

## Egg-related *Salmonella enteritidis*, Italy, 1991

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### SUMMARY

In recent years, *Salmonella enteritidis* has become an increasingly important public health problem in Italy. In some parts of the country, the fraction of total human salmonella isolates accounted for by *S. enteritidis* has risen from 3–4% in the mid-1980s to more than 30% in 1990. Between 1990 and 1991, the number of reported *S. enteritidis* outbreaks increased more than sixfold. The 33 outbreaks reported in 1991 occurred in seven contiguous regions in northern and central Italy and were clustered in time between June and October: in the majority, products containing raw or undercooked shell eggs were implicated. Five of the egg-related outbreaks that occurred within a 30 kilometre radius over a 7-week period were investigated in detail. A phage type 1 strain containing a 38·9 MDa plasmid appeared responsible for three of the outbreaks, while in the remaining two a phage type 4 strain, also with a 38·9 MDa plasmid was isolated. Efforts are being made to enhance epidemiological surveillance and laboratory evaluation, and the use of pasteurized eggs has been recommended for high-risk populations.

### INTRODUCTION

The number of reported *Salmonella enteritidis* cases has increased substantially in recent years in many European countries. In England, the number of cases increased sixfold between 1982 and 1988 [1]. France experienced a sevenfold increase between 1986 and 1990 [2], and in Switzerland, the number of reported salmonella isolates has increased an average of 51% per year between 1984 and 1989, with the increase attributable almost entirely to *S. enteritidis* [3]. Spain also

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documented a 10-fold increase in *S. enteritidis* isolates between 1983 and 1987 [4], and substantial increases have also been noted in Hungary, Sweden, and Finland beginning in the early 1980s [5].

In most of the countries, much of the increase is attributable to the consumption of products containing raw or undercooked eggs [1–5]. Infection may occur with eggs that are externally contaminated or cracked, but trans-ovarian transfer of *S. enteritidis* also appears to play an important role [6].

In Italy, by contrast, egg-related outbreaks have been identified [7] but until recently remained relatively rare. Only six *S. enteritidis* outbreaks were reported to the Laboratory of Epidemiology and Biostatistics at the Istituto Superiore di Sanità (ISS; National Institute of Health) in 1990. In 1991, however, a total of 33 outbreaks was reported, most of which were caused by products containing shell eggs.

In this paper, we describe trends in *S. enteritidis* isolates and reported outbreaks in Italy using data from laboratory and disease surveillance sources. We present the temporal and geographical distribution of the 1991 outbreaks and the likely vehicles of transmission. Finally, we present details of a series of five egg-related outbreaks that took place over a 7-week period within a 30 km radius in the Emilia Romagna region of Northern Italy.

## METHODS

### *Trends in S. enteritidis in Italy and characteristics of 1991 outbreaks*

No single source of data was available to examine trends in human *S. enteritidis*. Therefore, a combination of data from the national infectious disease surveillance system and the laboratory-based enteric pathogen surveillance system has been used.

*National infectious disease surveillance system.* Salmonella infection is a notifiable disease in Italy, but prior to 1990, cases were not reported by strain. Since 1990, data are available by strain, but only outbreaks, rather than individual cases, are now reported. Because of the lack of strain-specific data and the modifications in reporting practice, we limited our analysis of these data to the years 1990 and 1991.

In the revised epidemiological surveillance system, outbreaks are notified by local health units. Each of the health units covers an estimated population of 50 000 to 200 000 and has one or more hygienists responsible for investigation of food-borne outbreaks in its jurisdiction. The definition of an outbreak is left to local discretion. At a minimum, food histories are taken from the cases, a reasonable hypothesis formed about the vehicle, and appropriate cultures are obtained from cases, possible vehicles, and from the environment. In those outbreaks occurring in 'closed' settings where participants in an implicated meal can be readily identified, all attendees or a sample of attendees are usually interviewed using standardized forms widely circulated in Italy that allow for rapid gathering of data on the onset of illness and symptoms and the calculation of food specific attack rates, and at a minimum the differences between the rates among those who ate and did not eat a given food are compared to determine the most likely vehicle. As with most infectious disease surveillance systems, there is

likely to be considerable underreporting, although the levels vary from region to region. The magnitude of the underreporting with the new outbreak-based surveillance system has not been assessed, although no interventions were undertaken to improve surveillance and levels were unlikely to have changed between 1990 and 1991.

