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Eurosurveillance

Europe's journal on infectious disease epidemiology, prevention and control

Special edition:

Gastrointestinal infections

March – June 2011

This special edition of *Eurosurveillance* features the first peer-reviewed articles on the outbreak of enteroaggregative, Shigatoxin-producing *Escherichia coli* O104:H4-related haemolytic uraemic syndrome (HUS) in Germany and France. It introduces a paper that demonstrates new microbiological findings which will facilitate coordinated investigations by European public health laboratories.

The edition also focuses on outbreaks and surveillance of infectious gastroenteritides caused by a wide range of pathogens such as *Listeria Salmonella, Shigella* and rotavirus.

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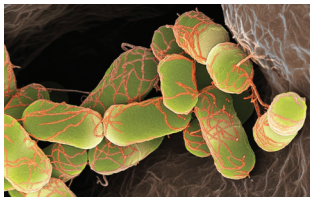

Escherichia coli bacteria, coloured scanning electron micrograph (SEM). *E. coli* bacteria are a normal part of the intestinal flora in humans and other animals, where they aid digestion. However, some strains, for instance E. coli O157, can produce a toxin that leads to severe illness, or even death. Normal strains can also produce infections in weakened or immunosuppressed people.

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Enteroaggregative, Shiga toxin-producing *Escherichia coli* O104:H4 outbreak: new microbiological findings boost coordinated investigations by European public health laboratories

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In the past weeks, we witnessed the unfolding story of one of the largest ever reported outbreaks of haemolytic uremic syndrome (HUS) and bloody diarrhoea caused by Shiga toxin-producing *Escherichia coli* (STEC), also commonly referred to as verocytotoxin-producing *E. coli* (VTEC) and enterohaemorrhagic *E. coli* (EHEC) [1]. This outbreak has caused considerable suffering and resulted in a strain on healthcare and public health systems in parts in Germany. It has shown a number of striking features: an unusually large proportion of HUS cases as compared with diarrhoea cases [1]. Furthermore, whereas usually HUS triggered by STEC infection predominantly affects young children, the great majority of cases in this outbreak are adults and two thirds are women. Between 2 May and 14 June 2011, 3,332 STEC cases, including 818 cases of HUS, were reported from 13 European Union(EU)/ European Economic Area (EEA) Member States and 36 patients have died [2]. Over 95% of STEC cases have been reported from Germany and the vast majority of cases reside in or have a history of recent travel to northern Germany. Additional cases related to the outbreak have been reported from Switzerland, the United States and Canada [3]. However, since 10 June, there has been a clear signal that the number of newly reported HUS and STEC cases is gradually decreasing, which suggests that we may finally be reaching the tail end of the outbreak.

The search for the source and vehicle of the outbreak has been a long and arduous process. Initial epidemiological findings pointed to raw vegetables and salads consumed in northern Germany as likely vehicles of infection and consequently led to the recommendation to abstain from eating these vegetables raw in northern Germany [1]. Extensive investigations implicated an organic sprout farm in Lower Saxony near Hamburg. Sprouts produced at this farm had been distributed to many of the incriminated restaurants and catering facilities, and were thus identified as a likely vehicle of infection. On 10 June, German public health and food safety authorities issued a joint statement recommending people to abstain from consuming sprouts [4].

Initial laboratory analysis of clinical isolates from outbreak cases performed at the German National Reference Centre for Salmonella and other Bacterial Enteric Pathogens at the Robert Koch Institute, in Wernigerode, quickly revealed that the epidemic agent was an STEC strain of rare serotype O104:H4, with production of Shiga toxin 2 [1]. Moreover, it was further atypical in that it lacked the attaching/effacing pathogenicity island of virulent STEC strains, as indicated by negative PCR results for the intimin (*eae*) and haemolysin (*hly*) genes. All outbreak-related clinical isolates were found to be multidrug resistant and displayed indistinguishable genomic macrorestriction profiles by pulsed-field gel electrophoresis (PFGE) analysis.

In this issue of *Eurosurveillance*, a collaborative group of investigators, led by the WHO Collaborating Centre for Reference and Research on Escherichia and Klebsiella, report several intriguing and important new findings on the nature and possible origin of the epidemic strain [5]. Firstly, using well- validated genotyping methods, Scheutz et al. provide convincing evidence that the STEC strain causing the outbreak in Germany is in fact not a typical virulent STEC strain, but instead is a much rarer hybrid pathotype that harbours the phage-mediated Shiga toxin determinant with an enteroaggregative *E. coli* (EAggEC) background, more precisely described as enteroaggregative, Shiga toxin/verotoxin-producing *E. coli* (EAggEC STEC/VTEC). Secondly, they also identify in this strain the presence of the receptor for iron-chelating aerobactin, known to be a virulence factor associated with the extra-intestinal *E.*

coli pathotype. Thirdly, they provide new data attesting to a close genetic relatedness of the German outbreak strain to previously described similar EAggEC STEC/VTEC strains. These findings are relevant for identifying the ecological reservoir and evolutionary origin of the epidemic agent, gaining a better understanding of the biological determinants of unusual disease severity and clinical complications seen in outbreak cases and the design of specific diagnostic tools for detection and treatment of STEC cases, and identification of the epidemic strain for accurate outbreak monitoring.

So what do the findings tell us about the reservoir and origin of the pathogen causing this outbreak? EAggEC is a common pathogen causing diarrhoea in travellers and persistent diarrhoea in infants and young children living in countries with poor sanitation [6,7]. In contrast to STEC strains that have an animal reservoir, mostly ruminants, EaggEC strains have a human reservoir. Little is known about the pathogenic role and epidemiological features of infections caused by strains of the hybrid EAggEC STEC/VTEC pathotype. One HUS outbreak caused by a strain of this mixed pathotype, but associated with a distinct serotype, had been previously reported from France in 1998 [8]. Scheutz et al. report that seven previously reported cases of diarrhoea or HUS worldwide caused by EAggEC O104:H4 have been identified: from Germany in 2001, France in 2004, South Korea in 2005, Georgia in 2009 and Finland in 2010 [9,10]. By PFGE analysis of EAggEC O104:H4 strains that are positive and negative for the Shiga toxin (stx) gene, the authors further demonstrate that, in contrast to the diversity seen within this serotype, isolates from the 2011 German outbreak cases exhibit a level of genetic similarity, which is also seen in the EAggEC STEC/VTEC O104:H4 strain from an unpublished outbreak of HUS in Georgia, which was investigated jointly by the United States Centers for Disease Control and Prevention (CDC) and Georgian public health authorities in 2009. However, no epidemiological link between these two outbreaks has been reported as yet and therefore the meaning of this finding remains elusive. Additional comparison of genomic relatedness of the German 2011 epidemic strain with other previously detected STEC O104:H4 strains causing sporadic HUS cases in other parts of the world should provide a more complete understanding of the potential reservoir and possible origin of the 2011 epidemic strain.

Another fascinating development stems from comparative genomics, available in real time, to elucidate the ancestral origin of the 2011 outbreak strain. On 2 June, further information on the nature of the hybrid EAggEC STEC/VTEC pathotype of this strain came from whole genome sequence information generated by two groups of German academic investigators [11]. Sequence information from a third isolate from a patient was subsequently generated at the Health Protection Agency, United Kingdom. The data sets from these sequencing initiatives were instantly released for public access, resulting in data analysis among bioinformaticians and other researchers around the world. Results from these preliminary analyses have been rapidly communicated via blogs, Twitter and private web pages, outside the standard peer-reviewed scientific publication route. These initiatives have confirmed the microbiological characterisation of the outbreak strain made in the public health laboratories by targeted genotyping and phenotyping of facultative *E. coli* virulence genes. Most importantly, among compared *E. coli* genome sequences, the genome of the 2011 outbreak strain clustered closest to an EAggEC strain isolated in 2002, with the addition of stx2 and antibiotic resistance genes.

How do these microbiological findings help clinical and public health laboratories detect and confirm cases in a timely and reliable manner? Further to key information provided by the Robert Koch Institute on strain screening and characterisation, Scheutz et al. also propose an alternative simple laboratory screening tool for detecting the 2011 German outbreak strain: a bacterial cell slide agglutination assay with cross-reacting antiserum against the capsular K9 antigen. This test, depending on reagent availability, can be used for the primary laboratory detection of *E. coli* O104:H4 in faecal specimens from suspected cases. Therefore, this assay enhances the potential capability of microbiology laboratories to detect and report cases accurately to clinical practitioners treating the patients and to public health authorities investigating the outbreak.

In summary, from a scientific perspective, the major findings reported in this issue by Scheutz et al. shed light on the unusual pathogenic features, prior occurrence in human pathology and likely natural reservoir of the *E. coli* strain causing the ongoing HUS and diarrhoea outbreak in Germany. More studies are needed to understand which and how these biological features of the bacterium actually determined the unique clinical and epidemiological disease manifestations in this outbreak.

Furthermore, from a public health perspective, it should be emphasised that the microbiology findings and technical recommendation presented were immediately shared by the authors through EU and international public health and food safety laboratory alert networks. This timely dissemination of key data to those who need to know has included posting technical information on the European Centre for Disease Prevention and Control (ECDC)-supported Epidemic Intelligence Information System (EPIS) rapid exchange platform. The EPIS links together all EU/EEA public health laboratories in the Food- and Waterborne Diseases and Zoonoses network (FWD-Net). In parallel, the European Union Reference Laboratory for Verotoxin-producing *E. coli* rapidly developed a real-time PCR method to detect O104 somatic- and H4 flagellar antigen-associated genes in food samples and shared it with the EU veterinary and food safety reference laboratory network.

This approach illustrates how seamless collaboration between food and public health laboratories, as well the power of harnessing advanced molecular typing technology and electronic communication, can build the laboratory capacity needed to respond appropriately to the cross-border spread of a highly virulent food-borne pathogen.

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The new face of enterohaemorrhagic Escherichia coli infections

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The unprecedented outbreak of Shiga toxin/verotoxin-producing *Escherichia coli* (STEC/VTEC) O104:H4 in Germany in May and June 2011 displayed several novel epidemiological, microbiological and clinical features. Infection with STEC/VTEC, also referred to as enterohaemorrhagic *E. coli* (EHEC), with or without haemorrhagic uraemic syndrome (HUS), which is usually a disease of pre-school children and equally distributed among the sexes, affected in the current outbreak mostly women over the age of 20 years (87%). In addition, several intriguing microbiological characteristics of the new epidemic strain have just been published [1,2].

With regard to the clinical characteristics, STEC/VTEC O104:H4 again differed remarkably from previously described STEC/VTEC infections. During a telephone conference on 9 June, organised by the European Centre for Disease Prevention and Control (ECDC) with clinical experts and nephrologists from 16 Member States of the European Union (EU) and several European and national professional societies, German colleagues shared their first clinical experiences from their patients. Severe infection with STEC/VTEC O104:H4 usually presented as a disease in three phases. On admission, about 80% of the patients suffered from bloody diarrhoea and 20% from watery diarrhoea. In 25% of the cases with bloody diarrhoea, signs of HUS (based on laboratory parameters of haemolysis, thrombocytopenia, and renal function tests) evolved after 3–5 days [3]. Completely unexpected, however, was the observation that severe neurological symptoms developed after about 3–10 days in roughly 50% of patients with HUS, even though clinical and laboratory markers of HUS were improving. These patients who had at first seemed to improve or respond to therapy, deteriorated again. Some patients even had to be re-hospitalised 3–4 days after they had been discharged. Neurologists were very concerned about the severity of neurological symptoms, ranging from mild disorientation and cognitive dissociation to stupor or severe, life-threatening seizures. Despite the impressive clinical presentation, routine neuroradiological examination revealed only mild alterations, if any. Worryingly, especially patients with seizures seemed to respond only weakly to standard antibody-based treatment regimes.

In this issue of *Eurosurveillance*, Cordesmeyer et al. [4] report about an unusual case of STEC/VTEC O104:H4 infection associated with colonic ischemia, and Kuijper et al. [5] describe a case of household transmission of STEC O104:H4 from a mother to her child. In both cases, neurological symptoms were present, with severe manifestation and as yet unclear neurological outcome in the child. From a public health perspective, these and other rather unusual clinical presentations and sequelae of STEC/VTEC O104:H4 infections are of importance when it comes to supporting and guiding the identification of STEC/VTEC cases, providing recommendations for the follow-up of patients, or adapting existing case definitions for the disease. In order to share and disseminate relevant clinical data among European clinicians and to foster the dialogue between clinicians and epidemiologists, a clinical support initiative was established by the ECDC as a reaction to the outbreak. Nominated clinical contact points, and up to two additional clinical STEC/VTEC experts per EU Member State were invited by the ECDC to join this initiative. It comprises a password-protected internet discussion forum for timely exchange of information, expertise and best practices. In addition, an audio podcast (available through the ECDC website) has been produced, in which a clinical expert from Germany describes his experiences with the presentation, treatment, and outcome of patients infected with STEC/VTEC O104:H4.

This clinical support initiative is one more component of the European response against this devastating outbreak and the possible future establishment and spread of the new STEC/VTEC O104:H4 strain in Europe. It will add to and support the ECDCs ongoing efforts in the field of scientific advice, outbreak response and surveillance.

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Zoonoses in the European Union: origin, distribution and dynamics - the EFSA-ECDC summary report 2009

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We present a summary of the main findings of the latest report of the European Food Safety Authority and European Centre for Disease Prevention and Control on zoonoses, zoonotic agents and food-borne outbreaks in the European Union (EU), based on data from 2009. Zoonoses are prevalent and widely distributed across several countries in the EU. The most important highlight of this report was the continuous decrease of human salmonellosis since 2005, probably due to effective control programmes in livestock.

Background

The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2009, produced by the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) on 22 March 2011, describes the five-year trends (2005–2009) and occurrence of zoonotic infections and agents in humans, animals and foodstuffs in the 27 European Union (EU) Member States. Reported cases from countries of the European Economic Area (EEA)/ European Free Trade Association (EFTA), namely Iceland, Liechtenstein, Norway and Switzerland [1] are also included in the preliminary description but not in further analysis or trends.

Zoonoses are diseases that are transmissible between animals and humans. Humans can acquire these infections directly from contact with sick or carrier animals, or through the ingestion of contaminated foodstuffs or from other environmental sources. The severity of these diseases in humans can vary from mild symptoms to chronic sequelae or life-threatening conditions.

In order to prevent zoonoses from occurring in humans and to control such diseases, it is important to identify which animals and foodstuffs are the main sources of the infections. Thorough analysis and description of the distribution of zoonotic diseases among EU countries allows targeting of control measures and monitoring of the progress of food-safety policies in the EU. The annual EU summary report compiles information from human surveillance systems and from monitoring programmes for food and animals, with the aim of protecting human and animal health according to the Zoonoses Directive 2003/99/EC [2].

Assisted by the Zoonoses Collaboration Centre-Technical University of Denmark (ZCC-DTU), EFSA and ECDC jointly analysed the data and a summary of the main findings are presented in this article.

Trends in the main zoonoses and zoonotic agents

Campylobacteriosis

In 2009, as in the previous four years, campylobacteriosis was the most commonly reported zoonotic disease in humans (198,252 confirmed cases). There was a 4% increase in the number of reported cases compared with 2008. The notification rate was 45.6 cases per 100,000 population, with children aged under five years having the highest notification rate (128 cases per 100,000 population). The number of reports of human campylobacteriosis was stable over the five-year period, but the incidence was always higher during the summer months. This could be due to a seasonal effect that has not been addressed through traditional *Campylobacter* control programmes for food and animals.

In foodstuffs, as in previous years, *Campylobacter* was most commonly isolated from fresh broiler meat at different stages of production: 31% of samples (n=7,312) were positive. According to the recent scientific opinion of EFSA biological hazards panel, about 20–30% of human campylobacteriosis cases can be attributed to the consumption and handling of chicken meat [3]. In pig meat samples, *Campylobacter* was detected much less frequently (0.6%, n=1,006) than in broiler meat. However, there was high variability in the number of reporting countries and sample size, depending on animal species and type of meat.

C. jejuni was the most frequently reported species in humans as well as in poultry and cattle, while *C. coli*

was less prevalent in humans and was isolated mainly from pigs.

Salmonellosis

Salmonellosis was the second most commonly reported zoonotic infection in humans in 2009, with 108,614 confirmed cases reported and a notification rate of 23.7 cases per 100,000 population, which is 17% less than in 2008. There has been a statistically significant decreasing trend in the notification rate during 2005 to 2009, with a mean reduction of 12% per year. The decrease has been particularly sharp for the most commonly reported serovar in humans, *Salmonella* Enteritidis: notifications fell by 24% from 2008 to 2009. The second most common serovar, *S.* Typhimurium, was also reported less frequently in 2009 compared with 2008, presenting a decrease of 10%.

In food, *Salmonella* was the most commonly identified pathogen in fresh poultry and fresh pork meat, where 8.7% (n=30,544) and 0.7% (n=83,797) of samples were found positive, respectively. The bacterium was rarely detected in vegetables, fruit or dairy products.

Harmonized *Salmonella* EU control programmes in poultry have been implemented progressively since 1994, starting with primary production. In 2009, Member States had to meet the EU reduction target of having ≤1% of breeding flocks of *Gallus gallus* (chickens) infected with the five target serovars (*S.* Enteritidis, *S.* Typhimurium, *S.* Hadar, *S.* Infantis and *S.* Virchow) [4,5]. Control efforts at poultry-farm level in Member States are considered to have contributed remarkably to a positive public-health effect in reducing the number of reported human salmonellosis cases.

It is reassuring that the declining trend of human salmonellosis continued in 2009. This is likely to be the result of intensified control programmes of *Salmonella* in animal reservoirs, particularly in poultry, and better hygiene practices throughout the food production chain. The introduction of molecular surveillance at the EU level in the future will provide more clues about the importance of different animal and food sources of infection and the impact of *Salmonella* control programmes in livestock.

Yersiniosis

The number of reported human cases of yersiniosis in 2009 was 7,595, with a notification rate of 1.65 cases per 100,000 population. Although the notification rate decreased significantly ($p < 0.01$) since 2005 (2.6 cases per 100,000 population), the disease continues to be the third most frequently reported zoonosis in the EU.

In animals, *Yersinia* spp. were reported mainly in pigs and pork products. *Yersinia enterocolitica* was isolated from 4.8% of pork samples (n=2,134).

Listeriosis

In 2009, the notification rate of human listeriosis was 0.36 cases per 100,000 population. The number of confirmed cases increased by 19% in 2009 (n=1,645) compared with 2008 (n=1,381). Listeriosis is an important food-borne disease due to its severity: it can lead to a high risk of abortion in pregnant women and high levels of mortality in elderly people (a case fatality rate of 19% was reported in people aged 65 years and over). The highest notification rate was also reported in this age group (1.1 cases per 100,000 population), representing 59% of all reported cases. Only 4.2% of the reported cases were detected among children aged under five years.

Foodstuffs that are considered the main source of *Listeria* in the EU include ready-to-eat (RTE) products (fish and meat) and soft cheeses. According to the EU microbiological criteria, foodstuffs that contain less than 100 colony-forming units (cfu)/g of *L. monocytogenes* at the retail level are considered acceptable for human consumption [6]. In 2009, the highest proportions of non-compliant food products at retail level were found in RTE fish products, cheese (especially soft and semi-soft) and RTE products of meat origin, although the level was lower than in the previous two years.

The high proportion of deaths among elderly people as a result of *Listeria* infection is of particular concern. An EFSA-ECDC collaboration on typing of *Listeria* in RTE products and clinical cases of human listeriosis started in 2010 and continues to 2012. The results provided by this study will contribute to a better understanding of listeriosis epidemiology in the EU and should help to target effective control and preventive measures within both food safety and public health.

Verotoxigenic *Escherichia coli* (VTEC) infection

A total of 3,573 confirmed human cases of verotoxigenic *E. coli* (VTEC) infection (0.75 cases per 100,000 population) were reported in 2009, a 13% increase compared with 2008 (n=3,159). The notification rate has increased since 2007 (0.6 cases per 100,000 population). VTEC O157 was again the serotype most commonly reported, although VTEC isolates were not characterised at the serotype level in 28% of the cases in 2009. As in previous years, the notification rate was highest in children aged 0–4 years. A considerable increase (of 66%) in the number of reported cases who developed haemolytic uremic syndrome was detected in 2009 (n=242) compared with 2008 (n=146), occurring mainly among 0–4 year-olds. Several outbreaks of VTEC infection were detected in United Kingdom and the Netherlands in 2009 and have contributed to the increasing trend in Europe and increased the number of haemolytic uremic syndrome cases [7-9]

In animals, VTEC was mainly isolated from cattle and, to a lesser extent, from small ruminants such as sheep and goats. In food, VTEC was detected mainly in meat

from ruminants: 3.2% (n=248) of sheep meat samples, followed by 2.3% (n=9,285) of bovine meat samples. It was also isolated from raw cow's milk. The reported occurrence of VTEC bacteria in food was generally low, and the levels have been relatively constant between 2005 and 2009.

Q fever

A total of 1,987 confirmed human cases of Q fever were reported in 2009, representing a 25% increase compared with 2008 (n=1,594). However, the majority of cases (91%) was detected in two countries: the Netherlands (n=1,623) and Germany (n=190). Adults aged 45–64 years had the highest notification rate (1.2 cases per 100,000 population).

The continued increase in Q fever in 2009 was the result of several outbreaks in which people were exposed to infected sheep and goats, mainly in the Netherlands.

Trends in zoonotic parasitic diseases and zoonotic parasites

Trichinellosis

Reported cases of human trichinellosis increased by 12% in 2009 (n=748) compared with 2008 (n= 670). The distribution of reported cases was not homogeneous across EU Member States, as the majority of cases (94%) was reported by four eastern European countries (Bulgaria, Romania, Poland and Lithuania). The reason for this large proportion of human cases in these countries may be linked to particular regional habits, such as raising pigs in backyards for private consumption, for which official meat inspection for the presence of *Trichinella* spp. is not carried out.

The increased number of cases of trichinellosis in these countries is of major concern because the disease is easily preventable when appropriate veterinary meat inspection is carried out and preventive measures are taken.

Echinococcosis

There were 790 reported human cases of echinococcosis in 2009, which is 11% fewer than in 2008 (n=891). Among reported cases with a known species, the predominant species was still *E. granulosus* (77%) while *E. multilocularis* was reported three times less frequently.

In animal populations, 18 Member States submitted data on *Echinococcus* spp. found in domestic livestock (cattle, pigs, sheep, goats and solipeds) as part of routine screening at slaughter. In addition, 10 Member States reported data on foxes positive for *E. multilocularis* (15.6% of tested foxes carried this species). Control measures implemented for dogs, such as deworming treatment, can restrict the spread of echinococcosis. However, foxes remain a potential source of exposure and vehicle for spread in some EU countries.

Toxoplasmosis

In 2009, a total of 1,259 confirmed human cases of toxoplasmosis were reported . The highest proportion was recorded in women aged 24–44 years, probably due to routine screening for antibodies against *Toxoplasma* during pregnancy.

Sheep and goats were the animal species with the highest proportion of *Toxoplasma*-positive samples reported (24.4%, n=4,217).

Trends in other zoonoses: brucellosis, tuberculosis due to *Mycobacterium bovis* and rabies

In 2009, human cases of brucellosis (n=401) decreased by 35.2% compared with 2008 (n=619). The number of cases has been decreasing significantly ($p < 0.01$) in the EU since 2005.

Cases of human tuberculosis due to *Mycobacterium bovis* in 2009 were not reported to the European Surveillance System (TESSy) at the time of the report production. Therefore the trends and epidemiological analysis were based on 2008 data. The number of confirmed human cases of tuberculosis due to *M. bovis* increased in the EU by 7.5% in 2008 (n=115) compared with 2007 (n=108). However, this could be a normal variation in the disease occurrence. Overall, the numbers of human cases decreased during the previous four years, mainly due to effective disease eradication programmes implemented by Member States in cattle herds.

In 2009, one indigenous case of rabies – in a woman bitten by a rabid fox – was reported in Romania. This is the second autochthonous case of rabies that occurred in Romania in the previous two years.

Conclusion

In 2009, campylobacteriosis, salmonellosis and yersiniosis were the most commonly reported zoonotic infections in humans of those monitored for this report in the EU, as in previous years. Parasitic zoonoses – trichinellosis, echinococcosis and toxoplasmosis – are still present in the EU. While some diseases, such as salmonellosis, have continued to decline, probably due to effective EU control measures in animal reservoirs, others have increased considerably, such as trichinellosis, even though the disease can be easily prevented.

The results of this report highlight the importance of close collaboration between veterinarians and public health specialists and the need for robust surveillance systems, in the animal/food sector and in humans, in order to monitor the impact of EU-wide control measures, detect emerging trends and sources and unexpected changes in the disease dynamics of zoonoses in Europe.

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Outbreak of rotavirus gastroenteritis in a nursing home, Slovenia, December 2010

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A gastroenteritis outbreak affected 45 people (40 residents and five staff) in a nursing home for the elderly in the Celje region, north-east Slovenia, between 17 December and 31 December 2010. Rotavirus group A was laboratory confirmed in the stools of five ill individuals. The outbreak was identified when the number of affected persons was high but was successfully controlled after implementing preventive measures.

Background

On 28 December 2010, the regional epidemiologist of the Institute of Public Health Celje, North East Slovenia, was informed that several residents and staff of a nursing home in the Celje region had symptoms of acute gastroenteritis. Symptoms had first occurred in two residents on 17 December. On 26 December, an 88 year-old resident had been hospitalised for dehydration because of diarrhoea and vomiting. By 28 December, 32 people (four staff and 28 residents) were reporting one or a combination of symptoms including diarrhoea, vomiting, malaise and in four cases elevated body temperature. On 28 December, the Department of Medical Microbiology, Institute of Public Health, Celje confirmed the presence of rotavirus group A antigens in the 88 year-old resident's stool.

