

## Research Note

# Prevalence of *Salmonella enterica* and *Listeria monocytogenes* Contamination in Foods of Animal Origin in Italy

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## ABSTRACT

The present survey collected and analyzed the results of routine testing for *Salmonella enterica* and *Listeria monocytogenes* on foods of animal origin submitted for official controls in Italy during 2001 to 2002. *Salmonella* was detected in 2.2% of 71,643 food samples examined, and the isolation rates ranged from 9.9% for raw poultry meat to less than 0.1% for dairy products. Isolation rates were also high in raw pork (4.9%) and processed meats (5.3%), which often involved pork. Low rates were observed in seafood (0.5%) and in ready-to-eat foods, such as grocery products (0.7%) and ice creams (0.1%). Serotyping showed that approximately 50% of the isolates belonged to the serotypes most commonly isolated from humans in Italy, thus confirming that most cases of human salmonellosis have a foodborne origin. Levels of *L. monocytogenes* were higher than what is accepted by the current regulation in 2.4% of 42,300 food samples. The positivity rates ranged from 10.3% in raw pork to none in eggs and egg products. Contamination rates were higher in other meat products (between 2 and 5%) and fish (6.5%) than in cheeses (1.1%) and other dairy products (0.6%). Routine control activities on the microbial contamination of foods can generate data with statistical and epidemiological value. Such data can be used as a basis for estimating the exposure of consumers to foodborne pathogens, following the trends of contamination over time, and evaluating the effects of control measures on the contamination of food.

Foodborne infections are an important public health concern worldwide. According to reports of the World Health Organization (22) and the Centers for Disease Control and Prevention (6), every year a large number of people are affected by diseases due to contaminated food consumption. A wide spectrum of pathogens play a role in foodborne disease. Most of them have a zoonotic origin and have reservoirs in healthy food animals from which they spread to an increasing variety of foods. Therefore, foods of animal origin are considered major vehicles of foodborne infections (19), and in industrialized countries they are subjected to compulsory control plans to detect microbial contamination. Nontyphoidal *Salmonella enterica* serovars and *Listeria monocytogenes* are classic foodborne pathogens considered to be the leading causes of foodborne deaths caused by known pathogens in the United States (11). *Salmonella* infections are a frequent cause of foodborne outbreaks and affect several million people worldwide each year (18). In Italy, *Salmonella* causes approximately 15,000 cases of gastroenteritis per year (16), but these numbers are

probably underestimated. It also represents one of the most frequent causes of sporadic childhood diarrhea (5).

Listeriosis is a relatively rare disease, but fatality rates range from 15 to 30% (6). In immunocompromised or elderly patients, listeriosis usually presents as meningitis or septicemia. Infection during pregnancy causes mild flulike illness in the mother but serious complications for the unborn infant. In healthy individuals, the clinical manifestation is usually limited to a febrile gastroenteritis (noninvasive listeriosis) (17). In Italy, *L. monocytogenes* invasive infections occur sporadically (13, 14), but two large foodborne outbreaks of febrile gastroenteritis have been reported (2, 15).

Contamination with *Salmonella* and *L. monocytogenes* occurs frequently in food of animal origin. The detection of both these pathogens is part of the routine microbiological testing of foodstuffs, which is performed in Italy by a network of 10 regional veterinary laboratories of the Istituti Zooprofilattici Sperimentali (IZS), which is part of the Italian National Public Health Service. The aim of the present survey was to analyze the results of the routine testing performed during 2001 to 2002 on food samples submitted for official controls to evaluate the current level of contami-

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nation with *Salmonella* and *L. monocytogenes* in different categories of foods of animal origin in Italy.

## MATERIALS AND METHODS

**Background.** In Italy, testing for *Salmonella* and *L. monocytogenes* in foods is a compulsory part of the authorities' microbiological controls to protect the health of consumers. Sampling is usually performed at the retail level and at processing plants. The Italian regulatory policy requires the absence of *Salmonella* in 25 g of product, whereas for *L. monocytogenes* the absence in 25 g of product is required for ready-to-eat products in the final package. For products that must be consumed after cooking, a level not higher than 110 CFU/g is regarded as acceptable.

For food of animal origin, testing is mainly performed by the network of the IZS, which covers all the Italian territory. The IZS laboratories are accredited by the National System for Laboratories Accreditation according to standard UNI CEI EN ISO/IEC.

