A *Salmonella enterica* subsp. *enterica* serovar Enteritidis foodborne outbreak after consumption of homemade *lasagne*

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Abstract

In the latest year, and also in 2013, *Salmonella* was the most frequently detected causative agent in foodborne outbreaks (FBOs) reported in Europe. As indicated in EFSA report (2015) the serotypes mostly associated to FBOs are *S.* Typhimurium and Enteritidis; while *Salmonella* Typhimurium is generally associated with the consumption of contaminated pork and beef, FBOs due to *Salmonella* Enteritidis are linked to eggs and poultry meat. In this study it is described the investigation of a domestic FBO involving four adults and linked to homemade *lasagne*. Investigations were performed to determine the relatedness of *Salmonella* strains, identify the sources of infection, and trace the routes of *Salmonella* contamination in this FBO. *Salmonella* strains were isolated in 3 out of 4 patient stool samples and from *lasagne* and all of them were serotyped as *S.* Enteritidis. Pulsed-field gel electrophoresis (PFGE) analysis revealed the genotypical similarity of all the strains. Although serotyping and PFGE analysis identified the common food source of infection in this FBO, it was not possible to determine how or at what point during food preparation the *lasagne* became contaminated with *Salmonella*.

Introduction

Salmonella was the most common cause of foodborne outbreaks (FBOs) in the European Union (EU) in 2013, with 1168 FBOs of human salmonellosis reported by 22 member states, accounting for 22.5% of all notified outbreaks of foodborne illness in the EU according to the European Food Safety Authority (EFSA, 2015).

The genus *Salmonella*, which is closely related to the genus *Escherichia*, groups Gram-negative, non-spore-forming, is rod-shaped bacteria belonging to the *Enterobacteriaceae* family. Two species are distinguished: *S. enterica*, classified into six subspecies (Grimont *et al*., 2007), and *S. bongori*. *Salmonella enterica* subspecies *enterica* is an intracellular pathogen of warm-blooded mammals comprising over 2500 serovars (Baker and Dougan, 2007). Some *Salmonella* serovars, such as *S. enterica* serovar Enteritidis and *S. enterica* serovar Typhimurium, are non-specific host pathogens that can colonise a broad range of animals, rarely causing clinical manifestations. In humans, however, they can lead to gastroenteritis or occasionally septicemia (Galanis *et al*., 2006). The serovars most frequently associated with human illness in the EU are *S.* Enteritidis (39.5%), *S.* Typhimurium (20.2%), and monophasic *S.* Typhimurium (8.6%) (EFSA, 2015).

Salmonella is the most common causative agent, reason for hospitalisation, and cause of death tracked by the Foodborne Diseases Active Surveillance Network (FoodNet) (CDC, 2011). Beyond their health effects, foodborne illnesses may cause emotional and economic hardship. *Salmonella* alone is responsible for approximately 1 million foodborne infections (Scallan *et al*., 2011) and it costs 365 million $ in direct medical expenditures annually (US Department of Agriculture, 2011).

Common salmonellosis symptoms include diarrhea, fever, and abdominal cramps beginning 12 to 72 hours after the consumption of contaminated food or beverages (Shariat *et al*., 2013). More often, human *S.* Typhimurium cases are associated with the consumption of contaminated pork and beef, whereas *S.* Enteritidis cases are linked to eggs and poultry meat. Subclinical infection in animals leads to herd or flock contamination, with intermittent or persistent shedding of bacteria (EFSA, 2013).

In laboratory testing for *Salmonella enterica,* isolates are identified and typed by several phenotypic methods, including biochemical profiling, serotyping, and phage typing. Standard serotyping methods, based on the detection of somatic (O) and flagellar (H) antigens, are tedious and time-consuming. Although they lack the capacity to fingerprint strains in a sensitive manner, they remain useful in surveillance programmes (Herikstad *et al*., 2002).

More recently, different genotyping methods have been developed for the genetic discrimination of *Salmonella* isolates in outbreaks, such as multilocus sequence typing, multilocus variable-number tandem-repeat analysis, next generation sequencing. Pulsed-field gel electrophoresis (PFGE) was adopted for *Salmonella* surveillance and outbreak research in the 1990s. Because of its remarkable discriminatory power and high reproducibility, it has been successfully used in typing *Salmonella* strains isolated from human patients, foods and feed (Zou *et al*., 2013). Although PFGE is also labour-intensive, public health surveillance laboratories use it to determine strain relatedness and to confirm FBOs. Indeed, the US CDC recommends PFGE as the gold standard method for molecular characterisation in outbreak investigations.