Rotavirus infections are well documented in preschool children and present a problem in developed and developing countries alike. Worldwide, 870,000 children under five years old die from rotavirus infections every year [1,2]. In adults, symptomatic rotavirus infections are relatively rare, but can cause health problems and outbreaks in the elderly and in immunocompromised individuals [3,4]. For children under five, there are two licensed vaccines against rotavirus infections.

Rotaviruses are RNA viruses from the Reoviridae family; they are divided into seven serogroups (A to G) on the basis of antigen groups. Infections in humans are caused by serogroups A, B and C, serogroup A being the most common.

Outbreak investigation

On 28 December, an outbreak investigation was initiated. The nursing home for the elderly comprised 121 residents aged from 66 to 95 years, 85 females and 36 males. The residents were cared for by 30 of a total 62 staff which also included 14 kitchen staff and 18 support personnel (cleaners, drivers and janitor). Of the residents, 66 were fully mobile, 26 were wheelchair users and 29 were bed-bound. The rooms for residents are either equipped with one or two beds and are located in the basement, on the ground floor, at the first level, and in two lofts. In addition, there are four small kitchens on each respective floor, a dining hall and a living room. The nursing home does not have a separate unit for bed-bound residents. Mobile residents can go about freely in and around the nursing home.

Enterovirus infection was suspected based on the microbiological confirmation of rotavirus gastroenteritis in the hospitalised resident. Every resident and staff member (epidemiological link) who presented with at least one of the following symptoms and signs from 17 December was classified as a probable case: diarrhoea (›three loose stools/day), vomiting and elevated body temperature (›37°C). A confirmed case was considered as a case with clinical symptoms and laboratory confirmation.

A total of 151 epidemiological questionnaires were distributed to all residents and nursing staff with questions on the date of onset of symptoms if any, gastroenteritis-related health problems and their duration, treatments, and ingestion of food and beverages outside the nursing home. The residents were also asked to identify the room they occupied, and the nursing staff reported which residents they cared for and possible onset of symptoms of gastroenteritis in their family members, if applicable. In parallel, information on measures to prevent the spread of the disease and instructions on how and what samples to collect (vomit, stool) for microbiological analysis were distributed [5].

We received completed questionnaires for all nursing staff and all residents by 4 January 2011. Residents from all building levels of the nursing home felt ill; no level-based clustering was observed. All the staff affected had provided nursing care to symptomatic

residents. According to the probable case criteria, the two residents who became ill on 17 December 2010 (11 days before we were informed of the outbreak) were identified as the first two cases in the outbreak. Between 28 and 30 December, 15 residents became ill, and no further cases were identified after 31 December (Figure). A total of five of 30 nursing staff (16.7%), and 40 of 121 residents (33%) became ill during the outbreak. The overall attack rate was 30%. Only one resident was hospitalised. None of the kitchen staff and support personnel became ill as they were informed about the outbreak and asked to report if they had any symptoms. The staff did not report any symptoms of gastroenteritis in their family members.

Diarrhoea was reported by all 45 affected individuals, 19 experienced vomiting and four had elevated body temperature. Some patients also reported abdominal pain (Table). The median age of the affected staff was 35 years (mean: 35 years, age range: 23 to 44 years), the median age of the affected residents was 78 years (mean: 82.4 years, age range: 66 to 95 years). The average duration of symptoms of gastroenteritis was 2.4 days (from one to four days) in staff, and three days in residents (one to nine days). The outbreak affected 26 women and 19 men. The highest proportion of resident cases was among fully mobile residents (29 of 40 cases), followed by bed-bound residents (seven of 40 cases) and residents on wheelchairs (four of 40 cases).

Laboratory investigation

One stool sample was collected from the 88 year-old hospitalised resident on 26 December and was sent to the Department of Medical Microbiology, Institute of Public Health Celje. On 28 December, results of enzyme-linked immunosorbent assay (ELISA) testing for antigens of adenoviruses, astroviruses and group A rotaviruses were available. Routine diagnostic procedures for rotavirus infections usually include spectrophotometric enzyme immunoassay (EIA), which is highly sensitive and detects group A rotaviruses only. Qualitative EIA was used to confirm antigens of group A rotaviruses (ProSpectTM Rotavirus Microplate Assay, OXOID). Up to 28 December, cultures for *Salmonella* spp., *Shigella* spp., *Campylobacter* spp. *Yersinia* spp., *Clostridium difficile* toxin A and B, and *C. difficile* did not point to infection with these bacteria and were confirmed to be negative on 30 December.

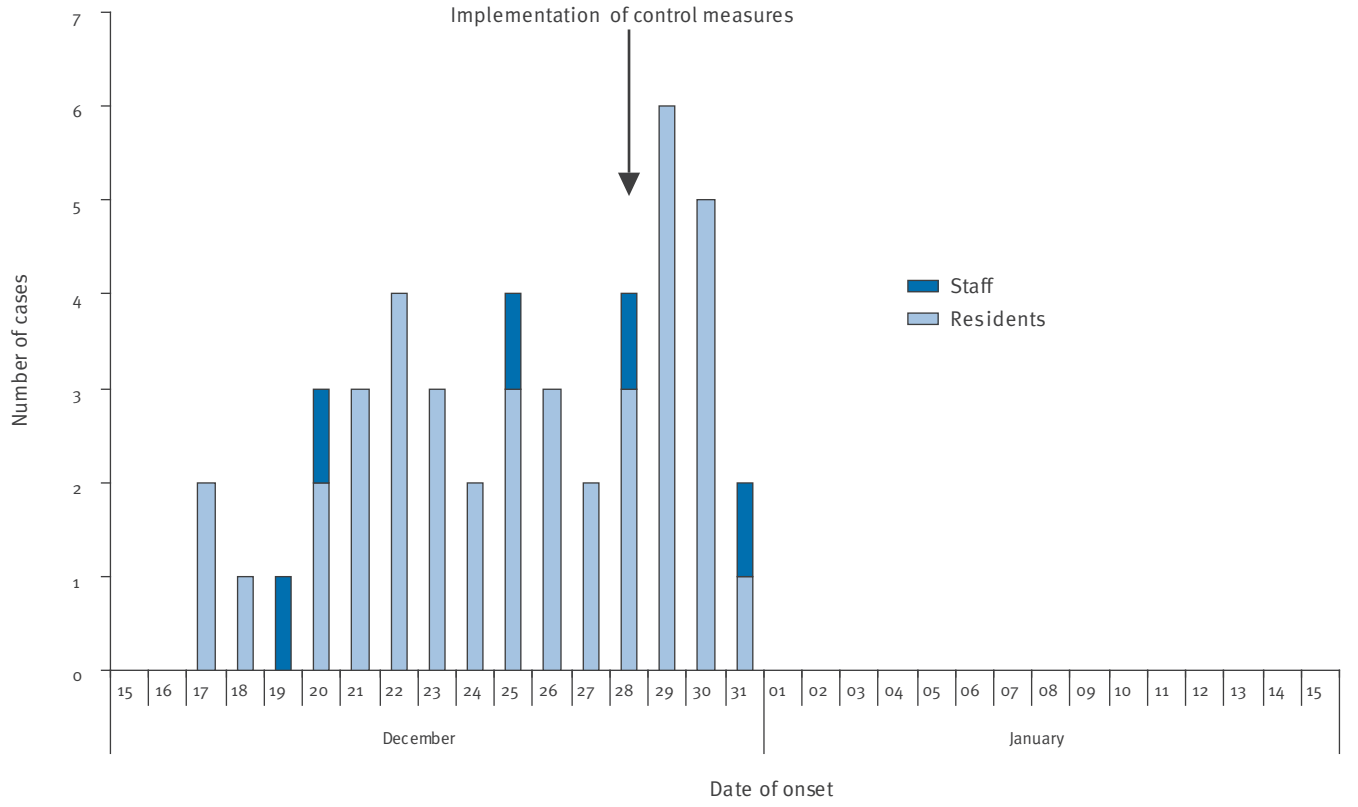
On 28 and 29 December, taking in consideration the result of the hospitalised patient, five additional stool samples from four symptomatic residents and one staff member were tested only for the presence of antigens of astroviruses, adenoviruses and group A rotaviruses. EIA was used to confirm rotavirus group A antigens in four samples, including three from the residents and one from the staff; one sample was negative. All individuals tested were negative for noroviruses.

Control measures

On 30 December, following the confirmation of a rotavirus outbreak, a special sanitary inspection of the

Figure

Epidemic curve for cases of rotavirus gastroenteritis in a nursing home for the elderly, Slovenia, December 2010 (n=45)



nursing home was performed. Measures to prevent the spread of viral diarrhoea were put in place; strict hand hygiene and cleaning with an appropriate disinfectant for viruses, cleaning and disinfection of equipment, surfaces and rooms. Regular airing of premises was recommended. Sanitary inspection of proper disposal of incontinence pads with excrements from residents was conducted. As a temporary measure, contacts between the affected and non-affected residents were limited; cohort isolation of the affected was not implemented. The affected staff were removed from work for a period of one to four days until they did not present any more symptoms [6,7].

Discussion

We describe an outbreak of rotavirus gastroenteritis in a nursing home for the elderly. On 17 December, two residents became ill at the same time; the first resident was bed-bound and the second was mobile and visiting the first one. The first member of the staff fell ill on 19 December (Figure). The outbreak, affected 40 of 121 residents and five of 30 nursing staff. All five affected members of the staff had provided nursing care to bed-bound residents. The most frequent symptoms were diarrhoea, vomiting and elevated body temperature. The average duration of illness was different for staff and residents, 2.4 and three days, respectively. All affected persons made full recovery; only one resident was hospitalised.

Rotavirus gastroenteritis symptoms usually accompany primary infection in childhood, which is followed by protection against subsequent symptomatic infection. For this reason, the ratio of symptomatic to asymptomatic infection decreases with age. In prospective studies, symptomatic infection rates were highest in children under two years, and lowest in those of 45 years of age [3]. Rotavirus infection in immunocompromised adults can have a variable course from asymptomatic to severe and sustained infection [4]. Vaccination for infants from six to 26 weeks of age, which has already been included in some national vaccination programmes, will serve to decrease the burden of rotavirus infections in the future [8,9]. In Slovenia, rotavirus vaccination for infants is available against payment [10].

Table

Clinical manifestation in ill individuals, rotavirus gastroenteritis outbreak, Slovenia, December 2010 (n=45)

Clinical manifestation	Number of individuals[a]
Diarrhoea	45
Vomiting	19
Elevated temperature	4
Stomach pains	1
Feeling unwell	3
Malaise	12

[a] Each individual could record up to six symptoms listed.

Before 2008, rotavirus gastroenteritis outbreaks in Slovenia were reported mostly in preschool and school environments [11]. In 2008, however, rotavirus gastroenteritis outbreaks in nursing homes for the elderly in Slovenia were first recorded in addition to norovirus infections [12]

Our investigation shows another outbreak of rotavirus gastroenteritis in an elderly nursing home, highlighting the potential of rotavirus outbreaks in such a setting. Our results are in agreement with other studies reporting that long-term residence in a closed community is a risk for rotavirus illness [13]. Noteworthy in our investigation, is that five of 30 younger nursing staff (ranging from 23 to 44 years) were affected. This indicates that rotavirus infections can occur in all age groups and affect caretakers of an elderly home, who in turn can contribute to the spread of the disease. This is not entirely unexpected as faeces and vomit from infected individuals can contain more than 10^{13} infectious infectious particles* per gram and only 10 to 100 of these are required to transmit infection [5]. Future epidemiological studies are needed to assess the impact of rotavirus infections in the elderly.

To this effect, outbreaks need to be not only registered, but also reported as close as possible to onset, so that microbiological diagnostic and complete monitoring can be implemented as fast as possible. In the present outbreak, public health authorities were only notified once the number of affected persons was high. This situation is likely to occur frequently because of the speed at which rotavirus gastroenteritis outbreaks can spread, so our investigation highlights the importance of a tight collaboration and dialogue between nursing home staff and public health authorities. More efforts need to be focused on increasing vigilance among caretakers for elderly or vulnerable groups and training caretakers to communicate outbreaks in a timely manner. This will prevent delays in putting in place containment measures and will allow for better care of vulnerable groups such as the elderly or immunocompromised patients.

*Erratum: The number 10^{13} was corrected on 09 April 2011.

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Yersinia enterocolitica O:9 infections associated with bagged salad mix in Norway, February to April 2011

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In March 2011, the Norwegian Institute of Public Health identified a possible outbreak involving 21 cases of *Yersinia enterocolitica* O:9 infection with similar MLVA-profiles. Preliminary results of epidemiological and microbiological investigations indicate bagged salad mix containing radicchio rosso (also known as Italian chicory) as a possible source. As a result of the investigation, bagged salad mixes of a specific brand were voluntarily withdrawn from the market by the producer.

Introduction

In March 2011, the Department of Infectious Disease Epidemiology at the Norwegian Institute of Public Health (NIPH) was informed by the National Reference Laboratory (NRL) for enteropathogenic bacteria of an unusually high number of *Yersinia enterocolitica* serotype O:9 isolates from geographically disparate areas in Norway. After being notified of five cases of *Y. enterocolitica* O:9, which is rare in Norway, a multidisciplinary investigation team was established on 18 March 2011 to find the source and prevent further illness.

Yersiniosis is a mandatorily notifiable disease and the fourth most commonly reported cause of bacterial diarrhoeal disease in Norway [1]. In the past 10 years, between 80 and 150 cases of yersiniosis were reported annually. More than 98% of yersiniosis cases in Norway are due to serotype O:3, which is also the dominant cause of yersiniosis in Europe, Japan, Canada and parts of the United States [2].

Descriptive epidemiology

A confirmed case was defined as an individual with laboratory-confirmed *Y. enterocolitica* O:9 infection with the outbreak MLVA-profile identified between 1 January and 5 May 2011. By 5 May, the reference laboratory had registered 21 cases with the outbreak strain of *Y. enterocolitica*. Of the 21 confirmed cases, 15 were female and six were male. The age range of patients was from 10 to 63 years with a median age range of 30-39 years (Figure 1).

Cases occurred in geographically disparate areas of the country, across ten different municipalities (Figure 2).

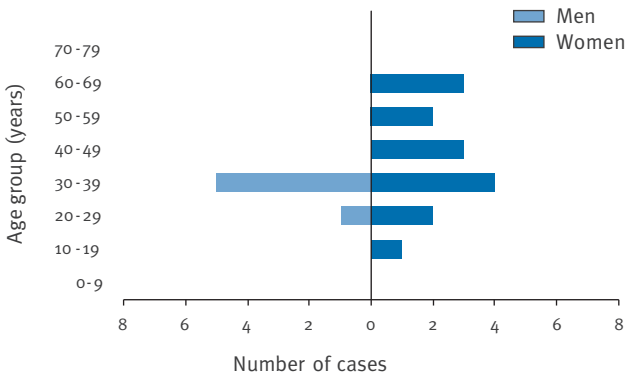
For four patients, the date of symptom onset was unavailable and the date of positive microbiological sample was used for the epidemic curve (Figure 3). Between week 6 (7–13 February) and week 11 (14–20 March), 17 patients with positive microbiological samples became ill.

Epidemiological investigation

When there are outbreaks in Norway where the cases are geographically widespread, the NIPH is responsible for coordinating the outbreak investigation. As is often done in foodborne outbreaks in Norway, after being notified of a microbiologically confirmed outbreak case, the NIPH contacted the respective municipal doctor and asked them to contact the patient in order to get consent for the district Food Safety Authority office to visit the home, collect food samples and conduct an interview. The first seven cases were interviewed using a trawling questionnaire, designed to collect information on food consumption in the seven days prior to onset of symptoms, animal contact and environmental exposures, as well as clinical and demographic

Figure 1

Age and sex distribution of cases of *Yersinia* O:9 infection, Norway, February–April 2011 (n=21)

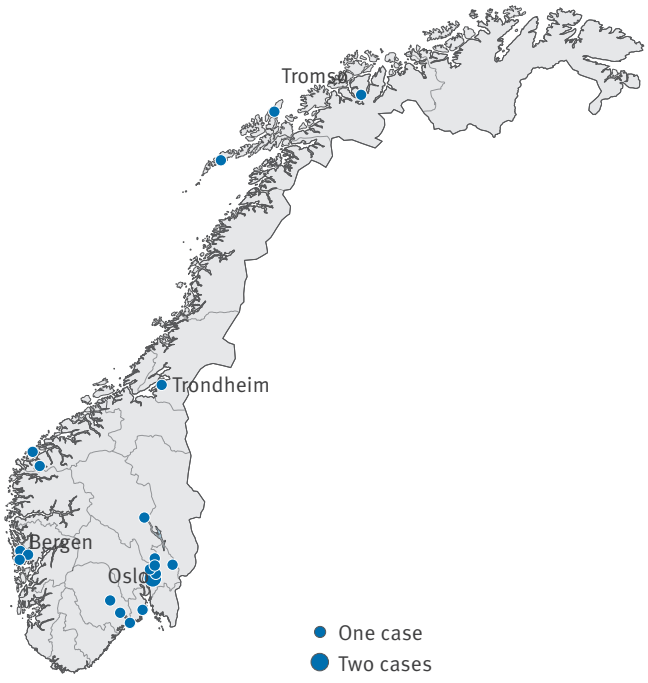


information. Following these interviews, the questionnaire was shortened to focus on categories of foods of most interest, and used to conduct a case-control study. In particular, from the trawling interviews, bagged salad mix was suspected as the source of infection. The case-control study was conducted in week 13 (28 March–1 April 2011). At that time, nine patients had been interviewed using the shortened questionnaire. In order to ensure enough statistical power in the case-control study given the small number of cases, three controls for each case were selected from the national population register. Controls and cases were matched by age, sex and municipality of residence. Potential controls were excluded if they reported having had diarrhoea during the last 14 days.

The results from the trawling interviews revealed that limited number of cases had consumed pork products. Salad mix and arugula were consumed by a notable number of cases, with at least four specifically stating they had consumed a specific brand of salad mix containing arugula. Preliminary results of the case-control study corroborate the hypothesis of bagged salad mix as the suspected source. Among the nine cases, six had eaten bagged salad mix in the week prior to onset of illness compared with three of 25 controls (matched odds ratio (mOR):13.7; 95% confidence interval (CI): 1.6–116.3). We included eight significant food items in a conditional multivariate logistic regression model. A forward selection procedure was used by starting with the most significant item and including the other items one by one. The only food item which remained significant in the model was the bagged salad mix.

Figure 2

Geographical distribution of cases of *Yersinia* O:9 infection, Norway, February-April 2011 (n=21)



International notifications

On 26 April 2011 the NIPH sent a message via the European Centre for Disease Prevention and Control (ECDC) Epidemic Intelligence Information System asking whether other countries had also experienced an increase in cases of *Y. enterocolitica*. The Norwegian Food Safety Authority sent a notification through the Rapid Alert System for Food and Feed (RASFF) on 15 April 2011. International requests for information produced no reports of similar yersiniosis outbreaks in European countries. However, it is possible that few countries routinely perform serotyping of *Y. enterocolitica*.

Microbiological investigation

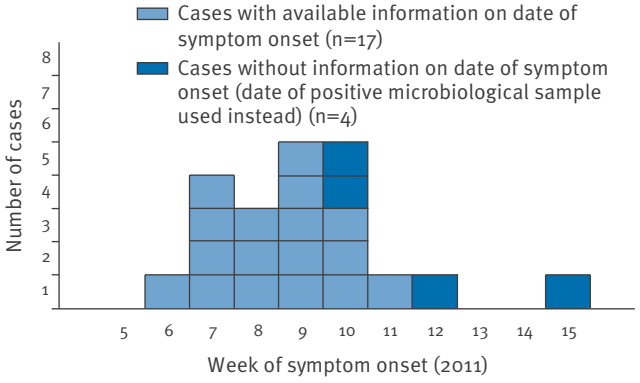
At the NIPH-located NRL all isolates of *Y. enterocolitica* from human patients are routinely characterised phenotypically, biotyped and serogrouped against O:3 and O:9 as well as a range of other serogroups. The *Y. enterocolitica* isolates were MLVA-typed by the method described by Gierczyński et al. [3], locally adjusted to capillary electrophoresis.

Food samples were sent to the Norwegian Veterinary Institute for analysis. A total of 61 samples consisting of two chicken meat products, two pork products and 57 diverse salad products and bagged salad mix products were collected from patient homes, retail and the company producing the bagged salad mix products. All products were analysed according to NMKL 117B, an adaptation of ISO 10273. Additionally, samples were cold enriched for 21 days according to NMKL 117. All enriched broths and colonies isolated were further examined for the *ail* gene, an indicator for pathogenic *Y. enterocolitica*, using PCR (NMKL 163, Part A (1998)). PCR positive colonies were characterised by biochemical reactions and their serogroup was determined.

Diverse *Yersinia* spp. including enterocolitica were isolated from 11 of the salad products of which two were consistently positive by PCR. These strains were

Figure 3

Cases of *Yersinia* O:9 infection by week of symptom onset, Norway, 7 February–20 March 2011 (n=21[a])



[a]Including the four cases for which information on date of symptom onset was not available.

isolated from one particular salad type, radicchio rosso, imported from Italy, and mixed salad products, which also contain radicchio rosso. However, these isolates were not serogroup O:9.

Discussion and conclusion

The geographically widespread occurrence of the yersiniosis cases and the illness onset dates indicate that the suspected source of infection is likely a product that was widely distributed but available only for a relatively short period of time. In addition, the number of female cases compared to male cases indicated that the source was a food product more commonly consumed by women. Radicchio rosso is the only variety of salad included in the suspected bagged salad mixes that keeps long enough to fit with the duration of this outbreak. Radicchio rosso is stored at –1°C before it is supplied to the market. The storage conditions may increase growth of *Y. enterocolitica* as this bacterium is able to grow down to –2°C.

Yersiniosis outbreaks are often associated with consumption of pork, as the pig is the only animal consumed by humans which regularly harbours the pathogenic serovars O:3 and O:9 [2]. Although most cases of yersiniosis in Norway are sporadic, there have been several previous outbreaks, including an outbreak of *Y. enterocolitica* O:9 in 2005-2006 due to a Norwegian ready-to-eat pork product ('sylte') [4]. Published literature on yersiniosis outbreaks linked to salad and/or fresh vegetables is limited. Although previous outbreaks of *Salmonella*, *Shigella* and *Escherichia coli* in Norway have been linked to the consumption of fresh vegetables [5-8], this is the first outbreak of yersiniosis in Norway to be linked to consumption of vegetables.

As of 5 May 2011, no new outbreak cases have been reported. The supplier voluntarily withdrew suspected bagged salad mixes containing radicchio rosso from the market based on the information collected through the interviews, case-control study preliminary results and positive PCR results, as well as their own risk assessments. Information obtained through RASFF indicates that while the exporter of radicchio rosso implicated in this outbreak also sends the product to the United Kingdom, the batch in question was only distributed in Norway. Although the epidemiological evidence incriminates bagged salad mix, the ongoing trace-back investigation of the product has been complicated. *Yersinia* is challenging to cultivate from food products [9] and microbiological testing is also still in progress.

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A cluster of *Listeria monocytogenes* infections in hospitalised adults, Midlands, England, February 2011

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Hospital-acquired listeriosis cases are not commonly reported but remain a significant public health problem. We report on three cases in patients with underlying conditions occurring during one week in February 2011. The cases had common exposure to pre-packed sandwiches and salads manufactured in compliance with regulations. Breaches in cold chain and shelf life controls at hospital level were identified as key contributing factors. Rigorous hospital food management systems remain important for patient safety.

Case description and clinical diagnosis

Listeria monocytogenes bacteraemia was confirmed in three patients admitted on 4, 5, and 6 February 2011 to a hospital in the Midlands region of England. Two were male and one was female. All lived in the same city served by the hospital but did not have any social links. Two cases were in the age range 50-59 years and one was older, over 80 years. All the three cases had underlying conditions which included malignancies and inflammatory bowel disease.

Cases were admitted in February 2011 to the same hospital where they had been hospitalised previously between 22 and 31 January 2011. Onset of symptoms leading to readmission of all three patients, ranged from 29 January to 3 February 2011, and these included fever, headache, confusion, abdominal pain and vomiting and *L. monocytogenes* was diagnosed in blood cultures three to four days after admission. All cases responded to antibiotic therapy with full recovery from infection.

Investigation and control measures

The 1,200-bed hospital is the only acute care facility in a district with approximately 500,000 inhabitants. A review of laboratory records for the preceding 12 months identified four unrelated (sporadic) community-acquired listeriosis cases. This background incidence rate is in keeping with national surveillance data, with 162, 180, and 139 non-pregnancy associated cases reported in 2008, 2009, and 2010 across England and Wales [1].

Following the identification of the three cases described above, an outbreak control team convened on 11 February 2011 to investigate the suspected outbreak and to advise on control measures.

Medical staff and management at the hospital were informed of the listeriosis cluster and the possibility of further cases. The hospital infection control team reinforced standard food avoidance advice for ready-to-eat foods commonly associated with listeriosis (such as pâté, smoked fish and mould ripened soft cheeses, or pre-packed sandwiches and salads) to patients with severe underlying conditions and/or on immunomodulating therapy, or pregnancy, and their families [2]. In addition, ward level food storage, distribution, and disposal practices were reviewed and staff reminded to follow existing protocols.

