

A community-based outbreak of *Salmonella enterica* serotype Typhimurium associated with salami consumption in Northern Italy

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SUMMARY

An outbreak of *Salmonella enterica* serotype Typhimurium belonging to phage type (PT) 193 occurred in autumn 1995 and involved 83 individuals in a large area of Northern Italy (Lombardy Region). Epidemiological and microbiological investigations of strains isolated from clinical and food specimens revealed that the vehicle was a batch of salami, produced on 4 September 1995 by a local firm. The outbreak was contained when the batch was withdrawn from sale. Insufficient ripening of the salami had allowed the salmonella to survive, emphasizing the need to define criteria for the ripening process of foods such as salami.

INTRODUCTION

Non-typhoid salmonellosis is a foodborne disease of primary concern in developed countries [1–4]. The spread of non-typhoid salmonella, which is favoured by the wide array of animal reservoirs [1, 5] and by the wide commercial distribution of both animals and food products, is one of the major public health problems in terms of socio-economic impact [6]. The implementation of surveillance systems with the capacity to identify the onset of an outbreak and to limit its spread is essential to the prevention of these infections. Nevertheless, recognizing and investigating foodborne-disease outbreaks of non-typhoid salmonella can be difficult, especially when the epidemic focus is community-based and the outbreak is due to

commonly consumed foods and/or involves frequently occurring serotypes [7]. Microbiological analysis using phenotypic and genotypic markers of strains isolated from infected individuals and/or from suspected food sources is extremely useful in testing epidemiological hypotheses [8, 9]. These methods have been used for various *Salmonella enterica* serotypes, and they are very useful for the two most frequent serotypes: *S. Enteritidis* and *S. Typhimurium* [10, 11]. *S. enterica* serotype Typhimurium represents the most commonly observed serotype, at least in the so-called ‘interepidemic periods’, that is, before and after the appearance of *S. enterica* serotype Wien in the mid-1970s and until the emergence of the ‘pandemic’ of *S. enterica* serotype Enteritidis in the second half of the 1980s [4]. In Italy, according to data (unpublished) from the Istituto Superiore di Sanità (ISS) (National Health Institute), *S. enterica* serotype Enteritidis and *S. enterica* serotype Typhimurium represented 60·1 %

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of the 12310 salmonella strains isolated from humans in 1995 (37.2% for *S. Enteritidis* and 22.9% for *S. Typhimurium*).

This paper illustrates the effectiveness, in terms of disease prevention, of the epidemiological intervention owing to the early recognition, by CEPIS†, of this outbreak, which occurred in the Lombardy Region (23858.7 km²) (Northern Italy) in October 1995 and was caused by a batch of salami contaminated with *S. enterica* serotype Typhimurium.

The epidemiological and microbiological investigations were begun after several reports of outbreaks were received by the Regional Health Authority of Lombardy from two different sources. On 18 October 1995, CEPIS reported the occurrence of two *S. enterica* serotype Typhimurium outbreaks in areas serviced by two Local Health Units‡ (LHU) south of the city of Milan; on 20 October 1995, one of these units reported three 'gastroenteritis' outbreaks. The events were probably associated with salami, which is widely consumed in Italy and which is considered to be safe for consumption, though the literature shows that pathogens such as salmonella can survive in dried sausages such as salami. In fact, the preparation of dried sausages is difficult to control and the product can undergo, sometimes for more than 90 days, a series of biochemical and microbiological degradations. Total inhibition of Salmonella, as with other pathogens, is normally achieved within 30 days [12], yet given the slowness of the process, it is more likely to be ensured when initial contamination is not high [13].

The present investigation was begun after the rapid identification of the salami responsible for the cases observed in one of the LHUs and after the isolation of *S. enterica* serotype Typhimurium [referred to as 'S. Typhimurium', according to Le Minor and Popoff (14)], in leftover salami produced on 4 September 1995. As a consequence, the Regional Health Authority withdrew from sale the batches of salami produced on or after 1 September 1995 by the VV & C company.

