Prevalence and risk factors for contamination with *Listeria monocytogenes* of imported and exported meat and fish products in Switzerland, 1992–2000

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Abstract

A total of 2053 import and 164 export samples from 425 production plants were examined over a 9-year period (1992–2000) for the presence of *Listeria monocytogenes* (*L. monocytogenes*) in Switzerland. Overall, 282 samples (12.2%) and 85 plants (20.5%) harbored the pathogen. The highest isolation risk was for marinated fish (38%); the lowest was in cured- and dried-meat products.

Unconditional fixed-effect logistic regression was used to identify the main hazards associated with the presence of *L. monocytogenes*. The plant-level model considered potential risk factors for a positive culture operating at the production-plant level by including a random effect of plant and year.

Food category was the only significant factor; sampling site, country of origin and season were not significant. Marinated fish was a strong predictor for positive culture, whereas cooked- and cured-meat products were protective. Plant and year effects were significant. Control measures should be focused on specific food items in each production plant.

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1. Introduction

*Listeria monocytogenes* (*L. monocytogenes*) is an opportunistic zoonotic agent with a worldwide distribution (Farber and Peterkin, 1991). In humans, listeriosis is mostly a...
food-borne disease (Rocourt et al., 2000). Although listeriosis is a rare disease, it is of great concern due to its high human case-fatality risk. A listeriosis outbreak in Switzerland in 1987 lead to the introduction of a regulation to consider this disease as part of the Swiss notification system (Bille, 1990). Since then, the annual incidence in Switzerland varied between 0.3 and 0.7 cases per 100,000 persons (Swiss Federal Office of Public Health, 2001).

Several food items have been associated with major outbreaks of listeriosis. The first documented food-borne outbreak suggested contaminated coleslaw as the source of infection (Schlech et al., 1983). Vegetables were incriminated also recently in Italy (Aureli et al., 2000). Milk and milk products were implicated several times (Fleming et al., 1985; Lyytikainen et al., 2000), including the Swiss outbreak—in which the infections were traced back to a contaminated regional soft-cheese specialty (Bille, 1990). In the 1990s, epidemics following consumption of contaminated meat products (e.g. pate or frankfurters) were reported (McLauchlin et al., 1991; Goulet et al., 1998; Anon., 2000). In addition, there is increasing evidence that fish and seafood have been the cause of smaller outbreaks (Farber et al., 2000; Tham et al., 2000). Most Listeria infections, however, occur sporadically.

*L. monocytogenes* has been isolated from all categories of food (Farber and Peterkin, 1991). In raw meat (including poultry meat), a prevalence of up to 40% was found, depending on the product type (Jemmi, 1990; Farber and Peterkin, 1991; Uyttendaele et al., 1999a). In fermented products (such as cured- and dried-meat products as well as fermented sausages), growth of *L. monocytogenes* is inhibited by a joint effect of low pH, low *a*<sub>w</sub>-values and the competitive flora (Jemmi, 1990; Farber and Peterkin, 1991). Nevertheless, contaminations occur quite frequently in these food products. Fish and seafood products are also contaminated frequently (Jemmi, 1993; Ben Embarek, 1994; Jemmi and Keusch, 1994). Smoked and marinated fish are of particular concern. Therefore, the behavior of *L. monocytogenes* during processing and storage of artificially inoculated fish was the subject of several studies (reviewed by Rørvik, 2000). Heated products such as hot-smoked fish (which harbor this pathogen due to post-processing contamination) are important because *L. monocytogenes* can multiply under storage conditions (Jemmi and Keusch, 1992; Farber and Peterkin, 1991).

In 1987 (after the listeriosis outbreak; Bille, 1990), Switzerland introduced a legal limit; *L. monocytogenes* may not be detected in 10 g of ready-to-eat food products. This regulation was changed in 1995, to the new limit of non-detection in 25 g of ready-to-eat food items (with the exception of cured-meat products with *a*<sub>w</sub> < 0.92, for which a quantitative limit of 100 g<sup>-1</sup> was introduced). A second modification was made in 1999, applying the same quantitative limit for cold-smoked fish and ice cream (Anon., 1995).

