Table 3

Comparison of common PFGE profiles with phage type, country of origin and sources of isolates, 2006-2008 (n=74)

STYMXB.	Number of isolates	Phage type (number of isolates)	Country of origin (number of isolates.)	Source (number of isolates)
0131	32	DT193 (30) DT120 (1) RDNC (1)	France (2) The Netherlands (6) England and Wales (16) Germany (8)	Humans (10) Pigs/pig meat (22)
0083	14	DT120 (10) DT104 (2) 18 var (1) DT193 (1)	France (2) England and Wales (9) Germany (2) Italy (1)	Humans (5) Pigs/pig meat (9)
0079	12	DT120 (5) RDNC (2) 18 var (1) 7 var (1) U302 (1) U311 (1)	England and Wales (2) Spain (1) Italy (9)	Humans (11) Pigs/pig meat (1)
0010	10	DT120 (7) RDNC (2) DT193 (1)	France (1) The Netherlands (1) England and Wales (1) Poland (3) Spain (1) Italy (1) Germany (2)	Humans (5) Pigs/pig meat (5)
0022	6	DT193 (4) DT120 (1) U311 (1)	France (2) England and Wales (1) Poland (1) Spain (1) Germany (1)	Humans (2) Pigs/pig meat (4)

PFGE: pulsed-field gel electrophoresis.

resistance(s) (Table 2). Six isolates were fully sensitive to all antimicrobials in the test panel. Eighty-three of 92 ampicillin-resistant iolates carried *bla*$_{TEM}$, 85 of 96 streptomycin-resistant isolates carried *strA-strB*,

Table 4

Subdivision of the five most common PFGE profiles using MLVA analysis, 2006-2008 (n=74)

PFGE pattern	Number of strains	MLVA profile (based on number of tandem repeats at each locus)[a]					
		SSTR9	STTR5	STTR6	STTR10	STTR3	
						27 bp	33 bp
STYMXB.0131	10	3	11	9	NA	2	11
	8		13	10			
	5		12				
	3		13				
	3			10			
	1			12			
	1			14			
	1			8			
STYMXB.0083	5	3	12	9	NA	2	11
	4			6			
	1		11	11			
	1		11	12			
	1		13	10			
	1		14				
	1	13	13	NA			
STYMXB.0079	2	3	12	11	NA	2	11
	2		13	10			
	1		11	8			
	1		12	12			
	1		12	9			
	1		13	12			
	1		13	9			
	2	11	15	NA			
	1	13	13	NA			
STYMXB.0010	3	3	14	9	NA	2	11
	2		12	10			
	1		12	7			
	1		12	9			
	1		13	7			
	1		14	10			
	1		15	10			
STYMXB.0022	2	3	12	9	NA	3	11
	1			13			
	1		13	11			
	1		14	9		2	
	1		15	10		2	

NA: locus not present; PFGE: pulsed-field gel electrophoresis; MLVA: multilocus variable number tandem repeat analysis.

[a] Number of tandem repeats only listed where it differs from the most common repeat number in each PFGE profile.

88 of 99 sulphonamide-resistant isolates carried *sul2* and 93 of 105 tetracycline-resistant isolates carried *tet*(B) (data not shown). Of 84 R-type ASSuT strains, 68 possessed bla_{TEM}, *strA-strB*, *sul2* and *tet*(B) resistance genes. Eighty-two percent of RDNC isolates, 80% of DT193 and 74% of DT120 were of R-type ASSuT (with/without additional resistance(s)), with resistance encoded by genes bla_{TEM}, *strA-strB*, *sul2* and *tet*(B) in 78%, 75% and 56% of isolates respectively. Isolates of R-type ASSuT were negative for both class 1 and 2 integrase genes; these were found only in strains expressing resistance to aminoglycosides and/or trimethoprim. Among the remaining 16 R-type ASSuT strains from the present study that did not carry bla_{TEM}, *strA-strB*, *sul2* and *tet*(B), 11 strains lacked only one of *tet*(B), bla_{TEM-1} or sul2, one strain each lacked bla_{TEM-1} and *tet*(B) or *strA-strB* and *tet*(B), one strain lacked bla_{TEM-1}, *strA-strB* and *sul2* and one strain lacked all four genes. These strains belonged to phage types DT120 (five strains), DT193 (four strains), RDNC (two strains), and one each belonged to phage types DT104, DT18 variant, U302, U311 and UT.

PFGE analysis identified 36 unique banding profiles among 114 strains; two strains were untypable. These were grouped into 12 clusters of two or more strains and 23 patterns corresponding to a single isolate (data not shown). Sixty-five percent (74/114) of strains were represented by one of five banding patterns (STYMXB.0131, n=32, STYMXB.0083, n=14, STYMXB.0079, n=12, STYMXB.0010, n=10, and STYMXB.0022, n=6) that shared more than 90% similarity (Figure, Table 3). Strains from humans and pigs or pig meat were represented in each common PFGE pattern. The majority of strains with PFGE patterns STYMXB.0131 and STYMXB.0022 were phage type DT193, while patterns STYMXB.0083, STYMXB.0079 and STYMXB.0010 were dominated by phage type DT120 (Table 3). Some country-specific differences were noted within the distribution of PFGE patterns: nine of the 12 STYMXB.0079 strains were from Italy, three of the five Polish strains were STYMXB.0010 and six of 10 strains from the Netherlands were pattern STYMXB.0131 (Table 3). STYMXB.0010 was the only profile identified in all seven countries. However, larger numbers of strains need to be analysed to determine whether these country-specific distributions hold true. Patterns STYMXB.0131 and STYMXB.0010 were dominated by R-type ASSuT strains (representing 81% and 80% of strains, respectively), whereas resistance profiles of the other common PFGE profiles were more variable (Table 2).

MLVA typing identified 45 different profiles that differed by loss or addition of tandem repeats at loci STTR9, STTR5, STTR6 and STTR3, and was able to further subdivide the five most common PFGE profiles (Table 4). Ninety-one percent (105/116) of strains failed to amplify a fragment from the Typhimurium-specific virulence plasmid pSLT-bound locus STTR10. The five most common MLVA profiles (3-11-9-NA-211, n=12; 3-12-9-NA-211, n=13; 3-13-10-NA-211, n=12; 3-14-9-NA-211, n=7; and 3-13-9-NA-211, n=6) accounted for 43% of strains and differed by only one to three tandem repeats at locus STTR5 and one repeat at locus STTR6.

The most frequently occurring combination of phenotypic and genotypic characteristics was that 51 of 116 (44%) serovar 4,[5],12:i:- isolates belonged to phage type DT193. Of these 51, 48 were PCR-negative for *fljB*. Among the 51 DT193 isolates, 37 were of R-type ASSuT (plus additional resistance to chloramphenicol, aminoglycosides and/or trimethoprim in four isolates) encoded by bla_{TEM}, *strA-strB*, *sul2* and *tet*(B), and 36 exhibited PFGE profile STYMXB.0131, which could be further divided into eight related MLVA profiles. Isolates bearing these characteristics were isolated in England and Wales (including one isolate from a patient with history of recent travel to Thailand), France, Germany and the Netherlands.

Discussion

Antimicrobial resistance is a serious public health problem limiting the therapeutic options available to clinicians treating complicated *Salmonella* infections. In recent years there has been an overall decline in the level of resistance in serovar Typhimurium in several European countries as a result of a reduction in the number of isolates of penta-resistant DT104 [14]. To some extent this reduction has been counteracted by an increase in prevalence of serovar 4,[5],12:i:- isolates expressing resistance to ampicillin, streptomycin, sulphonamides and tetracyclines [8,17].

One of the first reports of serovar 4,[5],12:i:- in Europe was of an isolate grown in the late 1980s from a chicken carcass in Portugal [26]. This serovar emerged in Spain in strains from humans and pork or pork products during 1997, and subsequently became the fourth most common *Salmonella* serovar identified from 1998 to 2000 [11]. All isolates belonged to phage type U302. These isolates were classed as monophasic variants of serovar Typhimurium due to presence of an IS*2000* fragment located in a Typhimurium-specific location within the *fliB-fliA* intergenic region and amplification of a Typhimurium DT104- and U302-specific region [3]. All 116 monophasic isolates in this study harboured the Typhimurium-specific fragment of the malic acid dehydrogenase gene, suggesting that these strains are monophasic variants of serovar Typhimurium. However, the majority (97%) were negative for the DT104- and U302-specific region, suggesting that these monophasic isolates may not be related to the serovar 4,[5],12:i:- strain(s) that emerged in Spain. This was confirmed by phage typing, which identified DT193 as the most common PT, followed by DT120, thereby adding to the diversity of phage types of serovar 4,[5],12:i:- linked to serovar Typhimurium. DT193 and DT120 have consistently fallen within the top five phage types of serovar Typhimurium from cases of human infection in England and Wales in recent years (HPA *Salmonella* database, unpublished data). It is plausible that at

least some of this increase may be attributed to the emergence of serovar 4,[5],12:i:- DT193 and DT120 strains. Putative Typhimurium isolates sent from primary diagnostic laboratories to the HPA *Salmonella* Reference Unit are only phage-typed and not routinely subjected to further serological examination. This may result in misclassification as serovar Typhimurium and under-reporting of this serovar in England and Wales, and in other countries where phage typing is used *in lieu* of full serotyping to identify strains as serovar Typhimurium. Serovar 4,[5],12:i:- DT193 strains have previously been isolated from human cases of infection and/or pigs in Luxembourg and Spain [6,13], while monophasic DT120 strains were identified in Italy [8].

The Spanish PT U302 serovar 4,[5],12:i:- strains were PCR-negative for H:1,2 [11], as were the majority (81%) of monophasic isolates in this study. Previous published work has shown that the lack of phase-2 flagellar expression may be due to different mutations (including point mutations) and partial or complete deletions in *fljB* and adjacent genes [4,27]. Monophasic strains in which the phase-2 flagellar antigen is not detected serologically but can be detected by PCR may contain deletions in a part of *fljB* that leave the H:1,2-specific PCR primer binding sites intact, or they may represent 'serotype inconsistent' strains [27]. These are serovar Typhimurium strains in which serological detection of the phase-2 flagellar antigen may be inconsistent. This may be due to problems with flagellar phase reversal, which is a time-consuming and technically demanding procedure that may result in misclassification of Typhimurium strains as serovar 4,[5],12:i:-. Alternatively, the invertible promoter controlling expression of *fljB* and *fliC* may have become locked in one position allowing only expression of *fliC* in these strains [4]. The range of mechanisms that can result in non-expression of the phase-2 flagellar antigen make definitive identification of serovar 4,[5],12:i:- problematic. It is possible that molecular serotyping could be used as a basis to define such strains as serovar 4,[5],12:i:- or Typhimurium, but as yet such methods lack standardisation, are not in place in most countries and may not be suitable for laboratories other than reference facilities. Given that there may be discrepancy in detection of the phase-2 flagellar antigen between classical and molecular serotyping, an international agreement both on the definition of monophasic strains and on detection methodology is required. Without reaching such a consensus the true incidence of such Typhimurium-like strains is difficult to assess; only the harmonisation and the sharing of methods will allow accurate comparison of reported data.

In contrast to the monophasic variants isolated in Thailand and Spain, which commonly expressed additional resistance to gentamicin and trimethoprim-sulphamethoxazole and/or chloramphenicol [10,11] and to serovar 4,[5],12:i:- strains isolated in Brazil and New York City, which were infrequently MDR [7,9], the countries participating in this study observed an increase in isolates of serovar 4,[5],12:i:- with resistance to ampicillin, streptomycin, sulphonamides and tetracyclines only. Characterisation of the resistance genes responsible for this phenotype identified bla_{TEM}, *strA-strB*, *sul2* and *tet*(B) in 81% of isolates. Such genes have also been identified in isolates of Typhimurium DT193 R-type ASSuT obtained during 2005 in England and Wales from raw beef and a human case of infection, although the majority of strains tested harboured *tet*(A) rather than *tet*(B) (unpublished data). Analysis of a 10 kb chromosomal region of a Typhimurium DT193 revealed the presence of an *strB-strA-sul2-repC-repA* region derived from plasmid RSF1010 located upstream of bla_{TEM-1} and downstream of a class 1 integron [28]. The resistance genes encoding the tetra-resistant phenotype in isolates of serovars Typhimurium and 4,[5],12:i:- from Italy, Denmark and the UK were also identified as bla_{TEM-1}, *strA-strB*, *sul2* and *tet*(B), but all isolates were negative for class 1 integrons [17]. Transfer experiments were unsuccessful and probes specific for these genes bound to a 750 kb I-CeuI digest fragment, suggesting a chromosomal location and existence of a new resistance island. As in the present study, strains with other R-types than ASSuT, but with related PFGE profiles and harbouring one or more of bla_{TEM-1}, *strA-strB*, *sul2* and *tet*(B) were identified. This suggests that rearrangements or deletions may occur within the resistance island leading to partial resistance patterns [17]. In contrast, resistance to ampicillin, streptomycin, sulphonamides and tetracyclines was mediated by plasmid-borne bla_{TEM-1} and *tet*(A), and a class 1 integron harbouring *aadA2* and *sul1* in the Spanish serovar 4,[5],12:i:- U302 isolates [29].

Thirty-six profiles were identified among the 114 strains typable by PFGE, thereby supporting previous observations that serovar 4,[5],12:i:- can demonstrate considerable diversity, even among strains from a single country [4,9,13,27]. However, serovar 4,[5],12:i:- strains have been reported to be less heterogenic than serovar Typhimurium strains [9,27,30]. Serovar Typhimurium demonstrates considerable diversity as evidenced by phage typing and molecular typing, but with certain clonal strains such as multidrug-resistant DT104 [31]. The most common PFGE profile identified in our study was STYMXB.0131, which, together with four other closely related banding patterns (STYMXB.0022, STYMXB.0079, STYMXB.0010 and STYMXB.0083), accounted for 65% of isolates. Previously submitted STYMXB.0131 patterns in the PulseNet Europe database belonged to serovar Typhimurium DT193 and PT507 (according to the Dutch phage typing scheme) strains isolated from human cases of infection in Finland, the Netherlands and England and Wales. Patterns STYMXB.0131 and STYMXB.0022 have also been identified in Typhimurium DT193 strains from humans, cattle and raw beef in England and Wales (unpublished data), while patterns STYMXB.0083 and STYMXB.0010 have been identified in Typhimurium DT120 isolates in England and Wales and in Denmark [32]. These observations are consistent with previous

studies that serovar 4,[5],12:i:- strains are genotypically closely related to serovar Typhimurium [4,7,8,27]. Patterns STYMXB.0079 and STYMXB.0010 represented 58% of serovar Typhimurium R-type ASSuT strains in Italy [8]. Pattern STYMXB.0131 has also been identified among Danish serovar 4,[5],12:i:- strains [17]. Serovar 4,[5],12:i:- R-type ASSuT strains belonging to profile STYMXB.0131 were responsible for two major outbreaks in Luxembourg in 2006 where pork meat was suspected as the vehicle for the outbreaks [6]. In Italy, profiles STYMXB.0079 and STYMXB.0010 represented 83% of serovar 4,[5],12:i:- R-type ASSuT strains [8]. However, the majority of strains were phage type U302 or untypable; only 8% of the isolates belonged to DT120 and none were DT193.

