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Occurrence of *Listeria monocytogenes* and *Salmonella* spp. in meat processed products from industrial plants in Southern Italy

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ABSTRACT

The main goal of the present investigation was to assess the microbiological safety of two typical meat-derived products, i.e. raw pork sausages and entrails lamb rolls, produced in Salento (Apulia, Southern Italy). Analyses were carried out for 7 years (from 2008 to 2014) and a total number of 6720 samples was collected by specialized personnel. The presence of *Listeria monocytogenes* and *Salmonella* spp. was detected by a PCR-based assay, combined with culturing in enrichment broth. The prevalence of *L. monocytogenes* was assessed in 2.4% entrails lamb rolls and in 4.2% raw pork sausages samples, whereas the occurrence of *Salmonella* spp. was revealed in 2.7% lamb rolls and in 3.5% pork sausages. A statistically significant seasonal variation was found in the occurrence of *L. monocytogenes*; in fact a higher number of samples contaminated by this pathogen was recorded in spring and autumn. On the contrary, no significant seasonal changes occurred in the prevalence of *Salmonella* spp. The data reported indicate that, due to the presence of these pathogens, the Italian food processors need to improve the microbiological monitoring of the processing chains, in order to guarantee health safety.

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1. Introduction

Foods of animal origin, and especially meat, are important component of human diet constituting an essential supply of valuable nutrients, however sometimes they also represent the source of serious food-borne infections. Epidemics related to meat products consumption produce both harsh health impact in the long term and important economic loss to the food industry (Anonymous, 2013; Olsen et al., 2005). Therefore, in order to ensure product safety, it is ultimate to detect and estimate the contamination sources of meat-derived foods in the raw materials and along processing plants (Rhoades, Duffy, & Koutsoumanis, 2009). Poor hygienic levels of meat raw materials may be a source of pathogenic bacteria such as *Listeria monocytogenes* and *Salmonella* spp., which have often been related with outbreaks of human salmonellosis (82,694 confirmed cases, 0.14% fatality rate) and listeriosis (1763 confirmed cases, 15.68% fatality rate) in 2012 in Europe (EFSA, 2014).

Salmonella is a Gram negative rod-shaped bacterium, it is one of the major food-borne pathogen and it is present in the gastrointestinal tract of animals. It can be found in foods of animal origin like raw meat, and its derivatives, causing infections and intoxications (Anonymous, 2012; EFSA, 2014). The number of salmonellosis cases is increasing and the inspection of food for the presence of *Salmonella* is becoming a routine all over the world.

Another microorganism whose presence is a concern for food industry is *L. monocytogenes*, a Gram positive pathogen contaminating unprocessed food like raw meat, fish and milk, since it is widely present either in the environment or along production plants (Larsen et al., 2014). This bacterium can be also found in some processed foods such as cheese, ice cream, and processed meat because of post-processing contamination.

Because of their ability to survive unfavorable conditions, these

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Table 1
Microbial quality of raw meat products for three processing plants in Salento (Southern Italy) during a seven-years period (2008–2014).

Plant	Product	Total	Detection	Negative samples (%)	Positive samples (%)
A	Rolls	3627	*L. monocytogenes*	3589 (99.0)	38 (1.0)
			Salmonella spp.	3612 (99.6)	15 (0.4)
	Sausages	559	*L. monocytogenes*	548 (98.0)	11 (2.0)
			Salmonella spp.	546 (97.7)	13 (2.3)
B	Rolls	1491	*L. monocytogenes*	1408 (94.4)	83 (5.6)
			Salmonella spp.	1382 (92.7)	109 (7.3)
	Sausages	932	*L. monocytogenes*	878 (94.2)	54 (5.8)
			Salmonella spp.	891 (95.6)	41 (4.4)
C	Rolls	303	*L. monocytogenes*	299 (98.7)	4 (1.3)
			Salmonella spp.	292 (96.3)	11 (3.6)
	Sausages	168	*L. monocytogenes*	164 (97.6)	4 (2.4)
			Salmonella spp.	164 (97.6)	4 (2.4)

pathogens represent a high concern for the meat industries (Sofos & Geornaras, 2010). It is important to note that the variety and amount of the above pathogen incidence in the processing plants are strongly related to the origin of raw materials and to the insufficient hygiene of staff involved in production (Sofos, 2014). The isolation of *L. monocytogenes* and *Salmonella* spp. from meat processing environments indicates the probable persistence of these pathogenic strains, thus confirming the need for sanitation procedures improvement (Vongkamjan, Roof, Stasiewicz, & Wiedmann, 2013; Yildirim, Gonulalan, Pamuk, & Ertas, 2011). Due to this reason, it is highly required to identify these pathogens combining traditional microbiological methods with modern molecular approaches, such as Polymerase Chain Reaction (Dalmasso et al., 2014; Jofré et al., 2005; Kawasaki et al., 2005) or Pulse Field Gel Electrophoresis (Senczek, Stephan, & Untermann, 2000). In particular, multiplex real-time PCR assay, performed after an enrichment step, has demonstrated to be a fast and trustworthy method for efficient screening of single or multiple pathogen occurrence in various meat products (Suo, He, Tu, & Shi, 2010; Wang, Jothikumar, & Griffiths, 2004).