**Questionnaire.** A standardized questionnaire to collect information on the laboratory methods used, the number and categories of food examined, and the results of the testing was prepared and sent to each IZS laboratory. In particular, the information requested included (i) the description of methods in use for *Salmonella* and *L. monocytogenes* detection in food items; (ii) the number of samples examined during the years 2001 to 2002 for each category of food according to the classification used by the Italian Ministry of Health (e.g., eggs and egg derivatives; raw meats, according to the animal species of origin; processed meats, which included hamburgers, raw sausages, and dry fermented sausages; seafood products, divided into fish, shellfish, and crustaceans; milk and cheeses; grocery products; and ice creams); (iii) the number of samples that tested positive for *Salmonella* or *L. monocytogenes*; and (iv) the serotypes of the *Salmonella* isolates.

**Data analysis.** Data on microbiological examination of food items and on the most common serotypes of *Salmonella* detected were collected in Microsoft Excel spreadsheets (Microsoft Corporation, Redmond, Wash.). The evaluation of the total number of examinations performed and the frequency of positive test results by type of food and animal species of origin were determined by EpiInfo statistical software, version 2002 (Centers for Disease Control and Prevention, Atlanta, Ga.).

## RESULTS

All 10 IZS laboratories provided the required information by filling in the questionnaire.

**Inventory of laboratory methods in use for *Salmonella* and *L. monocytogenes*.** All the IZS laboratories used standard operating procedures (SOPs) based on standard methods validated by the Association Française de Normalisation (AFNOR) and/or by the International Organization for Standardization (ISO). In particular, SOPs for *Salmonella* detection were based on ISO 6579:1993 and AFNOR V08/052:1997. The SOPs for *L. monocytogenes* detection were based on ISO 10560:1993 for milk and milk products, ISO 11290:1993 and AFNOR V08/055:1997 for samples other than milk and milk products, and ISO 7218:1996 for the enumeration.

Serotyping of the *Salmonella* isolates was performed by the slide agglutination method using commercial O and H antisera, according to the Kauffmann-White scheme. All

TABLE 1. Isolation of *Salmonella enterica* from different categories of foods in Italy (2001 to 2002)

Food category	No. of samples examined	No. (%) of positive samples
Raw meats by species		
Poultry	2,953	291 (9.9)
Swine	3,182	155 (4.9)
Bovine	5,037	49 (1.0)
Equine	273	6 (2.2)
Sheep and goat	151	0 (0.0)
Other species <sup>a</sup>	905	42 (4.6)
Not specified	3,103	50 (1.6)
Minced meats and sausages	14,266	751 (5.3)
Seafood products		
Shellfish	8,508	49 (0.6)
Fish	2,086	6 (0.3)
Crustaceans	879	4 (0.5)
Other	457	4 (0.9)
Not specified	2,465	11 (0.4)
Eggs	5,170	132 (2.6)
Egg-containing products (pasta all'uovo)	1,207	5 (0.4)
Milk and cream	3,725	4 (0.1)
Cheeses	11,981	12 (0.1)
Other dairy products	445	0 (0)
Gastronomy products	2,194	15 (0.7)
Ice creams	2,656	2 (0.1)
Total	71,643	1,588 (2.2)

<sup>a</sup> Mainly rabbit and pigeon.

laboratories participated in an external quality control program on *S. enterica* serotyping yearly organized by the National Reference Laboratory for *Salmonella*.

**Testing for *Salmonella*.** During 2001 to 2002, 71,643 food samples were examined for the presence of *Salmonella*. The highest number of tests (41.7%) was performed on meat products. Table 1 shows the number of samples examined for each category of food and the contamination rates observed. Overall, *Salmonella* was found in 1,588 (2.2%) of the samples, and the isolation rates ranged from 9.9% for poultry meat to less than 0.1% for milk, cream, and cheeses; other dairy products were free of *Salmonella*. High isolation rates were found in pork (4.9%), rabbit and pigeon meats (4.6%), and "processed meats" (5.3%), whereas lower rates were observed in seafood, cheeses, grocery products, and ice creams.

