An Easter outbreak of *Salmonella* Typhimurium DT 104A associated with traditional pork salami in Italy

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Salmonella enterica is a common cause of gastrointestinal illness in Italy. *S.* Typhimurium accounts for approximately 40% of isolates, and most of these strains belong to the phage type DT104. We describe the investigation of an outbreak of *S.* Typhimurium DT104A, a subtype never observed before in Italy, which occurred in Rome during spring 2004. We conducted a matched case control study between 24 July and 9 September 2004. Controls were matched for age and area of residence. Each case had between one and four controls. Odds of exposure to potential risk factors and vehicles for the outbreak were compared between cases and controls. A multivariate analysis was conducted to estimate adjusted Odds Ratios. Sixty-three cases of *S.* Typhimurium DT 104A infection with onset between 1 April and 5 May 2004 were identified. Sixty-one were residents of Rome and two were residents of a neighbouring region. Twenty-six cases (43%) were enrolled in the study. Their median age was 7.5 years. Fourteen of 26 cases and 16 of 62 controls had eaten pork salami (OR= 25.5; 95% CI 1.6- 416.8). No food samples were available for testing. In northern Italy, two months prior to the outbreak, the veterinary surveillance system identified the first isolation of *S.* Typhimurium DT104A in a pig isolate. Both human and pig isolates showed indistinguishable PFGE patterns. It was not possible to trace the pig after the sample was taken at slaughter. The epidemiological evidence on the implication of pork salami in this outbreak suggests that pork products can also be a vehicle for salmonella in Italy and underlines the importance of good manufacturing practices for ready-to-eat foods. This investigation highlights the value of laboratory-based surveillance in identifying community-wide outbreaks of uncommon pathogens. It also underlines the need to improve surveillance timeliness, for promptly detecting outbreaks, undergoing field investigation, and implementing control measures. Moreover, our study shows the usefulness of integrated human and animal surveillance in tracing the possible source of infection.

Introduction

Approximately 10,000 human salmonella cases are notified every year to Italy's mandatory surveillance system of infectious diseases [1]. Circulation of salmonella serotypes is monitored by the laboratory-based surveillance system Enter-net Italy, which is coordinated by Istituto Superiore di Sanità (ISS) and is part of Enter-net, the European network for the surveillance of salmonella and verotoxigenic *E.coli* (VTEC) infections [2]. Enter-net Italy collects epidemiological and microbiological information on salmonella strains isolated in 41 reference laboratories from 15 of the 21 Italian Regions, with the aim of describing the nationwide circulation of different salmonella serotypes. In addition, surveillance involves veterinary laboratories that collect data on isolates from animals and food items of animal origin (Enter-Vet Italy) [3]. Moreover, a sub-sample of strains of *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) and serovar Enteritidis (*S.* Enteritidis) serotyped in the regional laboratories, have been sent to ISS to be phagetyped and genotyped [4,5]. These two serotypes are the most commonly isolated from human infections in Italy, accounting for approximately 80% of total strains.

In 2004, Enter-net Italy reported over 5,000 human salmonella isolates: 41% were *S.* Typhimurium. In the same year, Enter-Vet Italy accounted for 4,600 isolates with 22% belonging to *S.* Typhimurium serotype.

As in other European countries, most *S.* Typhimurium strains in Italy belong to the phage type DT104 [6,7]. Within this phage type there are numerous distinguishable subtypes, identified as A,B,C,H,L [7]. In Italy, most human strains isolated between 2001 and 2006 were 104L and H [6]. We describe the investigation of an outbreak of *S.* Typhimurium DT104A, a subtype never observed before in Italy, which occurred in Rome during spring 2004.