METHODS

Epidemiological investigation

The Public Health Services of the LHUs of Lombardy

† CEPIS (Centro Enterobatteri Patogeni per l'Italia Settentrionale) (Enteric Pathogen Reference Laboratory for Northern Italy) is one of the three reference centres in Italy which participates in the Salm-Net programme and which closely collaborates with the ISS.

‡ Local Health Unit: it represents the district-level in the public health surveillance system.

were asked to examine carefully the cases of non-typhoid salmonellosis occurring after 30 September 1995 and to collect personal and epidemiological information on laboratory-confirmed cases. In the areas with the most widespread distribution of VV & C products, active case-finding was conducted using the following definitions:

(i) *exposed individual*: an individual who, in the period 1–31 October 1995, consumed salami purchased from retailers included in the list of clients of the VV & C company and/or produced by the same manufacturer (products hereinafter referred to as 'VV & C salami');

(ii) *probable case*: an individual consumed VV & C salami in the period 1–31 October 1995 and who presented at least one of the following signs: fever, diarrhoea, vomiting, within 72 h of consumption of the salami;

(iii) *confirmed case*: an individual who meets the criteria for a probable case and from whom *S. Typhimurium* was isolated from a stool sample or an asymptomatic individual who consumed VV & C salami in the period 1–31 October 1995, and who was epidemiologically linked to a symptomatic confirmed case and with the presence of *S. Typhimurium* in a stool sample.

The information on cases was acquired using a special data-collection form. Rates were based on the 1991 population census. A case-control analytic study was not conducted because of both logistic problems and the excessive homogeneity of risk factors hypothesized for cases and controls: the consumption of salami was very common during the period of the outbreak in this specific geographic area. It would have been even more difficult to determine the brand of salami consumed.

The Veterinary Services of the study area carried out a thorough investigation of the production process at the VV & C factory, with particular reference to the batch of salami produced on 4 September 1995.

Microbiological analyses

Microbiological analyses were performed on the strains of *Salmonella enterica* serotype Typhimurium, isolated from both clinical and food specimens. Sixty-six strains were isolated from stool samples, 4 strains were isolated from samples of left-over salami and 14 strains were isolated from samples of various types of dried sausages belonging to 200 batches withdrawn from sale. Overall, 84 isolates of *S. Typhimurium*, identified at the hospital and public health labora-

tories, were sent to CEPIS for the following additional analyses:

(i) *phage typing*: the phage typing was conducted on the 84 strains adopting the method described by Anderson and colleagues (15), using phages prepared by the WHO Reference Center for Phage Typing (Colindale, London, UK) and received through the ISS;

(ii) *ribotyping*: for 81 of the 84 strains molecular analysis was conducted at the Enteric Pathogen Reference Laboratory of the University of Palermo; the procedures used were those indicated by Grimont and Grimont [16]. DNA was digested with *HincII* restriction endonuclease.

RESULTS

Epidemiological investigation

The investigation identified 83 probable cases (40 males) among the 133 exposed individuals, with an attack rate of 62.4%. Of the 79 cases where the birth date was known, 26 were less than 14 years old. Twenty-one cases (25.3%) were hospitalized. Microbiological confirmation was obtained for 60 probable cases and for 5 individuals exposed and epidemiologically linked to one of the previous cases. Thus 65 individuals were considered as confirmed cases.

The epidemic curve (Fig. 1), constructed using the date of onset of signs in probable cases, shows that the cases were concentrated in the second and third weeks of October. Only four cases purchased and consumed the salami after 20 October, the date of its withdrawal from sale. No cases occurred after 31 October.

More than two thirds (56 of 83) of the probable cases lived in areas serviced by the two LHUs where active case-finding was conducted. Cases of non-typhoid salmonellosis not associated with this outbreak were also detected by the case-finding in these areas, and the rates of these non-outbreak associated cases were markedly higher compared to the rest of the region, especially for group B salmonella (Table 1).

