Journal of Food Protection, Vol. 80, No. 4, 2017, Pages 632–639
doi:10.4315/0362-028X.JFP-16-331

Research Note

Foodborne Salmonellosis in Italy: Characterization of *Salmonella enterica* Serovar Typhimurium and Monophasic Variant 4,[5],12:i– Isolated from Salami and Human Patients

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MS 16-331: Received 2 August 2016/Accepted 5 December 2016/Published Online 14 March 2017

ABSTRACT

Salmonella enterica serovar Typhimurium (STm) and its monophasic variant 4,[5],12:i:– (VMSTm) have been responsible for an increased number of foodborne infections in humans in Europe in recent years. The aim of this study was to investigate the origin of three foodborne salmonellosis outbreaks that occurred in Pavia Province (Lombardy region, northern Italy) in 2010. Phenotypic and genetic characteristics of the STm and VMSTm isolates from patients and from food that were recovered in the framework of the three outbreaks were evaluated through serotyping, phage typing, antimicrobial susceptibility testing, pulsed-field gel electrophoresis (PFGE), and multiple-locus variable-number tandem repeat analysis (MLVA). Salami from three artisan producers, which had all purchased meat from the same slaughterhouse, was the food source of infection in outbreak I. STm isolates were recovered from salami and patients with symptoms of gastroenteritis. These isolates had the same PFGE type and the same rare MLVA profile (3-18-9-NA-211). The same molecular profiles were found in an STm isolate from a salami, which likely was the source of another family outbreak (II). A VMSTm strain with common phenotypic and molecular profiles was isolated from three hospitalized patients and identified as the cause of another putative outbreak (III). During the following 3 years (2011 through 2013), 360 salami produced in Pavia Province were monitored for the presence of *S. enterica*. In 2011, no STm and VMSTm isolates were recovered from 159 salami tested. During 2012 and 2013, 13.9% of 201 tested salami harbored *S. enterica,* and half of the isolates were VMSTm, mainly in salami from those artisan producers involved in the previous outbreaks. These isolates were genetically variable, especially in terms of MLVA profiles. The data collected suggest that from 2012, VMSTm has replaced STm in the environments of the salami producers monitored in this study, and these data confirm the dominance of this emergent serovar along the pork supply chain.

Key words: Foodborne outbreak; Monophasic variant 4,[5],12:i–; Salami; *Salmonella* Typhimurium

Salmonellosis is the second most commonly reported gastrointestinal infection and an important cause of foodborne outbreaks in the European Union and the European Economic Area. However, salmonellosis rates in the European Union and European Economic Area show a significant 5-year decreasing trend, which has been largely attributed to the implementation of successful veterinary control programs in poultry farms. In 2013, *Salmonella enterica* serovar Typhimurium (STm) and the monophasic variant of *Salmonella* Typhimurium (VMSTm) were the second and the third most commonly reported serotypes, respectively, isolated from humans in the European Union. The reservoir of *S. enterica* is the intestinal tract of wild and domestic animals, and humans become infected usually through the consumption of contaminated raw or undercooked food *(12)*. Contamination often occurs when organisms are introduced into preparation areas and are allowed to replicate in food because of inadequate storage temperatures and/or cooking or cross-contamination of ready-to-eat foods *(8)*. In Italy, as in other countries, STm strains have been the most common cause of human

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infection since 2001 *(10),* and VMSTm strains have been the second highest since 2009 *(18)*. Together, these serovars account for more than 50% of the human *S. enterica* isolates during 2009 through 2011 *(20)*.

Among all animal-derived strains reported in Italy to the National Reference Laboratory for *S. enterica* in the framework of the Enter-Vet network between 2002 and 2010, the proportion of VMSTm strains increased from 3.5 to 10.3%. In 2010, VMSTm was the most common serovar isolated from food and animal samples *(4)*. The vast majority of these monophasic strains were recovered from pigs and pork products *(5)*.

In Italy, pork products, in particular salami, are widely consumed. Although these dry fermented sausages are traditionally considered safe because of their low pH, low water activity, and high salinity, *S. enterica* can survive fermentation and drying procedures, especially when the manufacturing processes or fermentation periods are inadequate *(7)*. In Italy, salami contaminated with various clones of STm have been responsible for a few documented human outbreaks. Reported cases include one in 1995 caused by STm DT 193 *(24)* and another in 2004 caused by STm DT 104A *(21)* and an outbreak involving Italy and Sweden in 2005 caused by a nontypeable (NT) STm *(14)*.

The aim of this study was to investigate three pork-related foodborne salmonellosis outbreaks caused by STm and VMSTm that occurred in Pavia Province (Lombardy region, northern Italy) in 2010. We also implemented a 3-year (2011 through 2013) *Salmonella* monitoring program in Pavia Province among salami producers either involved or not involved in the three outbreaks to monitor contamination over time.

