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Surveillance system and rapid tracing of primary sources in foodborne outbreaks by *Salmonella* spp. Part I: Identification of outbreaks in the Abruzzo region of Italy

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Summary

The determination of the origin of foodborne diseases is one of the top priorities for the world health community. Gastroenteritis caused by zoonotic *Salmonella* serovars is one of the major threats to human health. It is essential that surveillance systems are able to monitor the incidence of human cases and to provide useful data to plan and implement effective prevention strategies. Surveillance systems generate information that is of value both for the early detection of infection and for the identification of epidemiological trends and risk factors. The authors describe a surveillance system for the identification of the sources of infection foodborne disease outbreaks caused by *Salmonella* in the Abruzzo region of Italy between April 2000 and October 2002.

Keywords

Foodborne diseases, Italy, Public health, *Salmonella* spp., Salmonellosis, Surveillance, Zoonoses.

Introduction

The determination of the origin of foodborne diseases and the establishment of epidemiological trends of infections related to food consumption are of prime importance for public health worldwide (12). Diarrhoeal infections and with malaria are the most frequently encountered infectious diseases in the world (23). Each year, millions of people are affected by foodborne and waterborne diseases (24). In particular, gastroenteritis caused by zoonotic *Salmonella* serovars is one of the major threats to human health in extensive areas of the globe. Salmonellosis and campylobacteriosis are by far the most frequently reported zoonoses in humans not only in Italy, but also in the European Union (EU) (8).

The extensive spread of salmonellosis has been facilitated by the globalisation of food supplies and by changes in food consumption practices that have taken place in the last few decades. Public health authorities have to face new challenges due to emerging pathogenic agents, modifications in the routes of transmission and changes in food production systems. The possibility of transporting perishable foodstuffs to any destination in the world in just a few hours, together with the consumption of new food preparations, expose consumers to a wide range of infective agents that could have originated in many distant countries. Furthermore, the large demands of ready-to-eat preparations and the increasing number of servings consumed in the public catering system have modified the epidemiological trends of many foodborne diseases. In this context, it is essential

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that surveillance systems monitor the incidence among humans and provide data that can be used to plan and implement prevention strategies (1). Surveillance systems generate valuable information both for the early detection of infection and for the identification of epidemiological trends and risk factors (5). In the past, foodborne outbreaks were frequently associated with local outbreaks, they were limited geographically and were clearly defined. In addition, they were often household outbreaks linked to incorrect food handling by the final consumer. Epidemiological investigations were conducted and public health authorities have successfully collated the information required. Most foodborne diseases are caused by food that has been contaminated at the source which implies that contaminated batches have to be traced back over large geographical areas, involving several countries. In such a situation, competent food safety authorities have great difficulty correlating human cases with the primary sources of infections (25). Several surveillance systems have been implemented in recent years to address these issues.

In 1990, the World Health Organization (WHO) implemented the Surveillance Programme for Control of Foodborne Infections and Intoxications, collecting data from 51 countries on foodborne human cases and outbreaks, results of both laboratory tests and of specific projects in the food safety sector.

Since 2000, the Global Salm-Surv (GSS) project collects data and information from a network of institutions involved in the isolation, identification and surveillance of *Salmonella* infections (22). The project is the result of collaboration between the WHO, Danish Institute for Food and Veterinary Research (DFVF), Centers for Disease Control and Prevention (CDC), Institute Pasteur, the Public Health Agency in Canada and the Animal Science Health Group (ID-Lelystad).