Serotyping results were available for 1,240 isolates. The 10 most frequently isolated serotypes are listed in Table 2, according to their prevalence in the different food categories. The *S. enterica* serotype Typhimurium was the most common serotype; it was found in all the food categories but mainly in meat from rabbit and pigeon and in pork; it was also frequent in processed meats, which frequently involved pork. Other serotypes were mainly associated with a given category of food. *Salmonella* Enteritidis represented

TABLE 2. Serotypes of *Salmonella enterica* by category of food<sup>a</sup>

<i>Salmonella</i> serotype	Total	Minced meat and sausages	Raw meats				Eggs and products	Seafood	Other (ice creams, milk)
			Swine	Poultry	Bovine	Other species			
Typhimurium <sup>b</sup>	233 (18.8)	125 (21.3)	56 (36.8)	17 (8.5)	4 (14.3)	16 (39.0)	6 (4.4)	6 (10.9)	3 (7.5)
Derby <sup>b</sup>	130 (10.5)	92 (15.6)	28 (18.4)	0	4 (14.3)	3 (7.3)	0	1 (1.8)	2 (5.0)
Enteritidis <sup>b</sup>	123 (9.9)	4 (0.7)	0	2 (1.0)	0	0	110 (80.3)	3 (5.5)	4 (10.0)
Anatum	64 (5.2)	52 (8.8)	2 (1.3)	0	3 (10.7)	5 (12.2)	0	1 (1.8)	1 (2.5)
Infantis <sup>b</sup>	37 (3.0)	18 (3.1)	4 (2.6)	4 (2.0)	0	0	2 (1.5)	7 (12.7)	2 (5.0)
Panama	23 (1.9)	21 (3.6)	1 (0.7)	0	0	0	0	1 (1.8)	0
Blockley <sup>b</sup>	29 (2.3)	1 (0.2)	0	23 (11.6)	1 (3.6)	4 (9.8)	0	0	0
Hadar <sup>b</sup>	25 (2.0)	3 (0.5)	0	20 (10.1)	1 (3.6)	1 (2.4)	0	0	0
Bredeney	29 (2.3)	20 (3.4)	1 (0.7)	1 (0.5)	1 (3.6)	0	2 (1.5)	2 (3.6)	2 (5.0)
London	13 (1.0)	9 (1.5)	4 (2.6)	0	0	0	0	0	0
Others	534 (43.1)	243 (41.3)	56 (36.8)	132 (66.3)	14 (50.0)	12 (29.3)	17 (12.4)	34 (61.8)	26 (65.0)
Total	1,240	588	152	199	28	41	137	55	40

<sup>a</sup> Data are presented as number (percent).

<sup>b</sup> Serotypes included among the 10 most frequently isolated from human beings in Italy in the years 2001 and 2002 (data from Enter-net).

almost all of the isolates from eggs. *Salmonella* Derby was frequently found in pork and processed meats. *Salmonella* Blockley and *Salmonella* Hadar were both associated with poultry meat. *Salmonella* Infantis represented the most common serotype from seafood, and *Salmonella* Anatum was found in processed meats containing pork and/or turkey. Four of the five most frequently reported serotypes

were included among the five serotypes most commonly isolated from human beings in Italy in the years 2001 and 2002 (3, 4).

TABLE 3. Presence of *Listeria monocytogenes* in different categories of foods in Italy (2001 to 2002)

Food category	No. of samples examined	No. (%) of positive samples
Raw meats by species		
Swine	1,024	105 (10.3)
Bovine	1,182	64 (5.4)
Poultry	1,269	24 (1.9)
Sheep and goat	31	1 (3.2)
Equine	78	4 (5.1)
Other species <sup>a</sup>	262	11 (4.2)
Not specified	3,005	35 (1.2)
Minced meats and sausages	8,943	404 (4.5)
Seafood products		
Fish and fish products	3,160	204 (6.4)
Shellfish	1,494	0 (0)
Crustaceans	347	0 (0)
Other seafood	778	5 (0.6)
Cheeses	13,858	148 (1.1)
Milk and cream	3,182	19 (0.6)
Eggs	431	0 (0)
Egg-containing products		
(pasta all'uovo)	425	13 (3.1)
Gastronomy products	1,097	18 (1.6)
Ice creams	1,734	5 (0.3)
Total	42,300	1,060 (2.5)

<sup>a</sup> Mainly rabbit and pigeon.

**Testing for *L. monocytogenes*.** During 2001 to 2002, 42,300 food samples were examined for the presence of *L. monocytogenes*. The highest number of tests was performed on dairy products (40.3%) and meat products (37.3%). Table 3 shows the number of samples examined for each category of food and the observed contamination rates. Overall, the presence of *L. monocytogenes* was higher than what is accepted by the current regulation in 1,060 samples (2.5%). The positivity rates ranged from 10.3% in raw pork to none in eggs, shellfish, and crustaceans. High isolation rates were also found in fish and fish products (6.4%), bovine and equine raw meats (approximately 5%), and "processed meats" (4.5%); lower rates were observed in seafood, cheeses, grocery products, and ice creams.