MATERIALS AND METHODS

Outbreak descriptions. Three human salmonellosis outbreaks occurred in Pavia Province, in the Lombardy region of northern Italy, in November 2010.

Outbreak I involved about 30 guests from one restaurant over a period of 1 month. Guests had become ill with symptoms of gastroenteritis, and the epidemiological investigation revealed that all had eaten salami at the restaurant.

Outbreak II involved the five members of a family who consumed salami bought from a local salami producer (producer p3). All five family members had symptoms of gastroenteritis. An *S. enterica* isolate from White-Kauffmann-Le Minor group B was recovered from four family members; the isolate from one hospitalized family member was serotyped.

The epidemiological investigation of the two outbreaks identified four artisan producers of salami (p1, p2, p3, and p6), which had all purchased meat from the same slaughterhouse (A), as potentially responsible for these outbreaks. In both outbreaks, each guest ate approximately 50 g of pork products, of which about half was salami.

In the same period, San Matteo Hospital in Pavia collected three *S. enterica* isolates from patients with symptoms of gastroenteritis. In this case, the epidemiological investigation did not ascertain a correlation between the foodborne episodes and the source of infection. Based on the subtyping approaches used, which identified these three strains as indistinguishable, these cases have been considered as belonging to another putative salmonellosis outbreak (III).

Sample collection and *S. enterica* isolates. Twenty-two samples of salami were collected from four involved producers (p1, p2, p3, and p6) in the context of the epidemiological investigations of outbreaks I and II from November 2010 to February 2011. All producers had purchased raw meat for salami production from artisan slaughterhouse A; producer p3 had also purchased meat from industrial slaughterhouse B.

From March 2011 to December 2011, within a Provincial Surveillance Program carried out to investigate the presence of *S. enterica* in salami from different producers, 25 salami from producers p1, p2, p5, and p6, which had purchased meat from slaughterhouse A, were sampled according to the criterion of one salami per production batch. In the same period, 134 salami were collected from epidemiologically unrelated artisan producers (91) and slaughterhouses, other than slaughterhouse A, according to the same sampling criterion.

In 2012 and 2013, within a Regional Surveillance Program conducted in Lombardy for the same purpose, 52 samples of salami were collected from 6 artisan producers: the four producers (p1, p2, p3, and p6) involved in the outbreaks of 2010 and two additional producers (p4 and p5) that had purchased raw meat from slaughterhouse A. The sampling plan of the Regional Surveillance Program was conducted according to Commission Regulation (EC) No 2073/2005 *(11)* by collecting five sampling units per batch of salami. Over the same period, 149 additional samples collected from epidemiologically unrelated producers, both artisan (48) and industrial (31), and from slaughterhouses other than A and B in Pavia Province were analyzed for the same purpose.

The distribution of *S. enterica* isolates from salami by scope of sampling, period, producer, and slaughterhouse is reported in Table 1. In the context of the three outbreaks, a total of eight *S. enterica* isolates were recovered from human feces: four from outbreak I, one from outbreak II, and three from outbreak III (Table 2).

***S. enterica* detection and serotyping.** *S. enterica* was isolated from salami according to method ISO 6579:2002/Cor1:2004 *(16)* at the Istituto Zooprofilattico Sperimentale della Lombardia e dell' Emilia Romagna (IZSLER) laboratories. Suspect *S. enterica* colonies were tested for biochemical properties with API 20E micro-substrate system (bioMérieux, Marcy l'Étoile, France). *S. enterica* serotyping was performed according to the White-Kauffmann-Le Minor scheme by slide agglutination with O and H antigen-specific sera (Staten Serum Institute, Copenhagen, Denmark). VMSTm isolates were definitively identified and differentiated from STm using a previously described PCR protocol *(3)*.

Molecular characterization of STm and VMSTm isolates. Pulsed-field gel electrophoresis (PFGE) was performed according to the PulseNet standardized protocol using restriction enzyme *Xba*I (http://www.cdc.gov/pulsenet/PDF/ecoli-shigella-salmonella-pfge-protocol-508c.pdf). *Xba*I-digested DNA from *Salmonella* Braenderup H9812 was used as a molecular size marker *(15)*. Gel images were analyzed by BioNumerics 6.6 software package (Applied Maths, Sint-Martens-Latem, Belgium). PFGE pulsotypes differing by one or more fragments were considered distinct *(6)*. The pulsotypes of the isolates were named according to the IZSLER coding system. Profiles were compared using cluster analysis based on Dice's similarity index, and a dendrogram was obtained by using the unweighted pair group method with arithmetic mean (1% tolerance, 1% optimization).