In the United States of America (USA) the CDC routinely collects data from the following courses:

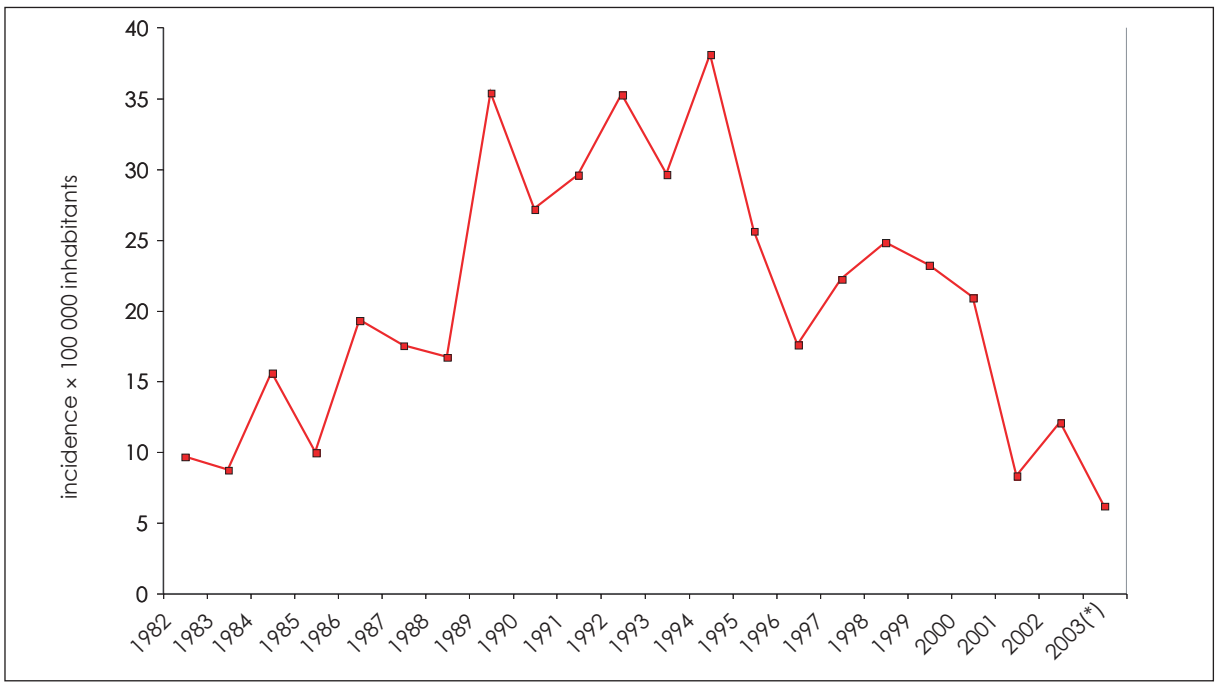
a) the Public Health Laboratory Information System (PHLIS), which is a passive surveillance system based on data from laboratory investigations
b) the National Electronic Telecommunications System for Surveillance (NETSS), which is also a passive surveillance system based on diagnoses of physicians in cases of suspected of infection
c) the Foodborne Diseases Active Surveillance Network (FoodNet), through which public health laboratories are regularly contacted to gather data on confirmed cases of diarrhoeal diseases (3)
d) the Foodborne-disease Outbreak Surveillance System, which analyses information on foodborne outbreaks investigated by State health departments (20).

A surveillance system for foodborne diseases (OzFoodNet), similar to FoodNet, has also been implemented Australia (2).

In the EU, the International Surveillance Network for Enteric Infections (EnterNet) collects data on diagnostic investigations on *Salmonella* and verotoxigenic *Escherichia coli* (VTEC) outbreaks from the laboratories participating in the network (6).

A surveillance system for salmonellosis infections in humans and animals was established in the Abruzzo region of Italy in 1992. This was part of a project that was completed in 1993 by the Public Health authorities in close collaboration with the Veterinary Services (16). Afterwards, a similar system was also implemented in the Lombardy region (21).

The present paper describes a surveillance system for the identification of sources of infection in foodborne outbreaks of *Salmonella* in the Abruzzo region.



* provvisional data
Source: Ministry of Health, Infectious Diseases Information System

Figure 1
Human incidence of salmonellosis in the Abruzzo region, 1982-2003 (× 100 000 inhabitants)

Materials and methods

Surveillance system

Salmonellosis in humans is the foodborne infection with the highest incidence in the Abruzzo region, although the number of cases has declined constantly since 1994 (Fig. 1). Although the causative agents of foodborne outbreaks in this region are often suspected (17), rarely the primary source of infection has been identified for zoonotic salmonellosis.