## DISCUSSION

*Salmonella* and *L. monocytogenes* are important food-borne pathogens; the recently issued European Directive 2003/99/EC on the monitoring of zoonoses (9) requires the implementation of mandatory control programs for both of them. Detection of the contamination of food of animal origin with *Salmonella* and *L. monocytogenes* is part of the compulsory routine microbiological testing of foodstuffs in Italy, and a large number of samples are examined each year as part of the official control activities performed by the National Public Health Service. This is the first time that the results of the testing performed in different regions are merged and analyzed to obtain estimates of the consumer exposure in our country. The present survey reviewed the activities performed in this field by 10 regional veterinary laboratories to evaluate the current level of contamination with *Salmonella* and *L. monocytogenes* in different categories of foods of animal origin in Italy.

The overall prevalence of *Salmonella* and *L. monocy-*

*togenes* in the foods examined during 2001 and 2002 was 2.2 and 2.5%, respectively. *Salmonella* was more frequently detected in meat products and eggs, whereas it was rare in fish and shellfish and almost absent in milk and dairy products. Among meat products, the highest contamination rates were found in poultry, followed by pork, and minor species such as rabbit and pigeon. All the categories of ready-to-eat products, such as cheeses, ice creams, and grocery products, showed prevalence rates lower than 1%. Figures reported for other countries included *Salmonella* prevalence rates in pork, beef, eggs, and poultry that ranged from 0 to 1.5% in Sweden, Norway, and Finland to 6.4% for pork in Spain and 12.7% for poultry in Greece and Germany (7). Other recently published studies reported similar rates for meat products in the United States (23) and for retail chicken in Northern Ireland (21). Much higher rates (36 to 60%) have been reported for poultry in studies conducted in Belgium (20), Spain (8), and Portugal (1). However, these differences could be due in part to the types of samples analyzed and the step of the food chain sampled.

Serotyping showed that approximately 50% of the *Salmonella* food isolates belonged to the serotypes most commonly isolated from human beings in Italy in the years 2001 and 2002 (3, 4), thus confirming the view that many cases of human salmonellosis have a foodborne origin (11, 18). Some of the serotypes were clearly associated with given food categories, likely reflecting their presence in the animal species from which the foods originated.

As for *Salmonella*, *L. monocytogenes* contamination was frequently detected in meat products and also in fish and fish products. Similar results have been reported in an Italian survey performed in the years 1990 to 1999 (10). Data reported for other European countries confirm that the highest contamination rates are observed in pork, other meats, and fish products, even if the range of prevalence is very broad (7). As in these other studies, the present survey showed a low prevalence in dairy products, which are usually considered products at risk for acquiring *L. monocytogenes* infection. Conversely, it appears that attention has to be paid to the contamination of ready-to-eat fish products, which could be considered an emerging issue (12).

In general, the results of controls performed in Italy by public laboratories show that the contamination rate of food items with *Salmonella* and *L. monocytogenes* is similar to that observed in other European countries. For both organisms, the contamination rates were highest in meat products and were related to the type of meat, with poultry and pork showing the highest rates. However, poultry and uncured pork products are usually eaten after cooking; therefore, they may be considered at low risk even in the event of contamination. Other contaminated items, such as rabbit and pigeon, have a much less quantitative impact on consumers.

A limitation of our study is that it does not take into account food preparation. It is well known that contamination of food items that are usually consumed after cooking represents a low threat from a public health point of view; however, more attention should be paid to the contamination of ready-to-eat products. Consumption of un-

dercooked meat products and cross-contamination during food handling and preparation must be avoided to ensure food safety at home and in the food service industry.

In conclusion, the present study shows that the Italian surveillance system for foodborne zoonoses is in place and that routine control activities on the microbial contamination of foods can generate data with high statistical and epidemiological value, considering that sampling is usually performed randomly and that large numbers of samples are examined on a nationwide basis. Such data may provide information to drive the sampling strategies on food items more at risk of contamination and to determine at which point of the food chain the contamination occurs. They can also be used as a basis for (i) estimating the exposure of consumers to foodborne pathogens, (ii) following the trends of contamination over time, and (iii) evaluating the effects that control measures can have on the contamination of food.

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