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**Research Note**

# Prevalence and Concentration of *Listeria monocytogenes* in Sliced Ready-to-Eat Meat Products in the Hellenic Retail Market

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## ABSTRACT

The aim of this work was to estimate the prevalence and concentration of *Listeria monocytogenes* in packaged precut (slices or cubes) ready-to-eat (RTE) meat products available in the Hellenic retail market. Samples of these RTE meat products ($n = 209$) were taken from local supermarkets during a 3-month period and analyzed for the presence of *L. monocytogenes* with an automated enzymatic qualitative immunoassay followed by biochemical confirmation of positive results. The concentration of the pathogen in the positive samples was also determined. Seventeen samples (8.1%) were positive for *L. monocytogenes.* Eight (47.1%) of these 17 samples were from the same manufacturer; 36.4% of the products tested from this manufacturer were positive for *L. monocytogenes.* When bacon samples were not considered, the estimated prevalence of *L. monocytogenes* in sliced RTE meat products was much lower (3.1%). The *L. monocytogenes* populations in all positive samples were low, ≤10 CFU/g. In 64.7% of the *L. monocytogenes*–positive samples, other *Listeria* species, including *L. innocua* and *L. welshimeri,* were also present at <10 to 690 CFU/g. These results indicate that *L. monocytogenes* is present in low numbers but is in a considerable proportion of the packaged precut RTE meat products that are sold in the Hellenic retail market. Cooked ham and bacon cut in cubes were the sample types most often contaminated with *L. monocytogenes.* The higher level of handling (e.g., cutting) associated with these products may further increase the risk of contamination with *L. monocytogenes.*

*Listeria monocytogenes* is a foodborne pathogen *(22)* widely distributed in nature *(29)*; therefore, contamination can occur at various steps of food production and distribution. The pathogen has been isolated worldwide from foods of plant or animal origin, and contaminated foods have been the cause of sporadic or epidemic cases of illness *(12, 23).* Controlling *L. monocytogenes* growth in contaminated foods is problematic because the bacterium is resistant to low pH, low water activity, and refrigeration temperatures, conditions that are employed as fundamental strategies in food preservation *(8).* Hence, several countries have adopted a zero-tolerance policy for *L. monocytogenes* in ready-to-eat (RTE) foods. Various researchers have documented the ability of *L. monocytogenes* to proliferate in cooked meat products *(3)* and other foods under refrigeration, a phenomenon aided largely through the pathogen's capacity for intracellular accumulation of cryoprotective compounds from foods *(2, 25).*

To estimate the risk to public health after consumption of different foods, both quantitative data (i.e., numbers of listeriae in *Listeria*-contaminated foodstuffs) and data from different countries are needed *(21, 30).* Such data are also of major importance in ongoing efforts for establishing sound microbiological criteria regarding the presence of *L. monocytogenes* in foods, such as the Commission of the European Communities draft regulation on the microbiological criteria for foodstuffs, which will become effective beginning 2006 *(9).* The aim of the present study was to estimate the prevalence and extent of *L. monocytogenes* contamination in packaged RTE meat products sold in the Hellenic retail market. The study was focused on sliced products because previous findings have indicated that such products are at higher risk for *L. monocytogenes* contamination *(13, 27).* To the authors' knowledge, this work constitutes the first international published report regarding the prevalence and concentration of *L. monocytogenes* in RTE meat products sold in the Hellenic market.

## MATERIALS AND METHODS

**Sampling.** From July to October 2004, 209 samples from 27 different manufacturers were collected from 13 retail stores located in and around the city of Thessaloniki. The sampled stores represent every major supermarket chain in Greece. One hundred thirty-six samples were obtained from nationally manufactured products, and the remaining 73 samples were from products imported from other European Union countries. Overall, the 209 sampled items represented 16 different types of meat products, which were classified based on their principal technology of manufacture as thermally processed (heat treated; $n = 160$) or fermented ($n = 49$) (Table 1). The samples were from products packaged under a modified atmosphere or vacuum packaged and stored under refrigeration with a shelf life of 2 or more months. With