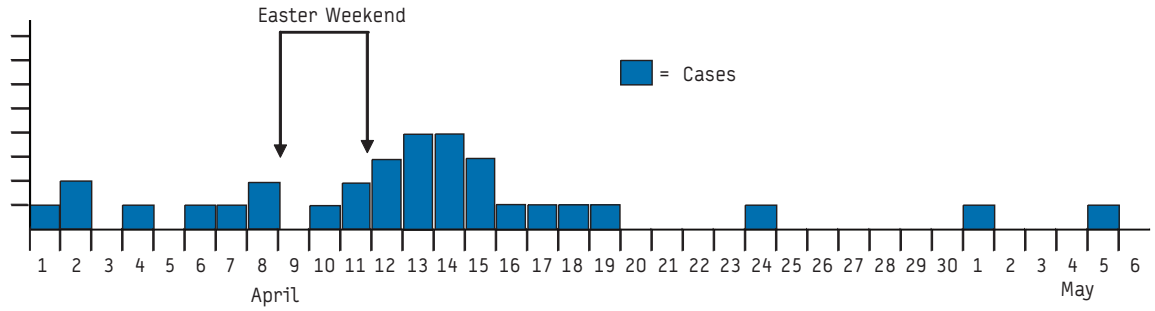
Methods

In June 2004, ISS typed 22 human isolates of *S.* Typhimurium as phage type DT104A [6]. The strains were sent by the Lazio regional references laboratory, and all were isolated by the laboratory of the Bambino Gesù Paediatric Hospital in Rome.

In order to verify if other cases related to the same serotype had occurred, in July 2004 ISS requested to laboratories participating in Enter-net Italy to send all the strains of *S.* Typhimurium isolated between 1 March and 1 June 2004. A request for information on DT104A *S.* Typhimurium strains eventually isolated in animals or food of animal origin were also sent to veterinary laboratories participating in Enter-vet.

FIGURE 1

Cases of *Salmonella* Typhimurium DT104a, by day of onset of symptom, Rome, 2004



***Salmonella* characterization**

Serotyping based on O and H antigens was performed according to the Kauffmann-White scheme [4]; phage-typing was performed in accordance with the methods of the UK's Health Protection Agency [5]. Susceptibility to 11 antimicrobial agents was assessed using the National Committee for Clinical Laboratory Standards (NCCLS) agar disk diffusion method [8]. Pulsed-field gel electrophoresis (PFGE) was performed after digestion of the DNA with XbaI according to a standardized protocol [9].

Matched case control study

In order to investigate risk factors for DT104A *S.* Typhimurium, a matched case control study was conducted between 24 July and 9 September 2004.

A case was defined as a person with a *S.* Typhimurium DT 104A infection, laboratory-confirmed between 1 March and 1 June 2004 in Rome. Demographic information on all cases was obtained from the Enter-net Italy database. We selected up to four matched controls for each case (assuming 25% exposure among controls, 80% power to detect a minimum Odds Ratio of 3.9, alpha error of 5%). We randomly selected controls from each case's general practitioner resident list matched for age (+/- 2 years), sex, and district of residence. Controls were excluded if they reported that they or any of their household members had experienced an episode of gastrointestinal illness (three or more loose stools in a 24-hour period, or vomiting, or abdominal pain) in the seven days prior to the onset of illness in the matched case.

Trained interviewers collected data using a structured questionnaire administered by telephone. The questionnaire collected information on clinical symptoms, food consumption during April 2004 (Easter month, with Easter falling on 11 April), travel (abroad and within Italy), contact with animals, and restaurants and food vendors visited. Interviewers made three attempts at different times of day to contact each case and the corresponding controls. If cases or controls were under 16 years old, parents or guardians were interviewed.

Statistical Analysis

All questionnaires were mailed to ISS, where the data were entered into an MSAccess 2000 (Microsoft, Redmond, Wash) database. Categorical variables were compared using the Chi^2 test; continuous variables were compared using the Wilcoxon Mann-Whitney test. In the univariate analysis, exposure to potential risk factors was compared between cases and controls calculating matched "Mantel-Haenszel" odds ratios (mOR), with exact 95% confidence intervals (95% CI).