In this paper it was reported the investigation of a household/domestic kitchen FBO of salmonellosis occurred in Biella on the 15th of January 2010 linked to homemade *lasagne*. Microbiological, serological, and molecular assays on *Salmonella* isolates from human and food samples were performed to determine the relatedness of the implicated *Salmonella* strains, identify the sources of infection, and trace the routes of *Salmonella* contamination in this circumscribed FBO. The study was conducted in collaboration between the Food Control Laboratory at the

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Institute for Experimental Veterinary Medicine of Piedmont, Liguria and Aosta Valley, Turin, the Biella Department of Public Health Prevention, and the Hospital of Biella *Ospedale degli Infermi*.

Materials and Methods

Epidemiological investigation

Four adults (1 man and 3 women; age range, 45-61 years) sought medical attention at the Emergency Room of the *Degli Infermi* Hospital, Biella because of gastrointestinal symptoms on the 16th of January 2010. From initial accounts, the physicians on duty at the time suspected a domestic FBO. All four persons were hospitalised, and the local Public Health and Prevention Department was notified. An epidemiological investigation was initiated. A standardised questionnaire was administered by the public health officials in order to obtain demographic and clinical data, history of recent travel, food and water consumption, exposure to animals, and other illness during the week before becoming ill. In the interviews, the patients stated that they had consumed a homemade baked *lasagne* dish consisting of boiled pasta in which cheese and tomato sauce are mixed and then cooked in the oven. Then, a mixture of raw eggs was added to homemade *lasagne* and left in switched off oven until the evening. Before eating, the homemade *lasagne* was reheated for a short time. In addition, the patients had also eaten salad, turkey meat, and cake.

People who consumed common meal on the 15th of January 2010 in Biella, who had gastro enteric symptoms from 15th to 16th of January, had not gastro enteric symptoms before the 15th of January 2010.

Microbiological investigation

Stool samples were collected from all patients. Only residues of the baked *lasagne* could be collected for sampling and subsequent analysis. The food sample was analysed to identify *Salmonella* spp.; the stool samples were tested for *Salmonella, Shighella* and *Campylobacter.*

Salmonella from the food sample was isolated according to ISO 6579:2002/COR 1:2004 (ISO, 2004). This method entails several steps: pre-enrichment in non-selective liquid medium (buffered peptone water; Biokar Diagnostic, Beauvais Cedex, France); enrichment in two selective liquid media [Rappaport-Vassiliadis medium with soya broth (Microbiol Diagnostici, Cagliari, Italy) and Muller-Kauffmann tetrathionate novobiocin – MKTTn – broth (LIOFILCHEM srl, Roseto, TE, Italy)]; and plating on xylose lysine deoxycholate agar (XLD) (Microbiol Diagnostici) and brilliant green agar (BGA) (Microbiol Diagnostici).

An internal method for detecting *Salmonella, Shighella and Campylobacter* was carried out on the stool samples. Specifically, for the isolation of *Salmonella* and *Shighella* have been used Hektoen Enteric Agar (Oxoid, Rodano, MI, Italy), Mac Conkey Agar (Oxoid) and *Salmonella Shighella* Agar (Oxoid); for the isolation of *Campylobacter* the stool samples were analyzed using *Campylobacter* Species Ag Rapid Test (Li StarFish S.r.l., Cernusco, MI, Italy) and *Campylobacter* selective agar (BioMérieux, Marcy l'Etoile, France).

All isolated strains were identified by conventional biochemical methods (API 20E; BioMériux) and the *Salmonella* spp. were serotyped according to the Kauffman-White-Le Minor classification scheme (Kauffmann, 1966; Le Minor and Popoff, 1987, Grimont and Weill, 2007).

The isolates were processed according to the PFGE protocol described by the US Centers for Disease Control and Prevention (CDC, 2013) using *Xba*I (Carlo Erba Reagents Srl, Cornaredo, MI, Italy) and *Bln*I (Roche Diagnostics Corporation, Indianapolis, IN, USA) restriction enzymes. PFGE using a single restriction enzyme (*Xba*I) is a standard method for genotyping *S.* Enteritidis (Dewaele *et al*., 2012). Thus, the ability to deduce the serotype of a *Salmonella* isolate based on its PFGE profile provides an alternative method for screening and identifying *Salmonella* serotypes (Zou *et al*., 2010).

The PFGE fingerprint patterns were analysed with BioNumerics software (version 7.1; Applied Maths, Sint-Martins-Latem, Belgium) and the PFGE patterns were normalised by interpolation to the nearest reference lane (*Salmonella* Braenderup strain ATCC H9812). For comparing the PFGE profiles, values of optimization of 1.0% and position tolerance of 1.0% were applied to both enzymes. Dice similarity coefficients were calculated on the basis of pairwise comparisons of the PFGE profiles. The complete linkage algorithm was used for dendrogram construction. Isolates were considered identical if their profiles showed 100% similarity.

Results

Epidemiological investigation revealed the main symptoms of nausea (50%), abdominal cramps (75%), diarrhea (100%), vomiting (100%), fever (100%), and low back pain (25%). The symptoms began 5 hours after eating the meal in the first case and 9 hours later in the last case. During the interview no history of recent travel, food and water consumption, exposure to animals, and other illness during the week before becoming ill was reported. A participant cooked the baked *lasagne* at the dinner and all the people ate the *lasagne*. The eggs were bought at the market.

