Rapid communications

SALMONELLOSIS CASES CAUSED BY A RARE *SALMONELLA* ENTERITIDIS PT6C ASSOCIATED WITH TRAVEL TO BULGARIA, JUNE-JULY 2008

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In June 2008 an outbreak of gastroenteritis was registered in Sunny Beach resort situated on the Black Sea coast in Bulgaria, affecting 14 employees of a hotel, five of whom tested positive for *Salmonella* Enteritidis. During June-July 2008 four sporadic *S. Enteritidis* cases were also reported and two of them were foreign tourists. In the same period *S. Enteritidis* cases connected with travel to Bulgaria were reported to the European Centre for Disease Prevention and Control (ECDC) from Finland, United Kingdom, Sweden, Germany and Norway. We describe a study performed to find out relatedness between Bulgarian and Finnish *S. Enteritidis* isolates using phage typing (PT) and pulse-field gel electrophoresis (PFGE). Fifteen *S. Enteritidis* isolates from Bulgaria and 195 from Finland (including 28 from travellers to Bulgaria) were phage typed. Within Bulgarian isolates four different PTs were found and PT6c with eight strains was predominant. Nineteen out of 28 strains isolated from the Finns visiting Bulgaria belonged also to PT6c. PFGE typing (with one enzyme) of all *S. Enteritidis* PT6c strains (8 Bulgarian and 19 Finnish isolates) showed indistinguishable PFGE profile. The typing results thus demonstrated a link between Bulgarian and Finnish *S. Enteritidis* isolates. We conclude that *S. Enteritidis* PT6c was the cause of a salmonellosis outbreak in Sunny Beach and was exported to Finland, and likely to the United Kingdom, Norway, Sweden and Germany.

Introduction

Background

Salmonella has long been recognised as an important food-borne pathogen which can cause symptoms in humans ranging from self-limited enteric infections to enteric fever. In the European Union (EU), serovars *Salmonella* Enteritidis and *Salmonella* Typhimurium are the most frequent causes of gastroenteritis in humans. In 2006, more than 160,000 cases of salmonellosis were reported in the EU resulting in an annual incidence of 34.6 cases per 100,000 population [1]. In 2001-2007, the annual number of salmonellosis cases in Bulgaria has varied between 800-1000 (incidence 9.3-15.4/100,000). Most of the Bulgarian cases have been sporadic. However, a few outbreaks have also emerged every year due to consumption of contaminated eggs and/or dairy products. Approximately 70% of the strains isolated in Bulgaria are of serovar *S. Enteritidis*.

Cases in Bulgaria

In the beginning of June 2008, a salmonellosis outbreak caused by *S. Enteritidis* occurred among personnel of a hotel in Sunny Beach resort situated on the Black Sea coast, in Nessebar municipality, Burgas region, Bulgaria. In all, 14 persons with symptoms of fever ($\leq 39.5^{\circ}\text{C}$), vomiting, abdominal pain and diarrhoea were reported of whom seven sought medical care in the local hospital. At the same time, during June and July 2008, four sporadic *S. Enteritidis* cases were also reported in the Burgas region two of whom were foreign tourists including an eight-year-old Finnish girl.

Cases in other countries

Finland: In June - July 2008, the Gastrointestinal Infection Unit of the National Institute for Health and Welfare (THL) in Helsinki identified 195 *S. Enteritidis* cases: 28 of them were connected to a trip to Bulgaria, including 19 that were of the phage type PT6c.

United Kingdom: On 8 July 2008 the Health Protection Agency reported an increased number of *S. Enteritidis* cases with an unusual phage type PT6c to the European Centre for Disease Prevention and Control (ECDC). Twelve patients were followed up and it turned out that all had been on holiday in Bulgaria preceding their illness.

Sweden: On 10 July 2008 Sweden reported to ECDC 29 *Salmonella* cases among travellers returning from Bulgaria during June and July. In Sweden, *Salmonella* strains related to travels abroad are not routinely serotyped. Nevertheless, 10 strains that were serotyped were all *S. Enteritidis*, and six of these cases were traced back to hotels in Nessebar and Sunny Beach. The 10 *S. Enteritidis* strains were also phage typed but the strains were not PT6c.

Germany reported to ECDC two *S. Enteritidis* cases linked to Bulgaria. One of these travellers had stayed in hotel at Nessebar from 23 to 30 May 2008 and had symptoms of salmonellosis starting from 28 May. More information about the other case was not available.

Norway reported a total of 76 salmonellosis cases in 2008 linked with travelling to Bulgaria, of which 48 isolates had been identified as *S. Enteritidis*. Of these isolates, eight were phage typed and four of them were of PT6c.