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TABLE 1. *Prevalence of* Listeria monocytogenes *in precut (sliced or cubed) ready-to-eat meat products in the Hellenic retail market*

| Product type | Product name | Product category[a] | Manufacturing company | No. of tested samples | No. of positive samples |
|---|---|---|---|---|---|
| 1 | Bresaola | F | F | 2 | 0 |
| 2 | Turkey breast | HT | H, I, K, S, U, X, ZA | 21 | 0 |
| 3 | Smoked tongue | HT | T | 1 | 0 |
| 4 | Ham (cooked) | HT | E, H, J, K, P, S, X, ZA | 27 | 1 |
| 5 | Ham (fermented) | F | E, I, N | 6 | 1 |
| 6 | Copa | F | E, F | 3 | 0 |
| 7 | Chicken breast | HT | Q | 4 | 0 |
| 8 | Mortadella | HT | C, D, E, H, I, S, X, ZA | 13 | 0 |
| 9 | Bacon | HT | B, C, D, E, H, I, J, M, O, S, U, W, X, Y, Z, ZA | 49 | 12 |
| 10 | Pork loin | HT | C, D, H, J, S, ZA | 10 | 0 |
| 11 | Pariza | HT | A, C, D, H, X, ZA | 14 | 0 |
| 12 | Pastirma | F | T | 2 | 0 |
| 13 | Prosiuto | F | F, M, R | 6 | 0 |
| 14 | Salami | F | D, E, F, G, I, M, P, R, S, U, V, X, ZA | 30 | 3 |
| 15 | Salami (cooked) | HT | K, L, Q | 6 | 0 |
| 16 | Pork shoulder | HT | D, H, M, P, S, U, X, ZA | 15 | 0 |
| Total | | | | 209 | 17 |

[a] F, fermented; HT, heat treated.

the exception of bacon, which in most cases (but not always) is consumed after some form of heat treatment, the sampled products do not require heating or cooking prior to consumption. The majority of the samples ($n = 196$) were sliced meat products, but some were cut into cubes ($n = 13$). With one exception (smoked tongue), each distinct meat product (i.e., a specified product of a certain manufacturer) was sampled and analyzed at least twice, ensuring that subsequent samples of the same product belonged to different lots. All sampled items had a net weight of at least 100 g. After purchase, samples were stored at 4°C overnight and tested by the same trained analyst in the same laboratory the following day.

**Detection, confirmation, and enumeration of *L. monocytogenes.*** For detection of *L. monocytogenes,* the VIDAS LMO2 (bioMérieux sa, Marcy l'Etoile, France) protocol was followed. Food portions (25 g), representing segments from several different slices within the package, were aseptically removed from their package and homogenized with 225 ml of half-Fraser broth (bioMérieux) in 400-ml stomacher bags for 2 min with a Bag-Mixer 400 stomacher (Interscience, St. Nom, France). The mixtures were then incubated at 30°C for 24 h (primary enrichment). At 24 h, 1 ml of the enriched culture was transferred to 10-ml Fraser broth (FB) tubes (bioMérieux) and incubated at 30°C for 24 h (secondary enrichment). These FB enrichment cultures (500 μl) were transferred to the sample well of the LMO2 strips and assayed for the presence of *L. monocytogenes* with the mini VIDAS apparatus (bioMérieux) according to the manufacturer's instructions. The VIDAS LMO2 is an automated and qualitative immunoenzymatic assay based on the enzyme-linked fluorescent assay principle and designed to detect *L. monocytogenes* in foods. For the samples presumed positive for *L. monocytogenes* from the VIDAS protocol, (i) biochemical tests were conducted to confirm the presence of *L. monocytogenes* in the FB tubes, (ii) the population of *L. monocytogenes* in the positive sample was determined, and (iii) the presence and concentration of other *Listeria* species were evaluated.

For enumeration of *L. monocytogenes* and other *Listeria* species in the VIDAS-positive samples, 25-g segments of the same slices used for detection were homogenized with 225 ml of sterile Ringer's solution (LAB M, Bury, UK), and the homogenate (0.2 ml onto each of five plates) and appropriate volumes of 10-fold serial dilutions of the homogenate in Ringer's solution were surface plated onto *Listeria* agar according to Ottaviani and Agosti (ALOA; Biolife Italiana S.r.l., Milano, Italy) *(19).* Plates were incubated at 37°C for 48 h, and blue-turquoise colonies surrounded by a precipitation halo (*L. monocytogenes*) or not surrounded by a halo (other *Listeria* species) were counted. Randomly selected colonies of both types were picked from ALOA plates and characterized to the species level.

For the biochemical confirmation of *L. monocytogenes* in the VIDAS-positive samples and for the detection of other *Listeria* species, the FB enrichment cultures were streaked onto ALOA agar plates and incubated at 37°C for 48 h. Five well-isolated colonies of each type were further streak purified onto tryptone soy agar plates (LAB M) containing 0.6% yeast extract (Merck, Darmstadt, Germany) (TSAYE) and incubated at 37°C for 24 h. Colonies from TSAYE plates were used for bacterial identification to the species level by testing for hemolytic activity on Columbia agar + 5% sheep blood plates (bioMérieux), assaying for catalase and Gram reactions, observing tumbling motility under wet mount at 22°C, and checking for enzymatic reactions and utilization of sugars with API *Listeria* strips (bioMérieux).