*Laboratory-based enteric pathogen surveillance system.* To examine trends in *S. enteritidis* between 1980 and 1990, we used data from the laboratory-based enteric pathogen surveillance system, which was started in 1973 and collected data until the late 1980s, when funding was temporarily discontinued. In this surveillance system, three inter-regional reference laboratories in Italy (north, central, and south) had the responsibility for final serotyping of salmonella isolates from the regions in their jurisdiction and reported their findings on an annual basis to the Laboratory of Bacteriology and Medical Microbiology at the Istituto Superiore di Sanità in Rome.

Although consistent data on the number of total human salmonella and *S. enteritidis* isolates was available for 1980–8, data were not available for the northern zone for 1989, and in 1990 and 1991, the data were incomplete. The south ceased reporting in 1989, and data from the central zone were incomplete for 1991. During the years in which the system was operating, the number of total salmonella isolates varied by as much as 85% from year to year, depending in part on the level of activity and staffing of the laboratories, complicating the interpretation of trends even for years with consistent reporting. Therefore, in addition to examining data on total human *S. enteritidis* isolates for 1980–8 by zone, we also calculated the number of human *S. enteritidis* isolates as a percentage of the total human salmonella isolates by region for all years between 1980 and 1991 for which either complete or partial data were available.

#### *Investigation of five geographically and temporally clustered egg-related outbreaks*

*Epidemiological investigation.* Six outbreaks occurred within a 7-week period between the last week of August and the second week of October in two adjoining provinces in the Emilia Romagna region. In five of the outbreaks, eggs were epidemiologically implicated. The remaining outbreak, which has been described elsewhere [8], occurred in a nursing home. Ground beef was implicated among three sub-populations for whom reliable dietary and illness history could be obtained. Cross-contamination with egg-containing meatballs prepared using the same ground beef was suspected but could not be definitively demonstrated.

Three of the five outbreaks in which eggs were implicated occurred in settings in which participants at the implicated meal could be readily identified and contacted. Two occurred in work-site cafeterias (outbreaks 1 and 2) and the third in a small hotel (outbreak 5). The remaining two were community-based, one involving a bakery (outbreak 3) and the other a pizzeria (outbreak 4).

In all but outbreak 3, a case was defined as a person with the acute onset of diarrhoea during the identified epidemic period, with or without laboratory confirmation. In outbreak 3, which was community-based, the case definition also included a culture positive for *S. enteritidis*.

For the three 'closed' outbreaks in which it was possible to identify those who had participated in the implicated meal, retrospective cohort studies were

performed to determine the likely vehicle. In outbreak 1, 38 of 60 participants at the implicated meal were located and interviewed; the remainder had gone on vacation or worked off-site. Detailed menus were available, and interviews were conducted on-site within 3 days of the outbreak. In outbreak 2, the company was less cooperative, and only 34 of an estimated 200 persons who ate regularly in the cafeteria were interviewed, including 14 who were ill and 20 who were well. Interviews were performed a week after the implicated meal, and menus were not available but were reconstructed based on the memories of the employees. Outbreak 5 was reported shortly after its occurrence. The hotel patrons and kitchen workers participating in the implicated meal were interviewed, and detailed menus were available.

In the outbreak involving a bakery (outbreak 3), outbreak-associated cases were identified through interviews with 17 sporadic cases reported during a one-week period. No further active case-finding was performed, although community awareness of salmonella was high because of the extensive publicity associated with the simultaneous nursing home outbreak in the same small town. No control group was examined in this instance. In the pizzeria outbreak (outbreak 4), two cases who had come to the attention of local health authorities were identified. Seven companions who had dined with the two ill persons at the pizzeria were interviewed, and one of the seven had also been ill. Food histories were obtained from the nine who had dined together, but no attempt was made to locate other restaurant patrons.