Multiple-locus variable-number tandem repeat analysis (MLVA) was performed according to the protocol previously

TABLE 1. *Distribution of* Salmonella enterica *isolates from salami by scope of sampling, period, producer, slaughterhouse, and serotype*

Producer	Slaughterhouse	No. of samples	No. of samples positive for:			
			Salmonella	STm	VMSTm	Other serotypes
Epidemiological investigation of the outbreaks (Nov. 2010–Feb. 2011)						
p1	A	3	2	2		
p2	A	10	5	3		2 (1 Derby, 1 Ohio)
p3	A	6	5	5		
p3	B	2	2	2		
p6	A	1				
Total		22	14	12	0	2
Pavia Province surveillance program (Mar. 2011–Dec. 2011)						
p1	A	6	1			1 (Derby)
p2	A	17				
p5	A	1				
p6	A	1				
Total		25	1	0	0	1
Other producers ($n = 91$)	Other slaughterhouses	134	1			1 (Derby)
Regional surveillance program in Pavia Province (Jan. 2012–Dec. 2013)						
p1	A	1				
p2	A	36	7		6	1 (NT[a])
p3	B	2	1		1	
p4	A	8	2		2	
p5	A	3	2		1	1 (Infantis)
p6	A	2	1		1	
Total		52	13	0	11	2
Other producers ($n = 79$)	Other slaughterhouses	149	15		3	12 (8 Derby, 2 Rissen, 1 Infantis, 1 NT)

[a] NT, nontypeable.

described *(19)*. The size measurements for each locus were estimated using a CEQ 8000 genetic analysis system (Beckman Coulter, Palo Alto, CA). MLVA profiles were assigned as a string of five numbers (STTR9, STTR5, STTR6, STTR10pl, and STTR3) representing the variable number of tandem repeats (VNTR) at the corresponding locus or as NA (no amplification, when PCR amplification results were negative) according to the MLVA nomenclature suggested by Larsson et al. *(17)*. For each isolate, the corresponding VNTR profile was imported as character data into the BioNumerics 6.6 software package.

Phage typing and antimicrobial susceptibility testing. The STm and VMSTm isolates were phage typed by the National Reference Laboratory for *Salmonella* (Istituto Zooprofilattico Sperimentale delle Venezie, Padova, Italy) using the protocol reported by Anderson et al. *(1)* and following the interpretative guidelines defined for STm by Public Health England (Colindale, London, UK) *(25)*. Isolates that did not react with any of the typing phages were considered NT.

Antimicrobial susceptibility testing of human and salami STm and VMSTm isolates was performed using the disk diffusion method according to the criteria established by the Clinical and Laboratory Standards Institute *(9)*. The antimicrobial agents (BD, Franklin Lakes, NJ) were tested using antibiotic disks: ampicillin (10 μg), cefotaxime (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), gentamicin (10 μg), kanamycin (30 μg), nalidixic acid (30 μg), streptomycin (10 μg), sulfonamides (250 μg), tetracycline (30 μg), and trimethoprim-sulfamethoxazole (1.25 and 23.75 μg, respectively). *Escherichia coli* ATCC 25922 was used as a control strain in the tests.

Statistical analysis. Fisher's test was used to evaluate whether the difference between the proportions of positive salami samples from the producers, both those related and those not related to the 2010 outbreaks within the Regional Surveillance Program, were significant at $P < 0.01$.

RESULTS

***Salmonella* detection and serotyping.** The four human isolates from outbreak I were serotyped as STm. From outbreak II, the single isolate available was serotyped as STm. The three isolates from outbreak III were serotyped as VMSTm (Table 2).

During the epidemiological investigations of outbreaks I and II, 14 (63.6%) of 22 salami from producers p1, p2, and p3, who bought pork from slaughterhouse A, harbored *S. enterica*. Of these 14 isolates, 12 (85.7%) were serotyped as STm and 2 belonged to other serotypes (Table 1).

Within the Provincial Surveillance Program conducted from March to December 2011, 1 of 25 salami from producers p1, p2, p5, and p6 was positive for *Salmonella* Derby. In the same monitoring program, 1 of the 134 salami sampled from producers and slaughterhouses epidemiologically unrelated to the 2010 outbreaks harbored *Salmonella* Derby.