Food history of patients

Interviews with the three cases and their close relatives excluded animal contact and travel as relevant exposures. Food histories of the preceding four months did not identify common food preferences, consumption or purchasing while living at home. None had preference for ready-to-eat foods commonly associated with *L. monocytogenes*, nor were these present in their home refrigerators. The cases had attended the hospital outpatient department on various occasions between the two admissions, prior to disease onset, but had not eaten ready-to-eat foods from on-site shops. Hospital food was not served to patients attending the outpatient department.

The patients reported that during their hospital stays they had not eaten food (including ready-to-eat foods and sandwiches) from home or any of the eight privately-owned on-site visitor/staff canteens and shops. They had all consumed food provided by the hospital, and this had not been kept at room temperature but consumed immediately. The food histories were supplemented by a review of patient menu choice records

kept by the hospital. The only risk factors (common food exposure) identified were pre-packed sandwiches and salads provided by the hospital during the common period of hospitalisation (22 to 31 January 2011). A wide variety of sandwiches and salads were eaten by all the cases, with no single sandwich or salad type being identified as unique common exposure. Salad types consumed included turkey, ham, cheese and coleslaw, and sandwich fillings included cheese, egg, ham, salmon, tuna, turkey, and tomato.

Isolates of *L. monocytogenes* from blood cultures of the three cases were identified as serogroup 4, and fluorescent amplified fragment length polymorphism (fAFLP) type V21. The isolates were thus indistinguishable by molecular typing, supporting a point source outbreak. In the absence of a common food exposure or source (no identified home-based common food exposure and no common food source that they used prior to the first hospital admission) the three cases were most likely exposed to contaminated food during their overlapping admission episode in January 2011. Based on this assumption, incubation periods were estimated to range from one-four days (minimum) to eight-13 days (maximum).

Investigation of food suppliers

An environmental health investigation confirmed that a single manufacturer supplied pre-packed sandwiches to the hospital for inpatient meals. Salad was prepared at the hospital central kitchen. At the hospital, samples for microbiological analysis were taken from ready-to-eat foods (pre-packed sandwiches, pre-packed meats, cheddar cheese, cottage cheese used in on-site salad preparation, and completed salads), and kitchen drains. No *L. monocytogenes* was isolated from a total of 27 samples taken from this hospital between 10 and 24 February 2011.

A review of ready-to-eat food management practices at the hospital revealed that storage temperatures generally did not exceed 5°C, but gaps in recordkeeping were found during evenings and weekends. Some instances were observed of ready-to-eat foods being accepted from the supplier at temperatures above 5°C. Salad preparation in the hospital kitchens revealed lapses of the procedure for washing and disinfecting vegetables using chlorine. In addition, prepared salads were commonly given a two- or three-day shelf life rather than the recommended one day. Measures were taken to rectify these issues and food safety procedures are being updated at this hospital.

The eight privately owned on-site visitor/staff canteens and shops were inspected. Each was found to have different suppliers, and none of them supplied the same food as that given to inpatients in the hospital. Despite the fact that the three cases reported not to have obtained food from these eight outlets, 15 samples were taken of pre-packed sandwiches and salads as a precaution. *L. monocytogenes* serotype 4 (<20 cfu/g) was isolated from one ham and cheddar cheese sandwich but the fAFLP type differed from the isolates of the three cases.

A full production hygiene investigation (focused on sandwich and salad component production) of the manufacturer supplying food for hospital inpatients was undertaken by local environmental officers. There was a fully documented hazard analysis and control system in place and the quality assurance programme included daily microbiological testing of sandwiches for indicator organisms at three days after production (end of shelf life), with enumeration testing for *Listeria* spp., including *L. monocytogenes*, approximately every ten days.

For the five months prior to 20 December 2010, none of 38 samples exceeded 10 cfu/g for *Listeria* spp. Due to adverse winter weather conditions routine sampling had ceased from 21 December 2010, to be resumed only after the detection of the three listeriosis cases early in February 2011. Based on hazard analysis control system documentation, no breach of production quality processes was detected during this period. Further independent sampling by environmental health officers on 23 February 2011 did not detect *L. monocytogenes* in ten sandwich samples and 15 environmental (food production sites and drains) samples. From March 2011, the company revised their microbiological sampling plan (including sampling sandwiches on day of production) and are now using both enumeration and enrichment techniques in *L. monocytogenes* detection. To date, no further *L. monocytogenes* isolates in sandwich samples have been detected from the supplier.

Discussion and conclusion

Detailed investigations identified the consumption of hospital supplied sandwiches and/or salads during the last ten days in January 2011 to be a likely risk factor for infection with *L. monocytogenes*. The cluster is unlikely to be due to a chance occurrence, as cases occurred close together in excess of background incidence, had overlapping hospital stay, and isolates were indistinguishable by fAFLP typing. Microbiological evidence that hospital supplied food was the source of infection could not be established.

The sandwich producer follows the British Sandwich Association target microbiological standard in finished products (at end of shelf life) of *Listeria* spp. at <10 cfu/g which is compliant with European Commission (EC) regulations [3,4]. Whilst the detection of <10 cfu/g of *L. monocytogenes* in sandwiches and salads supplied to patients provides some assurance, sample numbers were low and taken more than ten days after cases were likely to have been exposed. In addition, the break in sampling by the producer before and during the case exposure period coupled with significant cold chain breaches and extended salad shelf life at the hospital preceded the cases. Two extensive United Kingdom (UK)-wide microbiological surveys of

sandwich quality served at hospitals and healthcare institutions reported 2.7 - 3.1% of samples containing *L. monocytogenes* [5]. In both studies, the presence of *Listeria* spp. and *L. monocytogenes* was associated with sandwiches produced outside the hospital, and where storage above 8°C had occurred.

In our experience sandwiches are commonly consumed by all patients in the study hospital, as well as most hospitals across the UK [6,7]. Even low levels of *L. monocytogenes* in sandwiches and ready-to-eat foods pose a risk to certain immunocompromised patients and pregnant women. The vast majority of sandwiches are safe, and hospital incidents and outbreaks of listeriosis are relatively infrequent, with six outbreaks reported in England and Wales from 1999 to 2008 [7]. However, listeriosis is a serious disease in compromised patients, and despite low numbers it remains a significant patient safety concern. Leading investigators have therefore recommended that food served to hospital patients to be free from potential pathogens, including *L. monocytogenes* [5-7].

Although this hospital food manufacturer supplies food to many other hospitals in the UK, no further laboratory confirmed cases of listeriosis of the same fAFLP type (V21) were identified in the UK more than 10 weeks after the cluster was detected and there were no other outbreaks of listeriosis or sporadic cases of this unique fAFLP type. Even though the ready-to-eat foods in this study were manufactured in accordance with the European Union regulations [4], it is possible that lack of temperature and shelf-life controls at the study hospital were key factors leading to increases in listeriosis and infection in vulnerable patients.

The investigation of hospital-based *L. monocytogenes* outbreaks is notoriously difficult due to low attack rates, incomplete case ascertainment, food histories spanning long periods, and food samples often being negative or taken long after the exposure time [7]. Such outbreaks are likely to be underreported, with publication bias towards larger outbreaks confirming microbiological food exposures. In order to develop appropriate future control strategies for this ongoing public health problem, we recommend that investigators make every effort to report and publish the full spectrum of hospital associated *L. monocytogenes* clusters and outbreaks.

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Toxin producing *Vibrio cholerae* O75 outbreak, United States, March to April 2011

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The Florida Department of Health, Florida, United States, is investigating a *Vibrio cholerae* O75 outbreak. Ten cases with disease onsets from 23 March to 13 April 2011, presented with gastrointestinal symptoms of diarrhoea, nausea, vomiting, cramps, chills, and/or fever, after consuming raw or lightly cooked oysters harvested from Apalachicola Bay, Florida. Symptoms were milder than those during outbreaks of epidemic (serogroup O1 and O139) *Vibrio cholerae*; no case required rehydration treatment or hospitalisation.

Outbreak report

On Friday 15 April, 2011 the epidemiology team of the Escambia County Health Department (CHD), Florida, United States (US), notified the Florida Department of Health's Food and Waterborne Disease Program (FWDP) of a case of *Vibrio cholerae* non-O1/non-O139. The man in his early 20s had fallen ill with cramps, fever, watery diarrhoea, and nausea on 12 April after consuming raw oysters on 6 April in a restaurant. The bacterial isolate was sent to the Florida Department of Health's Bureau of Laboratories (BOL) in Jacksonville for typing and toxin testing. The suspect toxin-producing *V. cholerae* O75 specimen was forwarded to the Centers for Disease Control and Prevention (CDC) and confirmed positive on 19 April.

In the US, the intra- and interstate regulation of oysters is performed by state (Florida Department of Agriculture and Consumer Services, Division of Aquaculture (DOACS)) and federal agencies (US Food and Drug Administration (FDA)), respectively. These agencies require the tracking of oysters through tags that note the harvest date and area. Attempts were made to collect the oyster tags but they were unavailable from the restaurant.

On 18 April, Nassau CHD reported two cases in their late 40s and late 20s respectively, who developed a gastrointestinal illness the day after having purchased live shell stock oysters and consuming them steamed on 10 April. Symptoms included nausea, vomiting, diarrhoea, and chills. One of the patients provided a stool sample that tested positive for toxigenic *V. cholerae* O75.The other, who had presented with the same symptoms, did not provide samples. This patient was included in our case count as a probable case. The tag for the oysters had been discarded but records at the seafood dealer indicated that they had been harvested from Apalachicola Bay area 1642.

The FWDP was notified of a Louisiana, US *V. cholerae* non-O1/non-O139 case on Monday 19 April. The case became ill on 9 April after consuming raw oysters at a restaurant in Okaloosa, Florida, on 7 April. Tags for oysters likely eaten by the case were retrieved from the restaurant on Tuesday 20 April. The oysters had been harvested from the Apalachicola Bay area 1642 on 3 and 6 April. The toxin status for that case is still unconfirmed by CDC so this remains a suspected case.

On Wednesday 20 April, the FWDP investigators contacted the Florida DOACS, the agency with oversight over the Florida oyster industry, notifying them of the *V. cholerae* non-O1/non-O139 cluster investigation.

On 21 April, the FWDP coordinator and investigators issued a state-wide alert to the Florida EpiCom system, a state-wide epidemiology electronic alert system, notifying public health officials of the toxigenic *V. cholerae* oyster related case investigation. A similar notification was posted nationally on the CDC EpiX notification system, the federal epidemiology alert system, on 28 April.

Ten cases (eight confirmed, one probable, and one suspect) were identified in this outbreak (Figure 1). Seven were Florida residents, the three other cases were from Indiana, Georgia and Louisiana (Figure 2). The cases ranged from 22 to 74 years of age; six of the ten cases were males. Most cases were in good health, with only one reporting to have pre-existing conditions (kidney problems and a coronary artery stent). Cases reported gastrointestinal symptoms of nausea (n=7), vomiting (n=4), diarrhoea (n=9), chills (n= 8), cramps (n=1) and/

or fever (n=1) and none required hospitalisation. Dates of exposure ranged from 21 March to 11 April and onset of symptoms occurred between 23 March and 13 April. The average time from exposure to onset of symptoms was 2 days (range: 1 to 6 days). Information gathered during the investigation, which was derived from three sets of oyster tags, implicated one single harvest area in the Apalachicola Bay, Gulf of Mexico, as the source of the contaminated oysters. No additional cases of *V. cholerae* have been reported since 13 April and no oysters harvested later than 6 April have been implicated in any related illnesses.

In response to the outbreak, DOACS conducted an investigation into the implicated oyster harvesting area, Apalachicola Bay area 1642 (Figure 2). The area was closed on 30 April and all dealers and retailers were asked to recall any implicated product still in commerce. The area was reopened to harvesting on 11 May 2011 after DOACS had 15 oyster samples from 10 different sections of the implicated harvest area tested for *V. cholerae* O75 at the FDA laboratory in Dauphin Island, Louisiana. All samples were negative.

Background

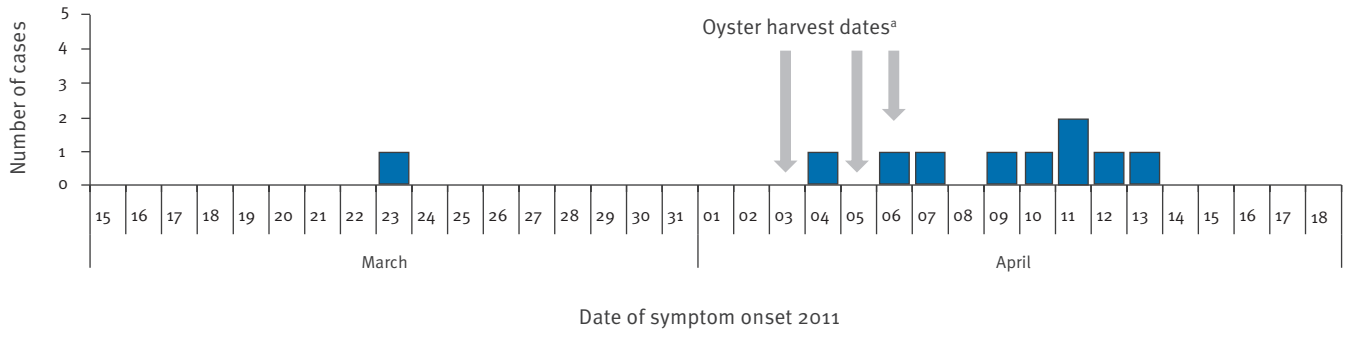
Vibrio spp. bacteria are common in the warm coastal waters of the state of Florida and are also found in local shell fish. *Vibrio vulnificus* is an important cause of gastrointestinal illness and wound infection in the state, causing 24 cases of illness and six deaths in 2009 [1]. Human illness from native, nontoxigenic non-O1 and non-O139 serogroups of *V. cholerae* are reported regularly [2] and all *Vibrio* diseases are notifiable in Florida. However, despite the favourable ecological conditions, and the fact that Florida receives many travellers from cholera endemic countries, toxigenic *V. cholerae* infections are uncommon. After the 2010 cholera outbreak in Haiti, ten cases of imported *V. cholerae* O1 serotype Ogawa were confirmed in state residents, with no secondary transmission detected (Ann Schmitz, personal communication, 16 April 2011) [3]. The subsequent heightened awareness of cholera has increased the number of human samples submitted to Florida Department of Health's BOL for *V. cholerae* testing, with more than 40 isolates having been evaluated since November 2010 [4]. At the same time, the turnaround time for test results has also been improved by adding capacity for the detection of cholera toxin. For *V. cholerae* testing, isolates or stools from ill persons, which were previously screened by hospital or private laboratories and suspected *V. cholerae* positive, are referred to the BOL as "suspect *V. cholerae*". Stools are tested by direct thiosulfate citrate bile sucrose agar (TCBS) and enrichment (alkaline peptone water 35°C incubation for 8 hours and 18 hours), then subculture to TCBS. Suspect colonies are checked biochemically for the matching *V. cholerae* pattern of reactions and then serology is performed with O1 and O139 antisera. All *V. cholerae* isolates are also checked for cholera toxin. When isolates do not agglutinate in O1 or O139 antisera but do produce cholera toxin, isolates are referred to CDC and serology for *V. cholerae* O75 is performed. This process takes from 10 to 14 days.

Discussion and conclusions

Toxigenic *V. cholerae* infections are highly unusual in Florida. This report describes the first *V. cholerae* O75 outbreak detected in the state, between 23 March and 13 April 2011. In Florida, the first isolate of toxigenic *V. cholerae* O75 to be identified was reported in November 2010, and originated from an immunocompromised Florida resident with a history of oyster consumption. Previously, the serogroup had been associated with sporadic cases of gastrointestinal disease after oyster and other seafood consumption in the south-eastern US [5]. Tobin-D'Angelo et al. [5] describe eight cases with a similar diarrhoeal disease to the ones reported here, who were identified in Georgia, Louisiana, Alabama and South Carolina between 2003 and 2007. These sporadic cases differ from the cases in the outbreak in question here. Four of five cases described in detail had underlying health conditions, whereas only one of the cases in this outbreak had underlying conditions. Two of the eight cases were hospitalised in the analysis of the sporadic cases whereas cases associated with this outbreak experienced milder symptoms

Figure 1

Cases of cholera O75 outbreak from oyster consumption, United States, March–April 2011 (n=10)



[a] When these dates were available from the oyster tags.

and did not require hospitalisation. Some of these differences may be explained by the increases in testing, which were also the result of recent concerns of imported cholera from Haiti. Persons displaying gastrointestinal symptoms are more likely to have cholera testing done than in the past. Historically, cases with severe illness may have been more likely tested than those with milder symptoms, resulting mainly in severe cases being reported. These factors may have allowed the current detection of this rare serotype of cholera in a relatively healthy population.

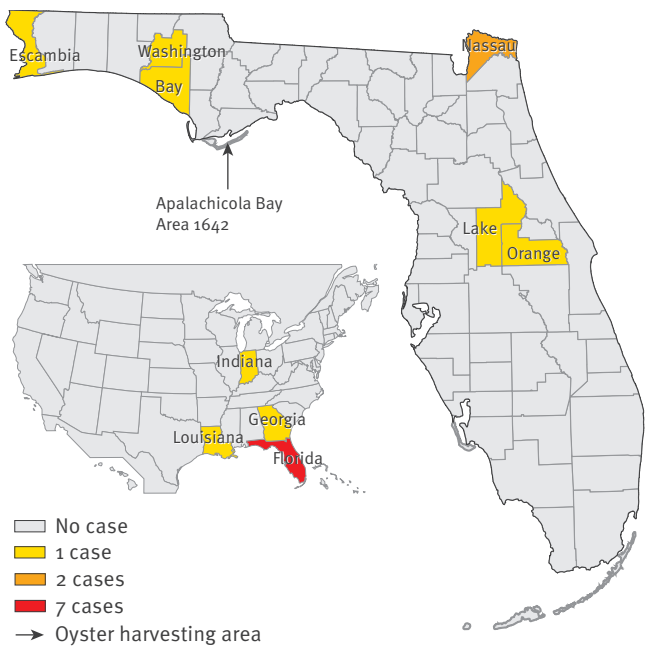
Epidemic cholera is an important cause of morbidity and mortality worldwide [6]. Outbreaks generally occur when there are significant deficits in the water sanitation and hygiene infrastructure in the communities, allowing for rapid spread of disease via food and/or water consumption. Gastrointestinal symptoms are generally mild, however about 20% of affected individuals develop acute, severe, dehydrating watery diarrhoea which can be fatal if left untreated. The *V. cholerae* O75 appears to cause a milder disease than infection with *V. cholerae* serogroups O1 and O139 and outbreaks in countries with universal access to health care are likely to have limited public health impact. The outbreak reported here is a reminder of the conductive environmental conditions for *V. cholerae* growth in Florida waters and the importance of maintaining sanitary and food safety practices to avoid future outbreaks.

This is the first recorded outbreak related to *V. cholerae* O75 and oyster consumption. The identification of the outbreak could be the result of an increase in testing for *V. cholerae* in human stool isolates, allowing unprecedented detection of a phenomenon that may have recurrently occurred in the past. Alternatively, the outbreak could be due to some change in the environment, acute or longer term, which has allowed this pathogen to establish or emerge in the oyster population. In the wake of this outbreak, there is concern around the oyster harvesting environment and the regulatory agency, DOACS, is continuing to look into incidents that could have influenced the presence or load of this pathogen in the oyster harvest areas.

Florida is a state of international significance given the large numbers of tourist that visit the state annually and this may raise concern internationally. Here we report preliminary results of the investigation to raise awareness among public health professionals worldwide about this rare oyster related *V. cholerae* outbreak. We will be monitoring for future cases of *V. cholerae* O75, and will continue our investigation into other factors associated with this outbreak.

Figure 2

Geographical distribution by state or county of residence of cholera O75 cases from oyster consumption, and oyster harvesting area, United States, March–April 2011 (n=10)



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Large and ongoing outbreak of haemolytic uraemic syndrome, Germany, May 2011

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Since early May 2011, an increased incidence of haemolytic uraemic syndrome (HUS) and bloody diarrhoea related to infections with Shiga toxin-producing *Escherichia coli* (STEC) has been observed in Germany, with most cases in the north of the country. Cases reported from other European countries had travelled to this area. First results of a case–control study conducted in Hamburg suggest an association between the occurrence of disease and the consumption of raw tomatoes, cucumber and leaf salad.

An unusually high number of cases of haemolytic uraemic syndrome (HUS) has been observed in Germany since early May 2011. This report presents the preliminary results of the investigation as of 26 May 2011

Haemolytic uraemic syndrome (HUS) is a serious and sometimes deadly complication that can occur in bacterial intestinal infections with Shiga toxin (syn. verotoxin)-producing *Escherichia coli* (STEC/VTEC). The complete clinical picture of HUS is characterised by acute renal failure, haemolytic anaemia and thrombocytopenia. Typically it is preceded by diarrhoea, often bloody. Each year, on average 1,000 symptomatic STEC-infections and approximately 60 cases of HUS are notified in Germany, affecting mostly young children under five years of age [1]. In 2010 there were two fatal HUS cases [1].

STEC are of zoonotic origin and can be transmitted directly or indirectly from animals to humans. Ruminants are considered to be the reservoir, especially cattle, sheep and goats. Transmission occurs via the faecal-oral route through contact to animals (or their faeces), by consumption of contaminated food or water, but also by direct contact from person to person (smear infection). The incubation period of STEC is between two and 10 days, the latency period between the beginning of gastrointestinal symptoms and enteropathic HUS is approximately one week.

Outbreak description

The Table lists the number of cases of HUS or suspected HUS notified to local health departments and communicated by the federal states to the Robert Koch Institute (RKI). Suspected HUS are included as the syndrome is a process and suspected HUS typically develops over the course of a few days into the full clinical picture.

Disease onset (regarding diarrhoea) in the 214 patients detected so far was between 2 and 24 May 2011. A total of 119 (56%) of the cases were communicated from four northern federal states (Hamburg, Schleswig-Holstein, Lower Saxony and Bremen). The highest cumulative incidence has been recorded in the two northern city states of Hamburg and Bremen. An additional 31 cases occurred in Hesse. They were connected to a catering company supplying the cafeterias of a company and a residential institution. It is likely that these cases constitute a satellite outbreak.

Besides the geographic clustering, the age and sex distribution of the cases is conspicuous: Of the 214 cases, 186 (87%) are 18 years of age or older (mostly young to middle-aged adults) and 146 (68%) are female. In the notification data for HUS cases from 2006 to 2010, the proportion of adults lay between 1.5% and 10% annually, and the sexes were affected equally.

Cases linked to this outbreak were also communicated from other European countries: On 25 May 2011, Sweden reported through the European Warning and Response System (EWRS) nine cases of HUS, four of whom had

travelled in a party of 30 to northern Germany from 8 to 10 May. Denmark reported four cases of STEC infection, two of them with HUS. All cases had a recent travel history to northern Germany. Another two HUS cases with travel history to northern Germany in the relevant period were communicated, one each by the Netherlands and by the United Kingdom.

So far two German HUS cases have died of the disease (both female, one in her 80s, one in her 20s) .

Laboratory investigations

Investigations at the National Reference Centre for Salmonella and other bacterial enteric pathogens at the RKI (Wernigerode) of isolates from two patients from Hesse and Bremerhaven suggests that the outbreak strain is an *E. coli* strain of serotype O104 with the following characteristics: Shiga toxin 2 (*vtx2a*, EQA nomenclature 2011, WHO Centre *E. coli* SSI Copenhagen)- producing, intimin (*eae*)-negative and enterohaemolysin (*hly*)-negative. The strain shows a high resistance to third generation cephalosporins (through extended spectrum beta-lactamases, ESBL, CTX-M-type), and a broad antimicrobial resistance to, among others, trimethoprim/sulphonamide and tetracycline.

Table

Cases of HUS and suspected HUS with onset of diarrhoea since 2 May 2011, Germany (n=214)

Federal State	Number of HUS cases and suspected-HUS cases	Cumulative incidence (per 100,000 population)
Hamburg	59	3.33
Bremen	11	1.66
Schleswig-Holstein	21	0.74
Mecklenburg-Vorpommern	10	0.61
Hesse	31	0.51
Saarland	5	0.49
Lower Saxony	28	0.35
North Rhine-Westphalia	31	0.17
Berlin	3	0.09
Baden-Württemberg	8	0.07
Bavaria	5	0.04
Thuringia	1	0.04
Rhineland-Palatinate	1	0.02
Brandenburg	0	0.00
Saxony	0	0.00
Saxony-Anhalt	0	0.00
Total	**214**	**0.26**

HUS: haemolytic uraemic syndrome.

Data as of 26 May 2011, 8am, communicated to the Robert Koch Institute by the federal states.

A further 13 isolates from Muenster, Paderborn, Hamburg and Frankfurt were analysed in the consulting laboratory for haemolytic uraemic syndrome in the Institute of Hygiene at the University hospital in Muenster. All were sequence-typed as ST678 (*stx1-, stx2+, eae-*, flagellin-coding gene *flicH4*), group HUSEC 41, also indicating serotype O104 [2,3]. Whether these results reflect the entire situation in Germany needs to be confirmed by the analysis of a greater number of isolates. As in the past most outbreaks of HUS in Germany and elsewhere were found to be connected with STEC O157 strains, the identification of serotype O104 in this context is highly unusual, although, *E. coli* O104 has previously been described as the cause of an outbreak in the United States in 1994 [4].