Salmonella spp. was isolated in three patients (stool samples) and in the baked *lasagne* (residues stored in the refrigerator); one patient was tested negative for all pathogens. *S.* Enteritidis was identified by serotyping in the four isolated strains. Four *S.*

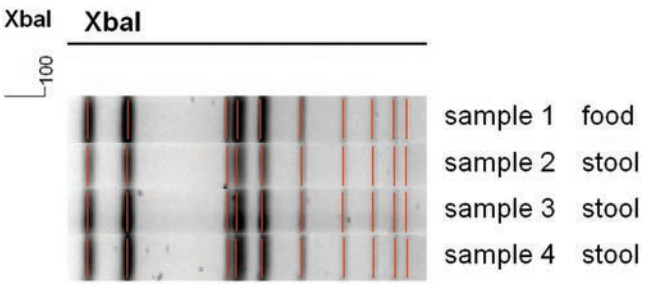

Figure 1. Single enzyme restriction analyses by *Xba*I distributed the four isolates into one cluster.

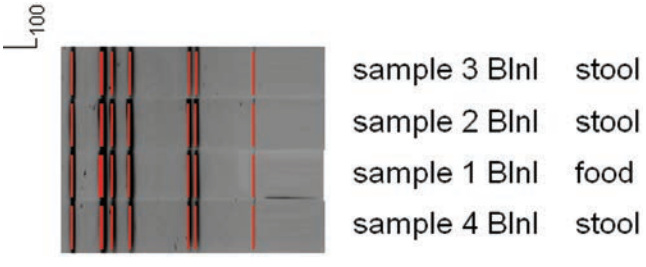

Figure 2. DNA digestion with restriction enzyme *Bln*I generated one cluster.

Enteritidis isolates were genotyped by PFGE using *Xba*I and *Bln*I restriction enzymes, which yielded consistent reproducible fingerprints. Single enzyme restriction analyses by *Xba*I distributed the four isolates into one cluster (Figure 1). DNA digestion with restriction enzyme *Bln*I also generated one cluster (Figure 2). Overall, the *S.* Enteritidis population appeared to be genotypically similar.

Discussion

S. Enteritidis is the major serovar associated with human salmonellosis linked to the consumption of contaminated poultry products, including eggs (Braden, 2006; Much *et al*., 2009). In 2013 207 strong evidence outbreaks were attributed to the consumption of eggs and egg products and 59.9% of FBOs were caused by *S.* Enteritidis (EFSA, 2015). A 2002-2003 US case-control study reported that a significant risk factor of *Salmonella* infections (OR 2.7, 95% CI 1.1-3.9) is consuming undercooked eggs or egg-containing dishes inside the home (Middleton *et al*., 2014). The household/domestic kitchen remains the most commonly reported setting in European FBOs (38.5%) followed by the public/commercial kitchens (restaurant, café, pub, bar, hotel categories) in 2013 (22.2%) (EFSA, 2015).

The domestic FBO outbreak described here was linked to the homemade *lasagne* contaminated with *Salmonella* spp. We identified a common source of infection by using PFGE, one of the most important molecular characterization methods of diagnostic testing to compare bacteria strains. How and at which point during food preparation the *lasagne* became infected with *Salmonella* cannot be definitively determined, but the data suggest opportunities for person to food, food to food, and equipment to food cross-contamination.

Generating a hypothesis about the likely sources, the most probable is that the eggs used in preparing the *lasagne* were probably contaminated with *Salmonella* Enteritidis and their adding after cooking process is responsible of the contamination of RTE product. Indeed the reheating process before consumption normally applied at low temperature is not enough to kill the bacteria and might even to promote the bacterial growth.

A plausible alternative hypothesis is that the *lasagne* was contaminated with *Salmonella* Enteritidis after cooking due to cross-contamination. Indeed, one of the most common causes of FBOs is cross-contamination during food handling due to the use of improperly washed utensils and handling by ill or healthy carriers.

Conclusions

Serotyping according to the Kauffman-White-Le Minor classification scheme and PFGE analysis allowed identifying the source of food poisoning in this domestic FBO. However, it was arduous to single out which ingredient of the meal was the source of *Salmonella* contamination. Collaborative efforts by the hospital physicians, public health department officials, and laboratories using different laboratory analysis methods during the outbreak investigation were essential for identifying the causative agent. Good sanitation, safe food handling and preparation practices in the entire food chain are key to preventing foodborne illness. Consumers should be made aware of the risks of FBO and learn how to minimise their chances of becoming ill by considering food safety at each step, from food purchase, to cooking, cleaning, and storing leftovers appropriately (International Food Information Council Foundation, 2014).

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