

## Foodborne outbreaks caused by salmonella in Italy, 1991–4

G. SCUDERI<sup>1</sup>\*, M. FANTASIA<sup>2</sup>, E. FILETICI<sup>2</sup> AND M. P. ANASTASIO<sup>2</sup>

<sup>1</sup> *Laboratory of Immunology, Istituto Superiore di Sanità, Rome, Italy*

<sup>2</sup> *Laboratory of Bacteriology and Medical Mycology, Istituto Superiore di Sanità, Rome, Italy*

(Accepted 8 January 1996)

### SUMMARY

This report summarizes studies on 1699 foodborne outbreaks, in Italy, reported to the *Istituto Superiore di Sanità* (ISS) (the National Institute of Health of Italy, Rome) during the period 1991–4. The most frequently reported foodborne outbreaks were caused by salmonellae (81%), in particular by *Salmonella enteritidis* and non-serotyped group D salmonella (34% and 33% of the total salmonella outbreaks, respectively). A vehicle was implicated in 69% of the salmonella outbreaks; eggs were implicated in 77% of the outbreaks for which a vehicle was identified or suspected. Salmonella strains isolated in 54 outbreaks were studied for phenotypic and genotypic characteristics. The isolates belonged to *S. enteritidis* (50 outbreaks), *S. typhimurium* (three outbreaks) and *S. hadar* (one outbreak). In the *S. enteritidis* outbreaks, phage type 4 was most frequently isolated (64·8%), followed by phage type 1 (14·8%). The virulence plasmid of 38 megadaltons was found in many different phage types of *S. enteritidis*.

### INTRODUCTION

In the wide spectrum of enteric infections, non-typhoidal salmonellosis has become of increasing importance in recent years in many European countries and in the US. Specifically, in the US, salmonella isolation rates for humans grew from 7 per 100 000 population in 1955 to 17 per 100 000 in 1994, peaking in 1986 (27 per 100 000) [1]. In the UK, salmonella, in laboratory surveillance, was the second most commonly reported microorganism in gastroenteric diseases in 1989 [2]; the number of salmonella isolates from humans rose from 10 761 in 1980 to 22 627 in 1991 [3] and to 23 367 for the period January–September 1994 [4]. Furthermore, according to laboratory surveillance of bacterial foodborne diseases in the UK, the serotypes most commonly reported from 1986 to 1988 were *S. enteritidis* (22 189), *S. typhi-*

*murium* (17 350), and other salmonellas (14 729), for a total of 54 268 [5].

A similar situation has been reported for foodborne outbreaks. In the UK, again in the years 1986–8, the number of foodborne outbreaks by cause was as follows: 477 by *S. enteritidis*, 432 by *S. typhimurium*, and 406 by other salmonellas [5], accounting for 84·2% of the foodborne outbreaks due to bacterial causes. In the period 1989–91 the number of salmonella outbreaks rose to 2766 (2374 family outbreaks and 392 general ones), the majority of which were due to *S. enteritidis* phage type 4 (1389) [4]. In the US there were 430 reported outbreaks of *S. enteritidis* during the 8-year period 1985–92 [6]: these outbreaks were caused by highly virulent organisms, resulting in many deaths [6].

In Italy, salmonella surveillance is carried out using two different systems:

(1) The Laboratory Surveillance System of Enteropathogenic Bacteria, which surveys enteropathogenic bacteria isolated from humans, animals, and foods [7]. This system consists of local laboratories which isolate

\* Correspondence and requests for reprints: Dr Gabriella Scuderi, Laboratorio di Epidemiologia e Biostatistica, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy.

salmonella strains and are responsible for their biochemical identification and serotyping; a central laboratory completes the phenotypic and genotypic characterization of the isolates.

(2) The National Infectious Disease Reporting System [8], which was set up in 1991 and which introduced compulsory notification, on the part of Local Health Units (LHU), using five types of reports, among which are: (a) class II notifications, for single cases of certain infectious diseases (ICD-9), including salmonella infections, and (b) class IV notifications, for outbreaks including foodborne diseases.

In Italy, isolation of salmonella from humans rose from 5759 (10.2 per 100 000) in 1987 to 13 600 (24.0 per 100 000) in 1993 (Fantasia, unpublished data). *S. enteritidis* in humans, which had been one of the five most commonly isolated serotypes since 1973, became the most frequently isolated in 1989, surpassing *S. typhimurium*. *S. enteritidis* isolates from humans ranged from 2.3% in 1982 to 7.4% in 1988 [9]; in 1992 it accounted for 57.1% of human and 22.8% of food isolates [10]. The statutory notification of single cases of salmonellosis, by class II forms, peaked in 1992 at 23 348 cases, declining to 20 385 and 21 350 in 1993 and 1994, respectively [11].