In 2012 and 2013 in the Regional Surveillance Program, the producers involved in the 2010 outbreaks and/or who purchased meat from the slaughterhouses involved were monitored. In this program, 13 (25%) of 52

TABLE 2. *Phenotypic and genetic characterization of 17 STm and 17 VMSTm isolates from salami and humans*

Origin	Producer	Slaughterhouse	Source	No. of isolates	Serotype	PFGE *Xba*I profile	MLVA STTR profile[a]	Phage type (no. of isolates)	Resistance type[b]
Outbreak I (Nov. 2010)			Human	4	STm	STYMXB_BS.0043	3-18-9-NA-211	DT120 (3), NT[c] (1)	Susceptible
Outbreak I epidemiological investigation (Nov. 2010–Feb. 2011)	1	A	Salami	2	STm	STYMXB_BS.0043	3-18-9-NA-211	DT120	Susceptible
	2	A	Salami	3	STm	STYMXB_BS.0043	3-18-9-NA-211	DT120	Susceptible
	3	A	Salami	4	STm	STYMXB_BS.0043	3-18-9-NA-211	DT120	Susceptible
		B		2	STm	STYMXB_BS.0043	3-18-9-NA-211	DT29	Susceptible
Outbreak II (Nov. 2010)			Human	1	STm	ND[d]	ND	ND	ND
Outbreak II epidemiological investigation (Nov. 2010–Feb. 2011)	3	A	Salami	1	STm	STYMXB_BS.0043	3-18-9-NA-211	DT120	Susceptible
Outbreak III (Nov. 2010)			Human	3	VMSTm	STYMXB_0131	3-13-10-NA-211	NT	ASSuT
Regional Surveillance Program (2012)	2	A	Salami	6	VMSTm	STYMXB.0131	3-14-9-NA-211	NT (2) DT120 (1) DT193 (3)	Susceptible
	6	A	Salami	1	VMSTm	STYMXB.0131	3-14-9-NA-211	DT193	Susceptible
	4	A	Salami	2	VMSTm	STYMXB.0131	3-13-10-NA-211 3-11-9-NA-211	NT	ASSuT
	5	A	Salami	1	VMSTm	STYMXB.0131	3-14-10-NA-211	NT	ASSuT
	3	B	Salami	1	VMSTm	STYMXB_PR.0538	3-11-14-NA-211	DT120	ASSuT
	7	C	Salami	1	VMSTm	STYMXB_PR.0550	3-12-9-NA-211	DT120	ASSuT
	8	D	Salami	1	VMSTm	STYMXB_PR.0553	3-12-10-NA-211	DT193	Susceptible
Regional Surveillance Program (2013)	8	D	Salami	1	VMSTm	ND	ND	ND	ND

[a] MLVA profile numbers are STTR9, STTR5, STTR6, STTR10pl, and STTR3. NA, no amplification (no PCR results).
[b] ASSuT, resistant to ampicillin, streptomycin, sulfonamides, and tetracycline.
[c] NT, nontypeable.
[d] ND, not done.

