NEW MICROBIOLOGICA, **33**, 249-252, 2010

Occurrence of *Listeria monocytogenes* in ready-to-eat foods from supermarkets in Southern Italy

Angela Di Pinto, Lucia Novello, Filomena Montemurro, Elisabetta Bonerba, Giuseppina Tantillo

Dipartimento di Sanità Pubblica e Zootecnia, Facoltà di Medicina Veterinaria, Università degli Studi, Bari

SUMMARY

The study provides data on the prevalence of *Listeria monocytogenes* in ready-to-eat (RTE) foods from supermarkets in Southern Italy. The pathogen was detected in 105/1045 (10%) RTE food samples. In particular, it was highlighted in 4/392 (1%) pastries, 23/112 (20.5%) vacuum-packaged sliced salami samples, 2/108 (1.9%) cream cheese samples, 31/115 (27%) mayonnaise based deli salads and 45/132 (34.1%) smoked salmon samples. The mozzarella samples were *L. monocytogenes* negative. Given the considerable public health implications, the study confirms that surveillance of listeriosis in Europe should be improved and coordinated between European Union Member States in order to better estimate the burden of disease and to prevent foodborne outbreaks, assessing the human health risk arising from RTE foods.

KEY WORDS: L. monocytogenes, RTE foods, Supermarkets

Received November 11, 2009 *Accepted* January 27, 2010

INTRODUCTION

L. monocytogenes is Gram-positive bacterial pathogen responsible for human listeriosis, which causes fever, muscle aches and sometimes gastrointestinal symptoms such as nausea or diarrhea. If infection spreads to the nervous system, symptoms such as headache, stiff neck, confusion, loss of balance, or convulsions can occur (Mead *et al.*, 2006). In healthy individuals, infection is usually mild. However, in pregnant women, infection can lead to miscarriage, stillbirth, premature delivery or infection of the newborn. People with a damaged immune system and the elderly are also at increased risk of more severe disease. The disease has a high fatality rate in the susceptible population (Garrido *et al.,* 2008). Transmission is generally through eating contaminated food, in particular dairy products made from unpasteurized milk and ready-to-eat meat and fish products (EFSA, 2009). In addition, *L. monocytogenes* has been found in a variety of raw foods such as uncooked meats and vegetables, as well as in processed foods that become contaminated after processing, such as soft cheeses and cold cuts at the deli counter (Cordano *et al.,* 2001; EFSA, 2009). Potential sources of *L. monocytogenes* contamination of foods include incoming product, food handlers, consumers and environmental sources, such as utensils and equipment, which may harbor pathogenic microorganisms or serve as vehicles of contamination if cleaning and sanitation are poor (Lianou, 2007).

The important impact that this disease has on public health is not always recognized, particularly since listeriosis is a relatively rare disease compared with other common foodborne infections. In addition, listeriosis likely is underreported due to its status as a non-notifiable disease in many countries and because of the absence of adequate surveillance programs.

Corresponding author
Angela Di Pinto
Dipartimento di Sanità Pubblica e Zootecnia
Sezione di Controllo e Sicurezza degli Alimenti
Facoltà di Medicina Veterinaria
Università degli Studi di Bari
Strada Prov.le per Casamassima, km 3
70010 Valenzano (Bari) - Italy
E-mail: a.dipinto@veterinaria.uniba.it

Foodborne diseases remain an important public health problem in the world. In fact, the Foodborne Diseases Active Surveillance Network (FoodNet) of CDC's Emerging Infections Program report that in 2008 *Listeria monocytogenes* was confirmed in 135 cases of infections, although the estimated incidence of *Listeria monocytogenes* did not change significantly compared with the preceding 3 years (CDC, 2009). According to the EFSA Report, in EU the number of confirmed listeriosis cases has increased from 2004 to 2006 with a slight decrease observed in 2007 (EFSA, 2009).