Investigation into the source of infection

The large number of persons suddenly affected, the geographical and demographic distribution as well as first interviews of patients suggested STEC-contaminated food as the vehicle of infection. Foods like raw milk and raw meat, which were identified as vehicles in former STEC outbreaks, appear not to be related to the current event. Preliminary results of a case–control study conducted by the RKI and the Hamburg health authorities demonstrate a significant association between disease and the consumption of raw tomatoes, cucumbers and leafy salads. This study collected food histories for the week before symptom onset for 25 patients hospitalised with HUS (n=20) or bloody diarrhoea with laboratory-confirmed STEC infection (n=5), who all had onset of disease between 9 and 25 May 2011. In addition, 96 controls matched by age, sex and residence were asked about their food consumption during the week before the interview. The food items they were asked about were those frequently mentioned in previous in-depth interviews of HUS cases. Consumption of each of the named food items was reported by around 90% of the cases in comparison to around 60% of the controls, yielding odds ratios between around 4 and 7, all statistically significant. Nevertheless it is possible that another or an additional food item is the source of infection. The results cannot necessarily be transferred to the whole of Germany because the study was limited to Hamburg.

Regarding the source of the suspicious food items the study showed a heterogeneous picture. It can be excluded that the source is a single shop or restaurant. Based on these findings, food trace-back investigations are currently ongoing.

Evaluation of the situation

The current events represent one of the largest described outbreaks of HUS/STEC worldwide and the largest in Germany, with a very atypical age and sex distribution of the cases. Incident cases of HUS or suspected HUS are continuing to be reported at least in Northern Germany, where the emergency room consultations for bloody diarrhoea remain elevated. Thus it has to be assumed that the source of infection is still

active. Many patients with bloody diarrhoea need to be admitted to hospital, and HUS patients often need intensive care with dialysis and/or plasmapheresis, which puts a severe strain on hospital resources in some areas. The epidemiological studies that were conducted in cooperation with regional and local health departments rapidly delivered important clues as to certain food items that could be linked to the outbreak. Further epidemiological studies, laboratory investigations and trace back of food items is needed to confirm these results and to narrow down the source of infection.

Recommendations for consumers and patients

Considering the ongoing outbreak that included many cases with a severe course of disease, the RKI and the Federal Institute for Risk Assessment (BfR) recommend to abstain from consuming raw tomatoes, cucumbers and leafy salads, especially in northern Germany, until further notice. Regular food hygiene rules remain in effect [5].

For persons with diarrhoea the importance of strict hand hygiene is emphasised. Patients with bloody diarrhoea should seek medical aid immediately. Physicians are reminded to initiate STEC stool diagnostics for these patients and to closely monitor them for the development of HUS. Patients suspected of developing HUS should be referred to appropriate stationary care.

Diagnostic laboratories are requested to send STEC isolates to the National Reference Centre for Salmonella and other bacterial enteric pathogens. The Protection Against Infection Act of 2001 renders both the laboratory confirmation of an STEC infection and the clinical diagnosis of HUS or suspected HUS notifiable to the local health department.

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Update on the ongoing outbreak of haemolytic uraemic syndrome due to Shiga toxin-producing *Escherichia coli* (STEC) serotype O104, Germany, May 2011

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Since early May 2011, a large outbreak of haemolytic uraemic syndrome (HUS) and bloody diarrhoea related to infections with Shiga toxin-producing Escherichia coli (STEC) has been observed in Germany. The outbreak is focused in the north, but cases have been reported from all German states and other countries. Since our report last week, the number of HUS cases has increased to 470 and STEC serotype O104 has been confirmed in many cases.

Description of the ongoing outbreak

Since the beginning of May 2011, 470 cases of haemolytic uraemic syndrome HUS have been notified to the Robert Koch Institute (RKI). Our initial findings have been presented [1], including background information on STEC infections and HUS. The clinical and laboratory case definitions used are available [2]. Here we give an update on the epidemiological characteristics of the outbreak concerning cases of STEC and HUS notified to the Robert Koch Institute as of 31 May 2011.

Of 470 HUS cases, 273 (58%) were clinical cases with laboratory confirmation of Shiga toxin-producing *Escherichia coli* (STEC) infection. The German National Reference Centre for Salmonella and other Bacterial Enteric Pathogens alone has detected STEC serotype O104, Shiga toxin 2 (*stx2*)-positive, intimin (*eae*)-negative in more than 60 samples from cases in the outbreak, indicating that this unusual serotype is the cause of the outbreak.

Geographical distribution of HUS cases

Cases of HUS have been notified from all German Federal states. The highest cumulative incidence of HUS, since 1 May 2011, continues to be observed in the five northern states: Hamburg, Schleswig-Holstein, Bremen, Mecklenburg-Vorpommern and Lower Saxony (Table). A total of 66% of HUS cases have been notified from these states.

Epidemiological development

From 1 to 8 May 2011, the number of new HUS cases was between one and two cases per day, based on the

Table

Notified cases and cumulative incidence of HUS since 1 May 2011, Germany (n=470)

Federal State	Number of HUS cases	Cumulative incidence (per 100,000 population)
Hamburg	97	5.47
Schleswig-Holstein	121	4.27
Bremen	22	3.32
Mecklenburg-Vorpommern	20	1.21
Lower Saxony	51	0.64
Hesse	33	0.54
Saarland	5	0.49
North Rhine-Westphalia	75	0.42
Berlin	9	0.26
Saxony-Anhalt	4	0.17
Thuringia	3	0.13
Baden-Württemberg	13	0.12
Brandenburg	3	0.12
Rhineland-Palatinate	4	0.10
Bavaria	9	0.07
Saxony	1	0.02
Total	**470**	**0.57**

HUS: haemolytic uraemic syndrome.
Data as of 31 May 2011, 3 pm.

date of onset of diarrhoea (Figure 1). From 9 May, we observed an initially steady increase in the number of cases. This increase gained in intensity over the following days and reached a maximum of 39 notified HUS cases on 16 May.

Age and sex distribution of HUS cases

As reported on 26 May 2011 [1], the age and sex distribution of HUS cases remain conspicuous: the majority of patients were aged 20 years or older (88%) and female (71%). Notably, between 2006 and 2010, the proportion of adults in reported STEC and HUS cases was only between 1.5% and 10%, and there were no marked differences in sex distribution [3]. Figure 2 shows the age- and sex-specific cumulative incidence of notified cases of HUS since 1 May 2011.

Fatal cases

To date, 13 deaths have been notified: in nine cases, the deaths were in connection with HUS; in the remainder, the cases had had symptomatic STEC infection that was laboratory confirmed. The cases who died were between 22 and 91 years of age: five were aged between 22 and 40 years and eight between 75 and 91 years of age.

Foreign cases with connection to the present outbreak

Further HUS cases have been communicated from Denmark, United Kingdom, France, Netherlands, Norway, Austria, Spain, Sweden (including one death), Switzerland and the United States. Nearly all of these cases had a travel history to northern Germany. For some cases, however, detailed investigations are ongoing. After a stay in northern Germany between 8 and 10 May 2011, 15 members of a Swedish travel group (30 members in total) developed symptoms of STEC infection and HUS was diagnosed in five of these cases.

Evaluation of the situation

The present situation marks one of the largest outbreaks ever described of HUS worldwide, and the largest outbreak ever reported in Germany. Because of the delay in notification and reporting of cases, the current notification data cannot be interpreted as a decrease in case numbers.

The age and sex distribution of cases in this outbreak is highly unusual, as is the identified outbreak strain: STEC O104, Shiga toxin 2 (*stx2*)-positive, intimin (*eae*)-negative. Serotype STEC O104 has caused food-borne outbreaks of diarrhoea and HUS, or isolated cases of HUS before [4,5], but is not known to have caused previous outbreaks in Germany.

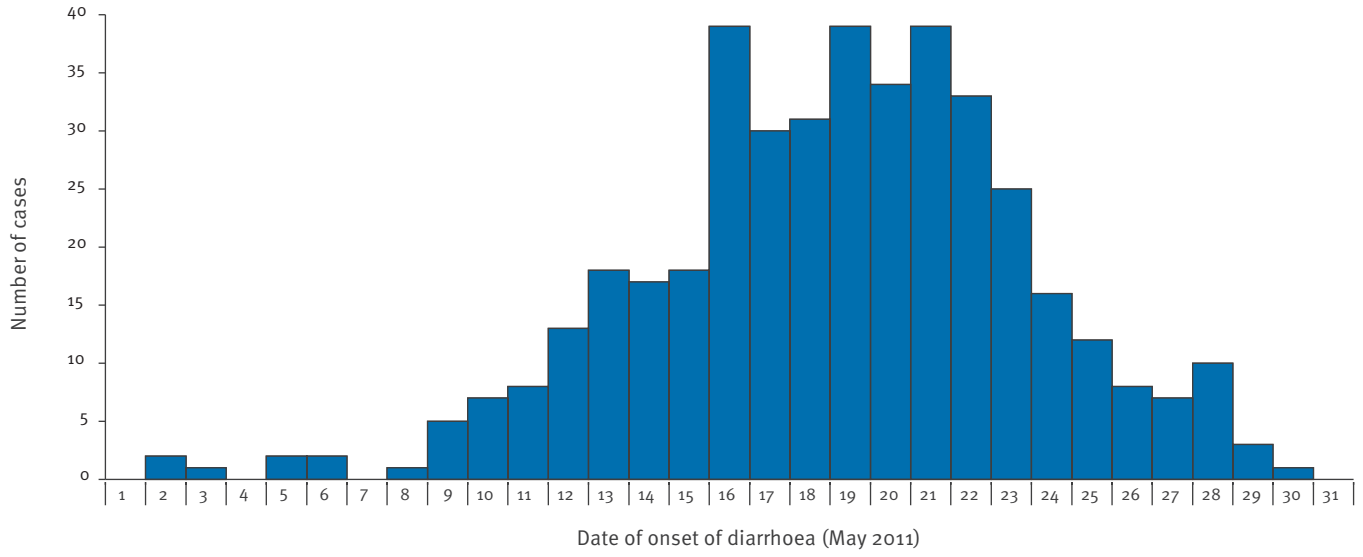
Current epidemiological activities

RKI is currently conducting the following studies to further investigate the outbreak:

- representative online survey within the German population to describe the disease burden;

Figure 1

Notified cases of HUS by date of onset of diarrhoea (only cases with a notified date of onset since 1 May 2011), Germany (n=421)



HUS: haemolytic uraemic syndrome.
Data as of 31 May 2011, 3 pm.

- case–control study in heavily affected hospitals, in Lübeck (in Schleswig-Holstein) and Hamburg;
- case–control study in hospitals that have observed a recent increase in cases numbers and had not been previously affected;
- analyses of questionnaires on cases completed by nephrologists treating the cases;
- Investigation of human-to-human transmission (and of information about purchases made by analysis of till receipts) within the setting of a special outbreak in a canteen;
- cohort investigations of various groups, in which several members developed symptoms of STEC infection after dinner in a restaurant (the members of the groups are questioned about the food products they consumed);
- exploration of several events and festivities that can be related to cases.

Furthermore, the RKI is cooperating with colleagues from Sweden and Denmark, who are performing cohort studies of groups in which several members developed symptoms of STEC infection.

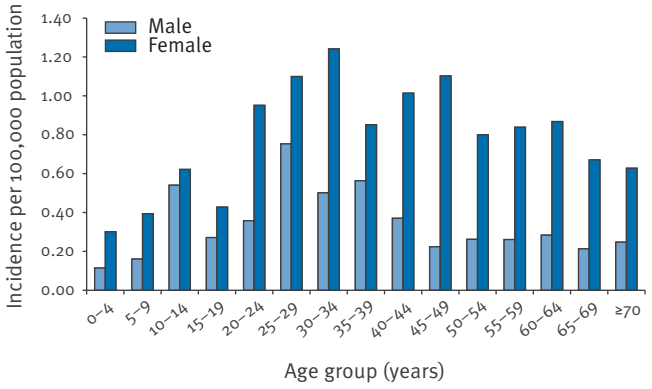
The Federal Institute for Risk Assessment (BfR) has recommended that consumers in Germany abstain from eating raw tomatoes, cucumbers and leafy salads (based on results from an epidemiological study, conducted by the RKI in cooperation with regional and local health departments from Hamburg [1]). As long as the studies outlined above do not lead to new evidence and as long as the outbreak is still ongoing, these recommendations – concerning goods available in northern Germany in particular – remain in effect.

Acknowledgments

We gratefully acknowledge the contribution of all local and state health departments, whose investigations and notifications were the data basis of this report, as well as the contribution of the health authorities of other European countries affected by the outbreak.

Figure 2

Cumulative incidence of HUS cases notified since 1 May 2011, by age and sex, Germany (n=470)



HUS: haemolytic uraemic syndrome.
Data as of 31 May 2011, 3 pm.

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Colonic ischaemia as a severe Shiga toxin/verotoxin producing *Escherichia coli* O104:H4 complication in a patient without haemolytic uraemic syndrome, Germany, June 2011

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An increasing rate of infections with Shiga toxin/verotoxin-producing Escherichia coli (STEC/VTEC) O104:H4 has been observed in Germany since May 2011, with unusually high numbers of patients suffering from haemolytic uraemic syndrome (HUS). We report a STEC/VTEC O104:H4 case without HUS, presenting with colonic ischaemia demanding surgery. This atypical clinical presentation of STEC O104:H4 infection might indicate new severe complications associated with this uncommon strain, and highlights the importance of immediate interdisciplinary assessment of STEC/VTEC patients.

Clinical presentation and initial evaluation

A woman in her 80s presented to our Emergency Department on 1 June with increasing abdominal pain and a history of nausea, primarily associated with dysuria that had been lasting for five days. On 29 May, the day after the onset of those symptoms, non-bloody diarrhoea had followed. The initial physical examination showed a mildly distended abdomen with diffuse pain and left upper quadrant tenderness without rebound. Blood tests showed a massive elevation of white blood cell count (29,300/µl; normal range: 4,300–10,000/µl) as well as an elevation of both C-reactive protein (32.42 mg/dl; normal range: ‹0.5 mg/dl) and lactate (2.5 mmol/l; normal range: 0.5–2.4 mmol/l). Ultrasound and computed tomography (CT) scans of the abdomen revealed distinctive amounts of ascites and wall thickening of parts of the transverse and descending colon, but did not show any disturbance of the main arteries and veins. Considering pseudomembranous colitis as a differential diagnosis, we decided to perform a colonoscopy.

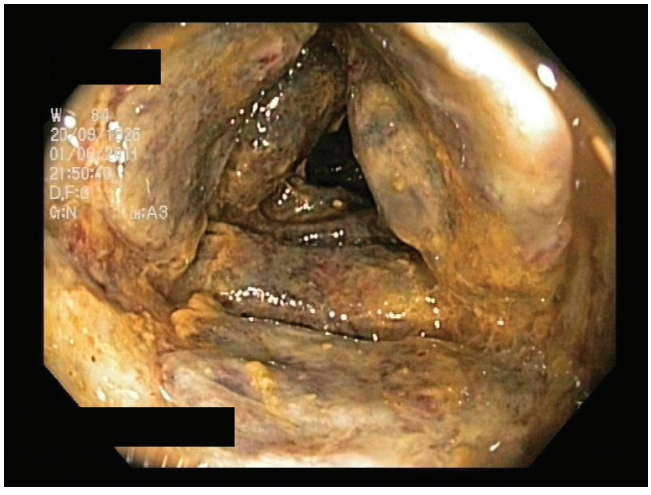
The endoscopy showed a normal rectum and sigmoid colon, macroscopically. The descending colon had a circularly swollen, partly pale, partly bluish mucosa, with no evidence of bleeding after biopsy, a combination of symptoms highly suspicious of ischaemia (Figure 1).

Surgical intervention

Following the confirmation of ischaemia by endoscopy of the descending colon on 1 June, surgery was performed immediately. Non-occlusive ischaemia of the descending colon with gangrenous bowel wall was detected during the operation, with patent macroperfusion of the medial colic artery and inferior mesenteric artery arcade, as well as the left colic artery up to the

Figure 1

Circularly swollen, partly pale, partly bluish mucosa of the descending colon, highly suspicious of ischaemia, endoscopy of STEC/VTEC O104:H4 patient, Germany, June 2011



STEC/VTEC: Shiga toxin/verotoxin-producing *Escherichia coli*.

gangrenous bowel wall. Therefore, a left hemicolectomy was performed, followed by thorough abdominal irrigation.

Pathology

Pathological examination of the removed part of the colon revealed wall thickening up to 1 cm and extensive necrosis throughout the entire intestinal wall with fibrinous-purulent exudation. The border area showed vital mucosa with erosive and phlegmonous inflammation, membranous-like fibrin exudation and crypt destruction, consistent with an ischaemic origin (Figures 2 and 3).

Postoperative course

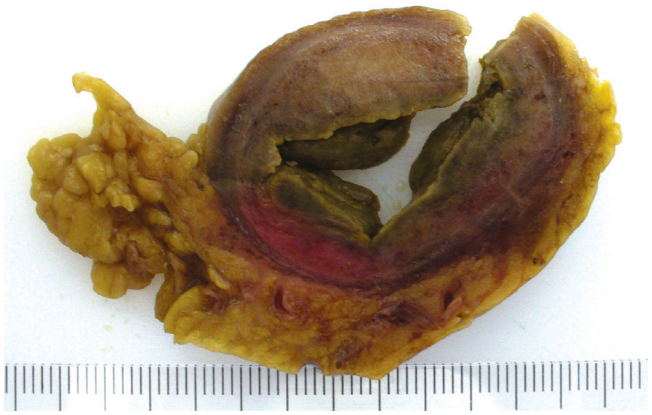
After the operation, which occurred late in the evening on 1 June, the patient was admitted to the intensive care unit. The next morning, as a precautionary measure, due to the ongoing Shiga toxin/verotoxin-producing Escherichia coli (STEC/VTEC) O104:H4 outbreak in Germany, the patient was placed in isolation and a stool sample was sent for further evaluation. The second day after surgery, seven days after the onset of the initial abdominal symptoms, neurological impairments were observed in terms of decelerated reactions of the patient, lack of orientation and intermittent response when addressed. Clinical examination was uneventful for abdominal, respiratory and haemodynamic findings. Laboratory studies were not suspicious for haemolytic uraemic syndrome (HUS): creatinine, elevated up to 1.7 mg/dl at admission (normal range: ‹1.2 mg/dl), had immediately revealed decreasing tendencies (0.8 mg/dl at day four) after intravenous fluid application, and was therefore most likely due to diarrhoea-induced hypovolemia. Lactate dehydrogenase (LDH) ranged between 139 U/l (normal range: 135-225 U/l) at admission and 318 U/l six days after, whereas platelets always presented within normal limits. The patient was monitored closely and improved neurologically during the next day. During the following 48 hours, however, noticeable neurological deficiencies with disturbance of vigilance, aphasia and apraxia were observed, as well as myoclonia of the extremities. Seizures did not occur. PCR analysis of the stool samples confirmed Shiga toxin 2-producing *E. coli* consistent with the strain responsible for the current outbreak, O104:H4, on 6 June. Monitoring the patient for an onset of HUS continued but has not eventuated as of 21 June.

Discussion

The ongoing outbreak of infections with STEC/VTEC, also commonly referred to as enterohaemorrhagic *E. coli* (EHEC), in Germany is one of the largest worldwide [1]. Besides causing non-bloody and bloody diarrhoea, the STEC/VTEC subtypes may also lead to HUS, a severe complication that is characterised by thrombocytopenia, microangiopathic haemolytic anaemia, and decreased renal function. So far, *E. coli* O157:H7 had been described as the predominant serotype causing HUS in approximately 10% of all cases to date [2,3], whereas the strain responsible for the current outbreak, which has been identified as *E. coli* O104:H4, is an extremely rare strain, hardly described during the last decade [4]. With adults and predominantly women being infected, the age and sex distribution in the ongoing outbreak is unusual, but might be related to gender-specific differences in dietary habits: vegetables, which are generally more often consumed by women, are still suspected to have been contaminated and at the source of this outbreak. In addition, an unusually high number of patients have developed HUS: The latest data account for 814 patients with HUS from a total of 3,587 infected patients in Germany [1]. Neurological complications, which were seen, on average, in about 25% of HUS patients in former outbreaks [2], could also be more severe in this outbreak. Indeed,

Figure 2

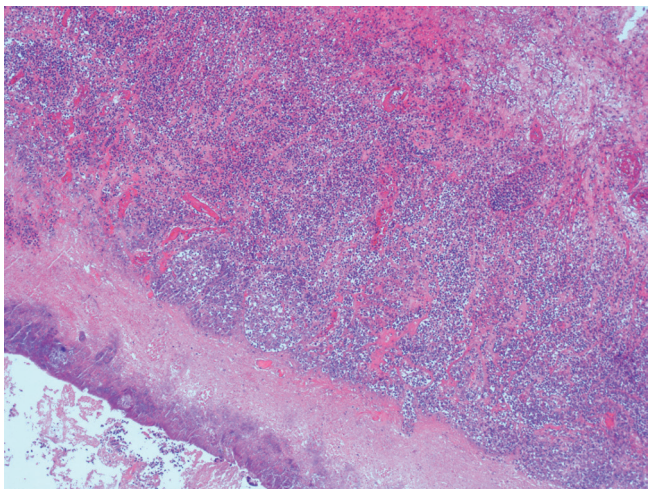
Left hemicolectomy specimen, revealing wall thickening up to 1 cm with induration and increased fragility, STEC/VTEC O104:H4 patient, Germany, June 2011



STEC/VTEC: Shiga toxin/verotoxin-producing *Escherichia coli*.

Figure 3

Extensive mucosal and submucosal necrosis affecting the muscular lining of the descending colon, with fibrinous-purulent exudation, left hemicolectomy specimen of STEC/VTEC O104:H4 patient, Germany, June 2011



STEC/VTEC: Shiga toxin/verotoxin-producing *Escherichia coli*.

the exchange among German clinicians, who set up a web-based platform to communicate clinical information in the context of the current outbreak, indicates higher numbers, but this has not been systematically evaluated so far. Taken together, the various aspects of the ongoing outbreak may suggest an increased virulent potential of the identified strain.

Besides HUS, STEC/VTEC-associated bowel ischaemia, as an additional severe complication, is rarely described in the literature. Very few reports of colonic necrosis and perforation due to Shiga toxin-induced intestinal damage exist, and in all these reports, this type of complication affected *E. coli* O157:H7-infected individuals. This complication was moreover mostly encountered in paediatric patients with concomitant HUS [3]. Only one case of ischaemic colitis in a non-HUS adult has been previously described [5].

Conclusion

Besides leading to its major complication HUS, infection with STEC/VTEC O104:H4 can also cause neurological complications and atypically present as bowel ischaemia, as shown in our patient. Since ischaemia-induced colonic wall thickening is difficult to differentiate from pseudomembranous colitis in CT imaging, endoscopy is essential and should be considered at an early diagnostic stage. Notably, our patient has not shown any signs of HUS to date, but obviously, even unexpected complications have to be considered as a differential diagnosis in STEC/VTEC O104:H4 infected patients, calling for interdisciplinary diagnostic investigations.

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Outbreak of haemolytic uraemic syndrome and bloody diarrhoea due to *Escherichia coli* O104:H4, south-west France, June 2011

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As of 12:00 28 June 2011, 15 cases of haemolytic uraemic syndrome (HUS) or bloody diarrhoea have been identified in the Gironde, south-west France. Investigations suggest the vehicle of transmission was sprouts, served at an event in Bègles on 8 June 2011. A strain of shiga toxin- producing *Escherichia coli* O104:H4 has been isolated from five cases. This strain is genetically related to the strain identified in the recent *E. coli* O104:H4 outbreak in Germany, and shares the same virulence and antimicrobial resistance characteristics.

Outbreak description

On 22 June 2011, the Cellule interrégionale d'épidémiologie (CIRE) Aquitaine, the regional office of the French Institute for Public Health Surveillance, was notified by the Robert Picqué Hospital in Bordeaux, south-west France, of eight cases of haemolytic uraemic syndrome (HUS) or bloody diarrhoea. Six of the cases lived in close proximity to one another in the commune of Bègles, in Bordeaux. Of these six cases, four were women (aged 41–78 years) and two were men (aged 34–41 years). Dates of symptom onset were between 15 and 20 June.

A case of HUS was defined as a person with acute renal failure and either microangiopathic haemolytic anaemia and/or thrombocytopenia. A possible outbreak case was defined as a case of HUS or a case of bloody diarrhoea without an alternative diagnosis in the French department (administrative region) of Gironde with a date of symptom onset since 10 June 2011. Active case finding has been carried out through contact with emergency, nephrology and intensive care departments of local hospitals, and general practitioners and out-of-hours doctors, and through the existing paediatric HUS surveillance network. Enhanced surveillance for cases of HUS or bloody diarrhoea in the rest of France has been implemented.

As of 12:00 28 June 2011, a further seven cases have been identified and investigated, bringing the total number of cases investigated to date to 15 cases of bloody diarrhoea, eight of whom have developed HUS.

Epidemiological investigations

The initial eight cases were interviewed using a standardised semi-structured questionnaire exploring food consumption, travel history and contact with other people with diarrhoea in the seven days before symptom onset. Initially no common food, visits to markets, restaurants or events, animal contact or leisure activity was identified. None of the cases reported eating sprouts. Only three of the cases shared the same municipal tap-water network. One of the cases had travelled away from home in France during the seven days before symptom onset and none had travelled abroad.

Given that a common exposure had not been identified, the predominance of adult women among the cases and the recent experience of the German sprout-related *Escherichia coli* O104:H4 outbreak in Germany [1,2], a second questionnaire was developed that included an in-depth exploration of vegetable consumption in the two weeks before illness.