The Swiss Federal Veterinary Office (BVET) is responsible for the control of imported meat, fish and seafood (including their products). The Border Veterinary Service obtains samples and sends them to the BVET laboratories for testing.

Our aim was to describe and analyze the data obtained from the import testing between 1992 and 2000 to determine the risk factors for the presence of *L. monocytogenes* in meat and fish products. The long-term goal was to develop guidelines for the control authorities at the border to determine high-risk products.
2. Material and methods

2.1. Source and description of the data

The database used for this study was retrieved from the collected information of testing of import meat, fish and seafood samples from the Swiss Federal Veterinary Office (BVET) for the period 1992–2000. These data were primarily aimed at monitoring the situation of the *Listeria* contamination in imported food, identifying positive production plants, and if necessary, implementing regulations and/or requiring an official certificate stating negative findings in the imports. In addition, food samples originating from Switzerland and intended for export were included in the final data set.

The following information was available:

- Date of sampling;
- Date of sample arrival in the laboratory;
- Sampling site (custom office): classified into six categories—airport, Basel, Geneva, northeastern border, southern border and domestic;
- Description of the sample (type of product, e.g. smoked salmon, tureen, salami);
- Classification of the sample into one of 10 food categories: raw meat and raw fish, cold-smoked fish, hot-smoked fish, marinated fish (e.g. gravad salmon), other ready-to-eat fish and seafood products, cooked- and cured-meat products, cured- and dried-meat products, fermented sausages, meat meals, and the environment in contact with the food;
- Country of origin of the food item, which was summarized into nine categories: Belgium and The Netherlands, Denmark, France, Germany and Austria, Great Britain and Ireland, Italy, Scandinavia (without Denmark), all other countries ("other countries"), and Switzerland;
- Name of the production plant;
- Culture result;
- Serotype of isolated *L. monocytogenes* strains.

2.2. Sampling techniques

Import samples were collected by border veterinarians, who are located at 80 inspection posts. Sampling was performed according to programs set up by the Federal Veterinary Office and aimed to identify positive samples at the production-plant level.

In general, two sampling techniques can be characterized:

- **Routine sampling**: only one specimen of a specific product per plant and sampling day is taken.
- **Cause of suspicion**: if *L. monocytogenes* was isolated from a previous sample, then the border veterinarian will obtain (on an average) three samples (range 2–5 samples) from the next shipment of the same producer that arrives at the border. This type of sample (regardless of the number) was considered as one sample for our analysis.

Exports of cured- and dried-meat products were also sampled by official veterinarians. In some cases, these samples included working surfaces in the production plants.
using a swab technique. These letter specimens were classified as environmental samples.

2.3. Detection of L. monocytogenes

2.3.1. Qualitative detection of L. monocytogenes

Ten grams (prior to 1995) or 25 g (from 1995 onwards) were mixed with 90 ml (225 ml) of University of Vermont (UVM) Broth I and incubated for 24 h at 30 °C; 0.1 ml of this first enrichment was transferred into 10 ml of UVM Broth II and incubated again for 24 h at 30 °C (McClain and Lee, 1988). Then, 0.1 ml of UVM Broth II was streaked onto PALCAM agar (van Netten et al., 1989) and Oxford agar (Curtis et al., 1989). Both plates were incubated for 48 h at 30 °C, Oxford in air, PALCAM microaerobically. Presumptive Listeria colonies were confirmed by Gram staining and catalase test, and differentiated to the species level using beta-hemolysis, CAMP-factor (using Staphylococcus aureus ATCC 25923) and fermentation of rhamnose (Jemmi, 1990). Hemolytic strains were not further differentiated and were instead considered L. innocua. Since 1997, species identification was performed by hybridization with a DNA probe (Accuprobe®, Gen-Probe, San Diego, CA). L. monocytogenes isolates were serotyped in the Swiss National Reference Center for Listeria, Lausanne, Switzerland.