A project was conducted from April 2000 to October 2002 with the objective of establishing a surveillance system to detect sources of infection in salmonellosis outbreaks.

The following institutions took part in the project:

a) Ministry of Health, General Direction of Veterinary Health and Food (DGVet)
b) Clinic of Infectious Diseases of the Faculty of Medicine, University 'G. D'Annunzio' of Chieti (Uni-CH)
c) Infectious Disease Departments of the following hospitals: Avezzano, Lanciano, Vasto, Teramo and Giulianova (Fig. 2)
d) Food Hygiene and Nutrition Services (FH&NS) and Veterinary Services (VS) of the following Local Health Units (LHU): Avezzano-Sulmona, Teramo, Lanciano-Vasto (Fig. 2)
e) Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale' (IZSA&M), in Teramo.

In the first phase of the project, the data set needed for the identification of human salmonellosis outbreaks was defined. One team was established in each LHU, including staff from the local hospital and from the Prevention Department (FH&NS and VS) of the LHU. The aim of the project was

Figure 2
Local health units in the Abruzzo region and location of hospitals participating in the project

to intervene in the suspected outbreaks in accordance with specific protocols (15).

An information system was established for the collection of data, as follows (Fig. 3):

a) hospitalised patients showing symptoms of salmonellosis
b) isolations of *Salmonella* spp. from samples of faeces or rectal swabs
c) sporadic and collective outbreaks of foodborne *Salmonella* notified to the Prevention Departments
d) epidemiological investigations performed by the Veterinary Services on suspect farms.

All cases of hospitalised patients with symptoms compatible with salmonellosis were notified to the competent FH&NS responsible for conducting the epidemiological investigations, primarily to