Further questioning of the initial eight cases and seven newly identified cases indicated that 11 of these 15 cases had attended an open day at a children's

community centre on 8 June, at which a cold buffet was served consisting of crudités (raw vegetables), three dips, industrially produced gazpacho, a choice of two cold soups (carrot and cumin, and courgette), pasteurised fruit juices and individual dishes composed of white grapes, tomatoes, sesame seeds, chives, industrially produced soft cheese and fresh fruit. The soups were served with fenugreek sprouts, a small amount of which were also placed on the crudité dishes. Mustard and rocket sprouts, still growing on cotton wool, were used to decorate the crudité dishes. One of the 11 cases has not yet been fully questioned because of a deteriorating clinical condition, but is known to collect their grandchildren from the centre and may have attended the event. The remaining four cases had no obvious links to the centre.

Among the 11 cases with links to the centre, nine reported consuming sprouts at the event on 8 June; two cannot yet be fully questioned. Of these 11 cases, eight have HUS and three bloody diarrhoea. Seven are women aged 31–64 years and four are men aged 34–41 years. Dates of symptom onset are between 15 and 20 June (Figure). For the eight cases with a well-defined date of symptom onset, the incubation period ranges from 7 to 12 days (median: 9 days).

Microbiological investigations

A strain of *E. coli* O104:H4 possessing the stx2 gene, encoding Shiga toxin, has been isolated from five HUS cases, all of whom consumed sprouts at the event at the children's community centre. The strain is negative for the genes coding for intimin (*eae*), haemolysin A (*hlyA*) and EAST1 toxin (*astA*) and positive for the *aggR* gene which regulates the expression of aggregative adherence fimbriae. The antimicrobial resistance pattern of the strain is similar to that seen in the outbreak strain in recent *E. coli* O104:H4 outbreak in Germany [3](ampicillin resistant (R), cefotaxime R, ceftazidime R, imipenem sensitive (S), streptomycin R, kanamycin S, gentamicin S, sulfamethoxazole R, trimethoprim R, cotrimoxazole R, tetracycline R, chloramphenicol S, nalidixic acid R and ciprofloxacin S). Our PCR analysis indicates the presence of the extended-spectrum beta-lactamase (ESBL) $bla_{\text{CTX-M-15}}$ (group 1) gene and the penicillinase bla_{TEM} gene.

Strains of *E. coli* O104:H4 isolated from two imported cases in France linked to the *E. coli* O104:H4 outbreak in Germany in May and June 2011 were compared by two molecular techniques (Rep-PCR [4,5] and pulsed-field gel electrophoresis (PFGE), using a standardised PFGE using either *Xba*I or *Not*I [6]) with strains of *E. coli* O104:H4 isolated from three patients in the Bordeaux outbreak. The results of these analyses show the genetic relatedness of the outbreak strains in France and Germany. The profile of the outbreak strains in the two countries differs from the profiles of two *E. coli* O104:H4 stx2 strains isolated in 2004 and 2009 and from two other strains of serotypes *E. coli* O104:H21 and O104:H12. Comparison by whole-genome sequencing and optical maps will be performed in the coming days.

Food trace-back investigations

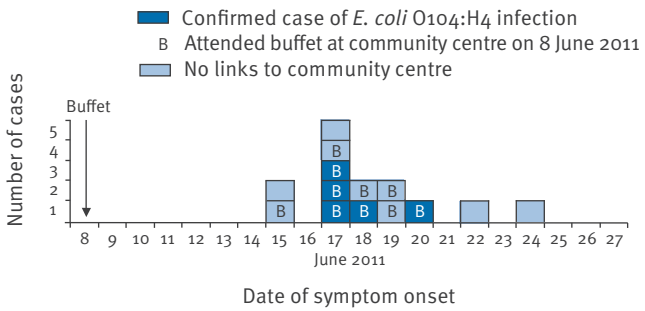
Food trace-back investigations were initiated on 24 June. The sprouts served at the event on 8 June had been grown from rocket, mustard and fenugreek seeds planted at the centre during 2 to 5 June. The fenugreek seeds were first soaked in tap water for 24 hours then placed in a jam jar topped with gauze and then rinsed with tap water two or three times a day. The mustard and rocket seeds were germinated on cotton wool moistened with tap water. They were harvested on 8 June to be served at the buffet. The seeds were purchased from a branch of a national chain of gardening retailers, having been supplied by a distributor in the United Kingdom. Leftover mustard and rocket seeds, gazpacho and tap water samples from the community centre have been sent for microbiological analysis, as have samples of rocket, mustard, fenugreek and other seeds from the French gardening retailer. Preliminary results are currently being analysed.

Control measures

Consumers have been advised by the French authorities not to eat raw sprouts, to thoroughly clean utensils used for germination and cooking, and to wash their hands thoroughly after contact with seeds and sprouts. Colleagues in other European countries were informed of this outbreak on 24 June via the Epidemic Intelligence Information System (EPIS) and Early Warning Response System (EWRS) of the European Centre of Disease Prevention and Control (ECDC). A European Food Standards Agency (EFSA) and ECDC joint rapid risk assessment has been carried out [7]. This assessment strongly recommends that consumers do not grow sprouts for their own consumption and do not eat sprouts or sprouted seeds unless thoroughly cooked.

Figure

Cases of HUS or bloody diarrhoea due to enterohaemorrhagic *Escherichia coli* O104:H4 with date of symptom onset since 10 June 2011, Gironde, France, June 2011 (n=14)



HUS: haemolytic uraemic syndrome.

Of the 15 cases of HUS or bloody diarrhoea, date of symptom onset was unavailable for one case, who attended the buffet on 8 June 2011.

Conclusions

Preliminary data indicate that this outbreak shares the same novel epidemiological, clinical and microbiological features identified in the *E. coli* O104:H4 outbreak in Germany [8], including a predominance of adult women among the cases, an unusually high proportion of HUS cases among identified possible outbreak cases, a longer median incubation period than expected for cases of Shiga toxin-producing *E. coli* infection, and a genetically related *E. coli* O104:H4 producing a CTX-M ESBL. The two outbreaks may share the same vehicle of transmission. A cohort study of those attending the event at the community centre and further epidemiological, microbiological and food trace-back investigations are underway. The possibility of similar outbreaks in France or elsewhere in Europe cannot be excluded.

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Characteristics of the enteroaggregative Shiga toxin/verotoxin-producing *Escherichia coli* O104:H4 strain causing the outbreak of haemolytic uraemic syndrome in Germany, May to June 2011

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The *Escherichia coli* strain causing a large outbreak of haemolytic uraemic syndrome and bloody diarrhoea in Germany in May and June 2011 possesses an unusual combination of pathogenic features typical of enteroaggregative *E. coli* together with the capacity to produce Shiga toxin. Through rapid national and international exchange of information and strains the known occurrence in humans was quickly assessed. We describe simple diagnostic screening tools to detect the outbreak strain in clinical specimens and a novel real-time PCR for its detection in foods.

Sequence of events

Having received the first Early Warning Response System (EWRS) alert issued by the Robert Koch Institute (RKI) in Germany on 23 May about an increase in the number of patients presenting with haemolytic uraemic syndrome (HUS) and bloody diarrhoea caused by Shiga toxin-producing *Escherichia coli* (STEC) with more than 30 possible cases reported since the second week of May, the World Health Organization Collaborating Centre (WHO CC) for Reference and Research on Escherichia and *Klebsiella* at Statens Serum Institut (SSI) in Denmark issued an alert to the Danish *E. coli* network of regional hospitals on the same day. On 24 May, Hvidovre University hospital reported a German patient who had been diagnosed with Shiga toxin/verotoxin-producing *E. coli* (STEC/VTEC) and referred the strain to SSI. The WHO CC found that this first isolate was of serotype O104:H4 and produced Shiga toxin (Stx)/verotoxin (VT) as also reported by RKI. Referral from other regional hospitals identified the German outbreak strain in further patients in Denmark during the next days. This information was immediately shared by postings on the Urgent Inquiry Network (UIN) Epidemic Intelligence Information System (EPIS) hosted by the Food- and Waterborne Diseases and Zoonoses (FWD) Surveillance Network of the European Centre for Disease Control and Prevention (ECDC), and emails to FWD, the European Union Reference Laboratory for *E. coli* (EU-RL) and the two associated networks including public health (ECDC) and food safety (EU-RL) reference laboratories, the Global Food-borne Infections Network (GFN), Food Safety, WHO Geneva and the WHO Regional Office for Europe, and PulseNet at the United States (US) Centers for Disease Control and Prevention (CDC).

Having verified the specific characteristics of eight of the Danish outbreak strains, the WHO CC sent the index strain and the reference strain for the O104 antigen to the EU-RL in Rome. The strains were received on 31 May and tested positive by the EU-RL using a novel real-time PCR developed at the EU-RL and its network for detection of *E. coli* strains of serotype O104:H4. Thus, within a week, screening tools and a novel PCR protocol for detection of the outbreak strain in clinical specimens and in foods were developed, tested and shared with national as well as international networks. In return, members of the networks contributed with their existing knowledge of *E. coli* strains of serotype O104:H4, thereby collectively adding to the existing knowledge of this pathogen and describing the relevant characteristics of the reported strains for public health investigation.

The outbreak strain

The Danish isolates were PCR-positive for the *aggR* gene, which is typical of enteroaggregative *Escherichia coli* (EAggEC). Further analysis showed that the outbreak strain (first eight isolates from Danish patients)

were also positive for the following genes: *sigA, sepA, pic, aatA, aaiC, aap*, as well as *aggA*, which encodes the major component of the AAF/I adhesin. AAF/I is a fimbrial organelle usually associated with a strong ability to form biofilms and haemagglutination with human erythrocytes. Preliminary testing at WHO CC showed that the isolates were moderate to good biofilm producers particularly in Dulbecco´s minimum essential medium (DMEM) supplemented with 0.45% glucose, which is typical and defining for EAggEC strains. The outbreak strain was a typical *E. coli*: lactose-positive, sorbitol-fermenting and beta-glucuronidase-positive. Furthermore, the strain was positive for iutA encoding an aerobactin receptor found in 80% of extraintestinal pathogenic *E. coli* isolated from urosepsis [2] and negative for the STEC-associated adhesin (saa) and cytotoxin subtilase (subAB).

Taken together, these data indicate that the outbreak strain is indeed a typical EAggEC strain that has acquired the bacteriophage encoding Stx/VT. Using a novel protocol for subtyping of *stx/vtx* genes [3], we have shown that the gene encoding Stx/VT is *stx2a/vtx2a*.

Sequence analysis of the published *stx2a/vtx2a* sequence (SRX067313 on http://www.ncbi.nlm.nih.gov/sra) showed 100% amino acid identity of the holotoxin to Stx2a/VT2a from *E. coli* O157:H7 EDL933 isolated from Michigan ground beef in 1983 (accession number X07865 [4]) but differed by one nucleotide at position 867 (C instead of T), making the nucleotide sequence identical to the sequence found in sorbitol-fermenting O157 strains from Germany in 2002 and 2005 (accession numbers AY143336 and AY143337, unpublished), DQ231589 and DQ231590 [5], and Scotland in 2006 (EU526759) [6]. This sequence variant of *stx2a/vtx2a* has also been detected in isolates from seagulls (accession number AB030484, unpublished) and human isolates of different serotypes: *E. coli* O121:H19 from Canada (DQ143182 and DQ143183) [7] and Idaho, US (EF441611) [8], and O111:H8 also from Idaho, US (EF441606) [8].

These findings could explain the unexpectedly high level of virulence in a STEC/VTEC strain negative for the attaching/effacing pathogenicity island. It is indeed conceivable that the enteroaggregative adherence phenotype could have allowed these *E. coli* O104 strains to colonise the intestinal mucosa of the affected patients as efficiently as the typical eae-positive STEC/VTEC strains. The different mechanism of adhesion might also explain why this strain is more likely to cause severe disease in adults rather than in children, as would be usual for typical HUS-associated STEC/VTEC: adults and children might differ in their susceptibility to the adherence and/or colonisation properties of this type of EAggEC strain. Obviously, elucidating this aspect requires dedicated studies and we cannot exclude that the different rates of HUS between adults and children observed in the current outbreak just reflect a difference in the exposures.

Screening for the outbreak strain

Plating clinical samples on extended-spectrum beta-lactamase (ESBL) plates, such as commercially available Tryptone Bile X-Glucuronide (TBX) medium will allow growth of the outbreak strain and inhibit the majority of other *E. coli* strains. Excellent growth of the index strain (only one of the strains has been tested so far) from the outbreak has also been observed as light red colonies on cefixime tellurite sorbitol MacConkey (CT-SMAC) plates at 37 °C, 41.5 °C and 44 °C (Jeppe Boel, personal communication, 3 June 2011). Since cefixime belongs to the class of cephalosporins, it seems likely that the strain can overcome the cefixime concentration in CT-SMAC, but apparently it is also able to overcome the tellurite concentration.

For quick screening of clinical samples, K9 antiserum for live slide agglutination can be used in both primary and secondary testing laboratories. This is because the O104 O antigen is identical to the K9 capsular antigen [9]. The K9 antiserum is readily available from SSI Diagnostica, Hillerød, Denmark (ivdorders@ssi.dk) and described on the SSI website [10]. At SSI, we have agglutinated culture from confluent growth but pools of 5 to10 individual colonies can also be agglutinated. Immediate positive reactions indicating the presence of *E. coli* O104 have all been confirmed by conventional serotyping of O and H antigen, presence of stx2a/vtx2a and lack of the eae gene. Based on our observations so far, all weak reactions have turned out to be negative for the outbreak strain. The strain can also be detected by a number of methods targeting the stx2/vtx2 gene by PCR, RT-PCR or commercial Stx/VT detection kits. The strain must also be negative for the eae gene and confirmed for O104.

Food samples should be enriched in Buffered Peptone Water (225 ml for 25 g test portion) and incubated for 18 to 24 h at 37 °C ± 1 °C. DNA extracted from a 1 ml aliquot is purified and tested for the presence of *stx/vtx* genes (first step of the real-time PCR procedure described in the ISO/TS 13136:2011(E) method [11]).

Samples positive for *stx/vtx* genes (regardless of the presence of the eae gene) are tested for the O104-associated gene (*wzx*O104) [12]. The *wzx*O104-positive enrichment cultures are plated onto two media: (i) MacConkey agar, or TBX, or any other medium suitable for *E. coli* isolation, and (ii) a more selective medium containing an antibiotic supplement. Colonies positive for *stx/vtx* genes are identified for the O104 antigen-associated gene *wzx*O104 and the gene encoding the H4 flagellar antigen, *fliC*H4 [12]. Conventional serotyping can be performed by standard methods [13]. Other markers can be tested by either conventional or real-time PCR for further characterisation.
DNA from an outbreak strain provided by the Robert Koch Institute to be used as positive control in the PCR assays can be obtained from Istituto Superiore di Sanità (ISS) in Rome (crl.vtec@iss.dk).

To the best of our knowledge, this unusual combination of virulence factors of STEC/VTEC and EAggEC has rarely been described in humans. A strain of serotype O111:H2 [14] caused a small outbreak of HUS in France in 1995, but the episode involved children, as is typical for STEC/VTEC [15]. As in the present outbreak in Germany, the association of the French strains with severe disease (HUS) supports the view that this unusual combination of virulence factors might confer a very high degree of virulence.

Serotype O104:H4

Sporadic cases of stx2/vtx2-positive *E. coli* serotype O104:H4 have been reported. These reports include two isolates from patients with HUS in Germany in 2001 [16], one in France in 2004 (data from the dedicated EU surveillance network Enter-net; not including clinical information), one from a case of HUS in Korea in 2005 [17], two HUS cases in the Republic of Georgia in 2009 (unpublished information provided via PulseNet, US CDC), and one uncomplicated case of diarrhoea in Finland in 2010 (reported to FWD on EPIS). The isolates from Germany 2001, Finland 2010 and the Republic of Georgia 2009 were EAggEC and STEC/VTEC.

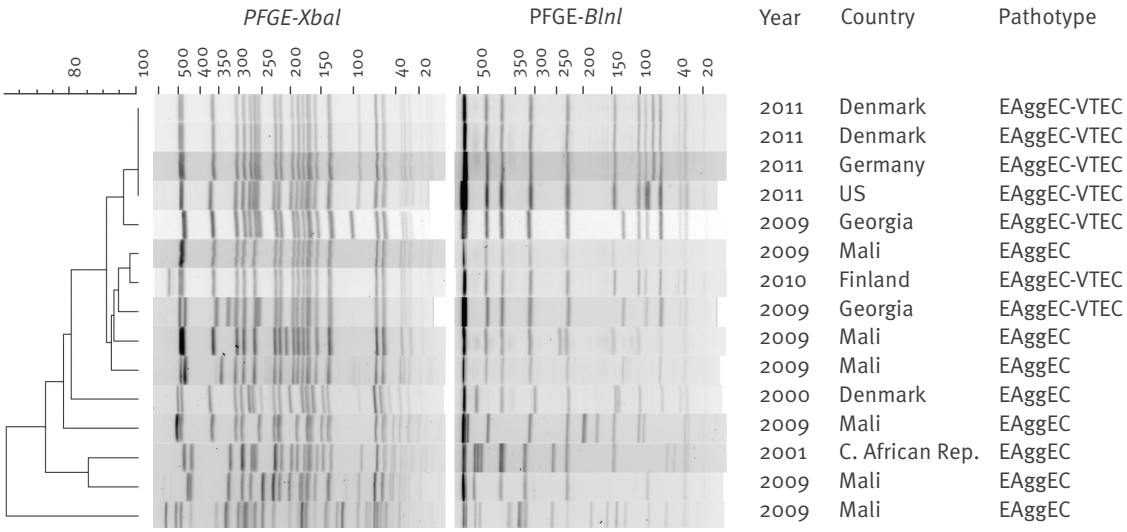
The strain from the Republic of Georgia had the following characteristics: serotype O104:H4, Shiga toxin subtype stx2a, eae-negative, haemolysin-negative, *aatA*-positive (EAggEC marker), susceptible to ceftriaxone (unlike the current outbreak strain), sorbitol-, lactose-, and beta-glucuronidase-positive, biochemically consistent with *E. coli*, Shiga toxin production on the low end of the spectrum, similar to that of the German strain (Peter Gerner-Smidt, personal communication 7 June 2011 from PulseNet, US CDC, and the Georgian team of investigators). At this time, we do not have further information on the remaining O104:H4 STEC/VTEC isolates from France and Korea.

In general, we have limited knowledge on EAggEC of this serotype: The archetype isolate for the aggregative adherence fimbriae type III (AAF/III, encoded by the *agg3A* gene) is strain 55989, which was isolated during a study of EAggEC as a cause of persistent diarrhoea in African patients infected with human immunodeficiency virus (HIV) [18,19]. In a recent study of childhood diarrhoea in Mali, we identified Stx/VT-negative EAggEC O104:H4 in three children with moderate to severe diarrhoea and from three healthy controls (unpublished data). The three EAggEC strains isolated from these cases were PCR-positive for different combinations of *aggR, aatA, aaiC, aap, astA, sepA, pic, sigA, aggA, agg3C* and *agg3A*.

We have compared the pulsed-field gel electrophoresis (PFGE) profiles of the available *E. coli* O104:H4 isolates to elucidate the diversity within this serotype, irrespective of the virulence profile. PFGE typing using the enzymes *XbaI* and *BlnI* showed that the serotype O104:H4 is diverse (Figure). For *XbaI*, a high similarity of ›95% was seen for the 2011 German outbreak isolates (isolated in Denmark, Germany and the US) and one of the isolates from Republic of Georgia. A large cluster of isolates with ›90% similarity included the German outbreak strain, the two Georgian cases from 2009, the isolate from the Finnish patient (all stx2a/vtx2a and EAggEC) as well as three of the *stx/vtx*-negative EAggEC isolates from patients in Mali. The profiles of five of the *stx/vtx*-negative EAggEC isolates showed

Figure

PFGE profiles (*XbaI* and *BlnI*) of *Escherichia coli* O104 compared with four isolates from the outbreak of haemolytic uraemic syndrome in Germany, May to June 2011



Year	Country	Pathotype
2011	Denmark	EAggEC-VTEC
2011	Denmark	EAggEC-VTEC
2011	Germany	EAggEC-VTEC
2011	US	EAggEC-VTEC
2009	Georgia	EAggEC-VTEC
2009	Mali	EAggEC
2010	Finland	EAggEC-VTEC
2009	Georgia	EAggEC-VTEC
2009	Mali	EAggEC
2009	Mali	EAggEC
2000	Denmark	EAggEC
2009	Mali	EAggEC
2001	C. African Rep.	EAggEC
2009	Mali	EAggEC
2009	Mali	EAggEC

C. African Rep: Central African Republic; CDC: Centers for Disease Control and Prevention; PFGE: pulsed-field gel electrophoresis; RKI: Robert Koch Institute; US: United States.

PFGE profiles (*XbaI* and *BlnI*) using the *E. coli* non-O157 PulseNet protocol (www.pulsenetinternational.com). Dendrogram based on analysis of the *XbaI* profiles. All isolates are EAggEC O104:H4 with and without *stx2/vtx2* gene. German outbreak isolates are from patients infected in May 2011 in Germany and diagnosed in Denmark, the US (profiles provided by PulseNet, US CDC) and Germany (strain provided by RKI, Germany). O104:H4 isolates from Mali are from children with and without diarrhoea.

major differences from the outbreak strain (Figure). The 11 Danish PFGE-typed isolates related to the German outbreak had indistinguishable *XbaI* profiles. One isolate from a case infected in Germany and diagnosed in the US had a minor variation in the *BlnI* profile (profile provided by PulseNet, US CDC) (Figure).

General characteristics of EAggEC

EAggEC is a pathotype of diarrhoeagenic *E. coli* defined as *E. coli* that do not secrete the heat-stable or heat-labile toxins of enterotoxigenic *E. coli* (ETEC), and by its characteristic aggregative or 'stacked brick' pattern (AA) of adherence to HEp2-cells in culture [20]. This property is usually due to the presence of aggregative adherence fimbriae (AAF), whose expression is regulated by the *aggR* gene, located on the large EAggEC virulence plasmid termed pAA [21]. EAggEC infections are usually associated with watery diarrhoea, which is often persistent [20]. Illness results from a complex interaction between pathogen and host, which implicates the initial adherence of the bacteria to the epithelium of terminal ileum and colon, by virtue of the aggregative adherence fimbriae (characteristic aggregative pattern), followed by a damage/secretion stage manifested by cytokine release, mucosal toxicity, intestinal secretion and induction of mucosal inflammation [22-26].

EAggEC is best known for its role in persistent diarrhoea (>14 days) in infants and children in developing countries. Studies in Mongolia [27], India [28], Brazil [29,30], Nigeria [31,32], Israel [33], Venezuela [34], Congo [35] and many other countries, have identified EAggEC as a highly prevalent (often the most prevalent) *E. coli* pathotype in infants. Further, the role of EAggEC as an important pathogen in AIDS patients continues to develop, and EAggEC now ranks among the most important enteric pathogens in this population group [36,37]. In a recent review of all published studies of traveller's diarrhoea, EAggEC was in aggregate second only to ETEC as the most common pathogen [38].

The first reported EAggEC outbreaks occurred in Mexico City before 1993 (year unpublished) where persistent diarrhoea was reported. Five of the infected children died as a consequence of the diarrhoea. Both outbreaks occurred in the malnutrition ward of a paediatric hospital [39], demonstrating that EAggEC is not exclusively a disease of infants under the age of 12 months [40]. Itoh et al. described a massive outbreak of EAggEC diarrhoea among Japanese children in 1993 affecting nearly 2,700 patients [41]. Another EAggEC outbreak was reported in a Serbian nursery in 1995 [42] in which 16 newborn babies (duration of illness 3–9 days) and three infants (18–20 days) developed diarrhoea accompanied by pyrexia and weight loss. Outbreaks have also been reported among adults in the United Kingdom [43] and a small outbreak of EAggEC serotype O92:H33 was reported in Italy in which pecorino cheese (unpasteurised milk) was epidemiologically implicated [44]. As these outbreaks suggest, EAggEC is capable of causing diarrhoea in adults and children, even in the absence of Stx/VT. We believe that this pre-existing diarrhoeagenic and outbreak potential, coupled with the highly virulent Stx/VT, has resulted in a hypervirulent strain currently circulating in Germany. It should also be noted that EAggEC are common in all populations of the world, industrialised and developing, but that no animal reservoir has been described. This observation suggests the startling possibility that this new O104 stain may have the capacity to persist among human populations, perhaps indefinitely.

Conclusions

The rapid exchange of information, strains and DNA fingerprints within existing national and international public health and food safety networks has been vital in the quick and alternative assessment of the public health significance of the strain causing the outbreak of HUS in Germany in May and June 2011. The combined contributions have resulted in major findings including:

- the characterisation of an unusual combination of pathogenic features typical of EAggEC combined with the capacity to produce Shiga toxin in the outbreak strain;
- recommendations for simple diagnostic screening tools for primary laboratory detection of the outbreak strain in clinical specimens;
- a novel real-time PCR protocol for detection of E. coli O104:H4 in foods;
- presentation of the known occurrence and clinical presentation in humans and the likely reservoir.

We hope that this report will help to strengthen existing networks, inspire the development of new networks and improve food safety in the future when new or emerging bacterial pathogens may occur in the food chain.