In light of the continued interest in salmonellosis infections both in Italy and worldwide, we analysed information on foodborne outbreaks due to salmonella, notified, using the class IV form, from 1991 through 1994, to the Infectious Disease Unit of the Laboratory of Epidemiology and Biostatistics (LEB) of the *Istituto Superiore di Sanità* (ISS) (the National Institute of Health of Italy), which is one of the central structures of the National Infectious Disease Reporting System. We also analysed epidemiological and microbiological characteristics of strains, isolated from some of the outbreaks, which were sent for phenotypic and genotypic characterization to the Pathogenic Enterobacteria Unit of the Laboratory of Bacteriology and Medical Mycology (BMM) of the ISS, which is the central laboratory of the Laboratory Surveillance System of Enteropathogenic Bacteria.

## METHODS

### Epidemiological methods

All class IV outbreak forms coming from LHUs were collected by the Infectious Disease Unit of the Laboratory of Epidemiology and Biostatistics, at the ISS, Rome. An outbreak of foodborne infection was

defined as two or more cases of gastrointestinal illness among persons who shared a common exposure.

The information requested on the class IV form included: date of notification, date of onset of symptoms of the first case, duration of the outbreak (days between the date of onset of the first and last case), place of the outbreak, outbreak setting (family or private home, restaurant, hospital, nursing home, etc.), number of persons exposed, number of cases diagnosed in the outbreak, aetiological agent and how it was identified (i.e. confirmed by bacteriological criteria or suspected on the basis of clinical criteria), and vehicle and how it was identified (i.e. confirmed by bacteriological analysis of the leftover foods or suspected on the basis of the statistical association, without microbiological confirmation).

Notification forms were transcribed in an electronic data base analysed by the statistical programme EPI INFO version 5.1 b [12].

### Microbiological methods

A total of 477 salmonella strains isolated from 54 outbreaks were sent to the Laboratory of Bacteriology and Medical Mycology, Pathogenic Enterobacteria Unit, at the ISS, for phenotypic and genotypic characterization. Serological typing was carried out by a slide-agglutination method using commercial sera (Behring; Biogenetics). Susceptibility to antibiotics was performed by the agar diffusion method [13] using a standard disk of ten antibiotics.

The phage type (PT) of *S. enteritidis* and *S. typhimurium* was determined by the method of Ward and colleagues [14] using phage sets obtained from the WHO Reference Center for phage typing (Colindale, London, UK). Plasmid DNA was extracted by the alkaline lysis method [15] and was visualized in horizontal agarose gel electrophoresis at 100 V for 3 h.

## RESULTS

### Epidemiological description

During the period 1991–4 a total of 1699 foodborne outbreaks due to any etiological agent were notified to the LEB, ISS. Of these, 1379 (81%) were due to salmonellae; 11% were of unidentified etiology. The remaining 8% of reported foodborne outbreaks were

Table 1. Foodborne outbreaks and number of cases caused by salmonella by serogroup, and year of notification, Italy 1991–4

Year	Serogroup					Salm. spp.	Total number of outbreaks (%)	Number of cases
	A	B	C	D	E			
1991	1	12	1	118	—	66	198 (14.4)	1775
1992	2	59	9	401	—	81	552 (40.0)	3495
1993	—	33	8	217	2	48	308 (22.3)	2209
1994	—	40	9	205	5	62	321 (23.3)	4291
Total	3	144	27	941	7	257	1379 (100.0)	11770
(%)	(0.2)	(10.4)	(2.0)	(68.2)	(0.5)	(18.6)	(100.0)	

Table 2. Notifications of foodborne outbreaks caused by salmonella by serogroup/serotype, Italy, 1991–4