Investigation of the VV & C factory

The VV & C factory is a family-run business that produces both ripe and fresh sausages. The factory, recently built, has since 1993 used equipment and facilities judged to be satisfactory according to the

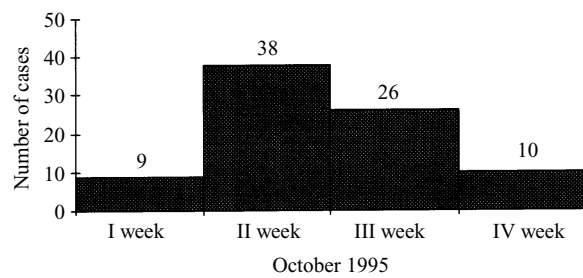


Fig. 1. Lombardy Region. Outbreak of *S. Typhimurium* by VV&C salami. Epidemic curve of possible cases

Table 1. Lombardy Region. Last quarter of 1995. Non-typhoid salmonellosis cases not associated with the outbreak by VV & C salami

	Spatial distribution	
	Case finding area	Rest of Lombardy Region
Non-typhoid salmonellosis cases		
Overall cases*		
Number	60	672
Rate/100000	18.0	7.9
Sporadic cases		
Number	46	535
Rate/100000	13.8	6.3
Salmonella B overall cases*		
Number	42	312
Rate/100000	12.6	3.7
Salmonella B sporadic cases		
Number	34	277
Rate/100000	10.2	3.3

* Sporadic and epidemic cases are included.

standards of the European Union. The production process, which follows a weekly cycle, was reconstructed. The fresh products are marketed after 48 h in the drying room; the ripe products, after being dried for 24 h, are matured for either 15 days ('cacciatori') or for 45 days ('salame'). For the batch of salami produced on 4 September 1995, the following was revealed:

(i) the raw meat used was provided by two large pork slaughterhouses located in the Lombardy Region, which are regular suppliers to the VV & C company;

(ii) the salami was produced following the usual methods: cleaning and dissection of the meat upon arrival and storage at 2 °C (4 September); grinding, salting, seasoning, sacking and cold storage for the next 24 h (5 September); binding and drying at 23 °C (6–8 September) and at 21 °C (until 11 September);

placement in the ripening room at 15 °C, with 85% humidity (12 September);

(iii) the ripening process had been discontinued before 45 days had passed. In fact, the product was confirmed to have been marketed on 7 October, after only 25 days of ripening;

(iv) during production, microbial starters are not used to accelerate the salami fermentation.

Microbiological analyses

Phage typing and ribotyping were conducted on strains from 66 infected individuals and from 18 food samples. PT 193 was the most frequently identified phage type: in 38 of the 39 confirmed cases (97.4%), in 18 of the 27 cases not meeting the confirmed-case definition (non-cases) (66.7%), in 4 samples of left-over salami, and in 8 samples of salami produced on 4 September and belonging to the batches withdrawn from sale.

Excluding the 3 strains with undetermined ribotype, 7 different ribotypes were identified: the most frequently observed was indicated by the number 14; it was identified in all the confirmed cases, in 10 of the 27 non-cases (37.0%) and in 10 samples of salami, produced on 4 September, consumed or withdrawn.

Finally, we identified the following 10 combinations of phage type-ribotype (the first number indicates the phage type and the second the ribotype):

(i) '193-14': This combination was observed in 38 confirmed cases and in 10 food samples of salami produced on 4 September, consumed or withdrawn; it was also identified in 10 non-cases: 7 of these non-cases, though not meeting the confirmed-case definition, were probably related to the outbreak. In fact, some lived in the same household as confirmed cases, and others had consumed sausages other than salami but purchased from retailers who were VV & C clients;

(ii) '104-14': this strain was isolated from one asymptomatic confirmed case; it represents the only exception, that is, the only strain linked to the present outbreak with a different phage type;

(iii) '193-21', '193-2', '193-55', '12-21', '22-7', '104-8', '104-13': these strains were isolated from 16 individuals who did not meet the case definition (unknown means of infection or consumption of various types of cold-cuts, of unknown origin or purchased from retailers who were not VV & C clients) and from 6 samples of various VV & C

sausages, belong to the withdrawn batches and produced after 4 September.

Two observations can be drawn from the above list:

(1) Strains of the phage type 193 were attributed to 4 different ribotypes, whereas ribotype 14, with a single exception, was always linked to phage type 193.

(2) Of the 58 strains of phage type 193 and ribotype 14, only in three cases was this strain isolated from individuals not associated with the present outbreak.