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Outbreak of Shigella sonnei infections in the Orthodox Jewish community of Antwerp, Belgium, April to August 2008

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In the beginning of April 2008 three cases of *Shigella sonnei* infection were identified among the Orthodox Jewish community of Antwerp, Belgium. We conducted a descriptive study and a household cohort study to identify potential risk factors. Stool samples were cultured and antibiotic susceptibility of the isolates was determined. Between April and August 2008, 42 cases were registered. All characterised isolates (n=20) shared an identical pulsed-field gel electrophoresis profile and were indistinguishable from one of the twelve main strains detected in Israel in 2008, where the index case's father had stayed before the outbreak. The secondary attack rate in households was 8.5% (95% confidence interval (CI): 4.3–12.7). Multivariate analysis identified the following risk factors for secondary spread: households with more than three children (adjusted relative risk (RR): 9.17; 95% CI: 1.21–69.13), children younger than five years (adjusted RR: 5.45; 95% CI: 2.44–12.62), and children younger than 12 years assisting in washing younger siblings (adjusted RR: 5.45; 95% CI: 2.44–12.17). Rigorous hand washing, use of disposable towels, information for parents and caregivers, and exclusion of symptomatic children from day care, preschool and school for a minimum of 48 hours were implemented.

Introduction

Infection with *Shigella sonnei* is a major cause of bacterial gastroenteritis and a leading cause of bacillary dysentery in Belgium [1,2]. Shigellosis is a highly communicable disease and requires a low dose for infection [1,2]. In industrialised countries person-to-person transmission accounts for most cases of *S. sonnei* infections, which occur commonly in children aged between six months and 10 years [2,3].

Shigellosis has been a statutorily notifiable disease for clinicians and microbiologists in Belgium since 1971 [4]. Between 2000 and 2009, the number of laboratory isolates of shigellae registered annually by the Belgian reference laboratory of salmonellae and shigellae varied from 316 to 500. *S. sonnei* has been the predominant agent causing 65% to 75% of all registered Shigella infections [4,5].

In the beginning of April 2008, a microbiologist of one of the town hospitals informed the Department of Communicable Disease Control of Antwerp that *S. sonnei* had been isolated from three children. The patients belonged to the Orthodox Jewish community of the town. The standardised post-notification interview with their general practitioners and parents showed that the patients had not been out of the country in the month before onset of symptoms. The father of the first case had just returned from a stay in Israel where he felt sick during three days before his return.

Antwerp has an Orthodox Jewish, highly insular community of approximately 10,000 persons living in one quarter of town. The community is characterised by relative social isolation and frequent international contacts especially with New York, London, and Israel [6]. Although sporadic cases of shigellosis have been identified among members of the Orthodox Jewish community in Antwerp before, a well documented outbreak in Belgium has never been described [4].

The first aim of the study was to describe the extent of the outbreak and to identify risk factors for secondary transmission. In addition, we tried to compare the strains from identified cases to confirm that they were genetically indistinguishable and to compare them to the circulating strains in Israel. Using the information

obtained from these objectives, we wanted to implement appropriate public health control and prevention measures in order to stop the propagation of the disease. An outbreak control team was established to oversee the coordination of this study.

Methods

Case definition

A confirmed case was defined as a person living in the town of Antwerp, who had a positive stool culture for *S. sonnei* in the period between 1 April and 31 August 2008. A probable case was defined as a person who had diarrhoea (three or more loose stools within 24 h), fever (≥38°C) and nausea, and who lived in a household where a confirmed case had been detected. An index case in a household or school was the first laboratory-confirmed shigellosis case in each household or school class. A secondary case was a confirmed or a probable case occurring within seven days after the detection of an index case in a household or in a classroom.

Case finding

Cases of shigellosis were reported by peripheral microbiological laboratories and clinicians in accordance to statutory notification of infectious diseases. Active case finding among members of affected households and school classes was performed by the local health authorities. General practitioners and paediatricians were asked to report cases to the outbreak control team.

Data collection

For each identified case, information on demographics, and clinical and microbiological characteristics was collected using a standardised questionnaire. The questionnaire also collected information on possible exposures including any recent travel, attending family gatherings, contacts with other cases, names of household contacts, and attendance at schools or day care centres. It was administered by telephone or by face-to-face interviews at home. Household contacts were followed prospectively for clinical symptoms during one week after contact with the index case. Social Service of the Antwerp Jewish community assisted in contacting people in order to avoid language and cultural barriers. Demographic data collected on household members were compared to data collected from the municipal registry office.

Secondary attack rate study

To identify specific risk factors for secondary transmission, a retrospective cohort study was conducted among the household contacts of the index cases. A household contact was defined as a person living in the same house as the household index patient. A secondary attack rate was calculated by identifying secondary cases in proportion to the number of household contacts after exclusion of the index case. Potential risk factors for transmission in the household were assessed as follows: the number of children in the household, the age of the children, the presence of children with nappies, the practice of hand washing after washing children, the number of toilets, whether children younger than 12 years (primary school children) were assisting their parents in washing siblings with gastrointestinal symptoms or assisting them at going to the toilet, whether the index case received antimicrobial treatment or whether the index case was admitted to hospital.

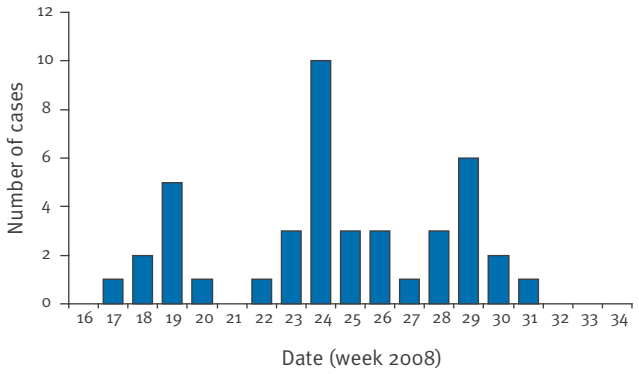
Laboratory investigations

Shigella strains isolated from patients in peripheral clinical laboratories were sent on a voluntary basis to the National Reference Centre for Salmonella and *Shigella* for serotyping by slide agglutination with commercial antisera (Denka Seiken Co). To evaluate antimicrobial susceptibility, *S. sonnei* specimens were tested by disk diffusion (Kirby-Bauer) following recommendations of the National Committee for Clinical and Laboratory Standards Institute (CLSI), formerly the National Committee for Clinical Laboratory Standards (NCCLS) [7]. Antibiotics tested (BioRad disks) were: ampicillin, amoxicillin/clavulanic acid, cefotaxime, chloramphenicol, tetracycline, naladixic acid, ciprofloxacin, streptomycin, kanamycin, gentamicin, sulfonamides, trimethoprim, and trimethoprim/sulfamethoxazole. Interpretation of inhibition zones was performed according to the CLSI criteria, and quality control was performed using the *Escherichia coli* ATCC 25922 reference strain [7].

S. sonnei strains were analysed by pulsed-field gel electrophoresis (PFGE) according to the PulseNet method and digested with the restriction endonuclease *Xba*I (New England Biolabs) [8]. *Salmonella enterica* serovar Braenderup H9812 was used as size marker. Fingerprinting II Informatix software (Bionumerics, BioRad) was used to compare the PFGE profiles. *Salmonella enterica* serovar Braenderup H9812 was used as size marker. FingerprintingII Informatix software (Bionumerics, BioRad) was used to compare the PFGE profiles. In addition, we included as internal reference five unrelated *Shigella* strains from national collections that had been isolated from Belgian patients

Figure 1

Onset of illness among *Shigella sonnei* cases, by week, Jewish community Antwerp, 17 April–31 August 2008 (n=42)



in 2008. The PFGE profiles of the outbreak strains in Antwerp were also compared to patterns, obtained with the same PulseNet method, of *S. sonnei* isolated from different orthodox Jewish community outbreaks in Israel between 2000 and 2008. The PFGE gel for them was provided by the Central Laboratories Ministry of Health of Israel. The bands had been analysed using the Dice coefficient and the unweighed-pair group method using average linkage with a tolerance of 1%.

Statistics

Univariate analysis was performed on data collected in the retrospective household cohort study and crude relative risk (RR) along with 95% confidence intervals (CI) were calculated to determine associations between potential risk factors and infection. Adjusted RRs were calculated using a binomial regression model. All statistical analyses were performed using Stata software version 11 (StataCorp).

Results

Outbreak description

Between 17 April and 31 August 2008, 42 cases of shigellosis were identified in Antwerp and all of them belonged to the Orthodox Jewish community, with the highest number of cases in week 24 (Figure 1). Thirty-two of them were confirmed cases and 10 were probable cases. Cases occurred in 19 Jewish families and in four confessional schools. Two additional reported cases of *S. sonnei* identified outside the Jewish community of Antwerp were excluded from the study because the disease started during a stay in Egypt. They were classified as travel-associated cases.

Of the 42 cases, 20 were male and 22 female. The arithmetic mean of age of cases was 4.4 years with a range from three months to 61 years. Four patients were younger than two years, 19 were between two and five years-old, seven were between six and 10 years-old, six cases were between 11 and 15 years-old, and six cases were older than 20. The affected families had an average of 4.6 children (range: 1–12). All patients had their residence in an enclosed area in the town centre.

Eighteen cases reported fever ≥38°C and bloody or mucopurulent diarrhoea and abdominal cramps, and 32 cases were hospitalised. The average duration of illness was eight days with a range from six to 11 days. The average stay at the hospital was 3.4 days.

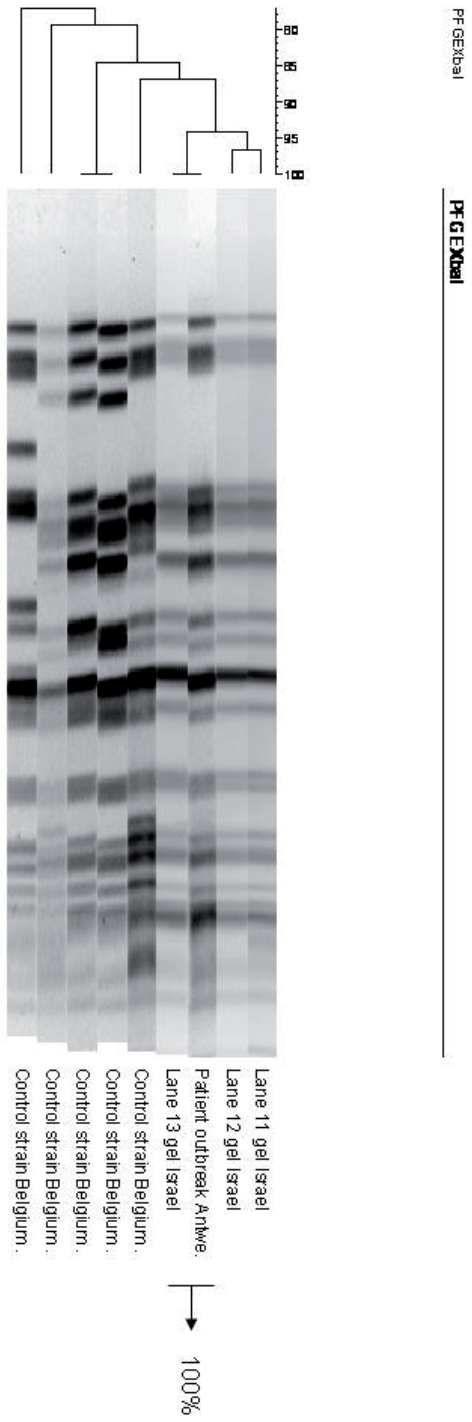
Of the 42 cases, 15 cases met the criteria for a secondary case. The generation interval was 3.5 days (range from one to four). Three children developed illness within two to five days of detection of a case in a classmate.

Laboratory data

We received antibiotic susceptibility results from 28 of the 32 confirmed *S. sonnei* isolates. All of them were resistant to amoxicillin and trimethoprim-sulfamethoxazole, but were susceptible to levofloxacin and

Figure 2

Cluster analysis of PFGE fingerprinting of *Shigella sonnei* isolated in Antwerp and Israel in 2008



'Lane 11, 12 and 13 gel Israel': *S. sonnei* from different outbreaks in Orthodox Jewish communities in Israel in 2008.

'Patient outbreak Antwerp': *S. sonnei* strain from the outbreak in Antwerp in 2008.

'Control strains': unrelated *S. sonnei* strains from the national collection of the Belgian national reference laboratory in 2008.

cefotaxim. PFGE was performed on 20 of the 32 isolates and showed that all strains isolated during this outbreak displayed the same restriction-fragment patterns, confirming the relatedness of these isolates. The outbreak strain in Antwerp was compared to 12 different outbreak strains detected in *Shigella sonnei* shigellosis outbreaks in Orthodox Jewish communities in Israel between 2000 and 2008. Figure 2 presents the results of a cluster analysis on the basis of PFGE fingerprinting of isolates from Antwerp and Israel. The isolate called 'Lane 13 gel Israel', *S. sonnei* isolated in 2008 in Israel, was indistinguishable from the Belgian outbreak strain. The isolates shown as 'Lane 11 and 12 gel Israel', also isolated in Israel in 2008, had a closely related profile with the Belgian outbreak strain. Five unrelated *S. sonnei* strains originating from national Belgian collections ('Control strain Belgium' from 2008) were used as internal reference.

Secondary attack rate study

For the 29 affected households with confirmed cases, we identified 175 household contacts, of whom 15 developed shigellosis. A secondary attack rate of 8.5% (95% CI: 4.3–12.7) was calculated. Information on hand washing, the number of toilets in the home and the use of disposable towels was only provided by four of the 25 interviewed households. These questions were excluded in the analysis. The calculated crude and adjusted RRs for the other risk factors are shown in the Table. In the uni- and multivariate analysis, having more than three children in the family, having children younger than 12 years who assisted their parents washing siblings and helping them go to the toilet, and having children younger than five years, were significantly associated with a higher risk of secondary transmission. Having more than three children in the household was associated with the highest risk, with an adjusted RR of 9.17 (95% CI: 1.21–69.13). Hospitalisation and treatment with antibiotics of the household index cases were not significantly associated with a lower risk of secondary infection, with a respectively adjusted RR of 0.88 (95% CI: 0.61-3.1) and an adjusted RR of 1.8 (95% CI: 0.80-4.34).

Control measures

To prevent further spread of the disease, parents of the affected families were advised of the importance of hand washing with running water and liquid soap after using the toilet or washing the children and also on the importance using disposable towels and cleaning the toilets with chlorine. The need to decontaminate toys was highlighted. In June 2008 educational presentations for parents, caregivers and teachers were organised. Information was also published in the local media. Physicians were informed via articles in the local medical infectious disease journal. Schools were informed on the hygiene of hand washing facilities. We insisted on excluding symptomatic children for a minimum of 48 hours after clinical recovery from day care centres, preschool and school attendance [9].

Discussion

We identified a cluster of 42 cases of shigellosis in the Orthodox Jewish community of Antwerp with 32 isolates laboratory-confirmed as *S. sonnei* with the same genetic profile. Temporal and spatial clustering in one area of town affecting one specific community supported the hypothesis of a single ongoing outbreak, maintained through person-to-person transmission. Statutory laboratory-based surveillance of shigellosis failed to identify concurrent cases outside this community. Two additional *S. sonnei* cases notified in the study period in the province of Antwerp in people who were not Jewish were most probably not linked to the outbreak. The disease started during a stay in Egypt and they were classified as travel-associated cases.

The index case was most probably infected by their father, who had suffered from gastrointestinal problems during a stay in Tel-Aviv, Israel until two days before symptom onset in the index case but did not seek medical care. No exceptional family gatherings could be identified except for synagogue attendance. The father also reported having been in contact with relatives coming from London.

To investigate a possible link between the outbreak in Antwerp and an ongoing outbreak in Israel [10], the circulating strains in both outbreaks were compared. Such a link was supported by the microbiological analysis in which the main strain circulating in Israel at the time and the outbreak strain in Antwerp were indistinguishable. The father of the index case also reported having been in contact with relatives coming from

Table

Risk factors of illness among household contacts of an index case with shigellosis, Jewish community Antwerp, 17 April–31 August 2008 (n=42)

	Univariate analysis		Multivariate analysis	
Exposure	Crude relative risk	95% confidence interval	Adjusted relative risk	95% confidence interval
>3 children in household	8.47	1.14-62.98	9.17	1.21-69.13
Children with nappies	2.41	0.90-6.48	1.59	0.84-3.01
Children <5 years in household	6.0	1.39-25.80	5.24	1.17-23.62
Children <12 years assisting parents washing siblings	6.54	2.59-16.51	5.45	2.44-12.17
Index case in household hospitalised	1.02	0.38-2.75	0.88	0.61-3.10
Index case in household treated with antibiotics	1.42	0.50-3.99	1.87	0.80-4.34

London. Addiman et al. reported on an outbreak of shigellosis in London starting a month before the onset of our outbreak in Antwerp in 2008 [11]. A strain from the outbreak of London 2008 could not be obtained for comparison.

Outbreaks of shigellosis with *S. sonnei* and recurrent increases in the number of cases in Orthodox Jewish populations have already been notified in 2008 and before in different countries. Calderon-Margalit et al. showed that between 1998 and 2006, outbreaks of shigellosis followed a biennial pattern in Israel with annual rates that ranged from 18 to 353 cases per 100,000 population [12]. Also in 2009 outbreaks of *S. sonnei* in Israel were still continuing [10]. Close contacts, day care attendance and having many young children in the families were considered risk factors. The characteristics of the outbreak in Antwerp are comparable with prolonged outbreaks of *S. sonnei* reported by Sobel et al. in North America in traditionally observant Jewish communities between 1994 and 1996 [13] , with outbreaks reported by Garret et al. in New York in 2005 [14] and with the outbreak in London in 2008 [11].

The secondary attack rate of 8.5% found in this study is comparable to those noticed in other studies [2,15]. In larger studies, secondary attack rate differed according to age and to the species of bacterium [2]. Due to the limited number of cases in our study, age-specific attack rates could not be calculated. Dupont et al. showed that for one to four year-olds, the secondary attack rate can reach 40% [2]. The combination of high communicability due to the low infective dose, crowding, and frequent contacts are known explanations for the high secondary attack rate for shigellosis [2,14,15]. In our study we analysed specific risk factors which might explain the noted secondary attack rate. Having more than three children in the household and having children younger than five years of age was significantly associated with the occurrence of secondary cases, which is consistent with data from other authors [14]. Contrary to what we expected, having children with nappies in the household was not a significant independent risk factor in our study. This could be due to good hygienic habits of the adults when providing care for their babies. That the index case of the family was hospitalised was hypothesised to be a protective factor, but the adjusted RR was 0.88 (95% CI: 0.61–3.10). The low number of cases and the different intervals between onset of the disease and moment of hospitalisation of the cases might have interfered with the association. Being treated with antibiotics was not significantly associated with a lower risk of secondary transmission either. Different delays in the start of therapy, the broad spectrum of used antibiotics and the small number of cases might explain the calculated RR of 1.8 (95% CI: 0.80–4.34).

However, it was remarkable that children younger than 12 years, helping their parents take care of babies, was associated with a higher risk of secondary cases (adjusted RR: 5.45; 95% CI: 2.44–12.17). In families with a high number of children, older children were asked to help. There is a risk that these young children are less sensitive or less knowledgeable than their parents on the risk and the practice of hand hygiene.

Visiting friends and relatives in areas with higher risk of shigellosis might be the seeding event leading to shigellosis outbreaks in especially susceptible communities. This is the case for Jewish communities in Antwerp that are more susceptible due to the high number of children in the families, the many social contacts, living in a relatively small community, and the frequent contact with relatives who live in areas of higher endemic prevalence of shigellosis, like certain neighbourhoods in London or Israel [11,12].

The high hospital admission rate in our study (32 of 42 cases) suggests that we have probably detected only the most severe cases, whereas milder cases could also have been expected. Presenting bloody or mucopurulent diarrhoea was noted in 18 of the cases. This is unusual compared to the expected picture of a *S. sonnei* infection which is normally associated with a milder disease [2,3,5]. We have therefore reason to consider under-diagnosis and under-reporting in this outbreak, which is also mentioned in similar outbreaks of shigellosis elsewhere [2].

Several limitations of the study especially for the secondary attack rate study should be noted. Firstly, the number of cases was limited. This raises concerns about the interpretation of the calculated relative risks. Secondly, we assumed that secondary cases acquired their infection at home. Alternatively they might have been infected during pre-school and school attendance or by visiting friends and relatives. Thirdly, personal questions like ‘did you wash your hands after toilet use?’ and ‘how many toilets do you use at home’ were often not answered, most probably due to their sensitive and private nature [16]. It is likely that not all possible risk factors could be explored in the study of this outbreak.

Early notification of shigellosis enabled prompt reaction, and implementation of the advice most probably put a stop to further propagation. We presume that especially the intensive hand washing campaign in families and schools, the educational presentations and specific information to physicians contributed to stopping the outbreak.

PFGE in studies of clusters has been shown to be a highly effective method of characterising *S. sonnei* and an important tool for outbreak investigations [17]. Provided that the same protocol is used, it allows comparison of strains detected in different outbreak. The genetic relatedness of the strains in this study provides strong evidence that this cluster was a single outbreak and associated with recurrent endemic shigellosis in Israel [10,12].

In conclusion, this is the first well-documented outbreak of *S. sonnei* in the Orthodox Jewish community of Antwerp and Belgium for which a direct link to an ongoing outbreak of endemic shigellosis in Israel could be identified. The combination of case finding, source tracing, and comparing different strains with PFGE was essential for confirming the hypothesis of import of an outbreak strain from Israel into the local community, and implementation of hand washing was important to stop the propagation of the epidemic.

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National outbreak of *Salmonella* Enteritidis phage type 14b in England, September to December 2009: case–control study

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We conducted an unmatched retrospective case–control study to investigate an upsurge of non-travel-related sporadic cases of infection with *Salmonella enterica* subsp. *enterica* serotype Enteritidis phage type 14b with antimicrobial resistance to nalidixic acid and partial resistance to ciprofloxacin (*S.* Enteritidis PT 14b $NxCp_L$) that was reported in England from 1 September to 31 December 2009. We analysed data from 63 cases and 108 controls to determine whether cases had the same sources of infection as those found through investigation of 16 concurrent local food-borne outbreaks in England and Wales. Multivariable logistic regression analysis adjusting for age and sex identified food consumption at restaurants serving Chinese or Thai cuisine (odds ratio (OR): 4.4; 95% CI: 1.3–14.8; p=0.02), egg consumed away from home (OR: 5.1; 95% CI: 1.3–21.2; p=0.02) and eating vegetarian foods away from home (OR: 14.6; 95% CI: 2.1–99; p=0.006) as significant risk factors for infection with *S.* Enteritidis PT 14b $NxCp_L$. These findings concurred with those from the investigation of the16 outbreaks, which identified the same *Salmonella* strain in eggs from a specified source outside the United Kingdom. The findings led to a prohibition of imports from this source, in order to control the outbreak.

Introduction

Infection with *Salmonella enterica* subsp. *enterica* serotype Enteritidis (*S.* Enteritidis) remains an important public health problem in Europe and other parts of the world [1-4]. Outbreaks caused by *Salmonella* infection have been associated with a variety of foods; however, outbreaks caused by *Salmonella* Enteritidis infection are closely associated with eggs and egg products [2,5,6]. In September 2009, the Department of Gastrointestinal, Emerging and Zoonotic Infections at the Health Protection Agency (HPA) reported a marked upsurge in the number of non-travel-related human cases of infection with *S.* Enteritidis phage type (PT) 14b with resistance to nalidixic acid and partial resistance to ciprofloxacin (*S.* Enteritidis PT 14b $NxCp_L$). Infectious diseases resulting from food poisoning are statutorily notifiable in England and Wales: cases are notified by registered medical practitioners and diagnostic laboratories to the HPA. In total, 572 cases of *S.* Enteritidis PT 14b $NxCp_L$ infection were reported between January and December 2009, compared with 141 in 2008. Between 1 September and 31 December 2009, there were 489 cases.

There were 14 recognised, discrete local outbreaks of *S.* Enteritidis PT 14b $NxCp_L$ infection in England and two in Wales between August and December 2009 (HPA unpublished data). All but one of these outbreaks were linked to food-service premises; the remaining outbreak was linked to a residential care home for the elderly. The total number of reported cases associated with these outbreaks was 152: six were hospitalised and two deaths were reported.

Preliminary investigations of these 16 outbreaks suggested putative links to infected eggs, with evidence of cross-contamination of *S.* Enteritidis PT 14b $NxCp_L$ to other foods, particularly ready-to-eat vegetarian foods. The outbreak strain was isolated from samples of eggs, egg mayonnaise, egg-fried rice, pooled liquid egg mix and work surfaces in the food-service premises investigated as part of the outbreak investigations. Eggs collected from these premises (five restaurants serving Chinese or Thai cuisine and two cafes) in seven of the outbreaks were from the same production establishment in Spain, as indicated by the stamp on the egg shells. We therefore conducted a case–control analysis to determine whether the likely source of infection in the apparently sporadic cases was the same as that for cases in the outbreaks.

Before the upsurge in *S.* Enteritidis PT 14b $NxCp_L$ infections in September 2009, there had been other sustained increases in the incidence of *S.* Enteritidis non-PT4 infections in England and Wales between 2000 and 2004 [7,8]. Epidemiological and microbiological investigations and a case–control study of primary sporadic indigenous cases found that consumption of eggs from food prepared outside the home was associated with being a case. The investigations identified

eggs sourced from Spain used in the food-service sector as the main cause of the increase [1,2,7,8]. In the United Kingdom (UK), the predominant PT responsible for egg-borne *S.* Enteritidis infection had been PT 4 between 1992 and 2002 [5]. Following large epidemics of *S.* Enteritidis infection in the UK in the late 1980s, mainly due to PT 4, a decline in human S .Enteritidis PT 4 infection in England and Wales occurred from 1997, largely because of industry control programmes in the poultry sector, including vaccination of layer flocks [9]. Since 2000, egg-associated *S.* Enteritidis PTs other than PT 4 causing human infection have emerged, with the greatest increases occurring in *S.* Enteritidis PT 1- and PT 14b-related infections [7].