Although *L. monocytogenes* infection is usually limited to immunocompromised individuals, the high mortality rate associated with human listeriosis makes *L. monocytogenes* the leading cause of death among bacterial pathogens acquired primarily through the consumption of contaminated foods (Jemmi *et al.,* 2006).

Given the considerable public health implications, monitoring of *L. monocytogenes* incidence in foods is important. This study evaluates the presence of *L. monocytogenes* in ready to eat (RTE) foods, known to be at risk for listeriosis, collected from supermarkets in southern Italy in order to verify the satisfactory application of GMP (Good Manufacturing Practice) during the food production chain.

MATERIALS AND METHODS

Sampling

Between February 2007 and January 2009, 1045 ready to eat (RTE) foods were sampled from supermarkets in Southern Italy. In particular, 132 smoked salmon, 115 mayonnaise based deli salads, 108 cream cheese samples, 112 vacuum-packaged sliced salami samples, 186 mozzarella samples and 392 pastries were analyzed. The samples were put in sterile bags, carried to laboratory in cooled container to 4 °C and analyzed within three hours from sampling.

L. monocytogenes isolation and identification

L. monocytogenes isolation and identification in RTE foods were performed following the UNI EN ISO 11290-1/2005 method, according to Commission Regulation (EC) No. 2073/2005. Analytical portions (25 g) were homogenized in 225 ml of half Fraser broth (Oxoid) in a stomacher bag and incubated at 30±1°C for 24 h. Then, 0.1 ml of the enrichment culture was added to 10 ml Fraser broth and incubated for 48 h at 37°C. The enrichment culture was streaked on the Oxford and ALOA differential selective-agars and incubated at 37°C for 24 and 48 h in order to isolate *Listeria* species. Then, five presumptive colonies from Oxford and ALOA agar plates were picked and subjected to morphological and confirmation tests including Gram-staining, mobility at 25°C on Mobility Agar (Oxoid), catalase test (Oxoid), oxidase test (Oxoid), CAMP test against *St. aureus* and *Rhodococcus equi* (Oxoid). The suspect colonies were further subjected to biochemical identification, which was performed using the API *Listeria* (bioMérieux® SA, Marcy l'Etoile-France) according to the manufacturer's instructions. The results were deduced using apiweb™ stand alone V1.2.1 (bioMérieux® SA, Marcy l'Etoile-France). Surveillance enumeration of *L. monocytogenes* levels was performed on positive samples. In order to enumerate *L. monocytogenes*, the MPN method was used. Analytical portions (10 g) of each sample was homogenized in 90 ml of buffer peptone water (BPW) (Oxoid). Then, 10-fold dilution (up to 10^{-3}) of homogenate were prepared in the same medium; 1 ml of the three dilutions (10^{-1}, 10^{-2}, 10^{-3}) was transferred, in triplicate, into tubes containing 9 ml of Fraser Broth (Oxoid) and then incubated at 30±1°C for 48 h. The broth cultures that changed color to back were streaked for isolation onto Oxford agar plates (Oxoid) and incubated at 37°C for 24 and 48 h. The results were calculated using the MPN table in the range of <3.0 to >1,100 MPN/g. Three unit collections (u.c.) were analyzed for each sample, and the presence of the pathogen was tolerated if the MPN of *L. monocytogenes* was lower than 110/g in two u.c. and 11 in the third u.c. (McCready scheme).