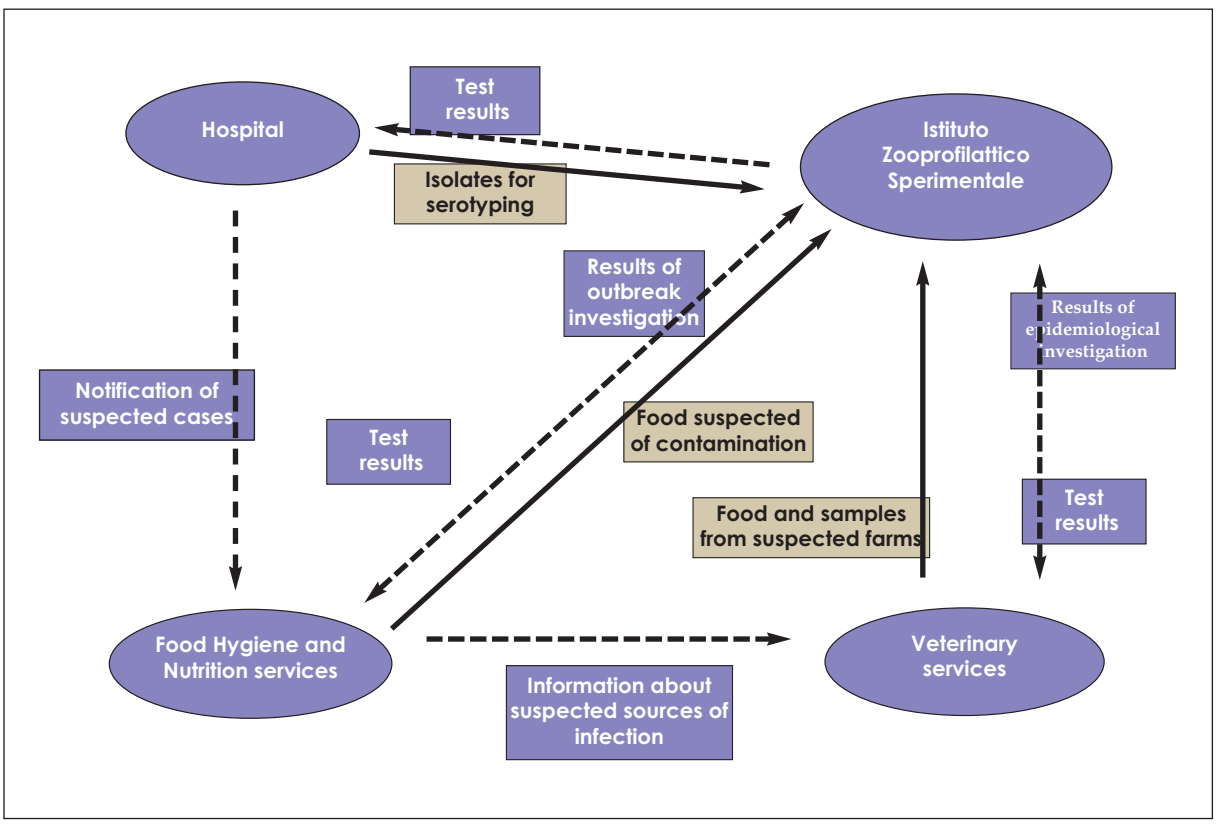


Figure 3
Data and sample flow scheme of the salmonellosis surveillance system in the Abruzzo region of Italy

confirm or reject the suspicion of foodborne outbreaks. In cases of confirmation, the FH&NS:

- define the extent of the outbreak with the identification of the involved peoples
- identified the suspected food and its origin
- collected food samples.

Two forms were used to collect the results of outbreak investigations: one for sporadic cases and another for collective episodes. A copy of all completed forms was sent to the IZSA&M where all data were entered into a central database.

The information on the origin of suspected food was transmitted to the competent VS for further investigation along the production chain (food suppliers and retail stores, food preparation and processing premises, slaughterhouses, animal farms, feed production plants) to trace the primary source of contamination. Epidemiological data collected by VS were recorded in the IZSA&M database.

Sample collection

Faecal samples were collected from hospitalised patients who had received no antimicrobial treatment in the preceding 48 h. Immediately after collection, rectal swabs were despatched to the laboratory in an appropriate transport medium (Cary-Blair, Stuart's or Amie's). All samples were delivered rapidly to the diagnostic laboratories of the same hospitals for initial examinations. If the samples could not be examined within 24 h of collection, they were kept frozen.

Food samples collected by the FH&NS and VS were examined by the IZSA&M. Each sample contained at least 100 g food or 6 units in the case of eggs.

Samples collected on farms by VS were carcasses of suspected animals, faecal material, eggs, feed, dust and other equipment. All samples were examined by the IZSA&M.

Isolation and typing

Faecal samples were inoculated directly on tetrathionate broth or selenite cystine broth at a ratio of 1:10, while faecal swabs were dipped into 10 ml of the same culture medium. Cultures were incubated for 18-24 h at 37±1°C. After the incubation period, two samples were removed from each broth culture and inoculated into two different agar plates. For this second phase, the following media were used: brilliant green agar, Hektoen enteric agar, MacConkey agar, Rambach agar, xylose-lysine-desoxycholate (XLD) agar. Cultures were incubated at 37±1°C for 18-24 h. The incubation period was prolonged to 48 h in the absence of bacterial growth.

Detection of *Salmonella* spp. from food samples was performed in accordance with ISO 6579:1993 (13). Samples from animal carcasses (liver, kidney, ovary, gut, etc.) were analysed in accordance with the Ministry of Health Decree dated 10 March 1997 (19) with the following variations: the modified semisolid Rappaport-Vassiliadis (MSRV) medium (0.2/20 ml) was used for the enrichment subcultures and subsequently the isolation was performed on three different selective media chosen among brilliant green agar, Hektoen enteric agar, MacConkey agar, Rambach agar, XLD agar and Gassner agar. Embryonated chicken eggs were analysed in compliance with the Ministry of Health Decree of 10 March 1997 (19). Biochemical confirmation of *Salmonella* colonies was performed using BBL™ Enterotube™ II (Becton Dickinson GmBh, Heidelberg-D) and 2 nitrophenyl-?-D-galactopyranoside (ONPG, Oxoid) or API® 20 E (Biomérieux® SA, Marcy l'Étoile, France).