Serogroup/type	Outbreaks			Number of cases
	Number	Serogroup (%)	Total (%)	
Salmonella group A not serotyped	3	100	0.2	57
Salmonella group B not serotyped	93	64.6	6.7	526
<i>Salmonella brancaster</i>	1	0.7	0.07	24
<i>Salmonella bredney</i>	2	1.4	0.15	5
<i>Salmonella paratyphi B</i>	1	0.7	0.07	4
<i>Salmonella typhimurium</i>	47	32.6	3.4	403
Salmonella group C not serotyped	21	77.8	1.5	70
<i>Salmonella bovis morbificans</i>	1	3.7	0.07	3
<i>Salmonella braenderup</i>	1	3.7	0.07	5
<i>Salmonella cholerae suis</i>	1	3.7	0.07	2
<i>Salmonella infantis</i>	2	7.4	0.15	19
<i>Salmonella ohio</i>	1	3.7	0.07	2
Salmonella group D not serotyped	456	48.5	33.2	2955
<i>Salmonella enteritidis</i>	473	50.3	34.4	5650
<i>Salmonella gallinarum</i>	1	0.1	0.07	3
<i>Salmonella panama</i>	6	0.6	0.4	18
<i>Salmonella typhi</i>	5	0.5	0.4	11
Salmonella group E not serotyped	7	100	0.5	65
<i>Salmonella</i> spp.	257	100	18.6	1948
Total	1379	100	100	11770

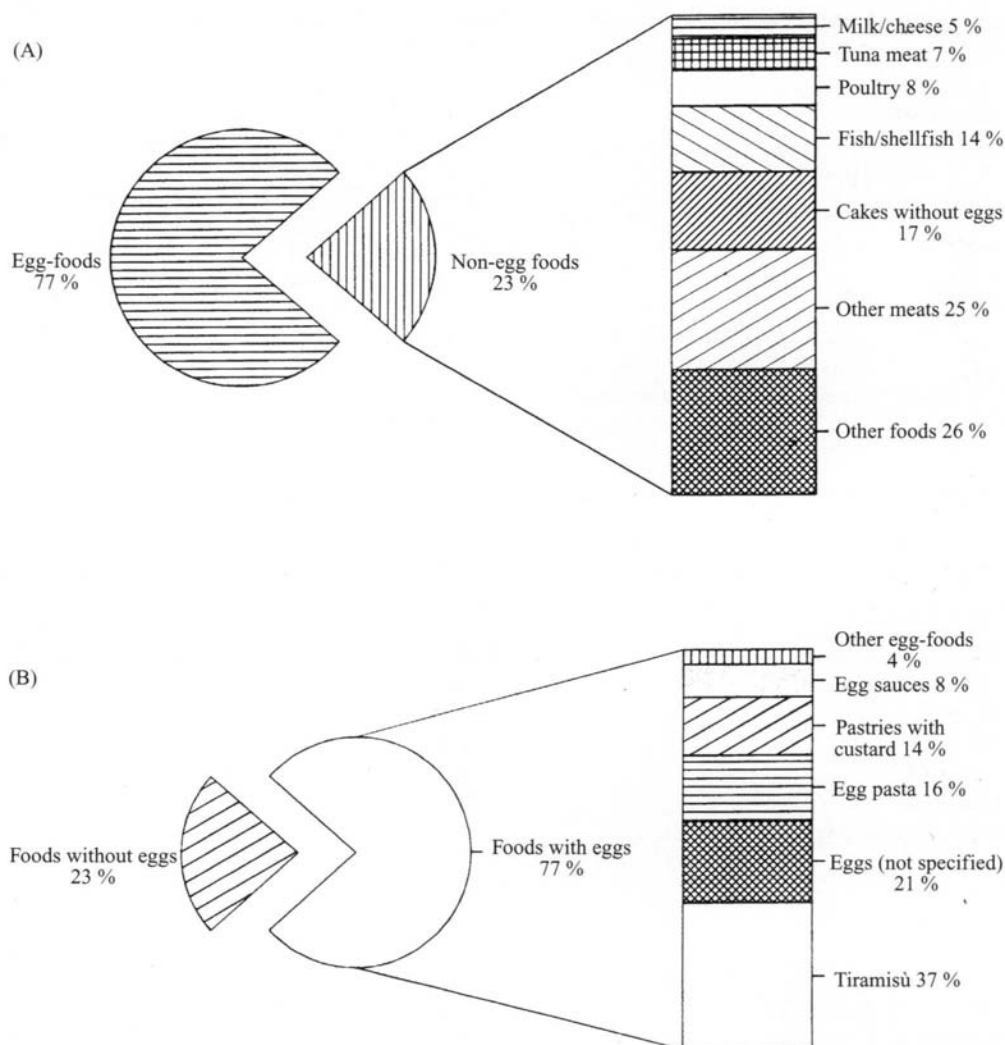
due to *staphylococci* (57 outbreaks), biotoxins (33), *Cl. perfringens* (19), *V. parahaemolyticus* (5), *B. cereus* (4), *brucellae* (3), *Cl. botulinum* (3), *E. coli* (2), *shigellae* (2), *Listeria monocytogenes* (1), viruses (7), and parasites (2).