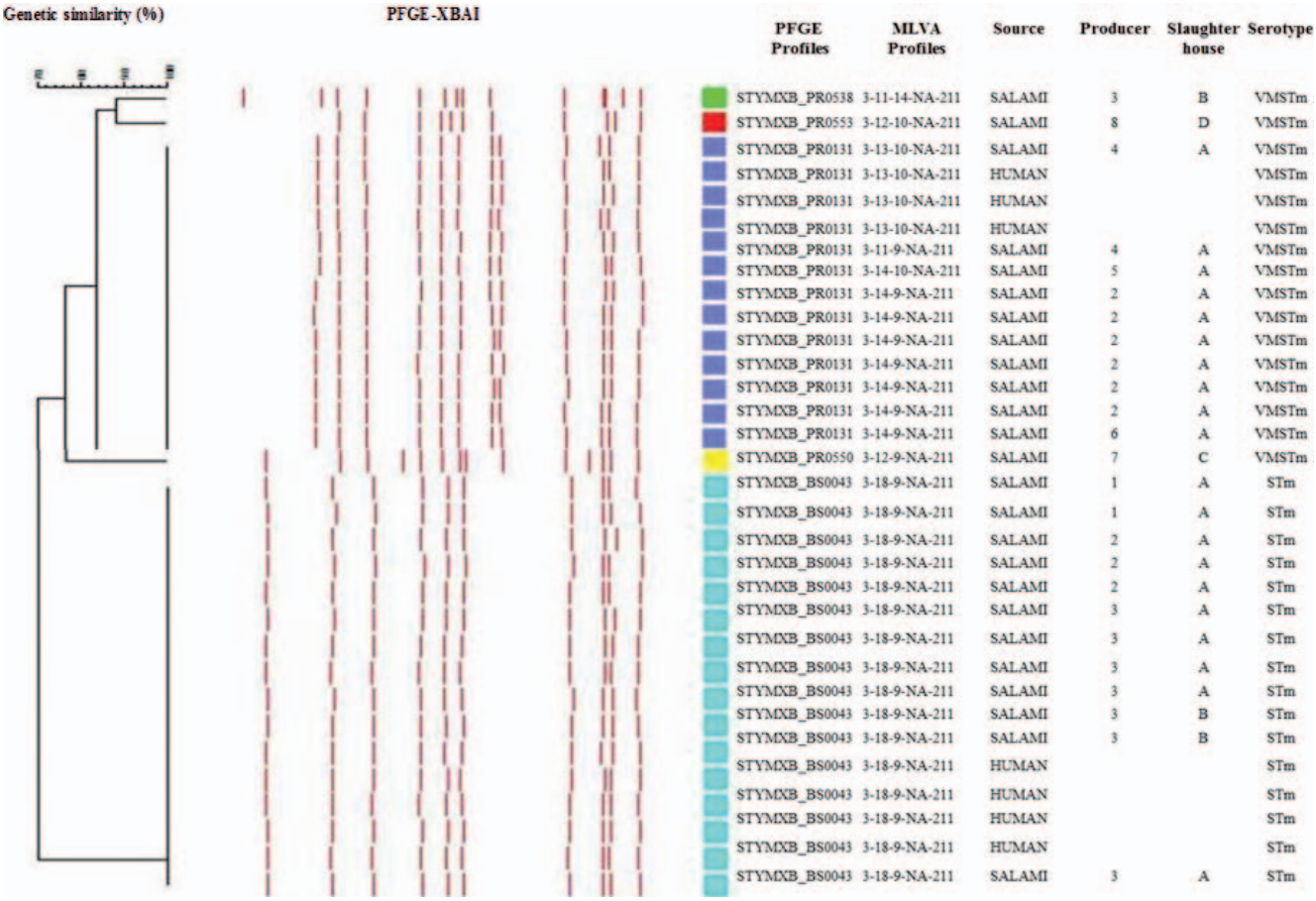

FIGURE 1. *Dendrogram of genetic similarity among the PFGE profiles of 32 human and salami* Salmonella enterica *serovar Typhimurium (STm) and 4,[5],12:i– (VMSTm) isolates. Similarity analysis was performed using the Dice coefficient (1.00% optimization, 1.00% tolerance), and clustering was performed using the unweighted pair group method with arithmetic mean.*

salami harbored *S. enterica* (Table 1). All producers except p1 had at least one positive sample. Eleven of the 13 isolates were serotyped as VMSTm. In the same period, 15 (10.1%) of the 149 salami from other producers and slaughterhouses were *S. enterica* positive, and 3 isolates were VMSTm. The difference between the proportions of positive salami from the producers involved and those not involved in the 2010 outbreak was significant.

Molecular characterization of ST and VMSTm isolates. The PFGE and MLVA profiles of all STm and VMSTm isolates are listed in Table 2, and the genetic similarity of PFGE profiles is shown in the dendrogram of Figure 1.

The four human STm isolates from outbreak I were PFGE type STYMXB_BS.0043 and MLVA profile 3-18-9-NA-211. The only human STm isolate from outbreak II was not available for genotyping, so the result for this isolate is listed as "not done" in Table 2. All STm isolates from salami that were sampled during the epidemiological investigation of outbreaks I and II from the producers involved had the same PFGE (STYMXB_BS.0043) and MLVA (3-18-9-NA-211) profiles as the outbreak I human isolates. This finding supports the hypothesis that salami from these particular producers was involved in the outbreaks. These findings were supported by answers to food questionnaires circulated during the epidemiological investigation, from which salami emerged as the suspect food.

The three VMSTm human isolates from outbreak III were PFGE type STYMXB_0131 and MLVA profile 3-13-10-NA-211.

During the Regional Surveillance Program of 2012 to 2013, seven VMSTm isolates from producers p2 and p6, both of which had purchased meat from slaughterhouse A, shared the same PFGE (STYMXB_0131) and MLVA (3-14-9-NA-211) profiles. Three isolates from producers p4 and p5 (slaughterhouse A) had the same PFGE type (STYMXB_0131) but had three different MLVA profiles (3-13-10-NA-211, 3-11-9-NA-211, and 3-14-10-NA-211). MLVA profile 3-13-10-NA-211 from producer p4 was the same type identified for the isolates from outbreak III, which had occurred 2 years previously.

Three VMSTm isolates from producers p3, p7, and p8 (slaughterhouses B, C, and D) differed in both PFGE and MLVA profiles. Three unique MLVA profiles (3-11-14-NA-211, 3-12-9-NA-211, and 3-12-10-NA-211) corresponded to different PFGE types (STYMXB_PR.0538, STYMXB_PR.0550, and STYMXB_PR.0553). Only sero-

typing was available for the single VMSTm isolate found in 2013.

Phage types and antimicrobial susceptibility. Detailed results of phage typing and antimicrobial susceptibility testing are listed in Table 2. Three of four human STm isolates, apparently representing the same clone, were phage typed as DT120 and the fourth was NT. Ten of 12 STm isolates from salami were phage type DT120, and the remaining two isolates were phage type DT29. Three human VMSTm isolates were NT. VMSTm isolates from salami were identified as phage types DT120 (three isolates), DT193 (five isolates), and NT (five isolates).