For the retrospective cohort investigations (outbreaks 1, 2 and 5), food preference and illness data were entered into Epi-Info Version 5.01, and the relative risk, 95% confidence interval, and *P* value associated with each food item were calculated [9]. Where appropriate stratified analyses were performed to control for potentially confounding variables, and Mantel-Haenszel summary relative risks and confidence intervals were calculated.

*Kitchen inspection and environmental evaluation.* Detailed inspections were performed at all of the implicated kitchens by public health physicians and/or hygienists from the local health units. Food preparation and storage were reviewed, and environmental and food cultures obtained. In most of the outbreaks, the implicated foods were no longer available, but in certain instances, analogous foods that had been prepared more recently were cultured, as were any eggs remaining in the kitchen.

*Investigation of the egg sources.* In each of the outbreaks, eggs were traced back to the laying farm, and the laying stock was further traced back to the hatchery of origin. As part of the investigation of the above-mentioned nursing home outbreak in which two deaths occurred, various types of cultures also were taken at several laying farms not directly implicated in the outbreak.

A total of three farms were implicated in the five outbreaks and investigated. One of the three produced its own laying stock; the remaining two were supplied by different hatcheries. Only one of the two was investigated because the veterinary authorities in whose jurisdiction the second hatchery was located declined investigation, stating that the hatchery supplied a large number of farms and no other problems had been reported.

The farm investigations were conducted between 1 and 7 weeks after the

outbreaks by public health veterinarians attached to the local health units. Investigations included environmental cultures and culture of manure scrapers or floor and nest boxes depending on the type of farm. *S. pullorum* serology and organ cultures were also conducted at the farm involved in outbreaks 1, 2 and 4.

*Laboratory investigations.* Most cases in the five outbreaks were laboratory-confirmed. Specimens were transported to hospital laboratories for the hospitalized cases or to the multi-zonal public health laboratory, where bio- and serotyping were performed using standard techniques.

A sample of human *S. enteritidis* isolates from outbreaks 3 and 5 was sent to the ISS in Rome for phage typing and plasmid analysis; isolates from the remaining outbreaks had been discarded and were not available for further analysis. Plasmid DNA extraction and electrophoresis were performed using methods described by Kado and Liu [10] and Birnboim and Doly [11]. The molecular size of the plasmids was estimated by comparing their electrophoretic mobility with those of plasmids of known molecular size from strains V517, pI40a, and R16. Phage typing was carried out according to the techniques described by Ward and colleagues [12] using reagents obtained from the Division of Enteric Pathogens, Public Health Laboratory Service, Colindale, London.

Food, environmental, and animal specimens were transported to the regional zoonophylaxis laboratory. Food, animal, and environmental specimens from the outbreaks were obtained and handled according to existing Italian national guidelines, which are subsequently being modified to improve specimen collection as well as pre-enrichment and enrichment techniques. *S. enteritidis* isolates were forwarded to the ISS for phage typing and plasmid analysis.

## RESULTS

### *Trends in S. enteritidis and characteristics of 1991 outbreaks*

Overall, the number of human *S. enteritidis* isolates reported by the laboratory-based surveillance system in Italy has increased gradually since the early 1980s, with steeper increases noted between 1984 and 1985 and between 1987 and 1988 (Fig. 1).

In the north and central zones, the proportion of total salmonella isolates that are *S. enteritidis* has risen substantially in recent years. In the north, *S. enteritidis* accounted for less than 6% of the human salmonella isolates for each of the years between 1980 and 1988 (Fig. 2). However, this fraction had risen to 13% in 1990 and to 18% in 1991. In the central zone, *S. enteritidis* accounted for no more than 5% of isolates prior to 1985, but by 1988, the percentage had doubled to 10%. It further increased to 19% in 1989 and to 31% in 1990, although it dropped slightly in 1991. In the south, by contrast, levels have remained consistently low, with *S. enteritidis* accounting for no more than 7% of the isolates in recent years.