A multivariate conditional logistic regression model was then performed to assess independent effects of the exposure variables and to estimate adjusted odds ratio (aOR); risk factors associated

TABLE

Matched univariate analysis (odds ratio: mOR) and multivariate conditional logistic regression analysis (adjusted odds ratio: aOR). Cases of *Salmonella* Typhimurium DT104A infection (n=26) and Controls (n=63) according to investigated risk factors, April-May 2004, Rome, Italy

			Univariate analysis			Multivariate analysis		
Risk factors*	Cases (%)	Controls(%)	mOR	95% CI	*p*-value	aOR	95% CI	*p*-value
Eating at a restaurant	2/25 (8)	17/48 (35)	0.1	(0.01-0.8)	0.03			
Consumption of:								
Crude eggs	5/26 (19)	3/63 (5)	3.4	(0.8-14.4)	0.1			
Sausage	7/25 (28)	33/63 (52)	0.2	(0.1-0.7)	0.01	0.04	(0.01-0.9)	0.04
Ham	3/25 (12)	1/63 (2)	8.9	(0.9-86.3)	0.06			
"Corallina" salami	14/23 (61)	16/63 (28)	4.4	(1.3-14.4)	0.02	25.5	(1.5 – 442.9)	0.03
Snacks	12/26 (46)	39/63 (62)	0.3	(0.1-1.0)	0.05			
Cow milk	12/26 (46)	57/63 (91)	0.1	(0.1-0.4)	<0.01			

**In the univariate analysis, only risk factors with p-value<0.20 are reported; in the multivariate analysis, only variables selected by the conditional logistic model according to a log-likelihood-ratio test for goodness-of-fit are reported*

with the outcome (P<0.20) in the univariate analysis, after testing for multicollinearity, were considered eligible to be included in the multivariate model, and retained in the final model, together with matching variables, according to a log likelihood-ratio test for goodness-of-fit. For each variable, the model excluded records with missing values. Analysis was carried out using STATA 8.2 (Stata Corp, College Station, Texas, US).

Results

Description of cases

A total of 242 *S.* Typhimurium strains were isolated from 1 March to 1 June 2004, and were collected by the Lazio regional reference laboratory in June 2004. Sixty-three (26%) of these strains belonged to DT104A; all were sensitive to the 11 antimicrobial agents tested.

Sixty-one isolates were from residents of Rome and two were residents of a neighbouring region (Umbria). All cases from Rome were distributed within the five districts of the municipality. Of the 63 patient with isolates of *S.* Typhimurium, 34 (54%) were male; the median age, available for 61 cases, was 7 years (range 1-78). Date of onset of symptoms was available for 32 patients (Figure 1) and ranged from April 1 to May 5 with a duration of symptoms of 1-30 days. The cases reported diarrhoea (93%), abdominal pain (73%), and fever (75%).

Matched Case Control Study

Of 61 cases identified in Rome, 35 (57%) could not be included in the case control study: 11 refused to participate, 10 could not be found because interviews took place over the summer period, and for 14 interviewed cases no controls could be identified. In total, 26 cases and 63 controls were enrolled in the study. The 26 cases included in the study did not statistically differ from the 35 cases who did not participate, in terms of sex (P=0.87), median age (7.5 years; P=0.16) and district of residence (P=0.32).

The matched univariate analysis revealed that cases were more likely than controls to have eaten "corallina", a fermented pork salami traditionally consumed during Easter in the Rome region. They were less likely to have eaten at a restaurant, to have eaten sausages or snacks, and to have consumed cow milk (Table).

In the multivariate conditional logistic regression analysis, to have eaten corallina become more strongly associated with illness (OR= 25.5; 95% CI 1.5- 442.9) while only to have eaten sausages (OR= 0.04; 95% CI 0.01-0.9) remained statistically inversely associated with illness.

Food investigation

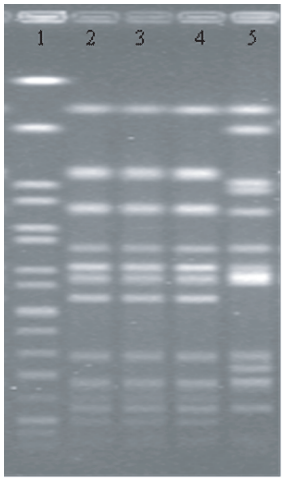
The epidemiological investigation could not identify a possible brand of corallina, as cases could not remember specific brands consumed. No samples of corallina were available for testing at the time of the study.