With regard to antimicrobial resistance, all STm isolates were pansusceptible to the panel of antimicrobial agents tested. Eight VMSTm isolates were fully susceptible, and eight were resistant to ampicillin, streptomycin, sulfonamides, and tetracycline (resistance type ASSuT), a pattern typical of this serovar, including the three human isolates of outbreak III and the isolate from salami from producer p4, which had the same PFGE and MLVA profiles as the outbreak strain.

For the only human STm isolate from outbreak II and for the single VMSTm isolate from the 2013 Regional Surveillance Program, no phenotypic data were available (listed in Table 2 as not done).

DISCUSSION

The epidemiological investigations of three human salmonellosis outbreaks caused by STm and VMSTm that occurred in November 2010 in Lombardy, Italy led to the isolation of *S. enterica* from salami. As a consequence, the salami manufactured by producers in the area were monitored over time for the presence of *S. enterica*.

A high proportion (>60%) of the sampled salami manufactured by the artisan producers involved in outbreaks I and II harbored *S. enterica*. Eighty-six percent (12 of 14 samples) were contaminated with STm isolates that had the same PFGE and MLVA profiles (STYMXB_BS.0043 and 3-18-9-NA-211, respectively) as the human STm isolates recovered during outbreak I. MLVA profile 3-18-9-NA-211 is rare, having been found only in these isolates of the 3,000 isolates examined for MLVA profile and listed in the IZSLER database (unpublished data). To our knowledge, isolates with this profile have not been reported previously anywhere in the world, reinforcing the evidence of a link between these salami and the human cases. These STm isolates with the rare MLVA profile were recovered from salami manufactured by three different producers and prepared in different environments and under different conditions during a short period of time (4 months), which suggests a common source of infection. The source was identified as the artisan slaughterhouse A, from where the producers had purchased the meat. The single finding of an STm isolate with the rare MLVA profile in a batch of salami from producer p3 made with meat from slaughterhouse B could have been due to cross-contamination by meat from slaughterhouse A at p3, who also obtained meat from slaughterhouse A.

The same PFGE, MLVA, and phenotypic profiles were also shared by an STm isolate recovered from a salami associated with outbreak II and purchased from producer p3. This finding supports salami as the source of outbreak II, although this association could not be confirmed because of the lack of subtyping data from the one available human isolate associated with this outbreak. The STm clone identified in outbreaks I and II was susceptible to all antimicrobial agents examined, and the isolates shared identical PFGE and MLVA profiles, although heterogeneity emerged with phage typing (three phage types were associated with this clone; Table 2). The main phage type identified within the clone (DT120) is quite common among animal-derived STm strains *(4)*. Although this evidence suggests a possible lack of reproducibility of phage typing, for outbreaks I and II the use of phage typing alone would not have allowed us to unequivocally link the human cases and the sources of infection because of the common occurrence of DT120.

Human *Salmonella* strains associated with outbreak III belonged to VMSTm and shared the same PFGE (STYMXB_0131) and MLVA (3-13-10-NA-211) profiles and resistance pattern (ASSuT). This phenotypic and genetic profile combination is common among VMSTm isolates recovered during IZSLER surveillance and is quite common among Italian human VMSTm isolates, as reported by Luzzi et al. *(20)*. For outbreak III, it was not possible to ascertain the source of infection. Nevertheless, a VMSTm isolate with the same PFGE and MLVA profiles (STYMXB_0131 and 3-13-10-NA-211, respectively) and the same ASSuT resistance pattern was found in a salami manufactured 2 years after outbreak III, which suggests that salami or pork meat could have been the source of the three human outbreak III cases.

In the monitoring conducted during March through December 2011, 1.3% (2 of 159) of salami from producers related and unrelated to the outbreaks of 2010 harbored *S. enterica,* and no STm or VMSTm were detected. Probably, good manufacturing practices and appropriate sanitation were successfully implemented in the slaughterhouses and production facilities after the outbreaks. In particular, the sanitation practices of slaughterhouses and production facilities were improved by increasing the frequency of sanitation and by separating the food processing areas. Monitoring conducted in 2012 and 2013 revealed that 13.9% (28 of 201) of salami harbored *S. enterica*. A significantly higher proportion of the positive findings originated from artisan producers that had been involved in the 2010 outbreaks and/or had purchased meat from artisan slaughterhouse A (12 of 50 samples), whereas only a minor proportion of contaminated samples (15 of 149) were from other producers. Overall, during 2012 and 2013 monitoring, 50% (14 of 28) of the isolates were VMSTm, and no STm was detected.

In the context of our investigations, the ASSuT antimicrobial resistance pattern was detected only in VMSTm isolates and not in STm, which is partially in agreement with data from the European Food Safety Authority and the European Centre for Disease Prevention and Control *(13),* who reported that more than 70% of STm

and VMSTm serotypes isolated from pork meat had multiresistance patterns.