The results reported below refer only to the 1379 foodborne outbreaks caused by salmonella; the number of these outbreaks peaked in 1992 (552), though many continued to occur in 1994 (321) (Table 1). The number of individual cases involved in outbreaks during 1994 (4291) was higher than that in the previous years.

Class IV forms were received from 17 of Italy's 20

geographic regions. The region of Emilia Romagna accounted for 55% (760) of the salmonella outbreaks notified. The other regions that also reported a high number of salmonella outbreaks were: Lombardia (142), Toscana (115), Trentino Alto Adige (111), Friuli Venezia Giulia (59), Marche (37), Liguria (35), and Umbria (31). The rate of notification in these regions remained stable over the 4-year period considered.

Serogroup D accounted for 68.4% of the notified outbreaks, followed by serogroup B (10.4%) and serogroup C (1.9%) (Table 1); serogroups E and A were rarely observed. In 18.6% of the notified



**Fig. 1.** Notification of salmonella foodborne outbreaks (946 notifications with vehicle identified or suspected) by vehicle in Italy, 1991–4 part A: distribution by non-egg containing foods; part B: distribution by egg-containing foods.

outbreaks, serogrouping of the salmonella isolates was not carried out, and strains were classified as *Salmonella* spp. It is noteworthy that serogroup D was notified particularly during 1992, with 401 foodborne outbreaks, as compared to 217 outbreaks in 1993 and 205 in 1994.

The distribution of outbreaks by serogroup and serotype is shown in Table 2. *S. enteritidis* accounted for more than 50% of the notified outbreaks caused by strains of serogroup D and for 34% of all notified salmonella outbreaks.

In 19% of the outbreaks, the vehicle was confirmed by bacteriological analysis, while in 50% the vehicle was only suspected. In the remaining 31% the vehicle was neither identified nor suspected.

The distribution, by type of food, of the 946 notifications with identified or suspected vehicles is

illustrated in Fig. 1. In 77% of the outbreaks, the implicated vehicles were egg-containing foods. For the remaining 23% of outbreaks, vehicles included: poultry and other kinds of meat (such as beef, lamb, and pork); cakes without eggs; fish/shellfish; milk/cheese; and tuna meat (veal with tuna sauce), which may or may not contain eggs (Fig. 1, part A).

The distribution by vehicle of the 726 outbreaks due to egg-containing foods is illustrated in Fig. 1, part B. Of these, the most frequently implicated food was 'tiramisù', a dessert made with raw eggs, which accounted for 37% of these vehicles, followed by shell-eggs or eggs in an unspecified form (21%), egg-pasta (16%), pastries filled with custard (14%), various foods with egg-sauces (8%), and other egg-containing foods (4%).

The rate of isolation for microbiologic confirmation

Table 3. Distribution of food vehicles for salmonella outbreaks by serotype, 1991-4

Vehicle	Serotype					
	<i>S. enteritidis</i>		<i>S. typhimurium</i>		Other serotypes	
	Number	Percent	Number	Percent	Number	Percent
Egg-containing foods	273	(57.7)	19	(40.4)	5	(22.7)
Cakes w/o eggs	8	(1.7)	0	(—)	0	(—)
Pasta w/o eggs	2	(0.4)	0	(—)	0	(—)
Poultry	10	(2.1)	0	(—)	0	(—)
Tuna meat	4	(0.8)	0	(—)	0	(—)
Other meats	14	(2.9)	3	(6.4)	5	(22.7)
Milk/cheese	3	(0.6)	2	(4.2)	0	(—)
Fish/shellfish	8	(1.7)	4	(8.6)	2	(9.1)
Other foods	13	(2.7)	3	(6.4)	0	(—)
Non-identified foods	138	(29.2)	16	(34.0)	10	(45.5)
Total No. of outbreaks	473	(100)	47	(100)	22	(100)

Table 4. Distribution of salmonella foodborne outbreaks by setting, Italy 1991-4

Setting	Outbreaks		Number of cases
	Number	(Percent)	
Family/private homes	1021	(74.0)	4009
Public eating places	194	(14.0)	3195
Restaurants	126	(9.1)	1927
Rotisseries	10	(0.7)	70
Hotels	39	(2.8)	920
Pastry shops	10	(0.7)	77
Ice cream shops	6	(0.4)	180
Bars	3	(0.2)	21
Canteens	90	(6.6)	3638
Work cafeteria	34	(2.5)	622
Homes for the elderly	12	(0.9)	221
Schools	26	(1.9)	2506
Military	8	(0.6)	229
Hospitals	10	(0.7)	60
Not identified	32	(2.4)	192
Other community	42	(3.0)	736
Total	1379	(100)	11 770

was dependent on the specific vehicle implicated. In particular, it was high (greater than 40%) for foods such as cakes, pastries with custard, and foods with mayonnaise sauces, while it was low for shell-eggs, poultry, and other meats.