## RESULTS AND DISCUSSION

Seventeen (8.1%) of the 209 samples were positive for *L. monocytogenes* with the VIDAS LMO2 method (Table 1). Of these positive samples, the FB enrichment cultures of two samples could not be biochemically verified as positive for *L. monocytogenes,* even after the FB cultures were reexamined after 24 h of storage at 4°C. The biochemical characterization of numerous ALOA isolates from these FB enrichment cultures (no isolate had the typical *L. monocytogenes* appearance on ALOA) consistently yielded *Listeria welshimeri* as the sole *Listeria* species. It is most likely, therefore, that these were false-positive results.

TABLE 2. *Concentration of* Listeria monocytogenes *and other* Listeria *species in the* L. monocytogenes*–positive samples of precut (sliced or cubed) ready-to-eat meat products in the Hellenic retail market*

| | Manufacturing company | Product | Population of *L. monocytogenes* (CFU/g) | Other *Listeria* species | Population of other *Listeria* species (CFU/g) |
|---|---|---|---|---|---|
| 1 | X | Bacon | <10 | | <10 |
| 2 | S | Ham (cubes)[a] | <10 | *welshimeri* | <10 |
| 3 | S | Bacon (cubes)[a] | 10 | *welshimeri* | <10 |
| 4 | E | Bacon (cubes) | <10 | *innocua, welshimeri* | <10 |
| 5 | E | Bacon (cubes) | 10 | *welshimeri* | 70 |
| 6 | E | Bacon (cubes) | <10 | | <10 |
| 7 | E | Bacon (cubes) | <10 | *welshimeri* | 60 |
| 8 | E | Bacon (cubes) | <10 | *welshimeri* | <10 |
| 9 | E | Bacon (cubes) | <10 | | <10 |
| 10 | E | Bacon | <10 | | <10 |
| 11 | E | Bacon | <10 | | <10 |
| 12 | P | Salami | <10 | *welshimeri* | <10 |
| 13 | P | Salami | <10 | *innocua* | <10 |
| 14 | I | Ham | <10 | | <10 |
| 15 | Z | Bacon | <10 | *innocua* | 690 |
| 16 | C | Bacon | <10 | *innocua* | <10 |
| 17 | R | Salami | <10 | *welshimeri* | <10 |

[a] The presence of *L. monocytogenes* in the Fraser broth enrichment cultures could not be confirmed biochemically.

*Listeria innocua* and *L. welshimeri* were isolated from four and eight of the *L. monocytogenes*–positive samples, respectively. No other *Listeria* species were isolated (Table 2). The populations of *L. monocytogenes* in the positive samples were low (<10 CFU/g in 15 samples and 10 CFU/g in the remaining two positive samples). A similar percentage of positive samples was found in fermented meat products (4 of 49, 8.2%) and heat-treated meat products (13 of 160, 8.1%) (Table 1). Eight (61.5%) of the 13 sampled products that were cut in cubes were positive for *L. monocytogenes*. This proportion was significantly higher ($\chi^2 = 52.91$, $P < 0.005$) than the proportion of sliced samples that tested positive (9 of 196, 4.6%). However, eight of the 17 positive samples were items imported by a major supermarket chain from the same manufacturer (manufacturer E from a European Union country). The percentage of samples from manufacturer E that tested positive for *L. monocytogenes* was 36.4% (8 of 22 samples). When these 22 items were excluded, the overall prevalence estimate was 4.8% (9 of 187). Also, 12 of the 17 total foods that tested positive were samples of bacon, a product that is usually (but not always) consumed after additional heat treatment. When the bacon samples were excluded from the analysis, the overall prevalence estimate of *L. monocytogenes* in sliced RTE meats was 3.1% (5 of 160).