Veterinary data

Two months prior to the outbreak, the veterinary surveillance system Enter-vet identified the first isolation of *S.* Typhimurium DT104A in a pig isolate, among 1021 animal and food *S.* Typhimurium isolates. This strain came from the intestinal content of a pig slaughtered in north-eastern Italy (Veneto region) in January 2004 during a monitoring program on the presence of *Salmonella* in swine herds. Both human and pig isolates showed indistinguishable

FIGURE 2

Pulsed-field gel electrophoresis profiles of three strains (two from patients and one from a pig) of *S.* Typhimurium DT104A after digestion with XbaI (Line 1:Molecular reference marker '*S.* Braenderup strain H9812'; Lines 2,3: *S.* Typhimurium DT104A human isolates; Line 4: *S.* Typhimurium DT104A pig isolate; Line 5: *S.* Typhimurium DT104L with the common penta-resistance pattern)



PFGE patterns (Figure 2). It was not possible to trace the pig after the sample was taken at slaughter.

Discussion

This widespread outbreak of new emerging phage type of *S.* Typhimurium involving 63 cases was identified through laboratory-based surveillance using serotype and phage typing.

The pattern of the epidemic curve, the long period over which the reports of confirmed cases increased and the geographic distribution of cases supports the hypothesis that the outbreak was due to a common source rather than a point source (e.g. food served at a large gathering). The common source could likely be a food product with a long shelf life that was widely distributed across the Lazio region. We suspect that it may have been a ready-to-eat item that did not require cooking, since food-borne infection with *Salmonella* species can usually be prevented with adequate refrigeration and cooking temperatures, and proper handwashing and food preparation practices [10].

The most likely hypothesis supported by the findings of this epidemiologic investigation was that illness was associated with corallina salami. However, just over half of the cases reported eating corallina. There are a number of possible explanations for cases not reporting having eaten the implicated salami. Despite the questionnaire listing a series of food items, interviews took place two to five months after the outbreak period, so it is possible that some cases could not remember their precise food consumption during the period of exposure. Six of the cases who did not report eating corallina were children aged between two and eight years and parents or guardians who answered may not have been aware of all food items eaten during a holiday period associated with social gatherings.

A further limitation of this investigation was the low proportion of cases who were enrolled in the case control study (43%). Even if their demographic characteristics did not statistically differ from patients who did not participate, this could have caused a selection bias.

It should also be noted that consumption of sausages was inversely associated with disease onset, which could be due to the fact that cases who did eat corallina salami were less likely to eat sausages.

Corallina pork salami is a plausible vehicle for infection as pig herds are frequently infected with *Salmonella* in Italy [11,12]. Several studies have shown that contamination of pork sausages with salmonella is common [13-16]. Salami are dry fermented sausages traditionally considered safe due to low pH, low water activity and high salinity, but *Salmonella* can survive fermentation and drying steps if the manufacturing process or fermentation periods are inadequate [15]. Survival of organisms in ready-to-eat products has the potential to cause illness, and salami has been previously identified as the food vehicle for *S.* Typhimurium in two geographically widespread outbreaks in northern Italy (PT 193) and England (definitive type 124) [12-13]. It is reasonable to assume that the corallina salami was commercialised before the optimal fermentation period, because of the high demand for this particular item during Easter banquets in the Lazio region.

A limitation of laboratory-based surveillance is that detailed microbiological analysis is performed on a periodic basis with a delay in recognising uncommon strains. Delays also occur when local laboratories wait to send in ISS samples or strain data until the end of the month or when there are an adequate number of samples.

In this outbreak, cases occurred during Easter, outbreak detection was in June and investigation was conducted during summer, the most difficult period to trace people for interview. As a result, no samples of the suspected salami were available for testing. We could not identify the brand, as cases could not remember which brands had been consumed. Furthermore, the 63 cases identified are probably an underestimation of the outbreak.

In conclusion, although food safety can be assured by good manufacturing practices and standards, the effectiveness of these practices should be monitored by sensitive surveillance of human cases, especially when dealing with the production of ready-to-eat foods. This outbreak highlights the need for timely surveillance and work is now underway to develop an online surveillance system in Italy enabling laboratories to input strain data immediately into the Enter-net Italy database. Finally, the usefulness of integrated human and animal surveillance was clear during this investigation, and is in line with the recent European directive [10].

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