2.3.2. Quantitative enumeration of L. monocytogenes

In 1999, due to the modifications in the regulation (Anon., 1995), a quantitative enumeration method for the analysis of cold-smoked fish was introduced: 10 g of sample was suspended in 90 ml of peptone broth and diluted decimally. Subsequently, 0.1 ml of each dilution was streaked onto PALCAM agar (van Netten et al., 1989). The plates were incubated microaerobically for 48 h at 30 °C. Presumptive Listeria colonies were confirmed as described above and counted.

2.4. Data analysis

An exploratory analysis was conducted to obtain the frequencies and descriptive statistics of the study variables. Analyses were performed at both individual-sample and production-plant levels. The culture result was considered the outcome for this study and it was coded as positive or negative for the presence or absence of L. monocytogenes, respectively. The individual-sample-level model considered risk factors associated with a positive culture using unconditional fixed-effect multivariable stepwise logistic regression for the whole data set. All variables associated with a positive culture at the univariable level (P < 0.3) and the country of origin (a potential confounding factor) were offered as categorical variables and were treated as dummy variables with a specific reference level. Odds ratios (ORs) and 95% confidence interval (CI) were estimated.

The production plant in which the samples were collected was considered a potential confounder for a positive culture. Therefore, a plant-level logistic model was constructed. Specifically, 221 out of 415 plants initially studied were included for further analysis of multiple observations. We eliminated the export samples (n = 358) from the model. To account for the potential production-plant effect on the outcome, we used a generalized
linear mixed model with logit link, accounting for plant-level variation in different years by including random effects of plant and year (Hu et al., 1998; Curtis et al., 1993). (Each sample within a cluster contributes only one data observation in such an analysis.) A distinguishing feature of such clustered data is that they tend to exhibit intercluster correlation (Shoukri and Pause, 1999). This method also allows inclusion of multiple observations per plant over time and accounts for the remaining variation among plants in the outcome. Random effects logistic regression models were fitted using the glimmix macro with dichotomous outcomes in SAS 8.0, which implements restricted maximum likelihood to get the variance-component estimates (Littell et al., 1996). The random effects of plant and year were assumed to have normal distributions, with means of zero and constant variances of $\sigma^2$. We used an exchangeable correlation for the vector of repeated observations from each plant (i.e. we assumed uniform correlation across time; Allison, 1999).

The log-linear model was used to examine whether a production plant that produced *L. monocytogenes*-contaminated cold-smoked fish also was associated with positive cultures from hot-smoked fish. To assess whether the risk of positive culture increases linearly with the total number of shipments, we categorized the shipments into six groups, and the linear relation of the variable was tested as a trend across the categories by considering the variable as an ordinal in the logistic regression (Szklo and Nieto, 2000).

The overall goodness-of-fit for the final fixed-effect model was assessed using the method of Hosmer and Lemeshow (1989) and testing the deviance (which is approximately chi-square distributed with $n - p$ degrees of freedom where $n$ is the number of observations and $p$ the number of independent parameters (Littell et al., 1996)). To test for the null hypothesis that the common variance of the random effect is zero (i.e. no variation among plants and/or year in the outcome), we compared (after adjustment for other covariates) deviances for the models with and without this parameter. This was done with a chi-square test with one degree of freedom for the single parameter (Breslow and Day, 1980; Allison, 1999). A $P \leq 0.05$ was considered significant, and all statistical analyses were performed with the SAS software version 8.0 (SAS Institute, Cary, NC).

3. *Results*

3.1. *Prevalence*


The highest isolation prevalence was for marinated fish (37.6%; Table 1); the lowest was in cured- and dried-meat products.

Quantitative enumerations of *L. monocytogenes* were below 100 colony forming units per gram (CFU/g), with the exception of one smoked-salmon sample with 1100 CFU/g.

Samples originated from 415 production plants from 36 different countries. The pathogen was found in 85 plants (20.5%). One hundred and ninety-four plants were sampled only once—but four accounted for more than one-fourth of the samples (Table 2).
Because of our interest in possible changes over the entire period of the study (9 years), we analyzed the data of all plants that were sampled >30 times or had ≥5 positive samples \( (n = 14) \). These were sampled during a period from 130 to 2998 days (median = 1403 days). In most plants, we could observe concentrations of positive findings during certain time periods. Seven plants, however, were contaminated again after negative periods (from 196 to 1634 days later; median = 497 days).