Aims of the study

Effective epidemiological surveillance of salmonellosis requires accurate subtyping of the strains in order to trace the potential sources of infection and the geographical distribution of different *Salmonella* serovars. A number of different phenotypic and genotypic methods have been used in microbiology laboratories for subtyping. Phage typing (PT) and pulsed-field gel electrophoresis (PFGE) are currently the only internationally standardised typing methods for *S. Enteritidis*. In order to find out relatedness between Bulgarian and Finnish *S. Enteritidis* isolates potentially associated with an outbreak occurring in Bulgaria, we initiated an investigation of those strains by phage typing and PFGE.

Methods

Surveillance

Salmonellosis is one of the notifiable communicable diseases in Bulgaria. The surveillance of salmonellosis in the country is laboratory-based. The primary diagnostics is performed by the regional clinical microbiology laboratories that are legally required to record and report all cases discovered in their regions. They send all outbreak-associated and some sporadic *Salmonella* strains to the National Reference Laboratory for Enteric Pathogens for confirmation, serotyping and antimicrobial susceptibility testing.

Outbreak investigation

Following notification of the outbreak in a hotel in Sunny Beach resort, field epidemiological investigation was performed including interviews with cases and contact persons, and active case-finding among hotel personnel. Stool samples were taken from 14 symptomatic employees of the hotel and 100 asymptomatic

contacts identified among personnel and families of cases, and were cultured by standard methods for *Salmonella*.

Collection and laboratory investigation of food samples

Food samples taken from five dishes prepared in the hotel restaurant and suspected based on the interviews (scrambled eggs with chopped peppers and tomatoes, chicken soup, chicken goulash, fish fried in egg and bread-crumbs, chicken giblets with rice) were tested for salmonellosis. Additionally, mash potatoes and two kinds of eggs, disinfected and not disinfected, were also examined in the microbiology laboratory at the Regional Inspectorate of Public Health Protection and Control in Burgas. ISO standard 6579 was used for the investigation of those food samples.

Pheno- and genotyping of the isolates

Subtyping of the *S. Enteritidis* strains was conducted at the National Reference Centre for *Salmonella* in Finland. *S. Enteritidis* isolates from Burgas region available at the National Reference Laboratory of Bulgaria (n=15) and all *S. Enteritidis* strains isolated in Finland during June and July 2008 (n=195) were examined. Isolates were phage typed using the method described in Ward et al. [2]. In addition, the 15 Bulgarian strains and the 19 Finnish PT6c isolates were analysed for genetic relatedness by PFGE using XbaI according to the Centers for Disease Control and Prevention (CDC) PulseNet protocol [3]. The PFGE patterns were named using international standardised nomenclature of PulseNet Europe.

Results

In the outbreak investigation, five of the 14 symptomatic persons among the hotel personnel and eight of the 100 contacts tested positive for *S. Enteritidis*. The food samples were all negative for *S. Enteritidis*. Nevertheless, scrambled eggs with chopped peppers and tomatoes were suspected as the most likely source of the

TABLE

Phage types and PFGE types of *S. Enteritidis* strains isolated in Bulgaria from stool specimens of 16 patients, June – July 2008

Date of receipt in NCIPD	Patient's nationality	National region	Occupation (Link to a Sunny Beach hotel)	Phage type	PFGE type
14/05/2008	Bulgarian	Burgas	other (sporadic)	1	SENTXB.0001
20/06/2008	Bulgarian	Burgas	other (sporadic)	6c	SENTXB.0010
20/06/2008	Bulgarian	Burgas	other (sporadic)	6c	SENTXB.0010
20/06/2008	Bulgarian	Burgas	other (sporadic)	6c	SENTXB.0010
20/06/2008	Bulgarian	Burgas	other (sporadic)	6c	SENTXB.0010
20/06/2008	Bulgarian	Burgas	other (sporadic)	4	SENTXB.0001
20/06/2008	Bulgarian	Burgas	other (sporadic)	4	SENTXB.0001
24/06/2008	Bulgarian	Burgas	personnel	6c	SENTXB.0010
24/06/2008	Bulgarian	Burgas	personnel	6c	SENTXB.0010
24/06/2008	Bulgarian	Burgas	personnel	6c	SENTXB.0010
24/06/2008	Bulgarian	Burgas	personnel	6c	SENTXB.0010
02/07/2008	Bulgarian	Burgas	other (sporadic)	1	SENTXB.0001
02/07/2008	Bulgarian	Burgas	other (sporadic)	21	SENTXB.0001
02/07/2008	Bulgarian	Burgas	other (sporadic)	4	SENTXB.0001
02/07/2008	Bulgarian	Burgas	other (sporadic)	1	SENTXB.0001
25/07/2008	Finnish		tourist	6c	SENTXB.0010

Abbreviations: NCIPD: National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria, PFGE: pulse-field gel electrophoresis

outbreak, since all persons affected reported having eaten this dish before onset of symptoms.