MLVA typing was also applied to the strain panel as the technique is reportedly more discriminatory than PFGE and provides unambiguous typing data that is free of the bias generated by differences in resistance genotype that reportedly affects PFGE [33]. Using the nomenclature of Larsson *et al.* allowed easy recognition of related profiles [25]. The five most common MLVA profiles identified in this study, and single locus variants thereof, have previously been identified in *S.* Typhimurium DT193 R-type ASSuT strains isolated from humans, pigs, cattle and beef products in England and Wales in 2005-2006 (unpublished data) and in isolates of Typhimurium DT120 R-type ASSuT associated with a putative outbreak in humans in the northeast of England in 2006 [32]. That all monophasic strains were typable by MLVA, using the Lindstedt *et al.* Typhimurium-specific scheme [24], and shared closely related profiles with these Typhimurium isolates provides tentative further evidence that monophasic 4,[5],12:i:- isolates derive from serovar Typhimurium.

The data presented here suggest that a serovar 4,[5],12:i:- DT193 R-type ASSuT clone with PFGE profile STYMXB.0131 has emerged from serovar Typhimurium and spread within several European countries, with pigs as a likely reservoir of infection. Isolates of serovar 4,[5],12:i:- DT120 R-type ASSuT with closely related PFGE profiles were identified in humans and pigs from five of the participating countries. The diversity of PFGE and MLVA profiles within serovar 4,[5],12:i:- DT193 and DT120 R-type ASSuT isolates, and the differences between these isolates and those previously described in Spain [30], suggests that serovar 4,[5],12:i:- is likely to represent several clones or strains that have emerged independently from serovar Typhimurium. Recent genotypic studies have shown that in addition to the Spanish 4,[5],12:i- clone, other 4,[5],12:i:- lineages exist [27].

In the first ten months of 2009, DT193 and DT120 accounted for 18% and 11% of Typhimurium isolates in England and Wales, respectively. In contrast, DT104 accounted for only 7% of Typhimurium isolates (HPA *Salmonella* database, unpublished data). Serovar 4,[5],12:i:- has already caused substantial outbreaks in several countries, with reports of severe infections and also deaths [6,7,9,10]. In order to prevent a global epidemic of these newly emerging clones or strains, as occurred with Typhimurium DT104, appropriate intervention strategies need to be put in place as soon as possible, particularly in pig husbandry throughout the EU.

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Risk of Salmonella infection with exposure to reptiles in England, 2004-2007

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Non-typhoidal *Salmonella* infections are a common cause of gastroenteritis in England. Non-Enteritidis, non-Typhimurium *Salmonella* serotypes have gained in relative importance in recent years, but their modes of transmission are poorly understood. In a large case-case study in England between 2004 and 2007, the association between exposure to reptiles and *Salmonella* illness was investigated using multivariable logistic regression. Recent reptile exposure was associated with *Salmonella* illness with an odds ratio of 2.46 (95% confidence interval: 1.57-3.85, p<0.001), with much stronger effects among children under five years of age. The exposure was rare, and a population attributable fraction was estimated as 0.9%. Among the *Salmonella* serotypes found in people exposed to reptiles, several non-Enteritidis, non-Typhimurium serotypes were strongly associated with exposure. Reptile exposure is a rare but significant risk factor for *Salmonella* illness in England, with much higher risk in children.

Introduction

Non-typhoidal *Salmonella* is the second most common bacterial cause of gastrointestinal infection in England and Wales. It was estimated to account for 116,000 cases of illness, 3,400 hospitalisations and 268 deaths in 1995 [1]. In recent years, there has been a decline in notified infections in England and Wales of the most common *Salmonella* serotype, *S.* Enteriditis, due to improved control of *Salmonella* in chicken flocks [2], meaning that non-Enteritidis non-Typhimurium serotypes of *Salmonella* are becoming of greater relative importance in the United Kingdom (UK) – see Figure 1 [3].

Epidemiological associations of *Salmonella* infections are mainly inferred from investigation of outbreaks [3], although these account for only a small proportion of notified cases. Furthermore, it is thought that as little as one in six cases of gastrointestinal illness are notified to public health authorities in the UK [4]. Therefore, understanding of the causes of *Salmonella* illness outside of recognised outbreaks is limited. Food- [5] and travel-related exposures [3] are believed to be the dominant causal factors. The role of other rarer modes of transmission at a population level is less well understood.

Salmonella are among the flora naturally found in the gastrointestinal tract of many reptiles [6]. Human infection with *Salmonella* acquired from contact with reptiles is a well-recognised phenomenon and a recent review article summarised recent reports of reptile-associated salmonellosis in Europe [7]: Some European countries (Belgium, Finland, France, Germany, Ireland, Latvia) had reports of confirmed or likely reptile-associated *Salmonella* cases. In the Netherlands, serotype attribution techniques based on past identifications were used to estimate the fraction of human isolates that could be accounted for by exposure to reptiles. It was concluded that although less than 1% of *Salmonella* isolates were attributable to exposure to reptiles and amphibians between 2000 and 2007, this proportion was increasing in recent years [7]. Other European countries reported no known cases of *Salmonella* associated with reptiles, although information on this kind of exposure might not have been available in notification data. In the United States (US), reptile-associated salmonellosis is well known: there are documented outbreaks of salmonellosis related to pet reptiles [8,9] and two case-control studies [10,11] have described contact with reptiles or amphibians as important risk factors for salmonellosis in children. We are not aware of any previous studies describing the population-wide effect of reptile-associated salmonellosis in the UK.

Salmonella taxonomy and nomenclature is complex. This study employs the standard Kaufmann-White serology-based naming system for serotypes described here. Serotypes are referred to by abbreviated versions of the full name: the formal title of *Salmonella* enterica serotype Enteritidis as is here abbreviated to *S.* Enteritidis.

The Co-ordinated Local Authority Sentinel Surveillance of Pathogens (CLASSP) study was conducted by the Health Protection Agency (HPA) to investigate the effects of a wide variety of exposures on the acquisition

of gastrointestinal illness in the general population in England. Among these exposures pet ownership in general and exposure to reptiles in particular were investigated. The CLASSP study used a case-case format [12] which is a variation of the standard case-control methodology where cases of another disease (here *Campylobacter* infections) are used as control cases for comparison with the disease cases under investigation (here *Salmonella*).

The main theoretical advantages of the case-case methodology are that it should be able to avoid introduction of notification bias and to minimise recall bias. The main disadvantages are that no apparent effect may be observed if the exposure under investigation is associated with both diseases, and that the control group will differ from the ideal study base (here the general population).

The most commonly described epidemiological associations of *Campylobacter* infection are with handling or consumption of inadequately cooked chicken meat and foreign travel, particularly to developing countries [13]. Consumption of some other foods has been described as a risk factor (RF) for *Campylobacter* illness [14,15]. *Campylobacter* is not among the commensal bacteria known to be carried by reptiles [6] and none of the many large epidemiological studies looking at RFs associated with *Campylobacter* (including those referenced above) have cited reptiles or amphibians as significant associations with this disease.

The aims of our study were to test the hypothesis that recent exposure to a reptile is associated with development of a *Salmonella* illness after accounting for all important confounding effects and to calculate a population attributable fraction (PAF) for reptile ownership on all *Salmonella* infections occurring in England.

Methods

Data collection

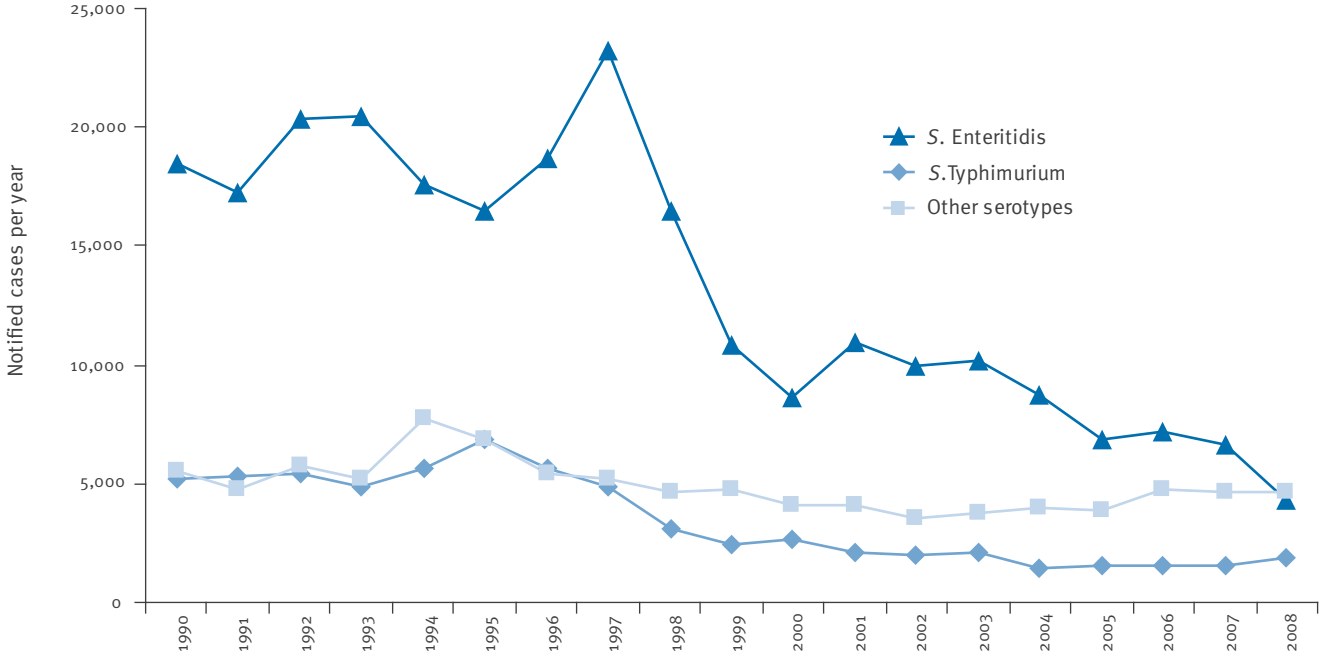
Health Protection Units of Local Authorities in England participated in the CLASSP study on a voluntary basis. Any individual resident in areas covered by these Local Authorities who had a microbiological isolate of either *Salmonella* or *Campylobacter* during the study period was eligible for inclusion. Information on exposures under investigation was collected using a standard study questionnaire covering a wide variety of plausible risk factors for acquisition of either *Salmonella* or *Campylobacter*. Questionnaires were filled in by Environmental Health Officers (who were unaware of the serotype of *Salmonella* isolates and to the hypothesis under investigation here) or were posted to the participants. Data entry and microbiological procedures were performed according to standard methods at HPA

Outcome and exposure variables

The outcome variable for this analysis was 'type of infection': either *Campylobacter* or *Salmonella*. Cases of *Campylobacter* were used as the control cases for this analysis with no matching of cases to controls. Questionnaires relating to infections with organisms other than non-typhoidal *Salmonella* or *Campylobacter* infections were excluded, as were records missing data for age, sex or cultural background.

Figure 1

Notified *Salmonella* infections in England and Wales, 1990-2008



Source: Health Protection Agency surveillance data.

A binary variable for the main exposure (ownership of reptiles) was derived by extraction of a variety of synonyms from the free text section of the CLASSP questionnaire relating to recent exposure to animals. The synonyms used for extraction were REPTILE, SNAKE, LIZARD, TORTOISE, TURTLE, TERRAPIN, DRAGON. Additionally, a manual search through all records was performed.

Other variables in the CLASSP questionnaire which represented potential RFs for acquisition of either *Salmonella* or *Campylobacter* infection were extracted from the study database These included variables relating to food and drink consumption, food handling, travel, pets (other than reptiles), visits to farms or zoos, recreational water activities, eating outside of the home. All of these study exposures were related to contact with the particular factor in the five days before development of illness. Age, sex and self-reported ethnicity were also included as study variables. Binary variables were created for each of these exposures, except for age and ethnicity which were categorical.

Missing data

For all binary exposures studied, we compared 'unexposed' individuals (those with no positive report of exposure) against 'exposed' individuals (reported exposure in questionnaire). Thus missing or unknown exposures were grouped into the 'no exposure reported' (baseline) group for each variable for the purposes of this analysis.

Statistical methods

All statistical analyses were performed using STATA v10.1.

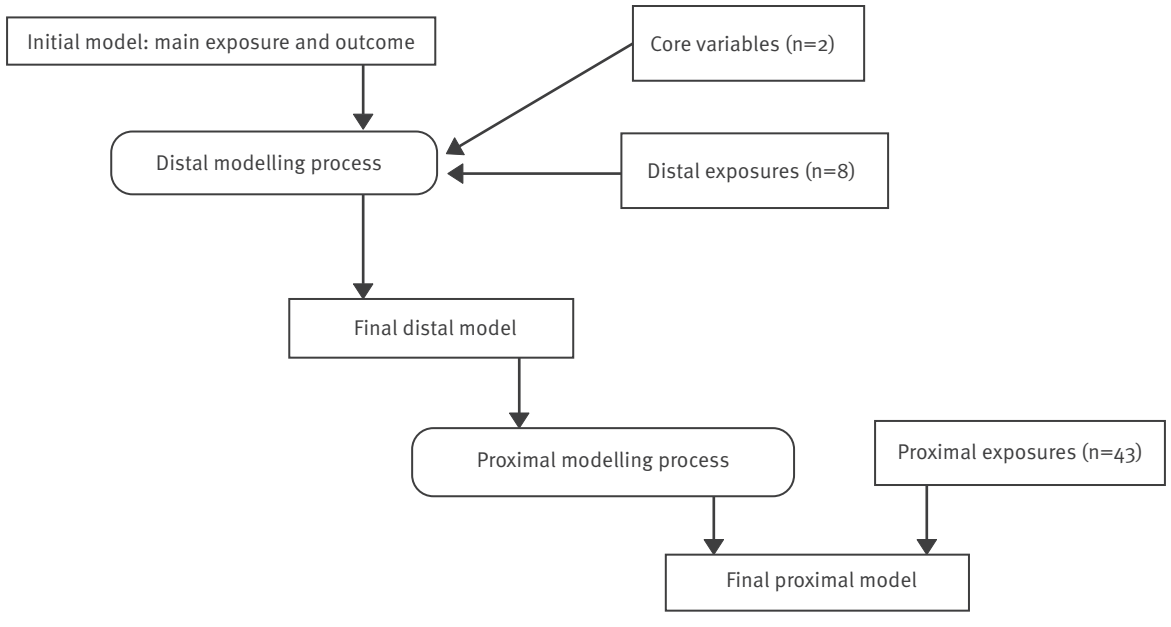
We described the demographic characteristics of study participants using Chi-square tests and Fisher's exact tests for association and Student's t-test for continuous variables. All exposure variables associated with the outcome with $p \leq 0.2$ in the bivariate analysis were included in the multivariable modelling process. Variables with $p > 0.2$ were considered not to have a direct effect on outcome, but were tested as potential confounders of the main exposure-outcome relationship.