The infectious disease surveillance system data for 1990 and 1991 confirm the recent increase in *S. enteritidis* in Italy. There has been more than a sixfold increase in the number of outbreaks reported to the system, from 5 in 1990 to 33 in 1991.

The 33 outbreaks reported in 1991 occurred in seven contiguous regions in north and central Italy and were closely clustered in time, with the vast majority

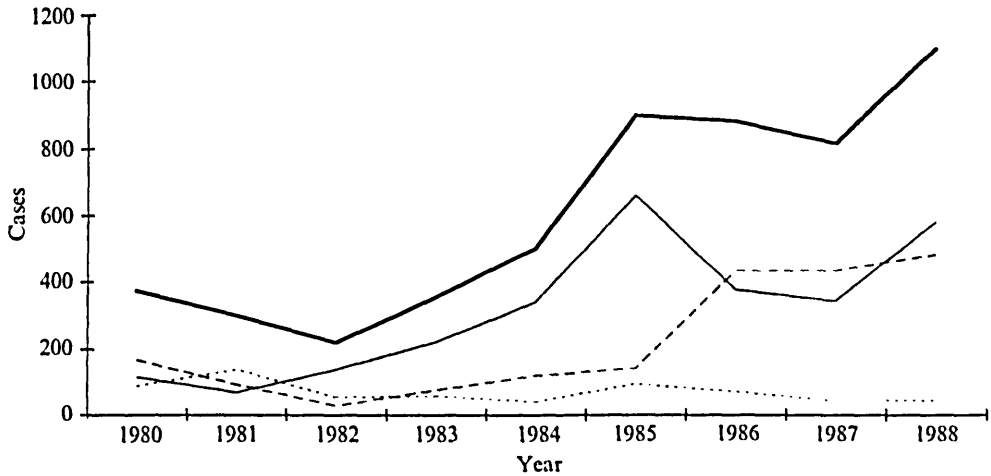


Fig. 1. Human *S. enteritidis* isolates for northern (—), central (---), and southern (.....) Italy and for the three areas combined (—), national laboratory-based surveillance system, 1980-8.

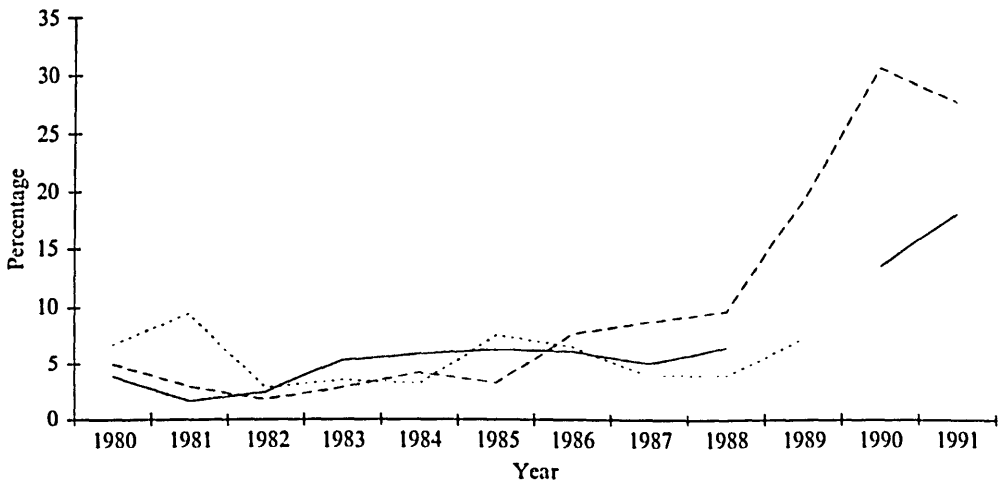


Fig. 2. *S. enteritidis* isolates as a percentage of total human salmonella isolates for northern (—), central (---), and southern (.....) Italy, national laboratory-based surveillance system, 1980-91.