The distribution by food vehicles (suspected or confirmed) for outbreaks with known etiology is shown in Table 3, grouped by outbreaks due to *S. enteritidis* (473 outbreaks), *S. typhimurium* (47 outbreaks), and the other 11 ascertained serotypes (22 outbreaks). For outbreaks due to the first two

etiologies the most commonly confirmed or suspected vehicles were egg-containing foods. The other serotypes which were isolated in outbreaks due to consumption of egg-containing foods were: *S. cholerae suis* (1 outbreak), *S. ohio* (1 outbreak), *S. panama* (3 outbreaks).

In 74.1% of the outbreaks the food was prepared and consumed in a family or private home (Table 4). In 14.1% of cases it was consumed in public eating places such as restaurants, rotisseries, hotels, pastry shops, or ice cream shops; 6.6% of the outbreaks

Table 5. Serotypes, phage types, plasmids, and resistance patterns of salmonella isolates from 54 outbreaks, Italy, 1991–4

Serotype	Phage type	Number (%) of outbreaks	Molecular weight of DNA plasmid (MDa)*	Drug resistance pattern†
<i>S. enteritidis</i>	4	35 (64.8)	38; 67; 38, 67	Sens
	1	8 (14.8)	38	Sens
	3	1	38, 25	Sens
	4a	1	38	Sens
	5a	1	ND‡	GM, NN, S, TE
	7	1	ND	Sens
	12	1	38	Sens
	RDNC1§	1	38	Sens
	RDNC2	1	67	CF
<i>S. typhimurium</i>	104	1	60; 37; 1.8; 1.5; 0.9	AM, C, S, TE
	193	1	60	AM, S, TE
	RDNC	1	—	Sens
<i>S. hadar</i>	26	1	3.1; 1.5; 1.3; 0.9	NA, TE
Total	—	54	—	—

\* MDa, megadalton.

† AM, ampicillin; C, chloramphenicol; CF, cephalothin; GM, gentamicin; NA, nalixic acid; NN, tobramycin; S, streptomycin; TE, tetracycline.

‡ ND, not determined.

§ RDNC, this culture reacts with typing phages but does not conform to a recognized pattern.

occurred in canteens (i.e. work cafeterias, nursing homes, schools, military facilities, and hospitals).

Very few of the outbreaks occurring in Italy involved individuals who had been recently exposed to an implicated meal in another country; imported cases were traced back to the following sources: two outbreaks in Spain in 1991 and 1992, one in Czechoslovakia in 1992, two in France in 1992, two in Greece in 1993, one in Slovenia in 1992, one in the Canary Islands in 1993, and one in Cuba in 1994. All but one, in which the isolate was not serogrouped, were due to salmonella group D or to *S. enteritidis*.

The outbreaks in our study were generally small. Specifically, the number of cases per outbreak was 2–3 for 49% of the outbreaks, 4–9 cases for 31%, and 10–50 cases for 14%; only 2% of the outbreaks involved more than 50 persons; the number of cases was not reported in 4%.

In 30% of the outbreaks, the number of cases ranged from 76–100% of the number of exposed persons; in 25% of the episodes the attack rate was between 51% and 75%; in 25% it was between 26% and 50%; and in 10% it was below 25% (in 10% the attack rate was not determined). The outbreaks lasted from 1–3 days for more than 50% of the episodes.

The interval of time between the occurrence of the epidemic and its notification was approximately 20 days in 50% of the cases and approximately 50 days

in 30%. Only 5% of the outbreaks were notified after 70 days and 2% between 100 and 200 days.

### Microbiological findings

The strains studied for microbiological characteristics were isolated from 54 outbreaks which occurred in the period 1991–4. Twenty were reported in Northern Italy, 30 in Central Italy, and four in Southern Italy. The outbreaks were classified as follows: 36 were general outbreaks involving a great number of individuals; 13 were confined to a family; and two occurred in hospitals; for three outbreaks this information was not available.

A total of 402 strains were isolated from humans, all from stool specimens, except for one, which was isolated from blood. The other 75 strains were isolated from eggs, egg-containing foods, vegetables, fish, and meat.