We used a multivariable logistic regression model to determine the effect of reptile ownership on outcome and whether other exposure variables (i) provided an alternative explanation for outcome or (ii) confounded the main exposure-outcome relationship. We formulated a simple hierarchical framework to describe the causal relations of exposure variables [16]. We thus divided variables into a main exposure variable (ownership of a pet reptile), core variables (age and sex) and potential distal (n=8) and proximal (n=43) exposure variables. Distal exposure variables were those that might alter the likelihood of pathogen acquisition through a wide variety of end transmission vehicles, such as travel outside the UK or eating outside of the home. Proximal exposure variables were those relating to a specific method of pathogen acquisition, e.g. exposure to a particular foodstuff (e.g. eating or handling chicken) or type of animal (e.g. contact with a dog) or particular high-risk activities (e.g. watersports). Questions regarded a wide variety of exposures covering all known or suspected vehicles of transmission of these infections.

Variables were progressively added to the initial model as shown in Figure 2. First the distal exposure variables were introduced to the core model using a step-wise

Figure 2

Flow diagram of multivariable modelling process



process if they were significantly associated with the outcome (p≤0.05) based on evaluation of the p value in the likelihood ratio test. This generated the preliminary distal model. All excluded distal variables were then tested one by one to see if their inclusion resulted in significant confounding (>10% alteration) of the odds ratio (OR) for the main exposure-outcome relationship. This gave a final distal model.

Following this, proximal exposure variables were then introduced to the final distal model by the same process. The preliminary proximal model was examined to

Table 1

Descriptors of study participants, CLASSP study 2004-2007

	Salmonella (n=2,310)	*Campylobacter* (n=11,204)	Chi-square value	p value
Demographic variables				
Mean age (years)	35.1	44.0	-	<0.001 (t-test)
Sex				
Male	1,113 (48.2%)	5,412 (48.3%)	0.01	0.91
Female	1,197 (51.8%)	5,792 (51.7%)		
Ethnicity				
White	2,130 (92.2%)	10,600 (94.6%)		
Asian	105 (4.6%)	393 (3.5%)	23.6	<0.001
Other	75 (3.3%)	211 (1.9%)		
Data collection variables				
Questionnaire method				
Personal interview	469 (20.3%)	397 (3.5%)		
Telephone interview	452 (19.6%)	941 (8.4%)	>1,000	<0.001
Posted	318 (13.8%)	5,112 (45.6%)		
Unknown	1,071 (46.4%)	4,754 (42.4%)		

Table 2

Main multivariable results, CLASSP study, 2004-2007

	Distal model		Proximal model	
	OR (95% CI)	p value	OR (95% CI)	p value
Main exposure				
Reptile as pet	2.49 (1.61-3.83)	<0.001	2.46 (1.57-3.85)	<0.001
Distal exposure variables with OR>1.0				
Travel abroad	4.02 (3.63-4.45)	<0.001	–[1]	
Eating out at parties or buffets	1.18 (1.05-1.33)	0.005	–[2]	
Proximal exposure variables with OR>1.0				
Eggs eaten at home			1.19 (1.05-1.35)	0.006
Eggs eaten outside the home			1.60 (1.39-1.83)	<0.001
Bacon eaten at home			1.30 (1.15-1.48)	<0.001
Cold meats eaten outside the home			1.23 (1.07-1.42)	0.005
Poultry other than chicken eaten outside the home			1.41 (1.18-1.69)	<0.001
Contact with an ill person			1.15 (1.01-1.30)	0.037
Swimming			1.22 (1.07-1.39)	0.003
Fishing			1.59 (1.05-2.42)	0.029
Total N	13,514		13,514	
Degrees of freedom[2]	16		35	

CI: confidence interval; OR: odds ratio.

[1] Variables from the distal model are included in the proximal model, but are not shown in the proximal model column as their effects are intended to be analysed in the distal model.

[2] Age, sex, ethnicity and any variables with a modelled OR of <1.0 are not shown in this table.

see if inclusion of any of the excluded proximal variables had a confounding effect (>10% alteration of OR) on the effect of the main exposure. We tested for interaction between the main exposure variable and age category, sex and history of travel abroad in the final model.

Results

Participating subjects

The CLASSP study took place in England between November 2004 and October 2007. There were 66 participating Local Authorities (or County Councils), covering approximately 20% of the English population.

Cases with mixed infections (both *Salmonella* and *Campylobacter*, or involving other organisms) were excluded from this analysis (n=140, 0.9% of questionnaires). All individuals with missing data for age, sex or ethnicity were also excluded (n=777, 5.4%). The remaining 13,514 questionnaires formed the basis for this analysis. There were completed questionnaires from 2,310 individuals with non-typhoidal *Salmonella* isolates (17.1%) and 11,204 with *Campylobacter* isolates (82.9%).

Questionnaires were completed by personal interview, telephone interview or by postal questionnaire. The method of data collection was only known for 7,689 of the 13,514 questionnaires (56.9%). In general terms, data for *Campylobacter* cases were more frequently collected by postal questionnaire (overall 46% *Campylobacter* versus 14% *Salmonella*), whilst questionnaires for *Salmonella* infections were more often collected by personal or telephone interview

Table 3

Interaction of main exposure and age in final multivariable model, CLASSP Study, 2004-2007

Age group (years)	*Salmonella* cases in reptile owners	*Campylobacter* cases in reptile owners	Multivariable OR	95% CI	p value
0	9	3	17.3	4.50-66.25	<0.001
1-4	6	1	44.6	5.17-385	<0.001
5-19	8	4	12.1	3.52-41.7	<0.001
20-49	9	43	1.23	0.56-2.68	0.61
50+	2	23	0.65	0.15-2.85	0.57
Total	**34**	**74**			

CI: confidence interval; OR: odds ratio.

Table 4

Salmonella serotypes in subjects with and without exposure to reptiles, CLASSP study, 2004-2007

	Number of cases (% of *Salmonella* cases)			
	Reptile ownership		Univariate OR	
Organism/serotype	No	Yes	(95% CI)	p value[1]
Campylobacter	11,130	74	1.0 (baseline)	-
Salmonella Arizonae	1 (0)	2 (5.7)	300 (26-3,400)	<0.001
Salmonella Blockley	3 (0.1)	1 (2.9)	50 (5.1-490)	0.027
Salmonella Chester	6 (0.2)	1 (2.9)	25.1 (3.0-210)	0.046
Salmonella Ealing	0 (0)	1 (2.9)	infinite	0.007
Salmonella Enteritidis	1,211 (53.2)	5 (14.3)	0.62 (0.25-1.54)	0.3[b]
Salmonella Java	12 (0.5)	2 (5.7)	25.1 (5.5-114)	0.004
Salmonella Kentucky	22 (0.9)	1 (2.9)	6.8 (0.91-51)	0.14
Salmonella Muenchen	4 (0.1)	3 (8.6)	112 (24.5-520)	<0.001
Salmonella Oranienburg	4 (0.1)	3 (8.6)	112 (24.5-520)	<0.001
Salmonella Panama	4 (0.1)	1 (2.9)	37 (4.1-341)	0.033
Salmonella Senftenberg	14 (0.6)	1 (2.9)	10.7 (1.4-82.8)	0.096
Salmonella Stanley	33 (1.4)	1 (2.9)	4.56 (0.62-33.8)	0.204
Salmonella Tel-El-Kebir	0 (0)	2 (5.7)	infinite	<0.001
Salmonella Typhimurium	311 (13.6)	2 (5.7)	0.97 (0.24-3.96)	0.96[b]
Unnamed serotypes	136 (5.9)	8 (23.5)	8.85 (4.18-18.74)	<0.001
Other named serotypes	515 (22.6)	0(0)	0	-
Total (all *Salmonella*)	**2,276**	**34**	**2.24 (1.49-3.38)**	**<0.001[2]**

CI: confidence interval; OR: odds ratio.

[1] p value for Fisher's exact test unless otherwise specified.

[2] p value for Chi-square test.

(20% *Salmonella* versus 4% *Campylobacter*), see Table 1. The study questionnaire was administered (or sent by post) on the same day as the case notification was received. The interval between reported onset of illness and administration of questionnaire was thus generally short (median interval: 9 days, interquartile range (IQR): 6-13 days).

Key demographic characteristics of the study population are shown in Table 1. In both pathogen groups, infections occurred most frequently in children under the age of five years, with reduced frequency between the ages of five years and 20 years and a plateau among adults over 20 years. Of all 13,514 included participants, 49.1% were male (49.3% of the *Campylobacter* cases, 48.2% of the *Salmonella* cases, chi-square: 0.1, p=0.91).

Risk factors for disease

Main exposure

A total of 34 of the 2,310 individuals (1.5%) experiencing a *Salmonella* illness reported ownership of a pet reptile, compared with 74 of the 11,204 (0.66%) individuals experiencing *Campylobacter* illness. Using *Campylobacter* as control cases, we calculated a crude OR for exposure to a pet reptile as 2.25 (95% confidence interval (CI): 1.49-3.38, p<0.001).

Types of reptiles were tortoises (n=51), snakes (n=30, various types), lizards (n=31, various types), turtles or terrapins (n=5), with some participants reporting exposure to more than one of these.

Multivariable analysis

The results of the multivariable modelling process are presented in Table 2 below. All exposure variables with an OR>1.0 are shown here, whilst age, sex, ethnicity and variables with a modelled OR<1.0 are included in the model but not shown. The main exposure was associated with the outcome with an OR of 2.46 (95% CI: 1.57-3.85, p<0.001) in the final proximal model. We identified 10 other exposures with association with *Salmonella* infection independent of the main exposure. These were consistent with known risk factors for *Salmonella* [3,5]. None of the identified risk factors related to pets, although fishing was identified as weakly associated with *Salmonella* infections. None of the variables investigated as potential confounders were found have a major confounding effect (>10%) on the main exposure-outcome association.

In the final multivariable model, there was evidence of interaction between the effect of the main exposure and age category (likelihood ratio (LR) test p=0.03) and between the main exposure and sex (LR test p=0.01). Children under the age of five years were at much greater risk when exposed to reptiles than other age groups. For infants (under one year old) the OR was 17.3 (95% CI: 4.50-66.25) and for young children (between one and four years old) the OR was 44.6 (95% CI: 5.17-385). The age-stratified effects of the main exposure are shown in Table 3. Males appeared to be at higher risk of *Salmonella* infection when exposed to reptiles than females.

Salmonella serotypes

Numbers of cases of *Salmonella* serotypes with one or more isolates among people with reported reptile exposure are shown in Table 4. Odds ratios are calculated in comparison to *Campylobacter* control-cases. There was a clear indication that the overall pattern of serotypes of *Salmonella* seen among people with exposure to reptiles was different to that seen among people without this exposure (chi-square: 654, p<0.001, data not shown). *S.* Enteriditis and *S.* Typhimurium were no more common among reptile owners than would be expected by chance, whilst several other serotypes appeared to have some degree of association with exposure to reptiles.

Population attributable fraction

A PAF is defined as "the proportional reduction in average disease risk (...) that would be achieved by eliminating the exposure(s) of interest from the population" [17]. To estimate PAF for *Salmonella* disease caused by reptile exposure, we needed a proportion of the (general) population with this exposure (ppe). We used the proportion of *Campylobacter* cases reporting reptile ownership to estimate this: 74/11,204=0.66%. OR was used as an approximation of risk ratio as this was a rare exposure. Using the formula

$$PAF = \frac{ppe(OR-1)}{ppe(OR-1)+1}$$

and the OR from the final multivariable model, we obtained a PAF value of 0.95% for reptile exposure on *Salmonella* infections in England. If such a PAF were calculated for under five-year-olds only, it would be significantly larger than this: note the very high multivariable OR for these age groups in Table 3. However, we feel it is not appropriate to actually calculate such a figure as it would be unreliable due to the small numbers of individuals in these groups.

Discussion

Main findings

In this large case-case study of the exposures associated with *Salmonella* acquisition, we hypothesised that contact with reptiles was associated with development of illness after adjustment for alternative modes of acquisition and confounding factors. In our final multivariable model, there was a strong association of reported exposure to reptiles with *Salmonella* illness with an OR of 2.46 (95% CI: 1.57-3.85, p<0.001). The risk of exposure to reptiles was strongly influenced by age: children under the age of five years with this exposure were at much greater risk of developing *Salmonella* infection whilst individuals over the age of twenty years with this exposure did not experience significantly elevated risk.

These findings are unlikely to have occurred by chance, although the precise size of the effects could be subject to minor variation due to the small number of exposed individuals. None of the other variables of acquisition of infection in the multivariable model explained or negatively confounded this effect, and the effect of travel abroad acted as a positive confounder on the effect of reptile exposure. The effect of exposure to reptiles is unlikely to be confounded by unmeasured aspects of pet ownership in general as none of the seven other types of animal exposure examined in this analysis (dogs, cats, fish, poultry, other birds, other pets and farm animals) showed an independent association with *Salmonella* illness.

These findings are consistent with two case-control studies of risk factors associated with *Salmonella* infections in children in the United States (US) [10,11] where the odds of *Salmonella* illness in children were increased in association with recent contact with reptiles or amphibians.

There was clear indication from analysis of *Salmonella* serotypes that they were of different relative importance among people with and without exposure to reptiles. In those without recent reptile exposure, *S.* Enteritidis and *S.* Typhimurium predominated, in line with prevalent patterns of illness in the UK. Among those with exposure to reptiles, these two serotypes were much less common – they are known to be rare in poikilotherms [18] – and a variety of unusual *Salmonella* serotypes predominated. Many of these serotypes are known to be mainly found in reptiles (*S.* Arizonae [19]) or have previously been reported in cases or outbreaks of reptile-associated salmonellosis (*S.* Tel-el-Kebir [20], *S.* Java [21]). The analysis of serotypes was based on small numbers, so some of these associations may be chance effects.