As a result of subtyping performed at the National Reference Centre in Finland, within the 15 Bulgarian *S. Enteritidis* strains, four different PTs were found: PT1, PT4, PT6c and PT21 (Table). The predominant PT was PT6c: eight out of 15 strains belonged to this phage type.

Among the 195 *S. Enteritidis* strains isolated in Finland, 15 PTs were found, the most common being PT21 with 54 strains. The cases were associated with trips to 25 countries, most commonly to Greece (34 cases), Bulgaria (28 cases), Turkey (27 cases) and Estonia (18 cases). The 28 strains isolated from Finns who visited Bulgaria in June or July were identified as PT6c (19 cases), PT6 (1 case), PT13a (2 cases), PT14b (2 cases) and PT22 (4 cases). PT6c strains were found only in samples of patients returning from Bulgaria.

PFGE analysis showed that the 15 *S. Enteritidis* strains from Bulgaria and the 19 strains from Finland could be assigned to only two characteristic PFGE patterns (SENTXB.0001 and SENTXB.0010) which were obtained after *Xba*I digestion (Figure). The *S. Enteritidis* PT6c strains (8 Bulgarian and 19 Finnish isolates) were indistinguishable from each other by this analysis and were classified as SENTXB.0010.

Discussion

Numerous reports of salmonellosis associated with foreign travel and caused by different *Salmonella* serovars have been published [4,5,6,7,8]. In this study, we report multinational cases of salmonellosis caused by *S. Enteritidis* PT6c associated with travel to Bulgaria.

S. Enteritidis PT6c is a rare phage type. None of the *S. Enteritidis* strains isolated from 978 patients and typed at the National Reference Centre in Finland in 2007 and 2008 (by June) belonged to this PT (A. Siitonen, unpublished data). Also, to our knowledge, there are no previously published reports on outbreaks caused by this phage type. Several European countries, namely

Austria, Norway, Hungary, Ireland, Finland, United Kingdom, Germany and Sweden reported to ECDC sporadic *S. Enteritidis* cases in 2008 among tourists returning from Bulgaria. United Kingdom, Finland and Norway found *S. Enteritidis* PT6c among *Salmonella* strains isolated from the samples taken from their citizens returning from Bulgaria.

In our study, 27 strains (8 Bulgarian and 19 Finnish) proved to be *S. Enteritidis* PT6c. The facts that i) the Bulgarian and Finnish isolates were of the same phage type and ii) among the Finnish *S. Enteritidis* strains, phage type PT6c was only found in isolates from patients who returned from Bulgaria, indicate an epidemiological link between them. Among Bulgarian *S. Enteritidis* PT6c isolates, four strains were taken from cases associated with *S. Enteritidis* outbreak in a hotel in Sunny Beach resort. *S. Enteritidis* PT6c was also isolated from an eight- year-old Finnish girl who had stayed in another hotel in Sunny Beach resort (Table). In the hotels of this resort, many Finns and people of other nationalities spent their summer holidays in 2008. After July, PT6c was still found in Finnish *S. Enteritidis* cases returning from Bulgaria in August (n=4), September (n=9) and October (n=1) but no cases were detected in November.

The finding that all tested Bulgarian and Finnish *S. Enteritidis* PT6c isolates had the same PFGE profile (SENTXB.0010) also suggests that these strains could have the same origin and be epidemiologically linked. However, this PFGE profile can be found in *S. Enteritidis* strains of several PTs, including common phage types PT4 [9], PT1 and PT21 (A. Siitonen, unpublished). This emphasises the importance and applicability of phage typing over genotyping in epidemiological surveillance of salmonellosis.

Tourism is the fastest growing industry worldwide. The globalisation leads to faster spreading of infectious diseases including salmonellosis and requires us to consider them from a global perspective. International networks worldwide and collaboration of the health authorities are essential for an effective control of salmonellosis.

FIGURE

Cluster analysis based on the PFGE profiles of *S. Enteritidis* isolates originating from Bulgaria and Finland, June-July 2008

In conclusion, the alert system administered by ECDC, the effective collaboration between EU countries and the use of internationally standardised subtyping methods such as phage typing and PFGE, enabled us to establish an international clustered record of *Salmonella* infections caused by a rare *S. Enteritidis* PT6c and its association with travelling to Bulgaria. We believe that *S. Enteritidis* PT6c was the cause of outbreaks of salmonellosis in resorts situated at the Bulgarian Black Sea coast and was exported to Finland and most likely to the United Kingdom, Norway, Sweden and Germany. In this study, the importance of a multinational approach for the determination of potential sources of salmonellosis and its geographical distribution was demonstrated.

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