The log-linear model showed no relationship between positive cultures in cold- and hot-smoked fish (chi-square = 0.12, d.f. = 1, \( P = 0.722 \)).

### 3.2. Serotypes

Of 282 \( L. \) monocytogenes isolates, 270 could be attributed to a specific serotype: 1/2a (153; 57%), 1/2b (28; 10%), 1/2c (24; 9%), 1/2 immobile (1; 0.4%), 3a (3; 1%), 3b
(4; 1.5%), 4b (26; 10%), atypical serogroup 4 (3; 1%), 7 (28; 10%). Serotype 1/2a was the predominant serotype over the whole period. No association was seen ($P = 0.87$) with the serotypes and the country of origin or the sampling site. Serotype 1/2c was more frequently isolated in fermented sausages (9; 43%) than in other food items.

Most of the plants that were sampled >30 times have one predominant serotype, e.g. all 28 serotype-7 isolates originate from the same producer. Other plants harbored several serotypes (more or less evenly distributed over time).

### 3.3. Logistic regressions

The final stepwise logistic model is depicted in Table 3. Relative to cold-smoked fish, the odds were lower for cooked- and cured-meat products, cured- and dried-meat products, and ready-to-eat fish and seafood products—but higher for marinated fish. Products originating from Scandinavia and Germany and Austria showed a significantly lower association to a *Listeria* positive culture than other countries. The final fixed-effect model fitted well; the Hosmer–Lemeshow chi-square was 0.33 (d.f. = 3, $P = 0.96$).

The final model was analyzed further by including random effects. Added separately, both the random effects of plant (deviance = 646.7, $P < 0.001$) and year (deviance = 457.6, $P < 0.001$) were highly significant. When these two random effects were included simultaneously, both remained significant. However, the random effect of the year was considered small (covariance-parameter estimate = 0.22) compared to that of the plant (covariance-parameter = 1.36). The largest differences in fixed-effects’ parameter estimates with and without the random effect terms were for the country of origin (Table 4); this variable was no longer significant ($F = 1.04$, d.f. = 7, 1628, $P = 0.40$). Cured- and dried-meat products and other ready-to-eat fish and seafood were also insignificant. The scaled
deviance for the model was 1553.7 on 1857 degrees of freedom (indicating that it fitted the data well).

4. Discussion

Our results confirmed previous findings that *L. monocytogenes* occurs widely in food, with high prevalences in meat, fish and seafood products. The overall prevalence of 12.7% (95% CI: 11.3, 14.1) is comparable to other studies (Farber and Peterkin, 1991; Jemmi, 1993; Ben Embarek, 1994; Heinitz and Johnson, 1998; Nørrung et al., 1999; Uyttendaele et al., 1999b). The contamination prevalence of the production plants of 20.5% (95% CI: 16.6, 24.4) is difficult to compare with other findings because of the nature and design of our data set. When stratifying according to the number of sampled lots, the more the plant was sampled, the more we found positive specimens. This can be explained by officials sampling positive plants more than usual (and therefore detecting consistent contamination problems in specific plants). This conclusion is stressed again when considering the concentrations of positive findings during certain times in specific
production plants—positive plants were sampled more frequently. When several consecutive samples were free of *L. monocytogenes*, the status of the plant was considered normal again and sampled accordingly.

Food category was the only risk factor with significant differences. The significance of random effects of both plant and year additionally suggests that control measures should be focused on specific food items in each production plant. It might also point at differences in the plant’s management and quality control (as we will discuss below). The total plant-associated variation in a positive outcome was 1.36 (which equals an OR of 3.2). Therefore, there is clear evidence of variation among plants in the positive outcome, even after adjustment for other potential confounding factors. However, because no information on plant-specific characteristics was available to us, we could not determine the magnitude of variation within-plant. Future studies are need on the characteristics of the plant to explain within-plant variation and time variation.