We calculated a PAF for reptile exposure on all *Salmonella* infections in England during the study period as being 0.95%. We believe that this is the first such estimation made for such a PAF in England. This is consistent with an observation of 0.7% of *Salmonella* cases being of reptile-associated serotypes in the Netherlands [7], but less than a PAF estimate of 6% in a study specifically investigating reptile-associated salmonellosis in the US [10]. Although this PAF for reptile-associated salmonellosis in England is small, it represents a part of a sizeable disease burden – approximately 12,000 cases of salmonellosis were reported in England and Wales in 2007 [22], and this may underestimate the true community incidence by as much as threefold [4]. Furthermore, reptile-associated *Salmonella* appears to predominantly affect infants and children and could represent an amenable target for public health interventions [10].

Strengths and weaknesses

An important strength of the case-case format adopted for the CLASSP study was that it should have minimised bias due to case notification [23] as both cases and control cases came through the same notification process. The median interval from illness to interview was similar for *Salmonella* (11 days) and *Campylobacter* (nine days), indicating that a significant degree of recall bias was unlikely. As interviewers and participants were blind to the main hypothesis of this analysis, report of exposure to reptiles is unlikely to have been affected by interviewer bias or purposeful misreporting.

We are not aware of any association between *Campylobacter* illness and reptile ownership. Therefore whilst this case-case methodology may not have detected all exposures conferring risk for either *Salmonella* or *Campylobacter*, we are confident that it has accurately assessed the risk associated with the main exposure.

An important limitation of this analysis is the method of ascertainment of exposures, including the main exposure. Study participants were questioned on a wide variety of exposures and there were no objective validations of such exposure. Recall and report of pet ownership is likely to be more accurate than food recall, particularly as this concerned a period (on average) 9-14 days earlier. We felt that accuracy of exposure classification was likely to be adequate for the purposes of this analysis, and any resulting bias would be more likely to lead to underestimation than overestimation of the true effect size for the main exposure. The CLASSP study did not investigate RFs relating to susceptibility to disease (except age and sex) – factors such as recent antibiotic usage [24] may have had an effect on the development of illness.

Some element of bias may have been introduced to this study by use of different questionnaire methods between pathogen types: *Salmonella* cases were more likely to have a personal interview and *Campylobacter* cases were more likely to have a posted questionnaire. If there was differential accuracy in report of exposure by different interview methods this could have led to over or underestimation of effect sizes. We believe that such influences are unlikely to affect our main findings.

We analysed this study by comparing people with positive report of exposure against those with no reported exposure, such that people with unknown exposure status were included with the baseline group. This was done as a high proportion (>70%) of study participants had an unknown value for ≥1 exposure. The tick-box format of the questionnaire makes it likely that some participants omitted to tick for negative responses, which would lead to the data being Missing Not At Random (MNAR). The effects of bias introduced by this pragmatic compromise are limited: Other analyses of this dataset using different strategies (complete-case only and missing-indicator approaches) both suggested very similar sizes of effect for the main exposure-outcome relationship [25].

Some caution is required for the interpretation of the PAF estimate. The estimate of exposure to pet reptiles in the general population (0.66%) was obtained from the *Campylobacter* control cases in this study. The age distribution of *Campylobacter* cases did not match the general population – children were over-represented. Precise information on reptile ownership in the UK is difficult to obtain. A conference presentation in 2008 indicated there were approximately one million households in the UK with one or more pet reptiles, based on estimates from pet food sales [26], suggesting the calculated PAF may be an underestimate.

Conclusions

Reptile ownership is an important risk factor for *Salmonella* illness, with the effect being much stronger among infants and children. Although this exposure is rare in the general population, it may account for approximately 1% of *Salmonella* infections currently occurring in the UK. The calculated effect of exposure to reptiles is supported by the serological data on specific *Salmonella* serotypes seen among people self-reporting this exposure – these individuals are much more likely to be infected with unusual serotypes of *Salmonella* known to occur in conjunction with reptiles. Public health measures to minimise the risks of reptile-associated salmonellosis have been discussed elsewhere [10]. The HPA has published a leaflet outlining risks associated with reptiles [27]. Ownership of reptiles represents a serious risk to children.

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Phage typing of Salmonella Typhimurium – is it still a useful tool for surveillance and outbreak investigation?

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Phage typing has for decades been useful as a phenotypical, definitive method for epidemiological characterisation of *Salmonella* Typhimurium. The system recommended by the World Health Organization (WHO) Collaborative Centre for phage typing of *Salmonella* has, however, become rather complex, and the present study illustrates the challenges of sufficient standardisation of the interpretation of lysis results to make sure that the same strain is assigned to the same phage type in different laboratories. Even though molecular typing methods will replace phenotypic characterisation methods in the future, it is our opinion that phage typing will remain for some time a useful tool to strengthen global *Salmonella* surveillance. Therefore, improved standardisation and quality assurance is essential to obtain a robust and harmonised method that allows comparison of results between laboratories.

Epidemiological characterisation of pathogens causing human outbreaks of food-borne disease is essential for many reasons. The outbreak is often identified by increased registration of a specific pathogen (the Epi-type) during routine diagnostics and surveillance, and is further confirmed by detailed molecular characterisations that support the hypothesis of a common source of origin. Furthermore, identification of similar strains in historical specimen collections from monitoring programmes of food and food animal production can contribute to the generation of a hypothesis for the outbreak investigation. During the outbreak investigation, epidemiological characterisation constitutes the basis for the comparison of strains from human cases and potential sources, and finally, when the actual food source is found, constitutes the final demonstration of the infection source.

In the last decades more and more food-associated outbreaks have involved more than one country and even more than one continent, primarily due to the ever increasing globalisation of the food supply [1-3]. Identification and control of food-borne disease outbreaks of international significance can only be performed when professionals work together and agree on common methods applicable for definitive characterisation of pathogens like, in our case, *Salmonella*.

Serotyping according to the Kauffmann-White scheme [4] has for more that 80 years been the primary characterisation of *Salmonella*. This method is widely applied all over the world and harmonised to a degree that allows results to be compared between laboratories and countries. Some serovars, e.g. *Salmonella* Enteritidis and *S.* Typhimurium, are, however, so dominant in especially Europe and the United States that further characterisation is needed for surveillance.

In Denmark, phage typing as described by the World Health Organization (WHO) Collaborative Centre for phage typing of *Salmonella* (Health Protection Agency (HPA), Colindale, United Kingdom) has been applied for surveillance of *S.* Enteritidis and *S.* Typhimurium in humans, food and food production animals. Phage typing has proven to be an important tool for strain characterisation and the results obtained have been used since the mid-90s in surveillance, source attribution and outbreak investigations [5,6]. Phage typing is, however, also a phenotypical method that depends very much on the experience of the individual laboratory and on support from the reference centre that coordinates the maintenance of phages and the updating of the system. Only when the phage typing method is harmonised and the performance in different laboratories is controlled, can the results be regarded as definitive and comparable between laboratories.

The challenges encountered when using phage typing have become clear in a number of outbreak investigations in Denmark. A year after a large outbreak of human salmonellosis in April 2008 [7], more than 1,300 cases have been registered, and despite intensive epidemiological and microbiological investigations it has not been possible to identify the infection source. The outbreak had been identified due to an increase in the number of laboratory registrations of human *S.* Typhimurium with a unique multiple-locus variable-number tandem repeat (VNTR) analysis (MLVA) type corresponding to phage type U292. The strains were

further characterised by pulsed-field gel electrophoresis (PFGE) which showed a unique *Xba*I-profile, confirming the epidemiological relationship between the human cases [8].

The strains from the outbreak described here were phage-typed at the National Food Institute as is routine for all *S.* Typhimurium strains from human cases, food items and food productions animals in Denmark. The lysis pattern obtained from the initial typing was characterised by strong reactions with phages 11 and 14, and weaker reactions with phages 26 and 35, although some variations in the extent of lysis were observed during the outbreak that did not influence the phage type. There was no reaction with the remaining routinely used phages. This lysis pattern was in acceptable agreement with phage type U292. In summer 2008 during the continued outbreak, representative outbreak strains were sent to the WHO Collaborative Centre for phage typing of *Salmonella*, where they were characterised as U276. The results obtained there were similar to those obtained at the National Food Institute, but the interpretation of the results was different. Identical strains from this outbreak were therefore assigned to two different phage types by two experienced laboratories. As the different interpretation was the result of weighing the interpretation of all lysis reactions against the others, it can be difficult to judge which is the 'right' type designation.

Even though phage typing during this outbreak has been a valuable tool for the national investigation, the disagreement between two laboratories puts into question its usefulness as a definitive typing system that would allow comparison and communication between laboratories and countries. Disagreement between laboratories has caused confusion in past outbreak investigations: In 2003, an outbreak of human salmonellosis was identified in Sweden, and Danish pork was pointed out as the most probable source of infection. The source of infection was identified as *S.* Typhimurium DT108, but because this *Salmonella* type had not been detected by the Danish surveillance of pigs and pork production, pork did not seem to be the likely source. However, based on further joint investigation by the two countries it emerged that the strain initially identified as *S.* Typhimurium DT108 in Sweden was known as *S.* Typhimurium DT170 in the Danish surveillance, and consequently Danish pork could not be ruled out as a probable source (unpublished results, Baggesen, 2003). Late in 2008, an outbreak of *S.* Typhimurium characterised by a specific MLVA-type was identified in Sweden, Norway and Denmark and traced back to Danish pork. Strains related to this outbreak was assigned to several phage types (U288 in Denmark, RDNC in Norway and U302 in Sweden), and only due to inclusion of MLVA results, these cases were recognised as a common outbreak [9].

Should these and similar experiences disqualify phage typing as method for definitive strain characterisation in relation to diagnostics and surveillance of *Salmonella* infections? Several authors have suggested that genotyping methods such as PFGE and MLVA, combined with harmonised computer-based evaluation of the typing results and electronic exchange of data, can fulfil the requirements for definitive typing [10,11].

There is no doubt that molecular typing methods, eventually whole genome sequencing, will replace phenotypic characterisation methods in the future. But is this time now? Compared with the genotyping methods, sero- and phage typing are cheap and less labour-intensive methods based on simple technology for which only limited equipment is needed. This opens an opportunity for screening a large number of *Salmonella* strains as part of human diagnostics and monitoring programmes in food and food production animals not only in the developed part of the world but also in developing countries. Nowadays, more and more countries contribute to the international food supply. A strengthening of the global *Salmonella* surveillance with improved characterisation of strains from humans, food and animals, and sharing of the results among professionals is essential for food safety.

The outbreaks described in this paper suggest that phage typing currently has limitations, which could become worse if it was to be implemented globally for *Salmonella* surveillance. Improved standardisation and quality assurance is essential in order to obtain a robust and harmonised method that allows comparison of results between laboratories. The phage typing system recommended by the WHO Collaborative Centre and applied in the studies described here was first described 1959 by Callow [12] and has since been extended. Today it utilises a comprehensive number of phages, which leads to a large number of different patterns and thereby phage types. Assignment of a lysis pattern to a specific phage type is based on interpretation of the individual lysis reaction and comparison to a standard scheme of lysis patterns and phage types, a procedure that leaves room for conflicting results.

In addition, experience from the WHO Global SalmSurv programme (http://www.who.int/salmsurv/en) has proven that the capacity and quality of *Salmonella* serotyping can be enhanced through regular training of diagnostics staff and implementation of international external quality assurance systems [13]. It is likely that similar capacity-building and harmonisation efforts could improve phage typing.

We believe that phage typing can, for a while yet, play an important role in surveillance and control of the common *Salmonella* serotypes. However, this requires strengthened efforts to make the system available to more laboratories internationally, possibly a simplification of the system to enhance its robustness even though this may slightly compromise its discriminatory power, and finally improved external and internal quality assurance systems.

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Listeriosis outbreak caused by acid curd cheese 'Quargel', Austria and Germany 2009

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We report an outbreak of listeriosis in Austria and Germany due to the consumption of 'Quargel' cheese produced by an Austrian manufacturer. At the time of writing this report, the outbreak was known to account for 14 outbreak cases in 2009, including four cases with lethal outcome. On 23 January 2010, the cheese product was voluntarily withdrawn from the market.

On 14 August 2009, the binational Austrian-German Consiliar Laboratory for Listeria in Vienna noticed the occurrence of a new pulsed-field gel electrophoresis (PFGE) pattern in human isolates of *Listeria monocytogenes* serotype 1/2a. This consiliar laboratory receives all human isolates from Austria as required by law. In Germany, submission of isolates is voluntary. According to the available information at the time of writing this report, the outbreak clone accounted for 12 of the 46 Austrian cases in 2009 (serotype 1/2a (n=29), 4b (n=9), 1/2b (n=8)). Onset of illness is shown in the Figure. The 12 Austrian outbreak cases (two of them fatal) affected six of nine Austrian provinces. The mean age was 74.5 years (range: 58-88 years), eleven patients were male. In addition, two of 92 available human isolates from Germany in 2009 (total number of cases 389) showed this new PFGE-pattern. The German outbreak cases were two women in their 70s who died in November and December 2009 respectively. They had not visited Austria during the likely period of incubation (up to 70 days).

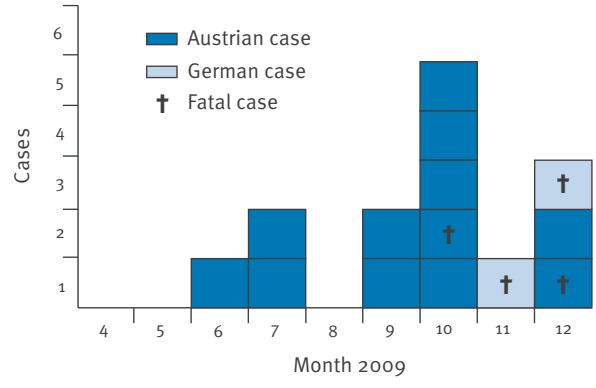
Since no reliable information was available on food consumed during the incubation period, all surviving Austrian outbreak cases were asked to collect grocery receipts for the three weeks after 3 December, i.e. after they were discharged from hospital, in order to collect information on routine food consumption behaviour. This epidemiological investigation revealed consumption of 'Quargel', a type of acid curd cheese available in different flavours, as a highly likely source of this outbreak. Three of seven outbreak cases providing receipts had bought product X produced by the Austrian manufacturer. Regular consumption of Quargel product X was confirmed by eight of nine participating outbreak cases, and consumption of Quargel cheese products was reported by heteroanamnesis for one German outbreak case (data on the second case remain unavailable).

Approximately 16 tons of Quargel per week are produced by the Austrian manufacturer. Fifty-three per cent of the product is exported to the German market and small amounts to the Czech Republic, Poland and Slovakia. This cheese is made of curdled milk, which ripens after addition of starter cultures for one day at 28°C, and after being sprayed with *Brevibacterium linens* for another two days at 14°C. The shelf life after packing and marketing is two months.