Serological identification was performed using the Kauffmann-White scheme (14), with commercial kits for the detection of somatic O and flagellar H antigens (Dade Behring, Marburg-D; Remel Europe Ltd, Dartford-UK).

Table I
Results of epidemiological investigations in foodborne outbreaks in the Abruzzo region April 2000-October 2002

Salmonella food poisoning outbreaks	Totale outbreaks: 19	Total sporadic cases: 49	Total: 68
One or more suspected foods	15	9	24
Food samples collected	8	3	11
Salmonella spp. isolated from food samples	4	0	4
Infected farms detected	2	0	2
Salmonella spp. isolated from human faecal samples	11	29	40

Results

A total of 19 foodborne outbreaks and 49 sporadic cases were notified during the project. *Salmonella* strains were isolated from food samples collected in 4 outbreaks and from 40 faecal samples taken from patients (Table I). The number of people in each outbreak varied from 2 to 11 (Fig. 4), while the total number involved in the outbreaks was 80.

Suspected food was identified in 9 out of 49 (18.4%) sporadic cases, whilst suspected food was incriminated in 15 out of 19 (78.9%) outbreaks (Table I). However, it was possible to take food samples only in 8 outbreaks (42.1% of total outbreaks). *Salmonella* spp. was isolated from food samples taken in 4 outbreaks (21% of total outbreaks). The contaminated foods were: mayonnaise, frankfurters and ketchup in the first outbreak, crêpes in the second, goose breast in the third and tiramisu (dessert made with raw eggs) in the fourth outbreak.

Fifty-eight *Salmonella* strains were isolated from 40 human faecal samples (11 taken in outbreaks and 29 from sporadic cases) (Fig. 5). *Salmonella enterica* subsp. *enterica* serotype Enteritidis (*S.* Enteritidis) was the most frequent serovar isolated from faecal samples (27 strains out of 58), followed by *Salmonella enterica* subsp. *enterica* serotype Typhimurium (*S.* Typhimurium) (16 strains).

Discussion

A thorough revision of the EU food safety strategies is in progress. In 1998, Decision 2119/98/EC of the European Parliament and of the Council established a network for the epidemiological surveillance and control of communicable diseases in humans (9). In 2000 the European Commission published the *White paper on food safety* (7), stating the intention of reviewing the relevant EU food

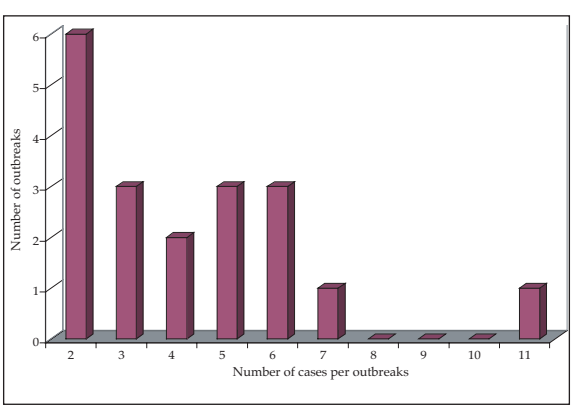


Figure 4
Number of salmonellosis human cases of salmonellosis per outbreak in the Abruzzo region April 2000-October 2002

safety legislation and requiring a risk analysis approach in the new policy to ensure the highest food safety standards in the EU. In the same document, the European Commission stated the need to establish an independent European Food Safety Authority, subsequently created in 2002 (10). Finally, in 2003, Directive 2003/99/EC of the European Parliament and European Council fixed new requirements for the monitoring of zoonoses and zoonotic agents (11), defining the plan of action for zoonoses control in subsequent years. In particular, Annex IV of Directive 2003/99/EC lists the minimum information that each member state must report to the EU each year. Regarding foodborne outbreaks the following data is required:

- total number of outbreaks
- number of human deaths and illnesses in the outbreaks
- causative agents of outbreaks, including, where possible, serotype or other definitive description of the agents (where identification of the causative agent is not possible, the reason for such absence of identification should be stated)
- foodstuffs incriminated in the outbreak and other potential sources
- identification of the type of place in which the foods were produced (or purchased, acquired, consumed)
- contributing factors (for example: deficiencies in food processing hygiene).

Each EU member state is therefore required to establish an effective national surveillance system for the rapid tracing of sources of infection in case of foodborne outbreaks.

The regional surveillance system described here may provide a valid example for the implementation of a wider national system. Although the project involved a limited number of institutions, strict collaboration between medical and veterinary

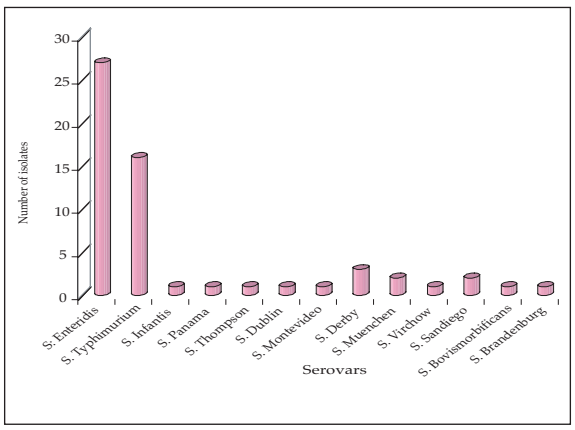


Figure 5
Distribution of salmonella serovars isolated from human faecal samples (n = 58)

services furnished accurate epidemiological investigations. Furthermore, the presence of a unique database, in which all relevant epidemiological information was stored, ensured standardisation and harmonisation of all data in terms of significance and procedures for collection. Finally, the database was always accessible to staff participating in the project, ensuring complete accessibility of information to the competent institutions.

A preliminary analysis of the surveillance system was conducted according to the recommendation for the evaluation of Public Health Surveillance Systems published by the CDC Guidelines Working Group (4). The surveillance system tested in this project proved to be highly flexible due to its linear and simple data flows, and respected the areas of competence of each institution stated by law (18). Despite the lack of full collaboration by staff of some institutions involved in the project, the percentage of outbreaks in which suspected food was identified (78.9%) and in which *Salmonella* spp. was isolated (21%) may be considered a good result. If one considers the Italian national official data for 2003 (17), the causative agent and contaminated food are only identified in 9.2%

(270 out of 2 948) of outbreaks. On the contrary, in case of sporadic cases, the sensitivity of the system was not satisfactory. The missed detection of sporadic cases might have been caused by a number of factors, as follows:

a) not all those involved in a foodborne disease asked for medical assistance
b) people sometimes hesitated to go to the hospital and/or commenced medication individually, reducing the probability of isolating the causative agent
c) emergency rescue units failed to recognise a significant number of infectious gastroenteritis cases, especially in milder cases.

The involvement of the Infectious Diseases Departments alone reduced the overall sensitivity of the system. A better result would have been obtained by also involving other hospital departments, such as emergency rescue, primary care, gastroenterology, paediatrics, etc.

Regarding the timing in the activation of the system, the delays in the hospitalisation of sporadic cases significantly reduced the promptness of response. The mean number of days between the appearance of the first symptoms and patient admission was 3, with a maximum of 32 days.

In conclusion, although the efficacy of the surveillance system established during the project was not entirely satisfactory, especially for the detection of sporadic cases, it represented a valuable first step in the organisation of a more comprehensive surveillance system on foodborne diseases. Further improvements should include more active participation of health institutions and physicians in order to increase the sensitivity of the system as a whole.

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