The serotype, phage type, molecular weight of plasmid DNA, and patterns of resistance to antibiotics of the isolates are reported in Table 5. The majority of *S. enteritidis* outbreaks (64.8%) were caused by phage type 4. These isolates showed three different plasmid profiles: one plasmid of 38 metadaltons (MDa), one plasmid of 67 MDa, and two plasmids of 38 and 67 MDa. All isolates belonging to this phage type were sensitive to all the antibiotics tested. Phage type

Table 6. Suspected or confirmed vehicles in 54 salmonella foodborne outbreaks (number of times in investigated outbreaks)

Sero-phage type*	Vehicle	
	Suspected	Confirmed
<i>S. enteritidis</i>		
PT4	Cake (2), cream custard filled pastries (2), tiramisù (2), cheese (1), ground beef (1), processed meat (1)	Eggs (8), ice-cream (5), tiramisù (4), cream (4), cake (2), vegetable (1), tomato sauce (1), salmon (1), lamb cutlet (1), timballo (1)
PT1	Tuna sauce (1), eggs (1), mayonnaise (1), pastries (1), unknown (3)	Tiramisù (1)
PT3	Cream (1)	—
PT4a	Unknown (1)	—
PT5a	Processed meat (1)	—
PT7	Unknown (1), cream (1)	—
RDNC1	Cream (1)	—
RDNC2	—	Tiramisù (1)
<i>S. typhimurium</i>		
PT104	—	Pork, veal meat, sauce and mushrooms (1)†
PT193	Unknown (1)	—
RDNC	Unknown (1)	—
<i>S. hadar</i>		
	Beef meat (1)	—

\* PT, phage type; RDNC, cultures which did not react conform to a recognized pattern.

† In the same outbreak.

1 was the second most common phage type, accounting for eight outbreaks (14.8%). These isolates harboured a plasmid of 38 MDa and were all drug sensitive.

The other phage types of *S. enteritidis* were responsible for one outbreak each. Only two isolates, one belonging to phage type 5a and one to phage type RDNC, were drug resistant. The DNA plasmid found in these phage type isolates weighed 38 MDa.

Of the outbreaks studied, three were caused by *S. typhimurium*. The epidemic strains differed in each episode; and included: phage type 104 (with plasmids of 60, 37, 1.8, 1.5 and 0.9 MDa), 193 (with plasmid of 60 MDa), and RDNC (without plasmids). Only the strain RDNC was drug sensitive; the other two were multidrug resistant. Recently, an outbreak was caused by a serotype which had never been previously involved in epidemics in Italy, *S. hadar*. This outbreak occurred in a canteen of a factory in Central Italy. A total of 450 individuals were affected, 90 of whom had confirmation by bacteriological analysis. All the strains isolated during this episode showed the same DNA plasmid profile, that is, four small plasmids weighing 3.1, 1.5, 1.3 and 0.9 MDa.

The suspected or confirmed vehicles of the outbreaks and the phage type involved are reported in Table 6. In the outbreaks caused by *S. enteritidis* of

any phage type, the vehicles involved, either suspected or bacteriologically confirmed, were eggs or egg-containing foods, such as mayonnaise, ice-cream, and 'tiramisù'; in rare cases, other foods, such as vegetables, lamb cutlets, and salmon, were involved. Ground meat was the suspected vehicle of the outbreak caused by phage type 5a. For the *S. typhimurium* outbreaks, the vehicles, when known, were meat and mushrooms. For the *S. hadar* outbreak, well-cooked beef, served cold and garnished with pickled vegetables, was implicated as the suspected vehicle by the epidemiological investigation.

## DISCUSSION

Though the number of reports of salmonella infections have been increasing worldwide (16), the literature only includes several papers on foodborne salmonella outbreaks. Mishu and colleagues (17) recently analysed data concerning 380 outbreaks which occurred from 1988–91 in the US and which involved 13056 individuals with 50 deaths. From 1986–93 150 outbreaks were reported in Argentina (18), where 6230 individuals were affected. In Italy, trends in outbreaks of *S. enteritidis* have been described (19), but the present study represents the first description of the general trend of foodborne outbreaks caused by

all serotypes or serogroups of salmonella. The data collected suggest that *salmonellae* are responsible for most foodborne outbreaks in Italy and that *S. enteritidis* is the most frequently implicated serotype.

In Italy, as in other countries (20), foods containing raw eggs are the most commonly implicated vehicle. For the *S. enteritidis* outbreaks studied, no differences were noted between suspected and confirmed vehicles: all were eggs or egg-containing foods. Similarly, egg-containing foods have also been implicated in *S. typhimurium* outbreaks, which is surprising. For the *S. hadar* outbreak the suspected vehicle was beef, though turkey has been reported as the most common vehicle (approximately 46%) for infection in outbreaks due to this serotype (21).