In conclusion, the results obtained in this study confirm that in 2012 and 2013, VMSTm replaced STm in the environments of Pavia salami producers. This finding is in keeping with the observation of an increase in the isolation of animal-derived VMSTm in Italy from 2010 *(4)*. The extended sampling method adopted in our study enabled us to detect *S. enterica* subtypes in salami, thus likely preventing the onset of foodborne outbreaks due to the good manufacturing practices and hygienic measures adopted as a result. Although the data presented in this study confirm the clear role of the pig as an important contributor to the burden of human salmonellosis, currently no control program for *Salmonella* in the pork chain has been implemented in Italy. The evidence collected in the present study and the results of the source attribution studies conducted in Italy *(2, 22, 23)* strongly support the need to urgently focus efforts on the pork supply chain through the application of control plans, as has been done for the poultry industry, with the aim of further reducing salmonellosis at the national level. Further studies are necessary to clarify the factors affecting the persistence of serovars, including STm and VMSTm, in pig farms, slaughterhouses, and pork production facilities.

ACKNOWLEDGMENTS

We thank Luisa Gennari, Paola Scotto di Fasano, and Anna Frulio for technical assistance and our Veterinary Service colleagues for fruitful cooperation. We also thank Dr. Simone Magnino for his proofreading and useful comments. This work was partly supported by a research grant from the Italian Ministry of Health (IZSLER PRC 2010-013).