An environmental *L. monocytogenes* 1/2a isolate from the production plant, collected in December 2009, became available in January 2010 and proved indistinguishable from the outbreak strain by genotyping. Quargel cheese products sampled at the plant on January 13 yielded three different strains of

Figure

Outbreak cases of listeriosis by onset of illness, Austria and Germany, 2009 (n=14)



L. monocytogenes 1/2a, including the outbreak clone, in numbers of less than 100 colony-forming units (cfu) per gram. Food products collected on 18 January 2010 yielded greater than 100 cfu/g *L. monocytogenes*. The product was voluntarily withdrawn from the market on 23 January. On the same day, the public was informed about the incident and warned about cheese already bought. The plant stopped production. Investigation of the source of contamination is ongoing.

Conclusion

Industrial food production combined with international marketing of food and the low attack rate of *L. monocytogenes* hinder epidemiological outbreak investigation with traditional concepts [1]. Genotyping of *L. monocytogenes* isolates from clinical specimens can discriminate single-source clusters of food-borne infection and contribute to the identification and investigation of outbreaks. The outbreak described in this report probably would not have been identified without molecular typing [2]. The effectiveness of microbiological surveillance is entirely dependent upon the consistent and timely submission of all Listeria isolates from clinical laboratories to public health laboratories. In Austria, clinical laboratories are required by law to submit all clinical isolates of *L. monocytogenes* to AGES for PFGE analysis. In Germany, submission of *L. monocytogenes* isolates from clinical specimens by clinical laboratories is not required. The high case fatality ratio of listeriosis makes a strong case for the importance and priority of improved surveillance in Europe [3]. Our outbreak report underlines the value of routine molecular typing of Listeria isolates and also points out the considerable potential of cross-border cooperation for elucidating chains of infections.

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Update: Multinational listeriosis outbreak due to 'Quargel', a sour milk curd cheese, caused by two different L. monocytogenes serotype 1/2a strains, 2009-2010

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We previously reported an outbreak of listeriosis in Austria and Germany due to consumption of 'Quargel' cheese. It comprised 14 cases (including five fatalities) infected by a serotype 1/2a *Listeria monocytogenes* (clone 1), with onset of illness from June 2009 to January 2010. A second strain of *L. monocytogenes* serotype 1/2a (clone 2) spread by this product could be linked to further 13 cases in Austria (two fatal), six in Germany (one fatal) and one case in the Czech Republic, with onset of disease from December 2009 to end of February 2010.

Clone 1

As reported earlier, the binational Austrian-German Consiliar Laboratory for Listeria in Vienna noticed a cluster of human isolates of *Listeria monocytogenes* serotype 1/2a in August 2009 with a new pulsed-field gel electrophoresis (PFGE) pattern [1]. Fourteen cases (12 Austrian and two German), including five with fatal outcome (two of them German), were identified. Onset of disease ranged from June 2009 to January 2010. An epidemiological investigation revealed 'Quargel' cheese produced by an Austrian manufacturer as the source of infection. The product was withdrawn from the Austrian, German, Slovakian and Czech markets on 23 January 2010 [1].

Microbiological investigations confirmed the presence of this new strain (clone 1) in 'Quargel' samples taken at the factory in 2010: Two of 64 isolates available for testing (44 isolates cultured from cheese produced in 2010 and provided by the manufacturer, 20 isolates cultured from samples officially gained during outbreak investigation) showed the new PFGE pattern associated with the outbreak.

Clone 2

The 62 remaining food isolates showed a different PFGE pattern, that had not previously been seen in Austrian isolates either (clone 2). It was indistinguishable from the pattern of a human isolate from a listeriosis patient hospitalised at the time, who claimed to have eaten 'Quargel' cheese. Only two of 46 human *L. monocytogenes* isolates documented at the Austrian Reference Centre in 2009 yielded this PFGE-pattern, both coming from patients with a food-history positive for 'Quargel'. Ultimately, this second outbreak clone of *L. monocytogenes* serotype 1/2a accounted for 13 Austrian cases (two with fatal outcome), six German cases (one death), and one Czech case; onset of disease ranged from December 2009 until end of February 2010. The epidemic curve shows all cases associated with the two different outbreak clones by onset of illness (Figure).

Outbreak analysis

In total, the outbreak involved 34 cases of invasive listeriosis: 25 outbreak cases originated from seven of nine Austrian provinces. Four of these patients presented with meningitis: two with clone 1 and two with clone 2. A further eight patients were from four of 16 German federal states, and one patient was from the Czech Republic. Eight of the 34 cases in this outbreak had a fatal outcome. The median age of the cases was 72 years (range: 57-89 years), and 26 patients were male. There were no materno-neonatal cases. Underlying diseases were not different from those generally described for patients with listeriosis [2].

A total of 63 food samples of the 'Quargel' cheese products were microbiologically analysed. 20 samples were found positive for *L. monocytogenes*. 11 of the 20

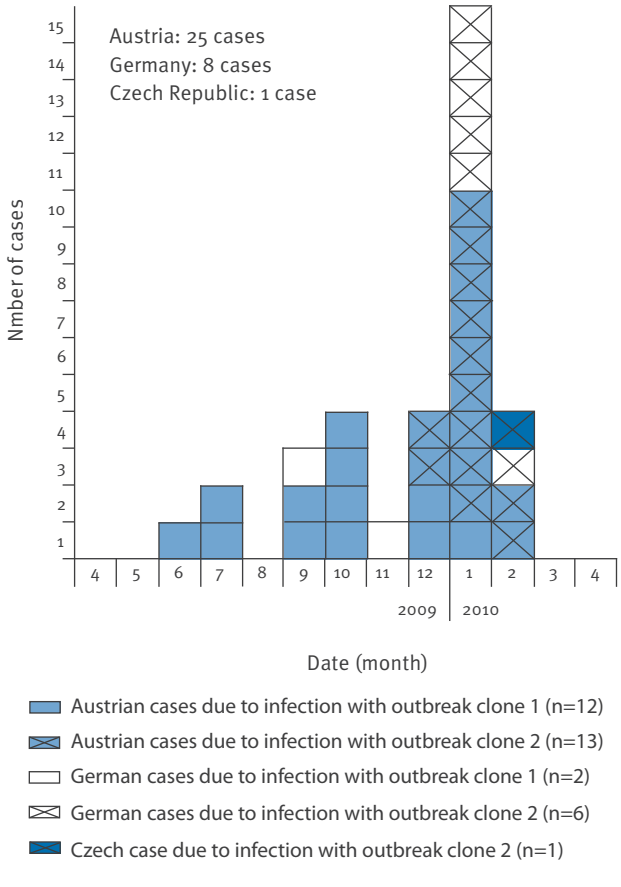
samples yielded less than 100 colony-forming units per gram (CFU/g), and nine samples harboured more than 100 CFU/g. All but one case can be explained by consumption of the contaminated product before it was withdrawn from the market on 23 January: one patient who was hospitalised for meningitis on 26 February 2010 had eaten the cheese (purchased before withdrawal from the market) on February 13. A leftover specimen, stored in the patient's refrigerator and sampled on 3 March, yielded 2,100,000 CFU/g of *L. monocytogenes*.

Case control study

The source of this outbreak was initially identified based solely on epidemiological findings [1]. We collected the cases' grocery receipts of purchases they made in December 2009, after their discharge from hospital, and compared them for matches. This generated a hypothesis to be tested by a case control study using case-case comparisons. For this study, a case was defined as a person in Austria from whom the *L. monocytogenes* outbreak clone 1 was isolated. Controls were patients from Austria with *L. monocytogenes* infections in 2009, whose isolates showed other profiles than the outbreak clone 1. Cases were asked about consumption of 12 cheese products in the six-month period prior to disease onset. Control persons were requested to provide information on consumption of the same cheese products in the year 2009. The overall response rate was 83.3% in the case group (ten of at the time 12 possible cases) and 72.2% in the control group (24 of at the time 33 possible controls, i.e. listeriosis-patients with isolates that showed other profiles than the outbreak clone 1). Consumption of the 'Quargel' cheese was identified as the only significant risk factor, highly associated with the illness in question. Nine of the ten cases with clone 1 had consumed the product; the tenth case provided no answer concerning this food item. Of 22 control cases (none with clone 2) all but two denied having eaten this specific cheese; the remaining two provided no answer concerning this food item. The computed odds ratio was 76.6 (95% confidence interval (CI): 9.3-infinity; P value <0.001).

Figure

Outbreak cases of listeriosis by onset of illness, Austria, Germany and Czech Republic, 2009-2010 (n=34)



Conclusions

The described outbreak provides some valuable lessons: Firstly, it underlines the considerable potential of molecular subtyping as a tool to identify outbreaks. Without routine PFGE typing of human isolates, this outbreak would have been missed. Secondly, it shows impressively that the waning of an outbreak (i.e. disappearance of an outbreak clone) does not necessarily imply that the underlying problem has disappeared. The shift to a different outbreak clone in December 2009/ January 2010 was probably caused by a change (in late November 2009) of the commercial ripening culture used in the cheese factory due to short supply of the original culture. Thirdly, our outbreak also emphasises the considerable potential of cross-border cooperation for elucidating chains of infections in multinational outbreaks. Industrial food production combined with international marketing of food and the low attack rate of *L. monocytogenes* hinder epidemiological outbreak investigations with traditional concepts [2]. Finally, the case of our patient with meningitis who had a leftover specimen of the causative food still in his refrigerator, underlines the importance of visiting households of listeriosis patients in order to obtain food samples and to advise other household members on precautionary measures. A single leftover food sample could prove an invaluable clue for elucidating the source of infection and thereby preventing further illness.

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Salmonella meningitis and septicaemia in an non-immunocompromised adult, associated with a cluster of Salmonella Enteritidis PT 14b, Ireland, November 2009

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We report a fatal case of meningitis caused by *Salmonella* Enteritidis phage type 14b in a middle-aged man who had no history or findings to suggest he was immunocompromised. To our knowledge, this is the first reported case of *Salmonella* meningitis in an adult in Ireland, and the first case of meningitis in an adult caused by phage type 14b. This case was associated with a nationwide cluster of salmonellosis which is still under investigation at the time of writing.

Introduction

Salmonella infection is recognised as a common cause of gastroenteritis which can result in large outbreaks [1]. Acute bacterial meningitis is a rare manifestation of *Salmonella* infection, and when it does occur, it is most commonly a disease of infants [2,3]. In adults, *Salmonella* meningitis, although rare, is most commonly seen in patients with impaired immunity, particularly in infection with human immunodeficiency virus (HIV) [4,5].

In this report, we describe a case of a non-immunocompromised adult with *Salmonella* Enteritidis meningitis and severe sepsis, with a rapid onset and a fatal outcome, that occurred in Dublin in November 2009.

This case is one of a cluster of at least 15 cases of *Salmonella* Enteritidis phage type 14b infection in Ireland that started in October 2009 [6; Health Protection Surveillance Centre, personal communication]. This variant has also been implicated in over 443 cases, including 14 outbreaks, in the United Kingdom since August 2009 [7,8].

Case description

The patient was a man in his late 40s who had a stable chronic mental illness and lived in a community psychiatric hostel in an urban area, supervised by 24-hour nursing staff. His regular medications included clozapine, amisulpride and valproate. Regular blood tests for potential side effects such as agranulocytosis did not show any abnormalities. He was a regular smoker. There was no abuse of alcohol or intravenous drugs and no known risk factors for HIV infection.

The patient woke in the early morning hours with a headache, which was relieved by treatment with 1 g paracetamol. After a quiet night he was found agitated and feverish six hours later. He was seen by a doctor and, due to rapid deterioration, was transferred by ambulance to the nearby acute hospital as an emergency with a seven-hour history of headache and a two-hour history of fever (38.5 °C), rigors, inability to stand and progressive reduction in level of consciousness. He was admitted to the intensive care unit on the same day.

On admission, a lumbar puncture revealed turbid cerebrospinal fluid, with a milky-brown colour. Three samples of cerebrospinal fluid (CSF) were taken at the time of admission. All of them showed a white blood cell count of >5,000 and a red blood cell count of zero. Microscopy of the CSF, performed urgently, showed abundant Gram-negative bacilli. A blood sample taken on admission showed a leukocyte count of 8.97 $x10^9$/L, with a neutrophil count of 8.13 x 10^9/L. Plasma urea, electrolytes, liver enzymes, total protein and albumin concentration were all within the normal range at that time.

A progressive neutrophilia was documented, with a count of 17.73 x 10^9/L on day 2, rising to 31.61 x 10^9/L on day 4 of hospitalisation. The patient developed acute renal failure on day 2 of hospitalisation.

The patient received one dose of 2 g cefotaxime and 2.4 g benzylpenicillin in the emergency department. After Gram-negative organisms were identified in the CSF samples taken on admission, this treatment was discontinued and the patient received 2 g meropenem

three times a day and 1 g vancomycin twice a day for the following three days. On day 4 of hospitalisation, cultures from the CSF samples and a series of three blood samples taken on the day of admission grew *Salmonella* sensitive to cefotaxime, and the patient's treatment was changed to 1 g cefotaxime every four hours.

In view of known association between *Salmonella* meningitis in adults and immunodeficiency, and despite the absence of risk factors, the patient was tested on day 5 for HIV, hepatitis B virus and hepatitis C virus infections, all of which were negative. Nor were there any incidental clinical signs, radiographic or laboratory findings to suggest underlying malignancy or opportunistic infection.

Despite treatment with appropriate antibiotics and interventions to support failing organ systems, he deteriorated and died five days after admission. The final *post mortem* report was not available at the time of writing this report.

Environmental investigation

In view of the fatal outcome, and despite the fact that no staff member or resident at the hostel had a history of gastroenteritis, stool samples from staff and fellow residents were obtained as part of the public health investigation. In addition to testing samples of food, eggshells and water from the hostel, other food premises where the patient was known or thought to have eaten were inspected, foods sampled and distribution chains traced. Particular attention was paid to foods containing chicken, eggs or egg products of any kind. None of the dozens of stool or food samples grew *Salmonella*.

The *Salmonella* species involved was nalidixic acid-resistant *Salmonella* Enteritidis phage type 14b. The same phage type has been identified in a cluster of cases notified to Irish departments of public health since November 2009, all of whom had gastro-enteritis alone and made a full recovery [6]. As with the above case, meticulous tracing of relevant foods through the distribution chains was and is being conducted for all cases, but to date the source of infection has not been identified.

Discussion

Human *Salmonella* infection is categorised into four manifestations: enteric infections, sepsis, non-enteric focal infections (including meningitis) and a chronic carrier state [3]. Bacterial meningitis is characterised by acute onset of fever, headache and one of the following signs: neck stiffness, altered consciousness or other meningeal signs [9].