Surveillance of salmonellosis from 1998 to 2003 also showed upsurges in *S.* Enteritidis non-PT4 infections in other European countries [1]. Between 1998 and 2003, the proportion of PT4 infections fell from 61.8% in 1998 to 32.1% in 2003, with a concomitant increase in *S.* Enteritidis non-PT4 infections (including PT1, 8, 14b and 21) in Austria, Germany, Spain, Denmark, Finland, England, Wales and Northern Ireland, Scotland, the Netherlands and Sweden [1]. Major upsurges are thought to be associated with substantive changes in market supply: during this time, eggs were imported from producers in EU Member States where there was a lack of vaccination of layer flocks against *Salmonella* or controlled food industry assurance schemes were not in place [1,10,11]. From 2000 to 2008, the mean incidence rate for *S.* Enteritidis PT 14b $NxCp_L$ gradually increased from 0.01 per 100,000 population in England to 0.4 per 100,000 population, respectively. In 2009, this rate more than doubled, to 1.1 per 100,000 population (HPA unpublished data).

This evidence, along with the findings of the 16 food-borne outbreaks, was used to formulate a hypothesis that *S.* Enteritidis PT 14b $NxCp_L$ infection of cases who were not part of the outbreaks was associated with consumption of eggs outside the home, within five days before symptom onset, particularly at restaurants serving Chinese or Thai cuisine.

Table 1

Demographic characteristics of sporadic cases of *Salmonella* Enteritidis PT 14b $NxCp_L$ infection (n=63) and controls (n=108), England, October–December 2009

Characteristic	Number of cases	Number of controls
Sex		
Female	28	72
Male	35	36
Age group (years)		
<10	9	2
10–29	19	8
30–49	14	38
50–69	11	38
≥70	10	22
Total	**63**	**108**

$NxCp_L$: resistance to nalidixic acid and concomitant reduced susceptibility to ciprofloxacin; PT: phage type.

Methods

A unmatched case–control study was carried out to analyse the apparently sporadic cases, recruiting two controls per case, to determine associations between potential risk exposures and symptomatic infection with *S.* Enteritidis PT 14b $NxCp_L$. Cases from the 16 food-borne outbreaks were excluded from our study.

Sample size calculations indicated that having data for 60 cases and 120 controls would enable us to detect an odds ratio of 3 (for 50% of the controls exposed) to 4 (for 10% of the controls exposed) as being significant at the 5% level with around 90% power.

Case definition

A case of *S.* Enteritidis PT 14b $NxCp_L$ infection was defined as a person in England with abdominal symptoms (diarrhoea and/or vomiting), with an isolate from their stool sample positive for *S.* Enteritidis PT 14b with resistance to nalidixic acid and concomitant reduced susceptibility to ciprofloxacin, and the isolate received by the HPA Laboratory of Gastrointestinal Pathogens between 1 September and 31 December 2009.

Recruitment and investigation of cases

Recruitment of cases for the study took place between 1 October and 31 December 2009. Before the data collection period, 12 cases reported in September 2009 were reviewed using local authority food-poisoning questionnaires ('trawling' questionnaires) to assist in generating hypotheses for the possible source of infection. All cases interviewed with this questionnaire were excluded from the study. Cases associated with the 16 discrete food-borne outbreaks were also excluded from this study, as were cases who had travelled outside the United Kingdom within five days of symptom onset and cases who were contacts of other reported cases.

Standardised data were collected on all patients infected with *Salmonella* (i.e. before the serotype/subtype was known), so that cases and outbreaks could be identified and investigated rapidly. This involved the completion of a standardised questionnaire for each person with presumptive *S.* Enteritidis or laboratory-confirmed *Salmonella* infection (all serotypes) by the Health Protection Unit or local authority. The extensive questionnaire included captured data on basic demographics, occupation, details of gastrointestinal illness and any other symptoms, history of travel, and details of food consumption and contact with animals within the five days before symptom onset. Questions on food consumption gathered details of the type and brand of each food consumed, place of purchase, whether the food was consumed in or away from the home, and type of food-service premises visited. The completed questionnaires were sent to the HPA Department of Gastrointestinal, Emerging and Zoonotic Infections for data entry, validation and analysis. Isolates were sent

to the *Salmonella* Reference Unit at the HPA Centre for Infections for further characterisation and antimicrobial susceptibility testing [12,13].

Recruitment and investigation of controls

We used cases' landline telephone numbers, which reflect the location of their domicile, as the basis of the selection of controls (cases who had been contacted by mobile telephone were asked for a landline number). For each case, two controls were recruited using random digit dialling [14]. Controls were therefore chosen from the same telephone exchange area and therefore lived in the same geographical area as the cases. Between 2 October and 2 December 2009, controls were recruited by telephone over five weekday evenings. The individual who picked up the telephone and who agreed to be interviewed was considered to be a control provided they were over the age of 18 years and they provided informed consent on the telephone before the interview.

All interviews were carried out using a standardised questionnaire for controls. This was similar to that used for cases, except that questions on contact with animals, travel history, food consumption and grocery-shopping habits related to the five days before the interview (rather than before symptom onset). Controls who had experienced any gastrointestinal symptoms in the two weeks before the interview were excluded from the study.

Data analysis

The data were analysed using STATA 11. For all exposures, estimated odds ratios and 95% confidence

Table 2

Single variable analysis of exposure variables for cases of *Salmonella* Enteritidis PT 14b $NxCp_L$ infection (n=63) and controls (n=108), adjusted for age and sex, England, October–December 2009

Exposure[a]	Odds ratio (95% CI)	P value
Eaten away from home		
Eaten away from home at any type of establishment	2.6 (1.1–5.9)	0.02
Eaten out at parties	1.5 (0.6–3.8)	0.4
Eaten foods from food-service premises		
Restaurants serving Chinese or Thai cuisine	4.1 (1.6–10.4)	0.002
Kebab houses	17.1 (1.7–172)	0.02
Restaurants serving Indian cuisine	2.7 (0.7–9.5)	0.1
Burger bars	0.5 (0.2–1.7)	0.3
Fried chicken bars	2.2 (0.3–17.4)	0.4
Public houses	0.6 (0.2–2.2)	0.5
Restaurants serving Italian cuisine	1.5 (0.4–5.0)	0.5
Food exposure		
Barbecued food	13.6 (1.4–129)	0.02
Eaten barbecued food at home	9.2 (0.9–93)	0.06
Eaten barbecued food away from home	ND	0.07
Pre-prepared sandwiches	2.5 (1.2–5.4)	0.02
Eaten pre-prepared sandwiches at home	1.5 (0.4–4.9)	0.5
Eaten pre-prepared sandwiches away from home	3.0 (1.3–7.2)	0.01
Vegetarian food	3.4 (1.3–9.2)	0.01
Eaten vegetarian food at home	1.7 (0.6–4.7)	0.3
Eaten vegetarian food away from home	13.6 (2.3–81)	0.004
Cold meats	1.9 (0.9–4.0)	0.08
Eaten cold meats at home	1.3 (0.6–2.5)	0.5
Eaten cold meats away from the home	8.0 (1.7–37)	0.008
Eggs	1.6 (0.7–3.6)	0.3
Eaten eggs eaten at home	1.0 0.5–2.1)	0.97
Eaten eggs eaten away from the home	7.0 2.0–24.8)	0.003
Environmental exposure		
Had contact with animals[b]	1.0 (0.5–2.0)	0.98
Lived on a farm or smallholding	4.4 (0.2–84)	0.3
Visited a farm	2.4 (0.5–11.5)	0.3

ND: not determined; $NxCp_L$: resistance to nalidixic acid and concomitant reduced susceptibility to ciprofloxacin; PT: phage type.

[a] The reference category for each exposure is having not eaten at the specified establishment or having not eaten the specified food, or having had the relevant environmental exposure.

[b] Occupational contact or contact with pets.

intervals were used as measures of association. In addition, all exposures were tested, singly, for association with the outcome variable (illness) using chi-square test or Fisher's exact test. Exposures exhibiting some evidence of an association ($p<0.2$) were deemed eligible for inclusion in the multivariable analysis. The $p<0.2$ cut-off was chosen so that important exposures would not be missed due to confounding effects. A logistic regression model was constructed using a forward selection procedure including the most significant exposure at each step (likelihood ratio test $p\leq0.05$). Potential confounding variables – age and sex – were included in the multivariable analysis regardless of statistical significance.

Results

A total of 489 *S.* Enteritidis PT 14b $NxCp_L$ cases distributed across all regions of England were identified by the HPA Laboratory of Gastrointestinal Pathogens during the study period. Of these, 101 were associated with the discrete food-borne outbreaks and were therefore excluded. Some cases not associated with these discrete outbreaks were also not included because they were interviewed with the initial trawling questionnaire in September, before the investigation, and others were excluded because they were identified after our investigation had closed. In total, 81 sporadic cases of *S.* Enteritidis PT 14b $NxCp_L$ infection completed the questionnaire. Of these 81 cases, 63 were included in the analysis: four were excluded due to recent travel history and 14 were excluded because they were contacts of other cases (although the index cases were included). There were reports of people with *S.* Enteritidis PT 14b $NxCp_L$ infection after December 2009, but the number reported had fallen to background levels.

A total of 108 controls were recruited: a mean of 3.6 calls (range: 1–32 calls) was needed to successfully recruit a control. Table 1 compares the basic demographic characteristics of cases and controls. Controls were more likely to be female ($p=0.004$) and older (mean age: 52.5 versus 36.8 years, respectively, compared with cases, $p<0.0001$). Due to these differences between cases and controls, single variable analysis was performed using logistic regression analysis adjusting for potential confounding by age and sex.

The cases had dates of symptom onset between 26 August and 16 November 2009, and the mean duration of illness in those who had recovered was 7 days (median: 7 days; lower and upper quartiles: 3 and 10 days, respectively). The predominant symptoms were diarrhoea (in 59 of 60 cases), abdominal pain (49 of 56), fever, defined as body temperature of at least 38 °C (32 of 55), nausea (29 of 55), headaches (26 of 55) and vomiting (20 of 59). Of the 63 cases, 15 reported having blood in their stool. A total of 50 visited their general practitioner, while 13 attended hospital accident and emergency departments and 12 were admitted to hospital. No deaths were reported among the study cases.

As there could be a delay in reporting (i.e. date of symptom onset was not necessarily the date the cases were reported) and to allow time for isolates to be sent for typing, the cut-off date for receipt of isolates at the HPA Laboratory of Gastrointestinal Pathogens was 31 December 2009.

In single variable analysis there was an association between having eaten away from home and symptomatic infection with *S.* Enteritidis PT 14b $NxCp_L$, particularly in restaurants serving Chinese or Thai cuisine and kebab houses (Table 2). Having eaten barbecued foods either at home or away from home, and pre-prepared sandwiches obtained away from home, was also associated with a higher risk of becoming a case. There was a very strong association between having eaten eggs away from home and becoming a case (Table 2).

As both eating away from home at any type of establishment and eating foods from restaurants serving Chinese or Thai cuisine were found to be significantly associated with being a case, a three-level factor was generated to determine any association between being a case and (1) not eating out, (2) eating out at restaurants serving Chinese or Thai cuisine, and (3) eating out at other restaurants. The final multivariable logistic regression model including the implicated exposure variables (Table 3) demonstrated no significant association between having eaten away from home but not at restaurants serving Chinese or Thai cuisine and becoming a case. However, having eaten foods from restaurants serving Chinese or Thai cuisine (including takeaways) was significantly associated with becoming a case. Among food exposures, eggs eaten away

Table 3

Multivariable logistic regression model of implicated food exposures, adjusted for age and sex, England, October–December 2009 (n=63)

Food exposure	Odds ratio (95% CI)	P value	Number of cases exposed
Had not eaten away from home	Reference	–	15
Eaten away from home but not at a restaurant serving Chinese or Thai cuisine	1.5 (0.5–4.1)	0.5	24
Eaten foods from restaurants serving Chinese or Thai cuisine	4.4 (1.3–14.8)	0.02	25
Eaten eggs away from home	5.1 (1.2–21.2)	0.02	12
Eaten vegetarian food away from home	14.6 (2.1–99)	0.006	6

from home and vegetarian foods eaten away from home were also identified as significant risk factors for becoming a case.

Discussion and conclusion

The case–control study presented here provides evidence of significant associations between eating in restaurants serving Chinese or Thai cuisine and eating eggs and vegetarian food away from home with becoming a case of *S.* Enteritidis PT14b $NxCp_L$ infection in a large national outbreak in England in 2009. The association between eating vegetarian foods and becoming a case may be related to the fact that vegetarian foods may contain eggs (which could be infected). These findings corroborated evidence obtained from concurrent investigations of 16 local discrete food-borne outbreaks of *S.* Enteritidis PT14b $NxCp_L$ infection. Our results indicated that the source of infection for the sporadic cases was likely to be the same as that for cases associated with the outbreaks. Information on eggs collected from food-service premises in seven of the 16 outbreaks indicated a common origin (a single production establishment in Spain). *S.* Enteritidis PT14 $NxCp_L$ obtained from eggs from this establishment, and also from environmental and food samples from the food-service premises were indistinguishable by molecular diagnostic testing from isolates obtained from human cases of *S.* Enteritidis PT14 $NxCp_L$ infection (cases associated with the outbreaks and the sporadic cases). *S.* Enteritidis PT1 $NxCp_L$ was additionally detected in eggs produced by this establishment in Spain as part of the outbreak investigations [15] providing further evidence of *S.* Enteritidis contamination within the laying flock.

Control measures

The United Kingdom Food Standards Agency was informed of the findings both from the case–control study and the 16 outbreak investigations and notified the European Commission and other EU Member States in October 2009 through the Rapid Alert System for Food and Feed (RASFF) of the eggs contaminated with *S.* Enteritidis PT 14b $NxCp_L$ and also PT 1 $NxCp_L$ sourced from an approved establishment in Spain (one of the conditions of approval is compliance with all the relevant legislation set out by the relevant EU Member State competent authority). This led to Spanish authorities investigating and identifying the affected flock. Eggs from this flock were prohibited from entering the fresh table egg market and were sent for heat treatment (as required by EU regulations [15,16], which state that eggs from flocks testing positive for *S.* Enteritidis or *S.* Typhimurium need to be treated in a manner that guarantees the elimination of *Salmonella*). After this control measure was introduced in early December 2009, the number of cases in England and Wales fell from a mean of 20 confirmed cases per week in November to nine and three per week in December and January 2010, respectively.

A decreasing trend in the notification rate of salmonellosis cases in the EU, particularly those caused by *S.* Enteritidis, has been seen over recent years. This has largely been attributed to the implementation of *Salmonella* national control programmes in the laying flocks [17]. Nevertheless, most of the reported food-borne outbreaks reported in the EU are still caused by *Salmonella*, with the most important food source being eggs and egg products [17]. Eggs have continued to be implicated as a source of or vehicle for cross-contamination in outbreaks of salmonellosis chiefly associated with the food-service industry in the UK [5-8]. Food-poisoning risks associated with eggs and egg dishes in the food-service industry, especially those serving Chinese cuisine, have included high-risk practices such as breaking, pooling and mixing shelled eggs [18,19]. One *Salmonella*-contaminated egg is capable of contaminating the whole batch of raw shell egg mix, and large numbers of consumers may be exposed to this contaminated raw material. The risk is increased if the egg mix is stored in a warm kitchen for later use during the day, as this would allow growth of the pathogen. Cross-contamination through egg mix aerosolisation during whisking and transfer to utensils and food preparation areas is also of concern [19]. The rates of *Salmonella* contamination have been linked to the origin of the eggs [20]. The food-service sector and consumers still need to be aware of this continuing hazard and adopt appropriate control measures and follow advice provided by national food safety agencies, in order to reduce the risk of infection.

Study limitations

Our case–control study had a number of limitations. Firstly, because of the limited time and resources available to recruit the controls, the final number of controls was slightly below the required number, based on our sample-size calculation (108 recruited as opposed to 120). In some of our analyses, small numbers led to large confidence intervals

Secondly, for the recruitment of controls we interviewed the person who answered the telephone (provided they were aged over 18 years), which may have introduced further bias, as we found that those who were most likely to answer were more likely to be older and also female. We did not use a method such as the 'last birthday' method (in which the adult in the household with the most recent birthday is requested for interview during the telephone call) – such an approach might help to increase variation in the demographics of the controls. However, we took measures to try to minimise response bias by varying the days of the week and the times that controls were telephoned. To minimise any potential confounding by age and sex, these were adjusted for in the regression analysis.

Thirdly, recall bias was a potential problem, particularly for controls. When cases were interviewed, they were asked about their food consumption in the five days before becoming ill whereas controls were asked

about their food consumption in the five days before the telephone interview.

Fourthly, the time period for recruitment of cases did not exactly mirror that for the recruitment of controls, as we recruited controls over five weekday evenings in October and December 2009, whereas cases were recruited over a continuous period throughout October and December 2009.

Finally, we recognise that there may have been further confounders relating to differences in occupation, socio-economic status and eating behaviours between cases and controls. We attempted to minimise these potential confounders by interviewing controls who were living in the same telephone exchange area as cases. We also note that cases were not over-representative of Chinese or Thai ethnic groups (data not shown), so this form of confounding is not relevant to our investigation.

Despite the limitations – most of which are common to case-control studies of outbreak investigations of gastrointestinal infection – the results of the study support our hypothesis.

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The proof of the pudding is in the eating: an outbreak of emetic syndrome after a kindergarten excursion, Berlin, Germany, December 2007

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An outbreak of food poisoning (emetic syndrome) occurred in three kindergartens (A, B and C) in Berlin, Germany, on 3 December 2007 after an excursion during which food was served. We conducted a retrospective cohort study among the kindergarten children and personnel who participated in the trip. The overall attack rate among the 155 participants was 30%. It was 31% among the 137 children (aged two to six years) and 17% among adults (n=18). The consumption of rice pudding was significantly associated with disease. Among those who ate rice pudding, the attack rate was 36%, compared with 0% for non-eaters (relative risk: infinite, p<0.001, aetiological fraction: 100%), but differed significantly between kindergartens A (43%), B (61%) and C (3%), probably because groups were served from different pots. *Bacillus cereus* sensu stricto was identified from one vomit sample. The clinical and epidemiological characteristics suggest that *B. cereus* emetic toxin (cereulide) was the causative agent, although it could not be proven in the single vomit isolate. Inadequate food handling most probably led to the outbreak. Single-portion ready-to-eat rice pudding was recommended for subsequent excursions and no further cases of food poisoning occurred.

Introduction

In some outbreaks of infectious gastroenteritis, emesis predominates. The emetic syndrome is characterised by acute-onset nausea and vomiting. The most common pathogens associated with emetic syndrome are enterotoxin-producing *Staphylococcus aureus* and emetic-toxin-producing *Bacillus cereus* [1-5]. Staphylococcal food poisoning results from the ingestion of enterotoxins preformed in food by enterotoxigenic strains of coagulase-positive staphylococci, mainly *S. aureus*. Several staphylococcal enterotoxins are heat-stable. The range of the incubation period is 0.5 to 8 hours. *B. cereus* is a spore-forming microorganism, which can cause both emetic and diarrhoeal types of disease. It occurs ubiquitously in the environment (e.g. in soil) and may also be found in various foodstuffs. The emetic type of disease is caused by a heat-stable peptide toxin (cereulide): the incubation period ranges from 0.5 to 6 hours. The illness usually does not persist longer than 24 hours but severe and fatal outcomes have been reported [6,7]. The toxin is produced in food when the organism multiplies at ambient temperature for several hours (e.g. if the food is inadequately stored after cooking) [5]. Emetic outbreaks due to *B. cereus* have mainly been linked to starchy foods such as rice, pasta and pastry [2].

Norovirus is also a common cause of outbreaks of acute gastroenteritis, with emesis as a prominent symptom. Infection can arise from contact with or airborne transmission from fomites, as well as faecal–oral and foodborne transmission.

Although outbreaks of acute gastroenteritis are notifiable in most countries, the number of toxin-related food poisoning outbreaks is largely underestimated because the disease is often mild and self-limiting, and laboratory detection (toxin testing) is not routinely performed.

On 3 December 2007, a kindergarten (A) reported cases of emesis among children and its personnel to the local health authority. In the morning of the same day they had been on an excursion on a local tram that included catering on the platform at the tram's final destination. Preliminary investigations by the local health authority confirmed the outbreak in this and

two other participating kindergartens (B and C) from another Berlin district.

We conducted an investigation immediately after the outbreak had come to our attention, to assess its scope, to identify the causative agent, and to determine the risk factors and the vehicle of infection in order to prevent further outbreaks.

Methods

Case finding

The tram excursion took place on the morning of 3 December 2007 between 09:00 and 10:00. On the following day, cases among the kindergarten groups were identified by the local health authorities. On 6 December, we obtained the addresses and telephone numbers of the kindergartens from the local health authorities. Food safety authorities provided the address of the caterer and the list of food items served during the excursion.

Exploratory interviews at the kindergartens were conducted on 7 December and showed that the staff who had accompanied the excursion clearly remembered the relevant epidemiological details (e.g., disease status and food consumption) of the children. Therefore we interviewed the kindergarten personnel using a standardised questionnaire on the children's and their own clinical symptoms, time of disease onset, type and duration of symptoms, secondary spread among family members, food consumption and demographic data.

Case definition

We defined a case as a person who attended the excursion on 3 December 2007 between 09:00 and 10:00 and presented with vomiting, abdominal pain or diarrhoea within 24 hours after the excursion.

Cohort study

We conducted a retrospective cohort study among children and personnel of the three affected kindergartens. We described cases by date and time of disease onset. Age group-specific and kindergarten-specific attack rates were calculated. We also calculated food-specific attack rates, aetiological fractions, relative risks and 95% confidence intervals. Data were also stratified by kindergarten to compare the results between the kindergartens. We used EpiData for data entry and SPSS software, version 15.0, for statistical analysis.

Laboratory methods

Human samples

Stool samples (n=10) and one available vomit sample were tested (at the Institute for Food Safety, Drugs and Animal Health) for various enteric pathogens (*Salmonella, Campylobacter, Escherichia coli* and other enterobacteria, *Yersinia enterocolitica, S. aureus, B. cereus* and viruses such as norovirus, adenovirus, rotavirus and astrovirus). For detection of bacteria, routine culture methods were used, and for viruses, PCR and antigen tests were carried out. In the routine laboratory investigations of the stool and vomit samples, no tests for staphylococcal enterotoxins or *B. cereus* emetic toxin were performed.

An isolate of presumptive *B. cereus* from the vomit sample was tested for *B. cereus* cereulide production using liquid chromatography-tandem mass spectrometry (LC-MS/MS), and for the presence of the cereulide synthetase (*ces*) gene using PCR. For species differentiation, we used Fourier transform infrared spectroscopy [8].

For LC-MS/MS analysis of cereulide, bacteria were directly extracted with methanol during ultrasonification [8]. Chromatographic separation took place on a C8 column with a buffer/methanol gradient, a triple quadrupole mass spectrometer with positive electrospray ionisation run in multiple reaction monitoring mode (mass-to-charge ratio 1,170.7 $[M+NH_4]+ \dashrightarrow$ 940.2; 1,170.7 $\dashrightarrow$ 741.4; 1,170.7 $\dashrightarrow$ 499.2; 1,170.7 $\dashrightarrow$ 357.2) was used for detection.

PCR to detect the *B. cereus ces* gene was performed using primers ces_TaqM_for and ces_TaqM_rev with probe ces_TaqM_probe (TIB-MolBiol, Berlin, Germany),

Table 1

Cohort characteristics with attack rates, outbreak of emetic syndrome following kindergarten excursion, Berlin, Germany, December 2007

Cohort characteristics	Number of participants (%)	Number of cases	Attack rate (%)
Sex			
Female	84 (54)	19	23
Male	71 (46)	27	38
Age group (years)			
2–6	137 (88)	43	31
≥18	18 (12)	3	17
Kindergarten			
A	96 (62)	34	35
B	23 (15)	11	48
C	36 (23)	1	3
Total	**155 (100)**	**46**	**30**

as described by Fricker et al. [9]. For more details, see Rau et al. [8].

Food leftovers

Two unopened tetrapaks of the rice pudding that had been used, and retain samples (obtained from the caterer) of spray cream, cinnamon–sugar mix, gingerbread and two opened bags of cocoa powder were tested for *Salmonella*, staphylococci, *B. cereus, Campylobacter, E. coli, Listeria monocytogenes, Clostridium perfringens*, Enterobacteriaceae, *Pseudomonas* and norovirus. Leftovers of heated rice pudding eaten on the tram platform were not available for testing.

Environmental investigation

Local health and food safety authorities inspected the caterer's facilities used on the tram platform and the cleaning facilities in the caterer's office. The caterer was interviewed regarding food purchase, transport and storage, the facilities on the tram platform during the excursion (stand, water and electricity supply), the preparation process of food items and drinks served during the excursions, and on the cleaning procedures of the cookware.