RESULTS AND DISCUSSION

The survey confirms that *L. monocytogenes* in RTE foods is a priority for risk assessment according to the Codex Committee on Food Hygiene (CCFH) in order to develop an international strategy for the reduction of illness from

TABLE 1 - *L. monocytogenes isolated from RTE foods.*

Type of Samples	*Positive samples (%)*
Pastries	4/392 (10%)
Salami	23/112 (20.5%)
Cream cheeses	2/108 (1.9%)
Mayonnaise-based deli salads	31/115 (27%)
Smoked salmon	45/132 (34.1%)
Mozzarella	0/186 (0%)

this source (CCFH, 2009). *L. monocytogenes* was detected in 105/1045 (10%) RTE food samples. In particular, the pathogen was highlighted in 4/392 (1%) pastries, 23/112 (20.5%) vacuum-packaged sliced salami samples, 2/108 (1.9%) cream cheese samples, 31/115 (27%) mayonnaise-based deli salads and 45/132 (34.1%) smoked salmon samples. The mozzarella samples were *L. monocytogenes* negative (Table 1). All *L. monocytogenes* positive samples showed contamination levels lower than 100 cfu/g in compliance with Commission Regulation (EC) 2073/2005.

The incidence of *L. monocytogenes* observed in RTE foods in this study was higher than the data reported by EFSA (2009): the percentage of *L. monocytogenes* observed was about 10%, whereas EFSA highlighted a prevalence rate of 4.4%.

This study reveals a less marked contamination of *L. monocytogenes* in pastries and cream cheese samples. The percentage of the pathogen detected in pastries were lower than the findings of Uhitil *et al.* (2004) who isolated *L. monocytogenes* in about 4% of pastries. For cream cheese samples, the data observed are in agreement with ILSI (2005). In cream cheese samples, the low incidence of *L. monocytogenes* may be due to high-temperature treatment of milk in order to eliminate the cells of the pathogens.

Although little information is available on mozzarella (Gattuso *et al.*, 2008), a traditional Italian stretched-curd cheese, the absence of *L. monocytogenes* was probably attributable to the initial high temperature processing of milk and to the cheese-making process (Kim e*t al.*, 1998). In particular, the stretching and kneading in hot water at 80-90°C to develop a stringy fibrous body inactivates the cells of the pathogen achieving a good health quality of mozzarella.

Furthermore, for pastries, mozzarella and cream cheese samples the data showed an adequate application of Good Hygienic Practices to prevent post-processing contamination (Kim *et al.*, 1998).

The incidence of *L. monocytogenes* in mayonnaise-based deli salads was about 27%. The results observed in this study highlighted a percentage higher than that reported b by Uyttendaele *et al.* (2009). Considering that these mayonnaise-based deli salads are prepared and packaged at large-scale industrial food processing plants, this high percentage is probably due in part to raw materials significantly contaminated and in part to inadequate application of Good Manufacturing Practices (GMPs). The data highlight the potential importance of prevention and control of *L. monocytogenes*, which may be achieved by appropriate implementation of Sanitation Standard Operating Procedures (SSOPs), Good Manufacturing Practices (GMPs) and Hazard Analysis and Critical Control Point (HACCP) systems (ILSI Research, 2005).

In sliced and vacuum-packaged salami samples the prevalence of the pathogen observed was mainly high. Although data are lacking, this high prevalence rate may be attributable to probable cross-contamination during slicing operation at large-scale industrial food processing plants. Simpson *et al.* (2008) observed that in inoculated sliced and vacuum-packaged salami the *L. monocytogenes* death rates increased with increasing the storage temperature. These data highlight the importance of implementing an assessment of the risk associated with *L. monocytogenes* in sliced and vacuum-packaged salami.

Compared with Uyttendaele *et al.* (2009) findings, the smoked salmon samples were less contaminated: Uyttendaele *et al.* (2009) observed 56.9% positive samples. Therefore the study confirmed that smoked salmon is considered to be a risk product for human listeriosis and *L. monocytogenes* contamination is of great concern for the smoked fish industry. The significant presence of *L. monocytogenes* detected in smoked salmon samples may be largely attributed to raw materials and post-processing contamination. In fact the manufacturing processes may not ensure elimination of the pathogen, especially if the raw

materials are heavily contaminated. Considering that the process involves a lot of handling by workers as well as the use of technically often complex equipment (Rørvik, 2000), post-processing contamination is considerable.