Analysis of our data has also provided some quality indicators for the system. First, it should be noted that the surveillance forms are generally completely filled-out. Secondly, the notification system seems to be timely. Thirdly, the percentage of outbreaks with an etiological agent identified in humans (nearly 90%) and for which a food vehicle was identified or suspected (almost 70%) may be indicative of the accuracy of the surveillance system. In contrast, microbiological confirmation of the implicated foods was obtained in less than half of them. This proportion appears low, but leftover foods are often difficult to obtain or to sample appropriately.

The surveillance system certainly suffers from underreporting: many outbreaks are notified only to the ISS, many others only to the Ministry of Health. The system also still needs to be unified. Moreover, although the National Infectious Disease Surveillance System became effective in April of 1991, many of the regions began adhering to the system at later dates and many others have yet to participate.

After the results of the second year of surveillance were made available, an advisory letter was written by the ISS in January 1992 to the Ministry of Health and to the department of health of each region, warning of the high incidence of *S. enteritidis* foodborne outbreaks due to consumption of foods containing raw eggs. The letter recommended the implementation of public education for the preparation of egg-containing foods and the use of pasteurized eggs for communities such as schools, hospitals, and elderly homes. To enhance surveillance, guidelines (22) for epidemiological and microbiological analysis to be conducted during salmonella foodborne outbreaks were developed and distributed to all Local Health Units. The data referring to the 2 years of surveillance following

the letter suggest that these measures led to a certain control in general community outbreaks; in fact, the highest proportion of outbreaks involved families or persons in private homes who do not use pasteurized eggs.

In Italy, as in other countries (23), PT4 is the predominant phage type in *S. enteritidis* foodborne outbreaks, and the majority of PT4 isolates carry the 38 MDa plasmid associated with virulence (24). In outbreaks caused by *S. enteritidis*, severe cases have been rare and deaths have occurred only among persons particularly at risk from infection, such as very young children and the elderly.

Generally, with a few exceptions, in each outbreak studied the isolates belonged to the same phage type; some differences were found in the DNA plasmid profile, but this was perhaps due to the non-stable character of these elements. However, the two tests used jointly could permit a good, although not completely accurate, evaluation of the clonality among the isolates.

Assuming that the serological identification of the microorganism is useful for epidemiological purposes when the serotype is rare, then one or more epidemiological markers should be used if the serotype is geographically widespread. Phenotypic and genotypic markers can be useful not only in determining if the isolates belong to a single epidemic clone, but also in characterizing strains for identifying the reservoir or the alimentary vector and for allowing the source of the infection to be traced (25).

Regarding *S. enteritidis* isolates, characterization by phage typing is a good identification method, yet it is not sufficiently discriminatory (26). Our study reveals that PT4 accounted for 64.8% of the outbreaks. Also, the virulence-associated plasmid of 38 MDa appears to be ubiquitous in strains of this phage type, in which two other DNA plasmid profiles are found. To discriminate between isolates within a serotype as widely diffuse as *S. enteritidis*, more than one typing method should be used, and new approaches at the molecular level should be developed for discriminating within this and other common types.

The pattern of antibiotic resistance concerns only a particular phage type, 5a. Other types are generally drug sensitive. For *S. typhimurium*, phage typing is a good epidemiological marker and is especially useful when used in association with plasmid profile and drug resistance testing.

In conclusion, from the epidemiological point of



view, the high number of notifications of foodborne outbreaks caused by *S. enteritidis* in Italy, though not alarming, should be considered an ever present phenomenon which should be monitored and compared to the situation in other countries; public health interventions may also be needed. From the bacteriological point of view, the use of phage typing, plasmid typing, and drug resistance typing for fingerprinting epidemic strains will improve the precision of surveillance and permit the clonality of isolates and the source and the means of diffusion of the epidemic strains to be defined. Our study demonstrates that the epidemic strains of *S. enteritidis* and *S. typhimurium* circulating in Italy and responsible for many different outbreaks do not belong to the same clone and very likely do not have the same origin.

#### ACKNOWLEDGEMENTS

The authors are grateful to Mr S. Arena, Laboratory of BMM, ISS, for phage typing of strains. The authors thank Mrs L. Ward, PHLS Laboratory, Colindale, UK, for phage typing of some atypical isolates.