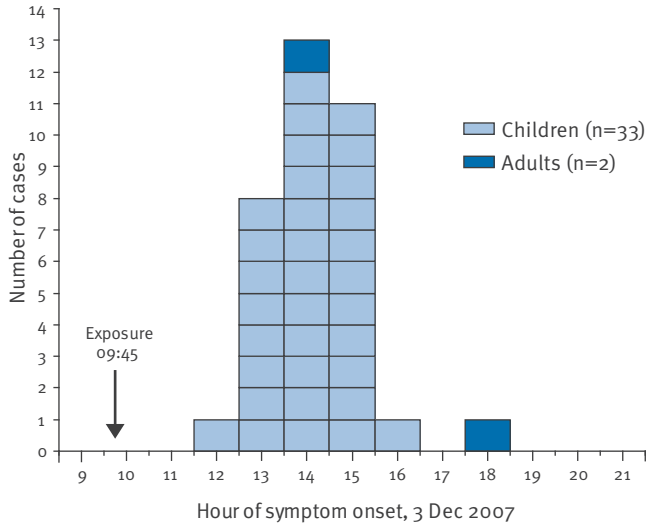
Results

Descriptive epidemiology

Overall, 155 persons (137 children, 17 kindergarten staff and one of the children's mother) from the three kindergartens participated in the excursion. The cohort characteristics are shown in Table 1. In total, 46 participants (43 children aged two to six years, and three adults) met the case definition (attack rate: 30%). The attack rate was higher among the children than among the adults, and differed significantly by kindergarten ($p<0.001$).

Figure

Cases by hour of symptom onset, outbreak of emetic syndrome following kindergarten excursion, Berlin, Germany, 3 December 2007 (n=35)



The predominant symptoms were vomiting (n=39), and abdominal pain (n=29). Diarrhoea was reported only in one person. Nobody was hospitalised and all cases recovered within one day. There were no secondary cases among household members.

The food items served during the excursion (at 09:45) were ready-to-eat rice pudding (from one-litre tetrapacks) that was heated before serving (served with cinnamon–sugar mix), cocoa drinks with and without whipped cream, and gingerbread. According to the personnel in Kindergarten C, the only person who became ill in this kindergarten recalled having been served from a different pot than that used for the other participants from this kindergarten. The participants had no other common meals before or after the excursion.

In all cases, symptoms started within a few hours after the end of the excursion. Detailed information about the time (hour) of symptom onset was available for 35 cases. Onset of symptoms began in the first case on 3 December, 2.5 hours after the meal had been served (Figure). The median time between the meal and symptom onset (the median incubation period) was four hours. No cases had onset of symptoms later than eight hours after the meal.

Cohort study

Of the food items served during the excursion, only consumption of rice pudding was significantly associated with illness in the cohort study. The relative risk was infinite (Table 2) with $p<0.001$, and all cases could be explained by the consumption of rice pudding (aetiological fraction: 100%). After stratifying by kindergarten (Table 3), the consumption of rice pudding remained associated with disease.

Laboratory results

One vomit sample was provided on the day of symptom onset (3 December 2007); 10 stool samples were provided after 6 December. 'Presumptive *B. cereus*' (collective name for *B. cereus* sensu strictu, *B. thuringiensis* and closely related bacilli), isolated from the culture of the vomit sample, was analysed by LC-MS/MS for cereulide production and for the presence of the *ces* gene by PCR: both analyses gave negative results [8]. No cereulide could be detected in the vomit sample itself. The isolate, initially described as presumptive *B. cereus*, was identified as *B. cereus* sensu stricto (non-cereulide producing) by Fourier transform infrared spectroscopy.

All stool samples taken within a few days after symptom onset and all food samples were negative for all tested pathogens.

Environmental results

All food items had been purchased by the caterer at the end of November 2007 and had been stored in the boot of the caterer's car until 1 December 2007. One similar excursion had taken place on 1 December 2007, with

the same tram and the same catering company, but no outbreak occurred.

On both excursions, pots in an electric water-bath were used to heat the rice pudding and to keep it warm on the tram platform. The caterer stated that after the meal of the first excursion (on 1 December), rice pudding remnants had been scraped out of the pots and the pots were cleaned superficially in a wash-hand basin in an improvised kitchen in the caterer's office. According to the caterer, no food leftovers were served on 3 December. On that day, since there were more participants than in the previous excursion, the caterer used three additional cooking pots to heat up the rice pudding. The electricity supply was temporarily interrupted (due to a blown fuse) during the food preparation on 3 December.

Discussion

There is strong epidemiological evidence that the vehicle of the outbreak was rice pudding served during the excursion on 3 December 2007: the narrow epidemic curve indicated a common source of infection. All cases of emetic syndrome could be explained by the consumption of rice pudding from some of the pots used, while other food items were not associated with illness. Unfortunately, only one vomit sample was available for testing: no leftovers of the rice pudding portions served were available. This substantially hampered the laboratory investigations and no causative agent could be unambiguously identified. However, the clinical characteristics of this outbreak – including the short incubation period (of only a few hours), vomiting as the main symptom and the short self-limiting course of the disease – are typical for *B. cereus* emetic toxins or *S. aureus* enterotoxin. The fact that rice pudding was the likely vehicle suggests that this outbreak was caused by *B. cereus* cereulide. Starchy food products, including rice dishes, have been described as typical vehicles in *B. cereus* toxin outbreaks [2,5,10]. However, *S. aureus* cannot be ruled out as the responsible pathogen. In both scenarios of *B. cereus* or *S. aureus* having caused the outbreak, the food contamination must have occurred at least several hours before serving because this minimum time is required for pathogen multiplication or germination (in case of *B. cereus*) and for toxin production [2,5]. It is very unlikely that the unopened commercial ready-to-eat tetrapacks were contaminated: had they been, more outbreaks would have been expected, given the wide distribution of these products. Since the cinnamon–sugar mix was added to the rice pudding only

Table 2

Food-specific attack rates, aetiological fraction and relative risks with 95% confidence intervals, outbreak of emetic syndrome following kindergarten excursion, Berlin, Germany, December 2007

Food items served during the excursion	Eaten by the person	Number of participants who developed symptoms and with available information about food items consumed n=46	Overall number of participants with available information about food items consumed[a]	Attack rate (%)	Aetiological fraction (%)	Relative risk (95% CI)
Rice pudding	Yes	46	129	36	100	∞
	No	0	24	0	–	–
Cocoa drink	Yes	37	124	30	79	1.0 (0.5–1.9)
	No	7	23	30	–	–
Whipped cream	Yes	5	22	23	11	0.8 (0.3–1.8)
	No	32	109	29	–	–
Gingerbread	Yes	2	22	9	4	0.3 (0.1–1.1)
	No	34	105	32	–	–

[a] For rice pudding: n=153; cocoa drink: n=147; whipped cream: n=131; gingerbread: n=127.

Table 3

Stratified analysis by kindergarten for rice pudding-specific attack rates, aetiological fraction and relative risks, outbreak of emetic syndrome following kindergarten excursion, Berlin, Germany, December 2007

Kindergarten	Rice pudding eaten by the person	Number of cases who consumed rice pudding and developed symptoms n=46	Overall number of participants with available information about rice pudding consumption n=153	Attack rate (%)	Aetiological fraction (%)	Relative risk
A	Yes	34	79	43	100	∞
	No	0	16	0	–	–
B	Yes	11	18	61	100	∞
	No	0	4	0	–	–
C	Yes	1	32	3	100	∞
	No	0	4	0	–	–

shortly before consumption it can be ruled out as the vehicle of the outbreak.

Unfortunately, in the initial microbiological investigations the human and food samples had not been tested specifically for the presence of *B. cereus* toxins and *S. aureus* enterotoxins. In such outbreaks, human and food samples should be obtained and tested in a timely manner, not only for the usual pathogens (bacteria and viruses) but also for the relevant toxins, using the appropriate tests. The *B. cereus*-like strain isolated from the only vomit sample tested negative for cereulide or the *ces* gene. However, it is conceivable that emetic-toxin-producing *B. cereus* strains as well as non-toxin-producing strains were present in the rice pudding, but could not be detected in the vomit sample. The presence of *B. cereus* in the vomit sample and the absence of this agent from the unopened package of rice pudding is consistent with a scenario of *B. cereus* spores (including toxin-producing and non-toxin-producing strains) having contaminated the rice pudding after the tetrapacks were opened. The spores may have germinated and multiplied in remnants of the rice pudding left in the pots during an inadequate cleaning and storage process between the first and second excursion. This scenario is supported by the fact that not all of the pots appear to have contained contaminated rice pudding.

The fact that children from three kindergartens participated in the excursion and were affected by emetic syndrome shortly afterwards (although with attack rates differing by kindergarten) clearly pointed to a common source related to the excursion. This epidemiological pattern narrowed the spectrum of causative agents to toxin-producing agents. This shift of focus when patients from more than one setting are affected is an important epidemiological practice that is not always appreciated. If only one kindergarten had been involved, the investigation would have needed to also examine potential earlier sources of exposure to other pathogens such as norovirus and rotavirus. In this outbreak, the epidemiological investigation started shortly after the outbreak had been detected and the kindergarten staff clearly remembered the few food items consumed by the children. However, in other outbreak investigations, if substantial time elapses between symptom onset and epidemiological data collection (e.g. standardised interviews) or if many different food items had been served recall bias may be a major problem.

Although the environmental investigations did not determine the source of the food contamination, it revealed several breaches in food hygiene regarding cleaning of the cooking pots between the first and second excursion, as well as incorrect holding times and temperatures of food.

The epidemiological findings in this outbreak are consistent with other published *B. cereus*-associated food-borne outbreaks [11,12]. It should also be noted that food can be contaminated at the same time by different strains of presumptive *B. cereus* (*B. cereus* sensu stricto, *B. thuringiensis, B. weihenstephanensis*), which can be difficult to discern in some cases of food poisoning [13-15]. Also contamination with mixed cultures of emetic and non-emetic *B. cereus* sensu stricto can occur that can only be revealed by the testing of several isolates [8]. Detection of the *B. cereus* toxin as well as *S. aureus* enterotoxin in human and food samples is not straightforward and may require advanced methods in specialised laboratories [13-15].

Mobile caterers and persons responsible for such excursions should be aware of the potential risk of outbreaks caused by bacterial toxins. In order to prevent *B. cereus* spores from germinating and producing heat-stable cereulide, caterers need to ensure that food leftovers are discarded or refrigerated at a temperature below 10 °C and, if stored, that they are reheated thoroughly (at least 65 °C) before consumption.

In presumed food-poisoning outbreaks, stool and vomit samples from a substantial number of patients as well as relevant food leftovers and their ingredients should be obtained and investigated, not only for pathogens but also for the relevant toxins by appropriate tests. If *B. cereus* is identified, it is useful to further analyse several isolates from the culture to identify toxin-producing *B. cereus* strains.

In the light of our study, we recommended using single-portion, ready-to-eat rice pudding packs during future kindergarten field trips. No further food-borne outbreaks related to such excursions were reported to the local health authorities.

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An outbreak of *Salmonella* Typhimurium traced back to salami, Denmark, April to June 2010

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Between April and June 2010, a small national outbreak of *Salmonella* Typhimurium with a particular multilocus variable-number tandem repeat analysis (MLVA) type was identified in Denmark through laboratory-based surveillance. The outbreak involved twenty cases, primarily living within the greater Copenhagen area. Half of the cases were children aged ten years or younger and 12 were male; three cases were hospitalised. A matched case-control study showed a strong link between illness and eating a particular salami product containing pork and venison, matched odds ratio (mOR):150, confidence interval (CI): 19–1,600. The salami had been produced in Germany. Microbiological confirmation in food samples was sought but not obtained. Danish consumers were notified that they should return or dispose of any packages from the suspected salami batch. Because the salami product had potentially been sold in other European countries, the European Centre for Disease Prevention and Control urgent enquiry and Rapid Alert System for Food and Feed systems were used to highlight the possibility of outbreaks in these countries. Case-control studies are a strong tool in some outbreak investigations and evidence from such studies may give sufficient information to recall a food product.

Introduction

Salmonella enterica is the second most common cause of bacterial gastroenteritis in Denmark. A series of national control and intervention programmes have reduced the annual incidence to less than 30 cases per 100,000 population in 2010 [1] compared to 96 in 1997 [2]. However, imported products, including products from other European Union (EU) Member States, are not monitored within the Danish national programmes, but are rather tested for *Salmonella* by random sampling at import and, less often, during retail product control. Ready-to-eat products sold in Denmark are required to be free of *Salmonella*.

The circulation of *Salmonella* serovars in humans and animals in Denmark is monitored by the mandatory national human laboratory surveillance system and by analysing data derived from isolates from animals and food items of animal origin as part of the control programmes. In 2010, 33% (521) of all registered human cases in Denmark were caused by *S.* Typhimurium [1]. Human isolates are sent from clinical laboratories to Statens Serum Institut (SSI) for typing, which for *S.* Typhimurium isolates include multilocus variable-number tandem repeat analysis (MLVA). MLVA is a typing method which has been shown to have good discriminatory power within *S.* Typhimurium [3]. Clusters of *S.* Typhimurium patient-isolates with identical MLVA profiles are treated as potential outbreaks. Routine MLVA typing is now standard practise for surveillance of human *S.* Typhimurium infections in Denmark. This allows the detection of outbreaks that would otherwise have remained undiscovered.

On 15 April 2010, a cluster of 11 cases with identical *S.* Typhimurium MLVA profiles was detected, with all cases notified in April. Cases were invited to respond to a hypothesis-generating questionnaire by telephone. Two affected families independently indicated having consumed a certain unusual type of salami, which led to a working hypothesis that consumption of this salami product was associated with infection with *S.* Typhimurium. Here we describe the investigations undertaken to confirm this hypothesis, identify the source of the outbreak and to trace-back the suspected product.

Methods

MLVA was performed using primers described by Lindstedt et al [4] in the widely accepted method for *S.* Typhimurium. Of the five loci, STTR9 and STTR6 were labelled with 6-FAM, STTR5 and STTR3 with HEX and STTR with NED [5]. The primers were used in a single multiplex PCR followed by detection on an ABI310 [6].

Phage typing was undertaken following the Anderson typing scheme [7].

Case definition and case-control study

For this investigation, a case was defined as a person residing in Denmark, who became ill with symptoms of gastroenteritis (diarrhoea and/or vomiting) after 1 April 2010, whose culture results yielded the outbreak strain and who had not travelled abroad between 25 March and 14 June. The outbreak strain was defined as *S.* Typhimurium having MLVA profile 3-14-12-NA-211.

Following initial hypothesis-generating patient interviews using a standard *S.* Typhimurium trawling questionnaire, a case-control study was initiated on 14 June, immediately after the discovery that a salami product may have been the source of infection. Controls were selected from the Danish population registry [8], matched by municipality, sex, and date of birth. To create a more robust statistical analysis, three controls were identified and interviewed for each case. Participants were interviewed by phone using a tailored questionnaire focusing on consumption of various types of meats, cold cuts, places where food was bought, as well as other exposures. Controls who experienced symptoms of gastroenteritis (diarrhoea and/or vomiting) or who had travelled outside Denmark during April and May 2010 were excluded.

Statistical analysis

Data from case and control questionnaires were entered into an EpiData database [9]. Statistical analyses were conducted in STATA 10 (StataCorp, TX). In order to examine relationships between each exposure and disease, odds ratios (ORs), matched odds ratios (mORs) and 95% confidence intervals (CI) were calculated.

International aspects

On 15 June, an urgent enquiry was published through the European Centre for Disease Prevention and Control (ECDC) Epidemic Intelligence Information System (EPIS) and on 16 June a Rapid Alert System for Food and Feed (RASFF) notification was issued. The RASFF notification led to inspection by the German food authorities of the factory where the salami was produced.

Results

During the time of the outbreak, 5 April to 6 June, 20 patient isolates (one isolate per patient) with the specific MLVA type were found over a period of nine weeks (Figure).

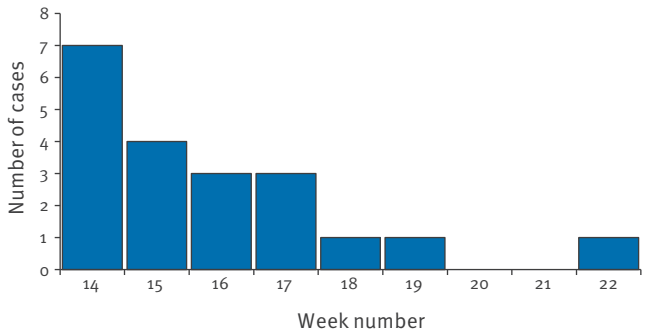
The median age of the patients was 20 years (range 1–69). Half of the cases (10) were children aged 11 years or younger and twelve were male. Three cases were hospitalised from symptoms caused by the infection, but no deaths occurred. The majority of patients (12 of 20) lived within the greater Copenhagen area which covers about 34% of the Danish population of approximately 5.5 million.

Trawling interviews using a generic *Salmonella* outbreak questionnaire led to a hypothesis concerning salami, when the majority of interviewed cases reported using the same supermarket chain when buying food and two families with cases mentioned having bought the same specific type of salami in that chain. The salami in question contained venison (meat from deer) and was traced back to a German producer which manufactures ready-to-eat products for the above-mentioned Danish supermarket chain. Considering the disease onset dates and the shelf life of the salami (one and a half months), it was determined that the potentially contaminated packages of salami were from a single batch labelled with a use-by-date between 6 April and 16 June 2010.

For the case-control study, 17 of the 20 identified cases and 79 controls were interviewed. A total of 16 controls were excluded from the analysis; five due to symptoms of gastrointestinal illness and 11 due to having travelled abroad. Consuming Brand X salami with smoked venison and pork was strongly associated with illness (Table). All cases but two reported having consumed this salami during the week before illness onset (mOR: 150; 95% CI: 19–1,600; P<0.0001). Illness was also significantly associated with consumption of salami

Figure

Cases of *Salmonella* Typhimurium with the outbreak MLVA type, by week of disease onset, Denmark, 5 April–6 June 2010 (n=20)



Table

Results of single-variate analysis of selected exposures from case-control study, Denmark 2010 (n=80)

Exposure	Cases n/N	Controls n/N	Matched Odds Ratio (95% CI)
Pork	2/17	29/63	0.2 (0.03–0.7)
Chicken	8/17	39/63	0.5 (0.2–1.6)
Turkey	2/17	8/63	1.3 (0.2–6.1)
Beef	7/17	29/63	2.0 (0.2–95.8)
Sliced rolled meat	2/17	4/63	1.1 (0.3–4.1)
Salami	15/17	34/63	6.4 (1.3–61.1)
Beef salami	4/17	17/63	0.8 (0.2–3.2)
Game salami	14/17	3/63	93 (13.9–723.9)
Brand X smoked deer and pork salami	15/17	3/63	150 (18.8–1626)

Multiple exposures to food items were possible.

in general (mOR: 6.4; 95% CI: 1.3–61) and answering yes to having consumed salami containing game meat (mOR: 93; 95% CI: 14–720) (Table).

The outbreak strain was initially phage typed to be a mixture of the biphasic *S.* Typhimurium DT120 and DT7 with resistance to ampicillin, streptomycin, sulphamethoxazole and tetracycline. Separate phage typing of several isolates at the World Health Organisation Collaborative Centre for phage typing of *Salmonella* (Health Protection Agency, Colindale, United Kingdom [10]), later confirmed that the type was actually DT193 with several non-specific reactions. These results were obtained after the EPIS and RASFF notifications had been issued. In contrast, the MLVA type was highly characteristic and non-varied and thus the case definition was purely based on the MLVA results. All patient isolates in the outbreak had the same MLVA type.

At the time of the discovery, the salami batch, which had most likely caused the outbreak, had passed its expiry date and therefore no recalls were made. Additionally, it was not possible for the regional food authorities to obtain any salami for microbiological examination and thus no samples of salami from the suspected batch were available for microbiological testing. The inspection by German authorities of the factory where the salami was produced did not identify problems in the factory nor a contamination of the meat in question. The Danish supermarket chain selling the salami also operates in other European countries, however investigation by the Danish Veterinary and Food Administration indicated that the particular salami from the same producer had only been sold in Denmark and Germany. There was no indication that the implicated batch of salami had also been sold in Germany, although similar products from the same producer were available in German stores of the same supermarket chain. The batch-focused food trace back yielded no information if and how the deer meat salami sold in German stores was related to the Danish batch.

Danish food authorities issued a warning for consumers to return or dispose of any packages of Brand X smoked deer and pork salami with a use-by-date between 6 April and 16 June 2010.

Discussion

A small national outbreak of *S.* Typhimurium DT193 was identified through laboratory-based surveillance using MLVA typing. The most likely conclusion, based on the findings of the case-control study, disease onset dates as well as product supply and distribution, was that the outbreak was caused by specific sliced salami containing smoked deer and pork meat. When the suspected outbreak source was detected in June, new cases had not been identified for several weeks. It was concluded that a single batch of infected salami had caused the outbreak. Survival of *Salmonella* in ready-to-eat products has the potential to cause illness and salami has on several occasions been identified as the food vehicle for *S.* Typhimurium [11-13]. A recent multistate outbreak of S. Montevideo in the United States was shown to have been caused by salami products containing contaminated red and black pepper, additionally highlighting the importance of post-processing contamination of ready-to-eat products [14].

Following the EPIS urgent enquiry, Germany reviewed the *S.* Typhimurium situation during April and May 2010. MLVA typing is not routinely performed in Germany; thus it would only be possible to detect a corresponding outbreak by looking at *S.* Typhimurium case numbers in general. During the time in question, a similar number of cases of *S.* Typhimurium were notified from Germany as a whole compared to April and May in previous years (1,479 vs a mean of 1,489 in 2007-9). However, restricting the comparison to only those six federal states where stores from the implicated Danish supermarket chain are widespread, a 24% increase in *S.* Typhimurium cases was observed, compared to the mean of 2007-9 (645 vs 519 cases).This excess was mainly in adult males above 18 years old and strongest between 12 and 18 April. Due to the time elapsed, these cases were not investigated further.

The observed regional increase in German *S.* Typhimurium cases, restricted to states with branches of the Danish supermarket chain, is suggestive of a link to the Danish outbreak. Differences in age distribution between the German and the Danish cases may be explained by the fact that food preferences for such an unusual sausage product may vary geographically. Without MLVA confirmation, we cannot be sure that the German regional increase was due to the same type of *S.* Typhimurium.

No further cases matching the outbreak profile were reported from Europe.

The investigations described in this paper highlight the usefulness of typing methods in combination with simple case-control studies to detect an outbreak and identify possible sources of infection. Although no samples were available for testing, it was concluded that the smoked pork and deer meat salami was the likely source of infection in this outbreak, solely based on the case-control investigation results. Vehicle identification was aided by the unusual type of salami, which made it stand out in the memories of the patients.

The international dimension of this outbreak in Denmark is not only demonstrated by the fact that the salami at the source was produced in Germany, but also because the Danish supermarket chain selling the salami operates in other European countries, including Germany and England. Therefore the ECDC urgent enquiry and the RASSF systems were used to highlight the potential for similar outbreaks in these countries. It is not unlikely that the particular salami had caused cases of salmonellosis in countries other than Denmark, however, the use of different national subtyping systems

made it difficult to detect such cases by the Danish definition in these countries. This is potentially a serious problem in a multi-national outbreak situation. To rapidly determine if disease outbreaks in several countries are caused by the same strain, methods for molecular typing should be standardised throughout the EU. Ammon and Tauxe [15] highlight the need for developing a consensus about which methods to use, their application in all laboratories as well as implementing additional methods, such as resistance testing and pulsed-field gel electrophoresis, in particular situations. Another potential challenge within the EU is the investigation of a food outbreak source by microbiology and trace-back in situations where the food is produced in a different country from the one where the outbreak takes place. Encouragingly, however, in this outbreak a food investigation was made by German authorities as a result of an RASSF notification from another country, based purely on the results of a case-control study, even though there were no confirmed cases in Germany.

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Notes

Notes

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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

Kansanterveyslaitos
Department of Infectious Disease Epidemiology, National Public Health Institute, Helsinki.
Monthly, print and online. In Finnish.
http://www.ktl.fi/portal/suomi/osastot/infe/tutkimus/tartuntatautien_seuranta/tartuntatautilaakarin_kommentit/

France

Bulletin épidémiologique hebdomadaire
Institut de veille sanitaire, Saint-Maurice Cedex.
Weekly, print and online. In French.
http://www.invs.sante.fr/beh/default.htm

Germany

Epidemiologisches Bulletin
Robert Koch-Institut, Berlin
Weekly, print and online. In German.
http://www.rki.de/DE/Content/Infekt/EpidBull/epid__bull__node.html

Hungary

Epinfo (az Országos Epidemiológiai Központ epidemiológiai információs hetilapja)
National Center For Epidemiology, Budapest.
Weekly, online. In Hungarian.
http://www.oek.hu/oek.web?to=839&nid=41&pid=7&lang=hun

Iceland

EPI-ICE
Landlæknisembættið
Directorate Of Health, Seltjarnarnes
Monthly, online. In Icelandic and English.
http://www.landlaeknir.is

Ireland

EPI-INSIGHT
Health Protection Surveillance Centre, Dublin.
Monthly, print and online. In English.
http://www.hpsc.ie/hpsc/EPI-Insight

Italy

Notiziario dell'Istituto Superiore di Sanità
Istituto Superiore di Sanità, Reparto di Malattie Infettive, Rome.
Monthly, online. In Italian.
http://www.iss.it/publ/noti/index.php?lang=1&tipo=4

Bolletino Epidemiologico Nazionale (BEN)
Istituto Superiore di Sanità, Reparto di Malattie Infettive, Rome.
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

Epidemiologijos žinios
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

Meldunki o zachorowaniach na choroby zakazne i zatruciach w Polsce
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

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

Romania

Info Epidemiologia
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.
Sporadic, print only. In Romanian.http://www.insp.gov.ro/cnscbt/index.php?option=com_docman&Itemid=12

Spain

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