Two typical products popular in the Salento area (Apulia, Southern Italy) are fresh-made pork sausages and entrails lamb rolls that constitute a good substrate for the growth of both pathogens. With the exception of two previous reports about the contamination level of different foods of animal origin in Italy (Busani, Cigliano, Taioli, et al., 2005; Di Pinto, Novello, Montemurro, Bonerba, & Tantilli, 2010), at the present, to our knowledge, there are no published data on whether and with which frequency *L. monocytogenes* and *Salmonella* spp. are present in meat-derived products in Southern Italy. Therefore, the objective of the present study was to supply the first evaluation of the presence of both pathogens in meat processing plants located in Salento.

2. Materials and methods

2.1. Sampling plan

Three meat processing plants located in Salento (Southern Italy) were selected. The selection was based on: i) capability to process large quantity of meat, ii) dissimilar processing lines (beef, pork, lamb) and iii) the variety of final products. A total number of 6720 samples were collected over a 7-year-period (2008–2014), through 504 independent sampling times. Samples were collected from two typical final meat products: fresh-made pork sausages and entrails lamb rolls. Sausages are made of pork intestine (gut) stuffed with minced pork meat, spices, salt, sugar (dextrose or saccharose). Entrails lamb rolls are made of pieces of suckling lamb liver, lung and heart wrapped into its omentum and tied with gut. All samples were transferred to the laboratory under aseptic and refrigerated conditions in portable insulated cold-boxes. Samples were kept at 4 °C and analyzed within 24 h.

2.2. Detection of L. monocytogenes and Salmonella spp. in meat samples

Samples were enriched before bacterial genomic DNA isolation, by separately adding 25 g of meat samples to 225 mL of buffered peptone water (*Salmonella* spp. enrichment) or *Half Fraser* broth (*L. monocytogenes* enrichment) in sterile plastic Seward filter bags (Norfolk, UK). The samples were then homogenized in a stomacher (Seward Stomacher 400 Lab System, Norfolk, UK) for 1 min. The homogenate was poured into sterile containers and incubated for 16–20 h at 37 °C (*Salmonella* spp.) or 14–26 h at 30 °C (*L. monocytogenes*). Aliquots of culture-enriched samples (1 mL for *Salmonella*, 1.5 mL for *Listeria*) were removed and transferred to 1.5 mL sterile tubes for DNA extraction. Bacterial genomic DNA was isolated from the enriched samples using either the iQ-CheckTM *Salmonella* spp. II or iQ-CheckTM *L. monocytogenes* II (Bio-Rad; USA) according to supplier's instruction. Extracted DNA was suspended in 500 μL and 5 μL of each sample were taken to perform real-time PCR. Monitoring of the reaction was carried out by CFX manager IDE software by Bio-Rad. Validation of positive samples was performed using the RAPID′ *Salmonella* spp. and RAPID′ *L. monocytogenes* (Bio-Rad, USA) according to supplier's instruction. Both the above methods according to the ISO 16140 standard, are an alternative method to the reference standards ISO 11290-1 (for *Listeria* spp detection) and to the ISO 6579 (for *Salmonella* spp detection) in all food products.

2.3. Statistical analysis

The chi-squared test was employed to evaluate the relative correlations between quantity of positive and negative samples. Comparison among months, years or seasons, based on the proportion of negative/positive samples, was performed with the Fisher exact test (alpha 0.05). The probability statistically significant value was identified as $p < 0.05$. The statistical analysis was carried out using PAST software (Hammer, Harper, & Ryan, 2001).

3. Results and discussion

3.1. Occurrence of L. monocytogenes

The presence of *L. monocytogenes* was determined in 5421 samples of lamb rolls over a seven-year-period, during which the samples were collected from three different production plants (Table 1). In total, the bacteria were present in 2.3% (125/5421) of the analyzed samples. Contamination distribution, along the seven-year-period, of the three monitored plants is reported in Table 2,

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