(87.9%) occurring between June and October. Tiramisù, a dessert made with raw eggs and mascarpone cheese, was the most common vehicle for the 27 outbreaks in which a responsible source could be clearly implicated (37.0%); other vehicles included mayonnaise (22.2%), ice cream (7.4%), eggs (7.4%), and egg-based pasta (7.4%).

#### *Investigation of five geographically and temporally clustered egg-related outbreaks*

*Epidemiological and laboratory investigations.* Results of the investigation of the five egg-related outbreaks occurring in Emilia Romagna over a 7-week period are detailed below.

Table 1. Food-specific attack rates, outbreak 1, Parma, Italy, August, 1991

Food	Ill	Well	Relative risk (95% CI)	P
Minestrone	4/6 (67%)	18/32 (56%)	1.2 (0.6-2.2)	1.0
Boiled fish*	16/21 (76%)	6/17 (35%)	2.2 (1.1-4.3)	0.03
Russian salad	15/15 (100%)	7/23 (30%)	3.3 (1.8-6.1)	0.00002
Eggplant	5/6 (83%)	17/32 (53%)	1.6 (1.0-2.5)	0.4
Potatoes	7/12 (58%)	15/26 (58%)	1.0 (0.6-1.8)	0.8
Meat salad	4/6 (67%)	18/32 (56%)	1.2 (0.6-2.3)	1.0
Vitello tonnato†	2/3 (67%)	20/35 (57%)	1.2 (0.5-2.7)	0.8
Fruit	8/14 (57%)	14/16 (88%)	0.4 (0.2-0.8)	0.004
Russian salad, meat salad or vitello tonnato	19/22 (86%)	3/26 (12%)	4.6 (1.6-13.0)	0.0001

\* Most also consumed Russian salad; summary relative risk after controlling for consumption of Russian salad = 1.4 (95% CI 0.4-4.8;  $P = 0.95$ ).

† Veal with a tuna- and mayonnaise-based dressing.

Outbreaks 1 and 2. Two outbreaks occurred in Parma within a 2-day period in work site-cafeterias served by a single caterer. In the first of these outbreaks, 22 of 38 persons eating lunch in a work-site cafeteria on 29 August and who were subsequently interviewed developed gastroenteritis (attack rate (AR), 58%). *S. enteritidis* was isolated from the stools of 5 of the 11 cases tested. In the second outbreak, 13 of 36 persons eating lunch in another work-site cafeteria on 30 August became ill with gastroenteritis (AR, 36%). Stool cultures were obtained on 2 of the 13 cases; both were positive for *S. enteritidis*. Positive cultures were discarded, however, and phage typing and plasmid analysis were not performed on the isolates.

Foods containing home-made mayonnaise prepared by the caterer from shell eggs were implicated in both outbreaks (Tables 1 and 2). However none of the mayonnaise remained at either site for culturing. One of the kitchen workers became ill during the outbreak and had a positive stool culture for *S. enteritidis*; the remaining workers were culture negative. Fifty eggs that remained in the kitchen were pooled and cultured, but cultures were negative.

Outbreak 3. Between 27 September and 1 October 1991, 13 people eating pastries with custard filling prepared from shell eggs by a single bakery in Fidenza developed the acute onset of gastroenteritis. Several were staff members of a neurological ward at the local hospital who consumed pastries brought in by the family of one of the patients. A case-control study was not performed because the outbreak occurred at the same time as the large nursing home outbreak. However, the bakery was one of six serving the town; no other common exposures were found among the cases. All 13 were culture-positive for *S. enteritidis*. None of the bakery employees were ill, and all were culture-negative. Environmental cultures and cultures of freshly prepared custard from the implicated bakery were negative. The single stool isolate submitted for phage typing and plasmid analysis was phage type (PT) 4 and had a 38.9 MDa plasmid.