From 1999 onwards, quantitative assays were included. Because the later test has a higher limit of detection, this could explain the decrease of contamination prevalence in 2000. Some authors (mainly Nørrung et al., 1999) also included quantitative analyses into their study. Another important point is that the detection method was changed in 1995, weighing in a sample of 25 g instead of 10 g. The data indicated that there is no significant trend in the prevalence over time (Cochran-Armitage trend test = 0.56, one-sided *P* = 0.29). However, if we assume that the detection level increased (25 g vs. 10 g), then the incidence risk after 1995 is lower than before the change.

According to Rocourt et al. (2000), there are 12 pathogenic serovars. For public-health purposes, all *L. monocytogenes* strains should be considered as potentially pathogenic for humans (although serotype 4b seems more likely to be involved in outbreaks). The most frequent serotypes isolated from food—as well as from human and animal cases—are 1/2a, 1/2b and 4b (Rocourt et al., 2000).

Most production plants have a predominant serotype—which might indicate a problem with a well-adapted in-house flora. That serotype-7 of *L. monocytogenes* is rarely isolated (Bannermann, personal communication) allows us to discuss the contamination of one specific plant in a little more detail, without the need of further subtyping. This plant mainly exports cold-smoked fish to Switzerland—but also marinated and hot-smoked fish and other ready-to-eat fish products. A total of 135 specimens were sampled between December 1993 and November 2000, of which 36 had *L. monocytogenes*—27 serotype-7. This producer has had a problem with this specific strain. It seems that it is very difficult to get rid of such in-house flora. To keep the export license to Switzerland, great efforts were made to get the problem under control. According to the managers of the plant, the introduction of a comprehensive HACCP concept (ICMSF, 1988) (including bacteriological process controls) and changes in their cleansing and disinfection system allowed the identification of the contamination sources.

From other plants, several serotypes of *L. monocytogenes* were isolated, suggesting that their products are not contaminated by specifically adapted clones. In those plants, we hypothesized problems with processing hygiene (such as lapses in cleansing and disinfection).

Boerlin et al. (1997) even showed that cross-contamination between different products of a single production plant can take place. We attempted to assess the association between
hot-smoked and cold-smoked fish with a log-linear model, but there was no association. Nevertheless, we believe that the problem of cross-contamination between processed raw food (such as cold-smoked fish) and cooked products (such as hot-smoked fish) is of great public-health importance. In heat-treated products, *L. monocytogenes* will find good growth conditions. Contamination of such products cannot be tolerated, because there is a great potential for the pathogen to develop at refrigeration temperatures; this represents an increasing health hazard for the consumers who store such products for a long time. It is therefore of secondary importance whether a positive finding was due to cross-contamination or undercooking. The mere fact of contamination will induce the control authorities to withdraw the product immediately from the market or—in the case of import—to declare an import ban.

In raw processed food, *Listeria* can be prevented from growing mainly due to technological steps used in processing and the effect of the competitive flora. This is true for various products such as fermented sausages or cured- and dried-meat products (Jemmi, 1990; Farber and Peterkin, 1991). This is underscored by the zero prevalence in these categories, when quantitative enumeration of *L. monocytogenes* was performed. Conversely, in cold-smoked and marinated fish, there is a potential for growth of the pathogen under storage conditions (Rørvik, 2000). Although Farber (1991) considered that such products should not represent a serious risk to human health, there have been recent outbreaks (Farber et al., 2000; Tham et al., 2000). In 1999, Switzerland introduced a quantitative limit of 100 *L. monocytogenes* per gram. Using quantitative enumeration, only one sample was positive so far. We believe that a future focus in import control has to be laid on marinated fish, analyzing quantitative levels of *L. monocytogenes* and assessing the potential growth of contaminated products during the storage period.

5. Conclusions

Our findings show that the risk of *Listeria* contamination is mainly associated with the product category and the production plant. Country of origin, sampling site or season had no detectable influence. Special emphasis should be laid in the control of heat-treated products, because they have the biggest potential impact on human health. Also, the frequent contamination of marinated fish should be assessed further. There is great need for quantitative data.

The current sampling protocol allows the identification of *Listeria* positive plants and the issue of eventual subsequent regulations.

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