REFERENCES

1. Anderson, E. S., L. R. Ward, M. J. De Saxe, and J. D. De Sa. 1977. Bacteriophage-typing designations of *Salmonella* Typhimurium. *J. Hyg. (Lond.)* 78:297–300.
2. Barco, L., F. Barrucci, E. Cortini, E. Ramon, J. E. Olsen, I. Luzzi, A. A. Lettini, and A. Ricci. 2015. Ascertaining the relationship between *Salmonella* Typhimurium and *Salmonella* 4,[5],12:i:– by MLVA and inferring the sources of human salmonellosis due to the two serovars in Italy. *Front. Microbiol.* 6:301.
3. Barco, L., A. A. Lettini, E. Ramon, A. Longo, C. Saccardin, M. C. Dalla Pozza, and A. Ricci. 2011. A rapid and sensitive method to identify and differentiate *Salmonella enterica* serotype Typhimurium and *Salmonella enterica* serotype 4,[5],12:i:– by combining traditional serotyping and multiplex polymerase chain reaction. *Foodborne Pathog. Dis.* 8:741–743.
4. Barco, L., M. Mancin, A. Roccato, V. Cibin, P. Zavagnin, K. Antonello, C. Minorello, A. A. Lettini, and A. Ricci. 2012. The Enter-Vet Network, summary of the data collected during the 9 years of activity, p. 5. *In* Proceedings of the VIII Enter-Net Italia National Workshop, Surveillance of Enteric Infections and Foodborne and Waterborne Diseases: Diagnosis and Epidemiology. Salerno, Italy.
5. Barco, L., M. Mancin, M. Ruffa, C. Saccardin, C. Minorello, P. Zavagnin, A. A. Lettini, J. E. Olsen, and A. Ricci. 2012. Application of the Random Forest method to analyse epidemiological and phenotypic characteristics of *Salmonella* 4,[5],12:i:– and *Salmonella* Typhimurium strains. *Zoonoses Public Health* 59:505–512.
6. Barrett, T. J., P. Gerner-Smidt, and B. Swaminathan. 2006. Interpretation of pulsed-field gel electrophoresis patterns in foodborne disease investigations and surveillance. *Foodborne Pathog Dis.* 3:20–31.
7. Boughton, C., F. C. Leonard, J. Egan, G. Kelly, P. O'Mahony, B. K. Markey, and M. Griffin. 2004. Prevalence and number of *Salmonella* in Irish retail pork sausages. *J. Food Prot.* 67:1834–1839.
8. Capuano, F., A. Mancusi, R. Capparelli, S. Esposito, and Y. T. Proroga. 2013. Characterization of drug resistance and virulotypes of *Salmonella* strains isolated from food and humans. *Foodborne Pathog. Dis.* 10:963–968.
9. Clinical and Laboratory Standards Institute. 2010. Performance standards for antimicrobial disk susceptibility tests. M02-A10. Approved standard, 10th ed. Clinical and Laboratory Standards Institute, Wayne, PA.
10. Dionisi, A. M., C. Graziani, C. Lucarelli, E. Filetici, L. Villa, S. Owczarek, A. Caprioli, and I. Luzzi. 2009. Molecular characterization of multidrug-resistant strains of *Salmonella enterica* serotype Typhimurium and monophasic variant (S. 4,[5],12:i:–) isolated from human infections in Italy. *Foodborne Pathog. Dis.* 6:711–717.
11. European Commission. 2005. Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. *Off. J. Eur. Union* L 338:1–26. Available at: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32005R2073&from=en. Accessed 18 January 2017.
12. European Food Safety Authority and European Centre for Disease Prevention and Control. 2015. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013. *EFSA J.* 13(1)3991:1–162.
13. European Food Safety Authority and European Centre for Disease Prevention and Control. 2015. EU summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2013. *EFSA J.* 13(2)4036:1–178.
14. Hjertqvist, M., I. Luzzi, S. Löfdahl, A. Olsson, J. Rådal, and Y. Andersson. 2006. Unusual phage pattern of *Salmonella* Typhimurium isolated from Swedish patients and Italian salami. *Euro Surveill.* 11(6):pii=2896.
15. Hunter, S. B., P. Vauterin, M. A. Lambert-Fair, M. S. Van Duyne, K. Kubota, L. Graves, D. Wrigley, T. Barrett, and E. Ribot. 2005. Establishment of a universal size standard strain for use with the PulseNet standardized pulsed-field gel electrophoresis protocols: converting the national databases to the new size standard. *J. Clin. Microbiol.* 43:1045–1050.
16. International Organization for Standardization. 2004. Microbiology of food and animal feeding stuffs—horizontal method for the detection of *Salmonella* spp. Technical corrigendum 1. ISO 6579:2002/Cor1:2004. International Organization for Standardization, Geneva.
17. Larsson, J. T., M. Torpdahl, R. F. Petersen, G. Sorensen, B. A. Lindstedt, and E. M. Nielsen. 2009. Development of a new nomenclature for *Salmonella* Typhimurium multilocus variable number of tandem repeats analysis (MLVA). *Euro Surveill.* 14(15):pii=19174.
18. Lettini, A. A., C. Saccardin, E. Ramon, A. Longo, E. Cortini, M. C. Dalla Pozza, L. Barco, B. Guerra, I. Luzzi, and A. Ricci. 2014. Characterization of an unusual *Salmonella* phage type DT7a and report of a foodborne outbreak of salmonellosis. *Int. J. Food Microbiol.* 189:11–17.
19. Lindstedt, B. A., T. Vardund, L. Aas, and G. Kapperud. 2004. Multiple-locus variable-number tandem-repeats analysis of *Salmonella enterica* subsp. *enterica* serovar Typhimurium using PCR multiplexing and multicolor capillary electrophoresis. *J. Microbiol. Methods* 59:163–172.
20. Luzzi, I., E. Filetici, I. Benedetti, S. Arena, S. Owezarek, C. Lucarelli, and A. M. Dionisi. 2012. Enter-Net—surveillance of the infection from enteric pathogens: results of the activity 2009–2011, p. 4. *In* Proceedings of the VIII Enter-Net Italia National Workshop, Surveillance of Enteric Infections and Foodborne and Waterborne Diseases: Diagnosis and Epidemiology. Salerno, Italy.
21. Luzzi, I., P. Galetta, M. Massari, C. Rizzo, A. M. Dionisi, E. Filetici, A. Cawthorne, A. Tozzi, M. Argentieri, S. Bilei, L. Busani, C. Gnesivo, A. Pendenza, A. Piccoli, P. Napoli, L. Loffredo, M. O. Trinito, E. Santarelli, and M. L. Ciofi degli Atti. 2007. An Easter outbreak of *Salmonella* Typhimurium DT104A associated with traditional pork salami in Italy. *Euro Surveil.* 12(4):E11–E12.

22. Mughini-Gras, L., F. Barrucci, J. H. Smid, C. Graziani, I. Luzzi, A. Ricci, L. Barco, R. Rosmini, A. H. Havelaar, W. Van Pelt, and L. Busani. 2014. Attribution of human *Salmonella* infections to animal and food sources in Italy (2002–2010): adaptations of the Dutch and modified Hald source attribution models. *Epidemiol. Infect*. 142:1070–1082.
23. Pires, S. M., L. de Knegt, and T. Hald. 2011. Estimation of the relative contribution of different food and animal sources to human *Salmonella* infections in the European Union. Scientific technical report submitted to the EFSA. Available at: onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2011.EN-184/pdf. Accessed 18 January 2017.
24. Pontello, M., L. Sodano, A. Nastasi, C. Mammina, M. Astuti, M. Domenichini, G. Belluzzi, E. Soccini, M. G. Silvestri, M. Gatti, E. Gerosa, and A. Montagna. 1998. A community-based outbreak of *Salmonella enterica* serotype Typhimurium associated with salami consumption in northern Italy. *Epidemiol. Infect*. 120:209–214.
25. Rabsch, W. 2007. *Salmonella* Typhimurium phage typing for pathogens. *Methods Mol. Biol*. 394:177–211.