Given that risk assessment reflects the state of knowledge on listeriosis and on contamination of foods with *L. monocytogenes,* surveillance programs of listeriosis in Europe should be improved and coordinated between European Union Member States in order to better estimate the burden of disease and to prevent foodborne outbreaks, assessing the human health risk arising from RTE foods.

REFERENCES

CCFH. (2009). www.fsis.usda.gov/Codex_Alimentarius/Codex_Committee_Food_Hygiene/index.asp

CDC. (2009). Preliminary foodnet data on the incidence of infection with pathogens transmitted commonly through food. **58**, 333-337.

Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *Offic. J. Europ. Union.* 22.12.2005.

Cordano A.M., Rocourt J. (2001). Occurrence of *Listeria monocytogenes* in food in Chile. Int. *J. Food Microbiol.* **70**, 175-178.

EFSA, The community summary report on trends and sources of zoonose and zoonotic agents in the European Union. (2009). The EFSA Journal.

Garrido V., Torroba L., García-Jalón I., Vitas A.I. (2008). Surveillance of listeriosis in Navarre, Spain, 1995-2005 - epidemiological pattern and characterization of clinical and food isolates. *Euro Surveill.* **13**, (49): pii=19058. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=1905

Gattuso A., Fiore A., D'Ottavio M.C., Casale M., Gianfranceschi M.V. (2008). Presenza di *Listeria monocytogenes* in mozzarella. *Ind. Alim.* **XLVII**, 3-7.

ILSI, International Life Sciences Institute Research Foundation, Risk Science Institute. (2005) Achieving continuous improvement in reduction in foodborne listeriosis - a risk-based approach. *J. Food Prot.* **68**, 1932-1994.

Jemmi T., Stephan R. (2006). *Listeria monocytogenes*: food-borne pathogen and hygiene indicator. revue scientifique technique (International Office of Epizootics). **25**, 571-80.

Kim J., Schmidt K.A., Phebus R.K., Jeon I.J. (1998) Time and temperature of stretching as critical control points for *Listeria monocytogenes* during production of mozzarella cheese. *J. Food Prot.* **61**, 116-118.

Lianoun A., Sofos J.N. (2007). A review of the incidence and transmission of *Listeria monocytogenes* in ready to eat products in retail and food service environments. *J. Food Prot.* **70**, 2172-2198.

Mead P.S., Dunne E.F., Graves L., Wiedmann M., Patrick M., Hunter S., Salei E., Mostashari F., Craig A., Mshar P., Bannerman T., Sauders B.D., Hayes P., Dewitt W., Sparling P., Griffin P., Morse D., Slutsker L., Swaminathan B. (2006). Nationwide outbreak of listeriosis due to contaminated meat. *Epidemiol. Infect.* **134**, 744-751.

Rørvik L.M. (2000) *Listeria monocytogenes* in the smoked salmon industry. *Int. J. Food Microbiol.* **62**, 183-190.

Simpson C., Geornaras I., Yoon Y., Scanga J., Kendall P.A., Sofos J.N. (2008). Effect of inoculum preparation procedure and storage time and temperature on the fate of *Listeria monocytogenes* on inoculated salami. *J. Food Prot.* **71**, 494-501.

Uhitil S., Jaksic S., Petrak T., Medic H., Gumhalter-Karolyi L. (2004). Prevalence of Listeria monocytogenes and the other *Listeria* spp. in cakes in Croatia. *Food Control.* **15**, 213-216

Uyttendaele M., Busschaert P., Valero A., Geeraerd A.H., Vermeulen A., Jacxsens L., Goh K.K., De Loy A., Van Impe J.F., Devlieghere F. (2009). Prevalence and challenge tests of *Listeria monocytogenes* in Belgian produced and retailed mayonnaise-based deli-salads, cooked meat products and smoked fish between 2005 and 2007. *Int. J. Food Microbiol.* **133**, 94-104.