#### REFERENCES

1. CDC. Summary of notifiable diseases, United States, 1993. *MMWR* 1994; **42**: 50.
2. CDSC, PHLS. Surveillance of gastrointestinal infections: 1980–89. *CDR* 1989; **89/52**: 1.
3. Sockett PN, Cowden JM, Le Baigue S, Ross D, Adak GK, Evans H. Foodborne disease surveillance in England and Wales: 1989–1991. *CDR Rev* 1993; **3**: R161.
4. CDSC, PHLS. Salmonella in humans. England and Wales: quarterly report. *CDR* 1994; **4**: 204–8.
5. CDSC, PHLS. Foodborne disease surveillance in England and Wales: 1986/88. *CDR* 1990; **90/15**: 3–8.
6. CDC. Outbreaks of *Salmonella enteritidis* gastroenteritis. California, 1993. *MMWR* 1993; **42**: 793–7.
7. Italy, Ministero della Sanità. DESIP Div II. Circolare n. 16–28 Dec. 1984. Sorveglianza delle Salmonelle e degli Enterobatteri.
8. Italy, Ministero della Sanità D.M. 15 Dec. 1990. Sistema informativo delle Malattie Infettive e Diffusive. *Gazz Uff* 8 Jan. 1991. Serie Gen. n. 6.
9. Fantasia M, Filetici E, Anastasio MP, Marcozzi MD, Gramenzi MP, Aureli P. Italian experience in *Salmonella enteritidis* 1978–1988: characterization of isolates from food and man. *Int J Food Microbiol* 1991; **12**: 353–62.
10. Fantasia M, Filetici E. *Salmonella enteritidis* in Italy. *Int J Food Microbiol* 1994; **21**: 7–13.
11. Italy, Ministero della Sanità. Direzione Generale Servizi Igiene Pubblica Divisione Profilassi Malattie Infettive. *Boll Min San*, 1995.
12. Epidemiology Programme Office, Centers for Disease Control, Atlanta, Georgia. EPI INFO version 5.1b. March, 1991.
13. Bauer AW, Kirby MM, Sherris JC, Turk M. Antibiotic susceptibility testing by a standardized single disc method. *Am J Clin Pathol* 1966; **45**: 493–6.
14. Ward LR, De Sa JDH, Rowe B. A phage typing scheme for *Salmonella enteritidis*. *Epidemiol Infect* 1987; **99**: 291–4.
15. Birnboim HC, Doly J. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res* 1979; **7**: 1513–23.
16. Rodrigue DC, Tauxe RV, Rowe B. International increase in *Salmonella enteritidis*, a new pandemic? *Epidemiol Infect* 1990; **105**: 21–7.
17. Mishu B, Koeler J, Lee LA, et al. Outbreaks of *Salmonella enteritidis* infections in the United States, 1985–1991. *J Infect Dis* 1994; **169**: 547–52.
18. Caffer MI, Eigner T. *Salmonella enteritidis* in Argentina. *Int J Food Microbiol* 1994; **21**: 15–9.
19. Binkin N, Scuderi G, Novaco F, et al. Egg-related *Salmonella enteritidis*, Italy, 1991. *Epidemiol Infect* 1993; **110**: 227–37.
20. St Louis ME, Morse DL, Potter ME, et al. The emergence of grade A eggs as a major source of *Salmonella enteritidis* infection. New implications for the control of Salmonellosis. *JAMA* 1988; **259**: 2103–7.
21. Rowe B, Hall ML, Word RL, De Sa JD. Epidemic spread of *Salmonella hadar* in England and Wales. *BMJ* 1980; **280**: 1065–6.
22. Greco D, Scuderi G, Fantasia M, et al. Linee guida per le indagini su epidemie di salmonellosi di origine alimentare. 1993; Istituto Superiore di Sanità, Rapporti ISTISAN 93/30.
23. Anonymous. *Salmonella enteritidis* phage type 4: chicken and egg. *Lancet* 1988; **i**: 720–2.
24. Nakamura M, Sato S, Ohya T, Suzuki S, Ikeda S. Possible relationship of a 36 megadalton *Salmonella enteritidis* plasmid to virulence in mice. *Infect Immun* 1985; **47**: 831–3.
25. Threlfall EJ, Hampton MD, Chart H, Rowe B. Use of plasmid profile typing for surveillance of *Salmonella enteritidis* phage type 4 from humans, poultry and eggs. *Epidemiol Infect* 1994; **112**: 25–31.
26. Rodrigue DC, Cameron DN, Pühr ND, et al. Comparison of plasmid profiles, phage types, and antimicrobial resistance patterns of *Salmonella enteritidis* isolates in the United States. *J Clin Microbiol* 1992; **30**: 854–7.