The first case of *Salmonella* meningitis in the literature was reported in 1907 by Ghon [10]. In a study of 7,779 infections identified at the New York *Salmonella* Centre, meningitis accounted for only 0.8% [3]. In adults, *Salmonella* meningitis is most commonly seen in patients with intercurrent illness [11,12], including particularly immunosuppression associated with HIV [4,5].

Irish legislation requires doctors to send notifications of infectious diseases, including *Salmonella* infections, to medical officers of health in regional public health departments. Notification of bacterial meningitis from any cause must also be given. These data are collated at the national level by the Health Protection Surveillance Centre (HPSC). In a review of national data for the ten-year period from 2000 to 2009, the number of notifications of bacterial meningitis in Ireland was 1,229 and the number of notifications of *Salmonella* infection was 4,395, including 88 cases of typhoid and/or paratyphoid. The data from this period include only one case of *Salmonella* meningitis, a three-week-old baby with S. Dublin (medical officer of health, personal communication). Before this period, Foley *et al*. published in 1980 one series of three cases of childhood *Salmonella* meningitis in Ireland; all were infants [13].

Of all *Salmonella* infections listed in 2008 in the database of enteric infections collected by Enter-net, the European surveillance network for human gastrointestinal infections, *Salmonella* Enteritidis was by far the most common serotype, while S. Typhi, Oranienburg, Paratyphi and Berta were not listed among the top ten [14]. However, the European literature includes only two previous case reports of adults with meningitis due to *Salmonella* Enteritidis, one of whom was immunocompromised [15,16]. Other cases of *Salmonella* meningitis in adults previously reported in the literature have involved a diversity of serotypes, including at least 19 cases of S. Typhi [17-22], two cases of S. Typhimurium [18,11], and one case each of S. Oranienburg [23], S. Virchow [24], S. Paratyphi [21] and S. Berta [25].

The case described here is the only notified case of *Salmonella* meningitis in an adult in Ireland in the last ten years, and the first published adult case in Ireland. Kauffman *et al*. reported that *Salmonella* meningitis, arising in association with a variety of serotypes, may present without preceding symptoms of gastroenteritis [11], as was also true in our case.

There were no clinical signs, laboratory or radiographic findings to suggest a compromised immune system in this case. While agranulocytosis is a recognised adverse effect of clozapine, the fact that the patient's white blood cell count was monitored regularly and that he developed a marked neutrophilia indicates that this was not a factor.

Conclusion

We describe the first reported case of *Salmonella* meningitis in an adult in Ireland, who was not immunocompromised. The association of this fatal case with a phage type that has also been implicated in a large

number of sporadic cases and several recent outbreaks in the United Kingdom indicates the need for continuing vigilance in terms of surveillance and investigation to reduce the risk of further such infections with *Salmonella* Enteritidis PT 14b.

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Cases of Salmonella Urbana in Finland, the Czech Republic and Latvia, January-February 2010

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A cluster of 14 cases of *Salmonella* Urbana cases in Finland, the Czech Republic and Latvia were identified in January-February, 2010. The majority of cases (11) were male and children under 16 years of age. The investigation is currently ongoing and comparison of pulsed-field gel electrophoresis (PFGE) profiles of the isolates suggests that the cases may have a common source of infection.

On 5 February, the Finnish National Salmonella Centre (NSC) in the Bacteriology Unit of the Finnish National Institute for Health and Welfare (THL) reported four laboratory confirmed cases of *S.* Urbana (30:b:enx) to the THL Unit of Epidemiologic Surveillance and Response. Isolates originated from different parts of the country. The samples were taken between 13 and 30 January. According to the patients' physicians, none of them had been travelling abroad prior to the onset of illness with symptoms of diarrhoea and fever. Three of the cases were children under four years. A link between the cases was suspected because of temporal association of isolates of a very unusual *Salmonella* serotype. During the last 30 years, only three human cases of domestically acquired *S.* Urbana were reported in Finland. According to the Finnish Food Safety Authority, *S.* Urbana was found once in peanuts (in 2003) and in dog treats (in 2008). [H. Kuronen; personal communication].

In order to build a hypothesis of the source of the infection, cases or their guardians were interviewed using an extensive questionnaire focussing especially on food items generally consumed by children and to animal contacts, or contacts to animal feed. To map the occurrence of *S.* Urbana infection in other European countries, an inquiry to detect potentially linked cases in other countries was conducted through the Programme on Food- and Waterborne Diseases and Zoonoses network [5].

Table

Clinical characteristics of *S.* Urbana cases, Finland, Latvia and the Czech Republic, 2010

Country	Age	Gender	Clinical picture	Sample	Hospital care
Finland	11 months	F	bloody diarrhoea	faecal	yes
Finland	1 year	F	bloody diarrhoea	faecal	yes
Finland	13 years	F	bacteraemia, no gastrointestinal symptoms	blood	yes
Finland	3,5 years	M	diarrhoea	faecal	no
Finland	2 years	M	bacterial arthritis, no gastrointestinal symptoms	faecal+synovial fluid	yes
Finland	13 years	M	diarrhoea	faecal	no
Finland	35 year	M	diarrhoea	faecal	yes
Latvia	2 years	M	diarrhoea	faecal	
Czech Republic	7 years	M	watery diarrhoea	faecal	yes
Czech Republic	4 years	M	diarrhoea	faecal	no
Czech Republic	6 years	M	vomiting*	faecal	yes
Czech Republic	1,3 years	M	diarrhoea	faecal	yes
Czech Republic	20 years	M	bacteraemia, no gastrointestinal symptoms	blood	yes
Czech Republic	2,5 years	M	diarrhoea	faecal	yes

* Vomiting since November 2009, no diarrhoea/abdominal pain, hospitalised 18.1.2010

Investigations to date

A case was defined as a person with *S.* Urbana (30:b:enx, PFGE profile SURBXB.0002 and SURBXB.0003) infection in the European Union (EU) with the date of sampling between 1 January and 14 February 2010. In total 14 cases met the case definition (Table 1).

Twelve of the cases were children under 16 years. The median age was five years (age range 11 months old to 35 years old). Eleven were males. Three cases had a bacterial invasive disease, *Salmonella* isolated from blood or synovial fluid. Ten cases were hospitalised. Seven cases were from different parts of Finland, six from different parts of the Czech Republic and one from Latvia. In Finland, the descriptive epidemiological study suggested that all cases could have been exposed to dogs and all children had eaten raisins. In the Czech Republic, the epidemiological investigation revealed contact with dogs only in two cases and consumption of raisins in one case. No potential common source was detected in the Czech cases. The Latvian case had had no contact with dogs and had not consumed raisins, but the family had a cat whose feed was sampled and tested with negative results. The dog faeces, dog treats and raisins collected from the homes of the Finnish cases tested negative for salmonella.

PFGE profiles from the three countries, Finland, the Czech Republic and Latvia, were indistinguishable when compared to each other (Figure 1) indicating that the infections might have had a common source.

One Finnish PFGE profile (SURBXB.0003) had an extra band. This minor difference might be caused by a plasmid which salmonellae can spontaneously lose or acquire. It is also possible that a recent point mutation, deletion or insertion in the DNA had occurred. *S.* Urbana strains were sensitive to all antimicrobial agents tested (ampicillin, chloramphenicol, cefotaxime, imipenem, mecillinam, nalidixic acid, neomycin, sulfonamide, tetracycline, trimethoprim, streptomycin, and ciprofloxacin).

Conclusions to date

An unusual *Salmonella* serotype leading to a high rate of hospitalisation and the severe clinical picture of the cases detected in Finland and in the Czech Republic were important reasons for triggering the epidemiological investigation. According to data from the Finnish Infectious Disease Registry data base gathered between 2000 and 2009, less than 2% of all non-typhoidal salmonella findings were from blood. Similarly, in a large Spanish study, 4.5% of the patients with salmonellosis had septicaemia [1]. In the current cluster of *S.* Urbana, three cases of 14 had an invasive extraintestinal disease; two with bacteraemia and one with hematogeneous septic arthritis.

S. Urbana is rarely described in the literature. In the 1990s, a large outbreak occurred in a neonatal ward in Thailand [2] and a case of *S.* Urbana encephalopathy was reported from Japan [3]. The inquiry to the experts in the Programme on Food- and Waterborne Diseases and Zoonoses revealed that *S.* Urbana is rare in Europe in general, and mostly reported in children. Some of these cases had been associated with contacts with reptiles [4]. *S.* Urbana has also been found in sesame and equsi (melon) seeds, black pepper, animal feed and sewage sludge, according to experts in the Programme on Food- and Waterborne Diseases and Zoonoses network.

Only one of the cases (in the Czech Republic) had had contact with a reptile. According to our investigations, neither animals nor their feed seem to be the source of the current infections. Milk products appear to be less likely to be the source of infection, since one of the cases suffered from severe milk allergy. Fish, nuts, soya products and health food items were rarely consumed by the Finnish cases. Most of the cases were males, but we were not able to reveal any exposure common to the cases that could have been linked to being male.

Since the beginning of February, no further cases of *S.* Urbana have been detected in the three countries. Most of the cases had accumulated in two weeks in January in all three countries. The cases detected in the beginning of February were in a cancer patient without gastrointestinal symptoms (*Salmonella* found in blood)

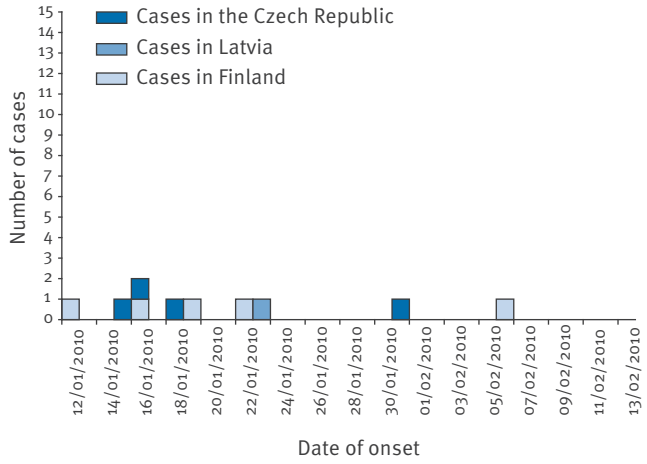
Figure 1

PFGE profiles of *S.* Urbana isolates from Finland, Czech Republic and Latvia when digested with *Xba*I enzyme.

PFGE; Pulsed-field gel electrophoresis

Figure 2

Cases of *S.* Urbana by date of onset of gastrointestinal symptoms and country, 12 January-7 February 2010



and an adult male who was considered a secondary case to his children that also suffered from gastrointestinal symptoms. When tested, however, the family members were negative for *Salmonella*. The accumulation of most cases with gastrointestinal symptoms in two weeks (Figure 2) suggests that the source of the infection could have been a product with a short shelf-life such as a batch of fresh produce, or a minor contamination of some other product. To date however, the source of the outbreak remains unknown.

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Upsurge of infections caused by Salmonella Concord among Ethiopian adoptees in Denmark, 2009

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Multidrug-resistant (MDR) *Salmonella* Concord has been associated with adoptees from Ethiopia. In 2009, Denmark saw an increase in MDR *S.* Concord infections: all eight cases reported in 2009 were among Ethiopian adoptees. The upsurge was linked to an increased number of infants adopted from Ethiopia. Data from other European countries suggests that they may face a similar problem.

Introduction

Two studies from seven European countries and the United States (US) have shown an increased number of infections with *Salmonella* Concord from 2003 to 2007 among mainly Ethiopian adoptees [1, 2]. Isolates associated with patients of Ethiopian origin were all multiresistant to antimicrobials including third generation cephalosporins.

In 2009, we recognised an increase in *S.* Concord infections in Denmark by querying the central *Salmonella* database at Statens Serum Institut. The recent investigation of this upsurge indicates that multidrug-resistant *S.* Concord continues to be imported from Ethiopia and therefore represents a concern for international public health. Additionally, we wanted to compare the upsurge of this specific pheno-/geno-type observed in Denmark with what has been reported in the rest of Europe through data from the European Surveillance System (TESSy) database at the European Centre for Disease Prevention and Control (ECDC).

Methods

A previously published study had indicated that in the US, the occurrence of *S.* Concord followed the number of Ethiopian infants adopted [1]. In 2009, an increased number of Ethiopian children adopted to Denmark were observed why this study was initiated to investigate if the same correlation between Ethiopian adoptees and the number of *S.* Concord cases were present in Denmark. In Denmark, all *Salmonella* cases are notified by the general practitioners to the Statens Serum Institut and archived in a central database; "Det Tarmbacteriobiologiske Register". By querying this database, the data on *S.* Concord were retrieved and the isolates further characterised. The patients or the parents of patients were interviewed to determine if the patients were adopted from Ethiopia, had travelled internationally or had any association with Ethiopians before onset of illness. Information about adoptions from Ethiopia was sought from national adoption agencies in Denmark [4, 5]. Serotyping and testing for susceptibility to antimicrobial agents was performed at Statens Serum Institut as minimum inhibitory concentration (MIC) determinations according to previously described methods [6], but with resistance (R) cut-off values for cefotaxime at R>2 mg/ml. Confirmatory testing for extended-spectrum beta-lactamases (ESBL) was applied on the eight isolates of Danish Ethiopian adoptees conferring resistance to cefotaxime and ceftiofur [1].

To get an overview of the situation in other European countries, we obtained data from the TESSy database at ECDC for 2006-2008 [3]. Case-based data on *S.* Concord serotype, importation status, probable country of infection and resistance against 11 antimicrobials were analysed from European Union (EU) and European Economic Area (EEA) countries. Country specific details are not included in the study.

Results

In Denmark, the number of *S.* Concord infections increased from none in 2007, two in 2008 and eight in 2009 (Figure).

Eight patients were female and age ranged from less than one year up to 30 years. Eight patients were infants less than one year of age and all were Ethiopian adoptees. The two adult patients were a 30 year old male who had been infected during a visit to Africa (country not specified) and a 23 year old female who had neither history of recent international travel nor contact with children or adults arriving from Ethiopia. General practitioners and parents of seven infants and the two adults were interviewed. Four infant patients were asymptomatic and were examined due to underweight,

relatives with diarrhoea and for general screening purposes. Five patients including both adults were suffering from non-bloody diarrhoea and one infant from urinary tract infection (UTI). Two infants, including the one suffering from a UTI were hospitalised. The link between the Ethiopian adoptees and the respective orphanages were not investigated. However, a previously published study reveal that the adoptees often originate from multiple orphanages or transit centres [1].