*L. monocytogenes* is ubiquitous in the environment and is often present on the hides and the in intestinal tract of clinically healthy domestic animals *(20).* Thus, meat can become contaminated during the process of animal slaughter and during the subsequent steps of meat processing, especially when hygiene practices are inadequate. RTE meat products have been associated with outbreaks of noninvasive febrile gastroenteritis *(24)* and with outbreaks of the more severe form of the disease, listeriosis, resulting in abortions, stillbirths, and deaths primarily in the young, elderly, and immunocompromised individuals. Contaminated hot dogs, sliced turkey meat, and other deli meats have been the foods implicated in recent listeriosis outbreaks *(4–6, 18).* In a recent risk assessment analysis, RTE deli meats ranked first among other food categories in terms of risk for listeriosis *(26).*

The reported prevalence of *L. monocytogenes* in RTE sliced meat products is quite variable. Possible reasons for this variability are (i) the differences among countries in the strictness and extent of measures and actions applied for controlling the contamination and proliferation of the pathogen in foods and (ii) the differences in the *L. monocytogenes* detection protocols used, in terms of their lower limit of detection and their epidemiological specificity and sensitivity. Hence, the reported prevalence estimates in the United States range from 0.89% *(14)* to between 4.2 and 8.0% *(16)* for sliced ham and luncheon meats. In Belgium, 6.65% of cooked and sliced ham, loin, and poultry products were reported to carry the pathogen *(27),* whereas in studies in the United Kingdom the presence of *L. monocytogenes* was between 0.4% *(13)* and 2% *(11)* of the cold sliced RTE meat samples tested. Higher estimates have been reported in Spain (8.8% of cooked meats and 6.7% of cured meats) *(28)* and Denmark (23.5% of preserved meat products and 5% of heat-treated meat products) *(17).* Therefore, the prevalence estimate (8.1%) for sliced RTE meat products in Greece is intermediate compared with those reported worldwide.

As has been the case in most studies, the populations of *L. monocytogenes* in the majority of the *L. monocytogenes*–positive samples from the Hellenic market were low and not measurable by plating (based on a sensitivity of pathogen detection of 1 cell per 0.1 g of food) (Fig. 1). *L. innocua* has been reported as the most common *Listeria* species isolated from foods *(1, 7, 10, 28, 31).* In our study,

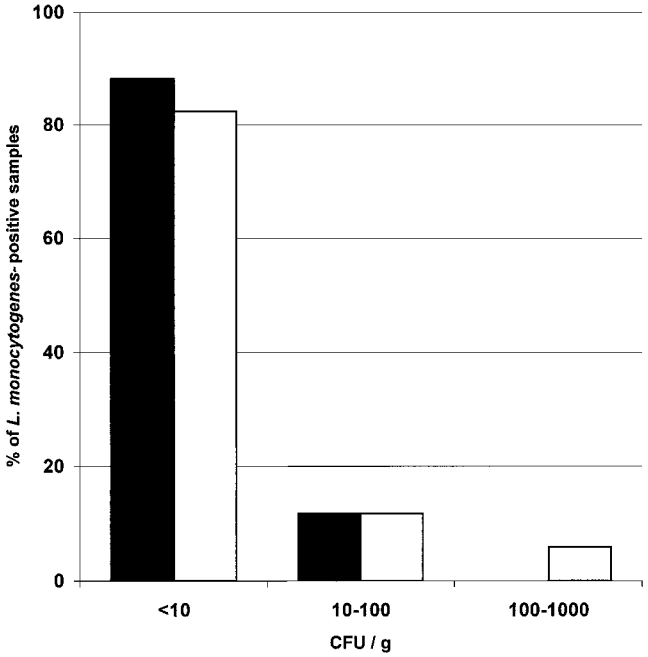


FIGURE 1. *Distribution of populations of* Listeria monocytogenes *(solid bars) and other* Listeria *species (open bars) in the* L. monocytogenes*–positive samples of precut (sliced or cubed) ready-to-eat meat products from the Hellenic retail market.*

both *L. innocua* and *L. welshimeri* were isolated in 64.7% of the samples that were also positive for *L. monocytogenes.* Other investigators also have reported the presence of *L. monocytogenes* in contaminated foods together with other *Listeria* species *(11, 13, 28, 31).* However, because in our study other *Listeria* species were assayed and enumerated only in the samples that were also positive for *L. monocytogenes,* the actual prevalence of these other *Listeria* species in sliced RTE meat products maybe higher. The high percentage of samples with the coincident presence of *L. monocytogenes* and other *Listeria* species emphasizes the need for corrective actions in cases where *Listeria* species other than *L. monocytogenes* are isolated from foods.

Because of the pathogenic potential of *L. monocytogenes* and the difficulties involved with documenting listeriosis outbreaks (i.e., the long incubation time of the disease), every effort should be made to avoid contamination of RTE foods so that food safety can be ensured. However, complete elimination of *L. monocytogenes* in such products may not be practically achievable. Therefore, efforts should also focus on the application of appropriate combination of hurdles associated with product formulation (e.g., pH, water activity, and nitrate concentration) *(15)* and on the application of effective storage-temperature controls. Such measures will inhibit the growth of the pathogen during the shelf life of these products and minimize the safety risk at the time of consumption.

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