Outbreak 4. Three cases of acute gastroenteritis occurred among patrons who had eaten at a pizzeria in Parma on 4 October 1991. Two had stool cultures which were positive for *S. enteritidis*, but specimens were not submitted for phage typing

Table 2. *Food-specific attack rates, outbreak 2, Parma, Italy, August, 1991*

Food	Ill	Well	Relative risk (95% CI)	P
Pasta with tomato sauce	4/7 (57%)	10/27 (37%)	1.5 (0.7-3.5)	0.6
Pasta country style	2/9 (22%)	12/25 (48%)	0.5 (0.1-1.7)	0.3
Rice	2/7 (29%)	12/27 (44%)	0.6 (0.2-2.2)	0.7
Beef with Russian salad	13/14 (93%)	1/20 (5%)	18.6 (2.7-126.2)	0.000002
Croquettes	0/6 (0%)	14/28 (50%)	not calculable	0.03
Chicken thighs	0/4 (0%)	14/30 (47%)	not calculable	0.2
Cooked vegetables	2/4 (50%)	12/30 (40%)	1.2 (0.4-3.7)	0.9
Raw vegetables	3/7 (43%)	11/27 (41%)	1.0 (0.4-2.8)	0.7
Fruit	5/13 (38%)	9/21 (43%)	0.9 (0.4-2.1)	0.9

Table 3. *Food-specific attack rates, outbreak 5, Tabiano, Italy, October, 1991*

Food	Ill	Well	Relative risk (95% CI)	P
Prosciutto	7/16 (44%)	10/16 (62%)	0.7 (0.4-1.4)	0.5
Lasagna	14/24 (58%)	3/8 (37%)	1.6 (0.6-4.0)	0.4
Roast veal	11/20 (55%)	6/12 (50%)	1.1 (0.6-2.2)	0.9
Guinea fowl	9/20 (45%)	8/12 (67%)	0.6 (0.4-1.3)	0.4
Tiramisù	17/28 (61%)	0/4 (0%)	not calculable	0.04

and plasmid analysis. All had eaten tiramisù prepared by the cook using shell eggs; their six dinner companions who did not eat tiramisù did not become ill (relative risk not calculable; 2-tailed Fisher exact *P* value = 0.01). Both ill and well subjects had eaten slices of pizza (vegetarian and with tomato and cheese); those who were well had also eaten cooked cream and gelato. Although none of the tiramisù served on the 4th remained, a second pan that had been prepared the same day and a swab of the refrigerator were positive for *S. enteritidis* that was PT 1 and contained a 38.9 MDa plasmid.

Outbreak 5. Between 6 and 9 October, 17 of 32 guests who were staying and taking their meals in a hotel located in Tabiano near Fidenza developed the acute onset of gastroenteritis with fever (AR, 53%). Of the 17 on whom cultures were obtained, 11 were positive for *S. enteritidis*. A retrospective cohort study (Table 3) of the hotel guests implicated tiramisù (relative risk not calculable, *P* = 0.04). The cook who had prepared and who had eaten some of the tiramisù had also become sick on the first day of the outbreak and was culture-positive; the remaining kitchen workers were negative. None of the implicated tiramisù remained and egg and environmental cultures were negative. Of the nine isolates submitted for phage typing and plasmid analysis, all were PT 4 and had the same 38.9 MDa plasmid seen in outbreaks 3 and 4.

*Investigations of egg sources.* Eggs from outbreaks 1, 2 and 4 came from the same farm, while for outbreaks 3 and 5, two additional farms were implicated. A PT 1 strain containing a 38.9 MDa plasmid was isolated from a manure scraper and the ovaries of two asymptomatic hens at the farm that supplied eggs for the two work-site cafeterias and the pizzeria (outbreaks 1, 2 and 4). In addition, *S. pullorum* serology was positive for 2 of 60 chickens sampled. Although none of the human



isolates from the three outbreaks was available for phage typing and plasmid analysis, the profile of the organism isolated at the farm was identical to that found in the tiramisù and the refrigerator of the pizzeria (outbreak 4). Environmental cultures from the farms implicated in outbreaks 3 and 5 and from the single hatchery investigated were negative.