Laboratory investigations

The eight *S.* Concord isolates originating from Ethiopian adoptees all conferred resistance to ampicillin, cefotaxime, ceftiofur, chloramphenicol, gentamicin, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim respectively. Additionally, resistance to colistin (n=1), florfenicol (n=6) and reduced susceptibility to ciprofloxacin (n=6) was observed in the samples. The strains were all susceptible to apramycin, amoxicillin+clavulanic acid, nalidixic acid, neomycin and spectinomycin. All eight isolates were confirmed as ESBL-producing according to the phenotypic characterisation.

The two strains belonging to patients older than one year of age and without association to Ethiopia were both pansusceptible.

A total of 256 children were adopted from Ethiopia to Denmark from 2007 to 2009. During this period, the number of Ethiopian adoptees increased by 220% from 39 adoptions in 2007 to 125 in 2009 (Figure).

Data from other European countries

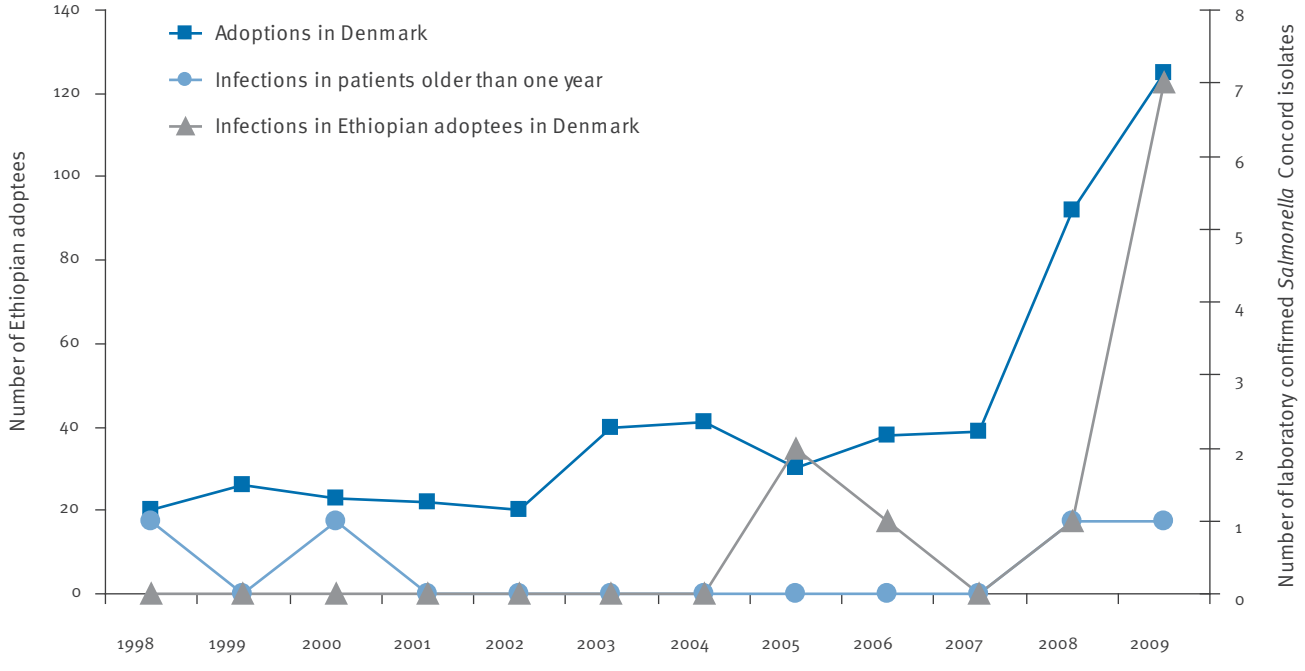
Data for 91 *S.* Concord patients were retrieved, as of 28 July 2009, from the TESSy database (2006: 21 patients from five EU/EEA countries; 2007: 37 from six EU/EEA countries; 2008: 33 from eight EU/EEA countries). In a pooled dataset from 2006-2008, 44 cases (48%) were reported in children under two years of age and 15 (16%) *S.* Concord cases were reported as acquired from outside of EU/EEA countries. The low proportion of isolates acquired from outside of EU/EEA are most likely biased due to the fact that many cases do not have the information on the country were the infection was contracted. Ethiopia was indicated as country of origin for eight cases in four countries and Kenya for one case. All eight cases originating from Ethiopia were below or one year of age. The one case with infection acquired in Kenya was a 24 year old male. Antimicrobial resistance data were available for 10 confirmed cases only, of which seven and four isolates conferred resistance to cefotaxime and ciprofloxacin, respectively; two of these cases were linked to Ethiopia.

Discussion

In Denmark and from a European perspective, *S.* Concord is a rarely reported serotype. From 2007 to 2009, an increased incidence of *S.* Concord infections among Ethiopian adoptees, most likely caused by the same multidrug-resistant clones from Ethiopia, was noted in Denmark [1, 2]. The increasing number of countries reporting cases of *S.* Concord and the suggested link between very young children and Ethiopia may be indicative of a noteworthy health problem in Ethiopia and an important route for the importation of *S.* Concord to Europe.

Figure

Number of children adopted from Ethiopia and the number of laboratory confirmed *Salmonella* Concord cases in Denmark, 1998 – 2009



In Denmark, the recent incidence of *S.* Concord has not earlier followed the adoption rate of Ethiopian infants as was described previously in the US [1]. There may be several explanations for this, one of which may be an increased awareness in Denmark of Ethiopian children being infected with *S.* Concord resulting in more testing. Another possibility could be an increased incidence of *S.* Concord in Ethiopian children.

In this study, the antimicrobial susceptibility data are consistent with the multidrug-resistant *S.* Concord widely spread among Ethiopian adoptees, whereas susceptible strains often can be traced to other parts of east Africa. It appears that the Danish strains from year 2009 are more resistant than previously observed, with six of seven strains from Ethiopian adoptees showing reduced susceptibility to fluoroquinolones. Previous studies of *S.* Concord isolates from Ethiopian adoptees have found that the strains harboured the following genes; $bla_{\text{CTX-M-15}}$ and $bla_{\text{SHV-12}}$ and *qnr*A and *qnr*B, respectively encoding resistance to third generation cephalosporins and reduced susceptibility to fluoroquinolones. Antimicrobial treatment of *S.* Concord from Ethiopian adoptees is hampered by the nature of the resistance pattern, which limits the options for treatment with traditional antimicrobials - including fluoroquinolones, which are not recommended for children [1].

Conclusions

The present study highlights the emergence of *S.* Concord isolates resistant to third generation cephalosporins among Ethiopian adoptees in Denmark during 2007 to 2009, and suggests that *S.* Concord continues to be a concern for international public health.

We recommend that physicians assess the health status of international adoptees, with special attention to Ethiopian adoptees, on arrival into the country of destination. If the health examination indicates that the child may have salmonellosis, specimens should be submitted for culture and subsequently for antimicrobial susceptibility testing if *Salmonella* is isolated. Unfortunately, it remains poorly understood why this *Salmonella* serovar seems to be linked in particular to Ethiopia, why the problem continues several years after its recognition, and how important this *Salmonella* serovar is for east African public health.

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Nationwide outbreak of Salmonella enterica serotype 4,12:i:- infections in France, linked to dried pork sausage, March-May 2010

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In May 2010, a nationwide excess of infections with the specific monophasic variant *Salmonella* enterica serotype 4,12:i:- was investigated in France. Subtyping with multilocus variable number of tandem repeats analysis revealed a distinct epidemic strain within this excess. Epidemiological investigations identified a dried pork sausage sold by a particular chain of supermarkets as the likely vehicle of transmission. The suspected batches have been withdrawn and recalled.

Introduction

On 7 May 2010, the National Reference Centre for *Salmonella* (NRC) alerted the French Institute of Public Health Surveillance (InVS) to a cluster of six cases of infection with *Salmonella enterica* subsp. *enterica* serotype 4,12:i:- in the area of Limoges, France, and to a nationwide increase of this specific monophasic serotype in comparison to previous years (Figure 1). At that time, 69 confirmed cases had been identified since the beginning of the year, compared with 37 in 2009 and eight in 2008 over the equivalent period of time. An epidemiological investigation was launched in order to determine the extent of the outbreak and identify the vehicle of transmission.

S. enterica serotype 4,12:i:- is one of a number of monophasic variants of the serovar Typhimurium, that have been emerging in Europe and elsewhere in recent years and are of increasing concern [1-3]. Information from the French Food Safety Agency (Agence francaise de securite sanitaire des aliments (AFSSA)) shows that this variant had been identified in a variety of foodstuffs, but most frequently in pork delicatessen.

Epidemiological and microbiological investigations

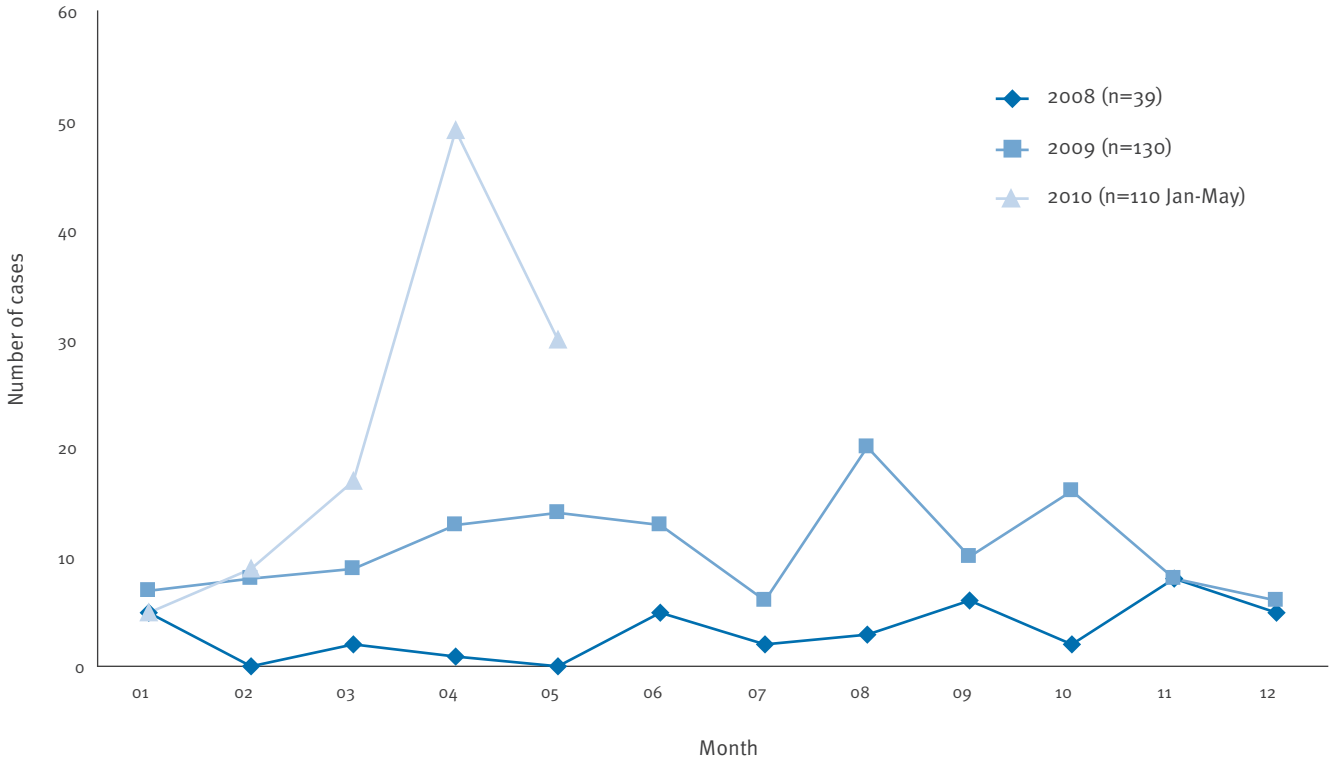
For this outbreak, a case was defined as a person resident in France with *S. enterica* serotype 4,12:i:- isolated from stool or blood in 2010, and with symptoms compatible with a *Salmonella* infection. The epidemic curve (by sample date, Figure 2) demonstrated an increase in the number of cases from week 12, with a peak in weeks 16 and 17. The investigation therefore focussed on the 90 (of 110) cases (as of 3 June) identified with a sample date from week 12 onwards. Among these cases, the median age was eight years (range 1–89 years), with a female:male sex ratio of 1.2. Cases were distributed throughout 49 of the 95 *départements* (administrative subdivisions) of mainland France, without any notable clustering (apart from the initial alert of six cases in Limoges).

As of 3 June 2010, 54 cases have been interviewed using a standardised semi-structured questionnaire exploring food consumption, travel history and other cases of diarrhoea in the household in the seven days before symptom onset. Dates of onset of symptoms for these cases ranged between 15 March and 16 May 2010. Twenty cases (37%) were hospitalised temporarily, with no deaths. Of these 54 cases, 53 (98%) reported buying pork delicatessen. Forty-two reported buying dried pork sausage (78%) and 33 reported shopping at supermarket chain A (61%). No other food types or activities were identified as likely sources of infection.

Multilocus variable number of tandem repeats analysis (MLVA) subtyping [4], using the latest nomenclature described by Larsson *et al*. [5], detected a major subtype, 3-13-15-NA-211, that allowed us to differentiate an epidemic strain from the sporadic cases. This profile differs from *S. enterica* serotype 4,12:i:- isolates from the beginning of 2010 and from 2007, as well as from other monophasic serotypes and serotype Typhimurium. To date, 53 of the 90 cases have been subtyped by MLVA, 32 of which had this specific subtype and have been retrospectively defined as 'epidemic cases'.