DISCUSSION

Identification of this outbreak was initially difficult, for the following reasons:

(1) the Typhimurium serotype has a worldwide distribution; it is widespread in animal reservoirs and is frequently observed in human cases of infection.

(2) the outbreak was associated with a commonly consumed food.

The literature mainly describes outbreaks characterized by rare or unusual serotypes of salmonella and/or by strains spread through unusual vehicles [7, 17–19]. Furthermore, this event did not occur in a closed or defined community, but consisted of small outbreaks in different areas of Lombardy, with no clearly apparent connection.

The investigation allowed us to confirm the association between the occurrence of cases of salmonellosis and the consumption of salami produced by VV & C on 4 September. Specifically, this assumption was verified by the following findings:

(i) the last cases occurred on 29 October, 9 days after the withdrawal from sale of salami batches produced beginning on 1 September, and only 4 of these cases reported having purchased the salami after 20 October. Thus, the withdrawal imposed by the Regional Health Authority was effective in preventing the further spread of the outbreak;

(ii) the microbiological analyses assigned to the same phage type-ribotype (193–14 "epidemic-type") 38 of the 39 strains isolated from confirmed cases and the 10 strains identified in the samples of VV & C salami produced on 4 September, among the strains isolated from food specimens; the only exception was represented by a strain isolated from an asymptomatic confirmed case, with a different phage type (104) but with the same ribotype (14) as the 'epidemic-type';

(iii) of the 10 non-cases characterized by the 'epidemic-type', 5 can be explained by a cross-contamination between the VV & C salami and other

sausages at the vendor and 2 by person-to-person transmission;

(iv) the number of combinations of phage type-ribotype identified among the 27 non-cases is very high ($n = 9$) when compared to the two combinations observed in the 39 confirmed cases.

Our investigation demonstrates that the main risk factor appeared to be the reduced ripening time, which had been shortened because of a strong market demand. This arbitrary reduction compromised the inhibition of pathogens, normally obtained by acidification, which can be accelerated by microbial starters and which is influenced by many factors: the bacterial species, producing lactic acid, present in the mixture; the ratio of fermentative microorganisms and pathogens; the temperature; the quantity and the speed of lactic acid production; the salting (inhibition when $> 6\%$) and the a_w (inhibiting when < 0.94) [20]. The literature reports the presence of salmonella in 17% of the salami samples of 'medium ripening' and *S. enterica* serotype Choleraesuis in 'cacciatori' salami at up to 50 days into the ripening process (20, 21), as well as the finding of a *S. Typhimurium* strain, responsible for an outbreak in England, also with a $pH < 5.2$ [22]. More recently, studies conducted in Washington State and in California reported the epidemic diffusion of a strain of *E. coli* O157:H7 through the consumption of 'dry fermented salami'; the investigations showed that *E. coli* O157:H7 can survive the current processes of salami production (use of microbial starters, 88 h in the fermentation room and 36 days in the drying room) (23). In our case, the lack of microbial starters and the shortening of the ripening process may have contributed to the survival of salmonella in the batch of VV & C salami produced on 4 September, because of insufficient acidification.

The source of contamination could not be ascertained, but it should be mentioned that other manufacturers in the area were not involved in this outbreak, despite the fact that they used the same suppliers for raw material.

In conclusion, this study revealed various points of interest: (1) the surveillance system, though imperfect, benefited from the collaboration among persons working in different fields (health care workers, veterinarians and microbiologists) in that it allowed for the early recognition of a community-based outbreak caused by a very common pathogen and carried by a largely consumed food; (2) the identification of the food vehicle and its immediate with-

drawal from sale was definitely effective in terms of disease prevention; (3) the typing methods greatly contributed to the study of the outbreak; (4) the microbiological results suggest that the phage typing/ribotyping combination has a greater discriminating power in comparison with phage typing alone, confirming the data in the literature [24]; and (5) the identification of the insufficient salami ripening highlights the fact that this type of food can be a vehicle of infection; thus the duration of fermentation and the consequent marketing of 'ripe' products, such as salami, should be defined with greater precision by proper guidelines.

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