Two other farms which were not implicated in any of the outbreaks but were visited as part of a broader inspection that occurred following the nursing home outbreak had positive isolates. At the first farm, a PT 4, 38.9 MDa was isolated from the manure scraper and egg belt, and at the second, the same organism was isolated from whole carcasses of chicks bred for meat.

#### DISCUSSION

As has occurred elsewhere in Europe, there has been a considerable increase in *S. enteritidis* infections over the past several years, although the analysis of trends is complicated by changes in the reporting system and the lack of continuity in the activities of the interzonal reference laboratories. In the laboratory-based surveillance system, for example, the 1984–5 peak may be spurious; an 85% increase occurred in total salmonella isolates during this period in the northern zone, and this zone accounted for nearly 3/4 of the 1985 *S. enteritidis* isolates. Nonetheless, Italy appears to have experienced a considerable increase in *S. enteritidis* beginning in the late 1980s. The number of outbreaks reported increased sixfold between 1990 and 1991, suggesting that this process may have accelerated dramatically over the past 2 years. As is the case elsewhere in Europe, eggs have been implicated in the vast majority of recent outbreaks.

*S. enteritidis* PT 4 with a 38.9 MDa plasmid was isolated in two of the three outbreaks in which strains from either cases or foods were available for testing. In the third, the PT differed, but the same plasmid was found. A PT 4, 38.9 MDa strain also appeared responsible for the nursing home outbreak in which cross-contamination with egg-containing products was suspected but could not be documented and was isolated from two chicken farms in the area not implicated directly in any of the outbreaks. Phage typing had not been performed in Italy prior to 1991, so that comparison data from previous years are not available. Plasmid analysis has been done in the past, however, and the 38.9 MDa plasmid found in isolates from the recent outbreaks has not been previously seen in either epidemic or sporadic human disease in Italy [7].

The majority of human *S. enteritidis* isolates in England and Wales [6] as well as in France [13] are PT 4 and have a 38 MDa plasmid; presumably this is the same organism responsible for two of the above-reported outbreaks and the nursing home outbreak. PT 4 differs from other phage types that infect chickens in that it can be isolated from the contents of intact, clean shell eggs [14]. The remaining three outbreaks were presumably due to a PT 1 organism with the same 38.9 MDa plasmid. This phage type is relatively rare in England and Wales, although it is reported to be the predominant type in the former Soviet Union [13].

Eggs for the five outbreaks came from three different farms. Efforts to isolate the organism at the production site were successful only for the farm implicated in outbreaks 1, 2 and 4, and an organism of the same phage type and plasmid as

that isolated from food and environmental cultures from one of the outbreaks was found. At the other two sites, delays in culturing, possible antibiotic treatment of flocks, and inadequate transport and isolation technique may have been responsible for failure to isolate an organism. A number of studies have suggested that the risk of shedding may be an episodic or intermittent phenomenon [14], decreasing the likelihood of isolating the organism if there is a delay in visiting the implicated layer farm. Nonetheless, the presence of a PT 4, 38.9 MDa plasmid *S. enteritidis* strain was demonstrated in two other farms in the same area, suggesting that the organism responsible for the remaining three outbreaks may have been circulating in local farms.

The recent increase in reported egg-related *S. enteritidis* outbreaks has been limited to northern and central Italy. This may be related to differences in surveillance and investigation activities. Alternatively, northern Italy has considerably greater commercial contact with other European countries, and the organism may have been inadvertently transported into the country from hens or perhaps contaminated feed purchased from one of the European countries experiencing a major *S. enteritidis* problem.

In response to the increase in egg-related outbreaks, regional authorities throughout Italy have been alerted and have been asked to report all outbreaks. Guidelines are being developed on the investigation and reporting of suspected *S. enteritidis* outbreaks, on microbiological techniques for the transport and isolation of the organism, on investigation of implicated farms, and on control measures such as the use of pasteurized eggs in day-care centres, schools, hospitals, and nursing homes. Such pasteurized eggs are already widely used in the production of commercial egg-containing products in Italy, including egg pasta.

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