Figure 1

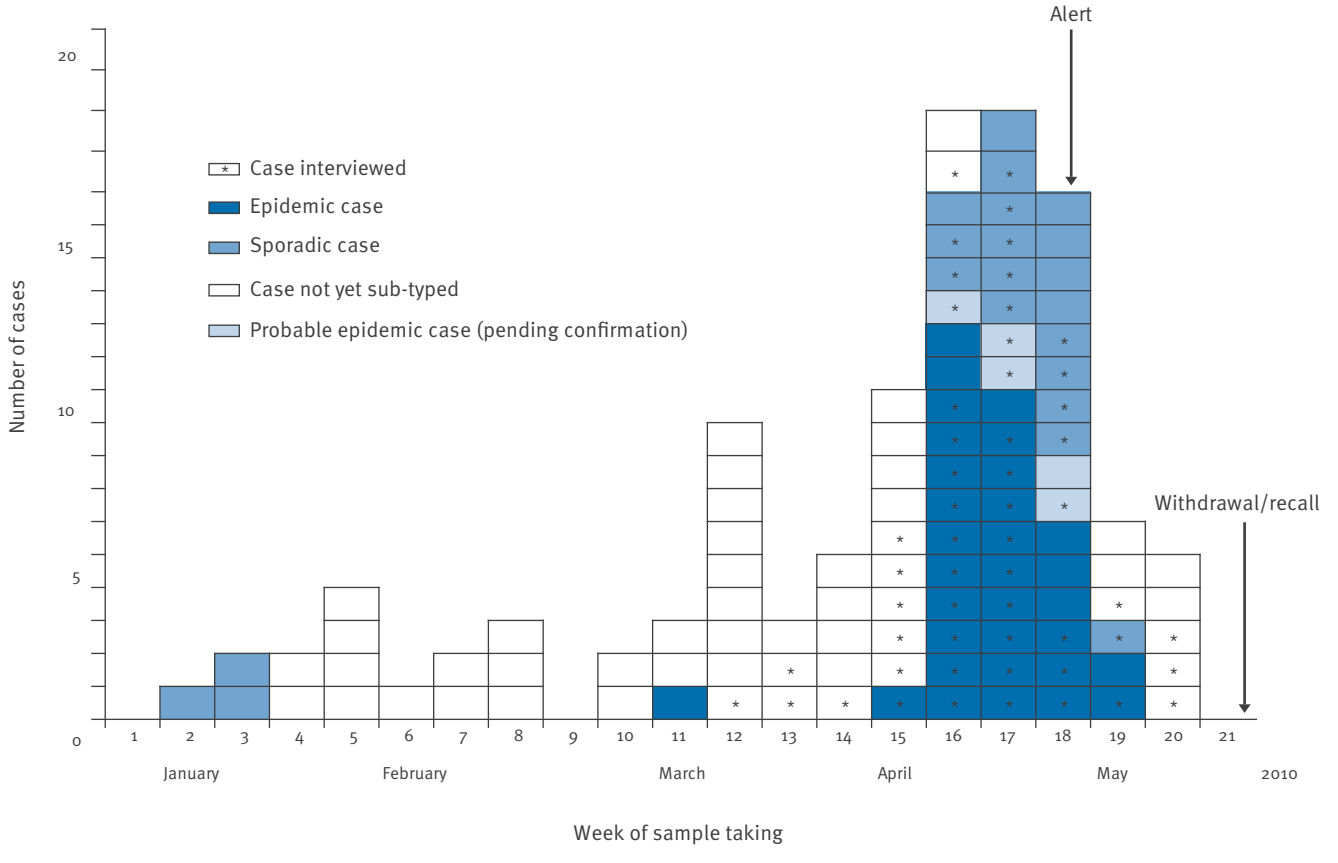
Number of *Salmonella enterica* serotype 4,12:i:- human isolates by month of sample collection, France, 2008-2010



Data as of 3 June 2010, National Reference Centre for *Salmonella*.

Figure 2

Number of human cases due to *Salmonella enterica* serotype 4,12:i:- by week of sample collection, France, 2010 (N=110)



Data reported by 3 June 2010.

Of the 53 subtyped cases, 36 have been interviewed. Of them, 24 (67%) were infected by the epidemic strain, two of whom were considered to be secondary cases and therefore excluded from further analysis. Twelve were considered to be sporadic. We noted that 20 of 24 epidemic cases shopped at a branch of supermarket chain A, compared with four of 12 sporadic cases (odds ratio 9.0, 95% confidence interval 1.41-61.7, p=0.0047). This reinforced the initial suspicions of an item purchased from supermarket chain A as the vehicle of transmission. Consumption of dried pork sausage was unusually high in both groups of cases (20 of 24 (82%) epidemic cases and nine of 12 (75%) sporadic cases), compared to previous outbreak investigations in France (range 33 of 67 (49%) to 21 of 33 (64%) in controls identified for outbreaks of *Salmonella* species linked to meat and cheese products since 2000 [6,7]).

Purchases of dried pork sausage made at branches of supermarket A in the three weeks prior to symptom onset were investigated by the French Directorate General for Food using data recorded through loyalty card numbers. Of the nine epidemic cases who used their card in the three weeks preceding symptom onset, all purchased the same type and brand of dried pork sausage produced by one manufacturer exclusively for supermarket A. *Salmonella* species had been isolated from a melee used to make a batch of this type and brand of sausage from this manufacturer in February 2010, but no failures in the production processes were identified. Later quality controls of this batch were negative for *Salmonella*. The isolate from the melee has been destroyed and is now unavailable for typing. Work is ongoing to identify any long term control measures to prevent future similar incidents.

Control measures

The batch of sausages ('use by' date up to 15 June) derived from this *Salmonella*-positive melee was subject to a national voluntary withdrawal and recall by the manufacturer on 27 May 2010, with a press release and posters in chain A supermarkets. A small proportion of the batch had been exported to Belgium, and the Belgian authorities were duly informed through the Rapid Alert System for Food and Feed (RASSF). Colleagues in other European countries were informed of this outbreak on 28 May via the Epidemic Intelligence Information System (EPIS) and Early Warning Response System (EWRS) of the European Centre of Disease Prevention and Control (ECDC). To date, no other European country has reported a current excess of cases of *S. enterica* serotype 4,12:i:-.

However, given that the suspected batch was delivered to supermarket A distribution platforms in the first two weeks of March, the relatively short turnover times at these platforms and at the supermarkets, and given that the last documented purchase from an epidemic case was made on 11 May (corresponding to a production date of 11 April at the latest), it is thought that the initial batch may not explain all the cases and that later batches may also have been contaminated. As a result, the French producer implemented a withdrawal and recall on 7 June of all batches available for purchase and produced before 12 April, accompanied by a press release from the authorities.

Conclusion

Epidemiological investigations identified one or more contaminated batches of dried pork sausage, produced by one manufacturer and supplied to branches of supermarket A, although *Salmonella* species were not isolated from a sample of the sausages. Incriminated batches have been withdrawn and recalled. Preliminary data suggest that the number of cases by week is decreasing. The investigation of this outbreak was assisted by the use of MLVA subtyping which was found to have an appropriate discriminatory power to identify a specific epidemic subtype. This outbreak of *S. enterica* serotype 4,12:i:- occurred on the background of the emergence of monophasic *Salmonella* strains in France and the rest of Europe, and future outbreaks due to this serotype are likely.

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Zoonoses in Europe: distribution and trends - the EFSA-ECDC Community Summary Report 2008

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On 28 January 2010 the European Centre for Disease Prevention and Control (ECDC) and the European Food Safety Authority (EFSA) launched their annual report on zoonoses and food-borne outbreaks for 2008. The report provides a comprehensive overview of zoonotic infections and disease outbreaks caused by consuming contaminated food. The number of reported human cases of the three most reported zoonotic infections, was lower in 2008 compared to 2007.

Campylobacteriosis was the most commonly reported zoonosis in the European Union (EU) for the last five years followed by salmonellosis and yersiniosis. The declining trend of salmonellosis continued, most likely as a result of the intensified control of *Salmonella* in animal populations, particularly in poultry, and better hygiene throughout the food chain.

The number of confirmed cases of listeriosis decreased by 11% in 2008 (1,381) compared to 2007 (1,554) in the EU. Foodstuffs that are considered the main source for human listeriosis in the EU include ready-to-eat (RTE) products (fish and meat), soft cheeses, salads and sandwiches. An EFSA-ECDC collaborative survey on Listeria in RTE products and in clinical cases of human listeriosis started in January 2010, the results of which will contribute to a better understanding about listeriosis in the EU.

Q-fever increased by 172% in 2008 (1,594) compared with 2007 (585). This was mainly due to several outbreaks in people entering areas with infected sheep and goats mainly in the Netherlands. In-depth investigations have been carried out in affected countries and it is suspected that the occurrence of Q-fever in humans and animals may be seriously underreported in Europe.

A total of 3,159 confirmed cases of Shiga-toxin/verotoxin producing *E. coli* (STEC/VTEC) were reported in 2008, representing an 8.7% increase from 2007 (2,905 cases). In animals VTEC was mainly isolated from cattle and, in lower proportion from small ruminants such as sheep and goats. In food, VTEC was detected in a considerable proportion of cow milk samples.

The 2008 annual Community Summary Report describes the five-year trends, distribution and 2008 figures for zoonotic infections and agents in humans, animals and foodstuffs in the 27 EU Member States, the European Economic Area and Switzerland [1]. Information aimed at protecting human health is collected and analysed according to the Zoonoses Directive 2003/99/EC [2]. Assisted by the Zoonoses Collaboration Centre (ZCC) in Copenhagen, Denmark, EFSA and ECDC jointly analysed the data. The results of this report highlight the importance of close collaboration between public health specialists and veterinarians and the need for robust surveillance systems in order to detect trends in zoonoses in Europe.

The full version with data per country and annexes are available on EFSA's and ECDC's websites.

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Notes

National Bulletins

Austria

Mitteilungen der Sanitätsverwaltung
Bundesministerium für Gesundheit Familie und Jugend, Vienna.
Monthly, print only. In German.
http://www.bmgfj.gv.at/cms/site/thema.html?channel=CH0951

Belgium

Vlaams Infectieziektebulletin
Department of Infectious Diseases Control, Flanders.
Quarterly, print and online. In Dutch, summaries in English.
http://www.infectieziektebulletin.be

Bulletin d'information de la section d'Epidémiologie
Institut Scientifique de la Santé Publique, Brussels
Monthly, online. In French.
http://www.iph.fgov.be/epidemio/epifr/episcoop/episcoop.htm

Bulgaria

Bulletin of the National Centre of Infectious and Parasitic Diseases, Sofia.
Print version. In Bulgarian.
http://www.ncipd.org/

Cyprus

Newsletter of the Network for Surveillance and Control of Communicable Diseases in Cyprus
Medical and Public Health Services, Ministry of Health, Nicosia
Biannual, print and online. In Greek.
http://www.moh.gov.cy

Czech Republic

Zpravy CEM (Bulletin of the Centre of
Epidemiology and Microbiology)
Centrum Epidemiologie a Mikrobiologie Státního
Zdravotního Ústavu, Prague.
Monthly, print and online. In Czech, titles in English.
http://www.szu.cz/cema/adefaultt.htm

EPIDAT (Notifications of infectious diseases in the Czech Republic)
http://www.szu.cz/cema/epidat/epidat.htm

Denmark

EPI-NEWS
Department of Epidemiology, Statens Serum Institut, Copenhagen.
Weekly, print and online. In Danish and English.
http://www.ssi.dk

Finland

Kansanterveys
Department of Infectious Disease Epidemiology, National Public Health Institute, Helsinki.
Monthly, print and online. In Finnish.
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France

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Weekly, print and online. In French.
http://www.invs.sante.fr/beh/default.htm

Germany

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Weekly, print and online. In German.
http://www.rki.de/DE/Content/Infekt/EpidBull/epid__bull__node.html

Hungary

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National Center For Epidemiology, Budapest.
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Iceland

EPI-ICE
Landlæknisembættið
Directorate Of Health, Seltjarnarnes
Monthly, online. In Icelandic and English.
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Ireland

EPI-INSIGHT
Health Protection Surveillance Centre, Dublin.
Monthly, print and online. In English.
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Italy

Notiziario dell'Istituto Superiore di Sanità
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Monthly, online. In Italian.
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Monthly, online. In Italian.
http://www.epicentro.iss.it/ben

Latvia

Epidemiologijas Bileteni
Sabiedribas veselibas agentura
Public Health Agency, Riga.
Online. In Latvian.
http://www.sva.lv/epidemiologija/bileteni

Lithuania

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Užkreciamuju ligu profilaktikos ir kontroles centras
Center for Communicable Disease Prevention and Control, Vilnius.
Online. In Lithuanian.
http://www.ulac.lt/index.php?pl=26

Netherlands

Infectieziekten Bulletin
Rijksinstituut voor Volksgezondheid en Milieu
National Institute of Public Health and the Environment, Bilthoven
Monthly, print and online. In Dutch.
http://www.rivm.nl/infectieziektenbulletin

Norway

MSIS-rapport
Folkehelseinstituttet, Oslo.
Weekly, print and online. In Norwegian.
http://www.folkehelsa.no/nyhetsbrev/msis

Poland

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Panstwowy Zaklad Higieny,
National Institute of Hygiene, Warsaw.
Fortnightly, online. In Polish and English.
http://www.pzh.gov.pl/epimeld/index_p.html#01

Portugal

Saúde em Números
Ministério da Saúde,
Direcção-Geral da Saúde, Lisbon.
Sporadic, print only. In Portuguese.
http://www.dgs.pt

Romania

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Centrul pentru Prevenirea si Controlul Bolilor Transmisibile,
National Centre of Communicable Diseases Prevention and Control, Institute of Public Health, Bucharest.
Sporadic, print only. In Romanian.
http://www.cpcbt.ispb.ro

Slovenia

CNB Novice
Inštitut za varovanje zdravja, Center za nalezljive bolezni, Institute of Public Health, Center for Infectious Diseases, Ljubljana.
Monthly, online. In Slovene.
http://www.ivz.si/index.php?akcija=podkategorija&p=89

Spain

Boletín Epidemiológico Semanal
Centro Nacional de Epidemiología, Instituto de Salud Carlos III, Madrid.
Fortnightly, print and online. In Spanish.
http://www.isciii.es/jsps/centros/epidemiologia/boletinesSemanal.jsp

Sweden

EPI-aktuellt
Smittskyddsinstitutet, Stockholm.
Weekly, online. In Swedish.
htpp://www.smittskyddsinstitutet.se/publikationer/smis-nyhetsbrev/epi-aktuellt

United Kingdom

England and Wales
Health Protection Report
Health Protection Agency, London.
Weekly, online only. In English.
http://www.hpa.org.uk/hpr

Northern Ireland
Communicable Diseases Monthly Report
Communicable Disease Surveillance Centre, Northern Ireland, Belfast.
Monthly, print and online. In English.
http://www.cdscni.org.uk/publications

Scotland
Health Protection Scotland Weekly Report
Health Protection Scotland, Glasgow.
Weekly, print and online. In English.
http://www.hps.scot.nhs.uk/ewr/index.aspx

Other journals

EpiNorth journal
Norwegian Institute of Public Health, Folkehelseinstituttet, Oslo, Norway
Published four times a year in English and Russian.
http://www.epinorth.org

European Union
"Europa" is the official portal of the European Union. It provides up-to-date coverage of main events and information on activities and institutions of the European Union.
http://europa.eu

European Commission - Public Health
The website of European Commission Directorate General for Health and Consumer Protection (DG SANCO).
http://ec.europa.eu/health/index_en.htm

Health-EU Portal
The Health-EU Portal (the official public health portal of the European Union) includes a wide range of information and data on health-related issues and activities at both European and international level.
http://ec.europa.eu/health-eu/index_en.htm

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