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From 24 April to 31 July 2011, nine cases of listeriosis were registered in the cantons of Aargau, Basel-Land and Zurich, Switzerland. In six of the cases, infection with *Listeria monocytogenes* was laboratory confirmed, while three remained suspected cases. The suspected cases were family members of confirmed cases with identical or similar symptoms. All confirmed cases were infected with a *L. monocytogenes* strain belonging to serovar 1/2a: all had an indistinguishable pulsotype by pulsed-field gel electrophoresis (PFGE). The same strain was detected in samples of cooked ham that were on sale from a particular retailer. Two samples of ham tested contained 470 and 4,800 colony-forming units (CFU) *L. monocytogenes* per gram respectively. Data of shopper cards from two confirmed cases could be evaluated: both cases had purchased the contaminated ham. The outbreak initiated a product recall and alert actions at national and European level, through the Rapid Alert System for Food and Feed (RASFF). Following the RASFF alert, the company producing the contaminated ham was inspected by the responsible authorities. Their investigations showed that the ham was not contaminated in the production plant, but in the premises of a company to which slicing and packing was outsourced.

Introduction

Infections with *Listeria monocytogenes* in animals have been known since the first studies in this field by Murray et al. in 1926 [1]. Soon after, the first sporadic infections in humans were detected [2]. In Switzerland, they have been reported mandatorily since 1975. At the beginning of the 1980s, the first outbreaks due to foods contaminated with *L. monocytogenes* were recognised in the United States: the foods concerned were coleslaw, pasteurised milk and Mexican-style soft cheese [1]. In Switzerland, beginning in 1983, an increase in the number of listeriosis cases was observed. After extensive outbreak investigations, an artisanal soft cheese (Vacherin Mont d'Or), produced in the winter months in the western part of the country, was identified as the source of infection [3].

The annual incidence of listeriosis in Switzerland has fluctuated over the last two and half decades, with a minimum of 0.3 cases per 100,000 inhabitants in 1990 and a maximum of 1.0 case per 100,000 inhabitants in 2006. The annual incidence in 2011 was 0.6 cases per 100,000 inhabitants [4].

During 1990 to 2008, the most prevalent serotypes were 1/2a and 4b. In contrast, other serotypes (1/2b, 1/2c, 3a, 4d) were comparatively rarely represented; only 1/b reached a prevalence of 15%, in 2007 [4].

In Switzerland, most cases of listeriosis are sporadic. An evaluation of patient data in the 1990s showed that in 115 (52%) of the 222 cases, an underlying disease was reported, 48 (22%) were mother–child cases, and in 59 (27%) of the cases, no underlying condition was reported. For pregnant women with the disease (n=28), the following symptoms were observed: abortion (n=8), endometritis (n=2), septicaemia (n=1), amnionitis (n=1) and minor symptoms such as gastroenteritis (n=8). Cases who were newborns (n=20) showed septicaemia (n=11), meningitis (n=3), pneumonia (n=3) and granulomatosis infantiseptica (n=2). For one newborn, there was no indication of symptoms. For patients who were neither pregnant women nor neonates (n=174), the following symptoms were reported: meningitis and meningo-encephalitis (40%, n=70), septicaemia (14% , n=25), pneumonia (11%, n=19), wound and joint infections (3%, n=5), endocarditis (2%, n=4), peritonitis (2%, n=3) and aggravation of general condition (2%, n=3). For 14 (8%) of the patients, minor symptoms such as diarrhoea occurred and in 31 (18%) of the cases, no symptoms were indicated [5].

Outbreaks of *L. monocytogenes* infections in Switzerland are rare and, until the outbreak described

in this report, had occurred only twice since 1983 [3,6]. Here, we describe an outbreak of listeriosis in 2011 due to contaminated imported cooked ham. The first notification of a possible ongoing outbreak was obtained through the mandatory reporting system for infectious diseases. The subsequent investigation was conducted mainly by the responsible food-control authorities, supported by other institutions. As outbreaks of listeriosis are almost exclusively food-borne [1], the aim of the investigation was to identify as quickly as possible the contaminated food-stuff that was the infection source, interrupt the infection chain and by taking adequate measures thereby re-establish food safety.

Methods

Outbreak case definition

A confirmed case was defined as person whose infection – reported to the Federal Office of Public Health – was laboratory confirmed as due to *L. monocytogenes* serotype 1/2a matching the outbreak pulsed-field gel electrophoresis (PFGE) pattern, with a test date between 24 April and 31 July 2011 in the cantons of Aargau, Basel-Land and Zurich, Switzerland.

A suspected case was a clinically compatible case of *L. monocytogenes* infection who had an epidemiological link to a confirmed case (family member), with a date of symptom onset between 24 April and 31 July 2011 in the cantons of Aargau, Basel-Land and Zurich, Switzerland.

The cases were detected through the mandatory reporting system for infectious diseases.

Patient interviews and evaluation of shopper cards

The occurrence of a cluster with four patients infected with *L. monocytogenes* 1/2a led the health authorities of canton Zurich to the decision to undertake patient interviews as soon as possible. Telephone interviews with seven patients (three couples and a single person), based on a standardised questionnaire, were carried out by a microbiologist of the local authority of food control in the canton of Zurich. The patients were asked which locations with collective catering (restaurant, party, etc.) they had visited in the two months before onset of symptoms. Furthermore, they were asked about their consumption habits during this time period, concerning categories of foods at major risk for the transmission of *L. monocytogenes* (raw milk, soft cheese, raw meat dishes, cured and fermented raw meat products and smoked fish). If high-risk products had been consumed, the interviewer tried to find out which brands had been purchased and from where. The patients were interviewed within 8 to 18 days after symptom onset.

In addition, data on shopper cards (client cards) of two of the couples were available for evaluation.

Microbiological tests

In the investigation, as a consequence of the interviews with the patients, samples of salami and samples of cooked ham were analysed. Testing for *L. monocytogenes* was done using the mandatory methods for official laboratories of food control based on the International Organization for Standardization (ISO) 11290-2 for quantitative detection [7] and ISO 11290-1 for qualitative detection [8]. Rapid detection was carried out by enrichment in half Fraser broth followed by real-time polymerase chain reaction [9]. Samples that were *L. monocytogenes*-positive by PCR were confirmed with culture tests according to ISO [8].

Serotyping was performed using a commercial set of Listeria O-factor and H-factor antisera from Denka Seiken (Pharma Consulting, Burgdorf, Switzerland).

Rapid alert system for food and feed (RASFF)

RASFF is a platform of the European Union (EU), used by the member countries for the exchange of information concerning foods and feeds that do not comply with the law. On the basis of a veterinary agreement, Switzerland is a part-member of RASFF and runs two official border inspection points at Zurich and Geneva airports and has therefore full access to border rejection notifications. Other notifications, such as alerts, are only distributed to the Swiss national RASFF contact point if Switzerland is directly concerned. This applies when a product has been delivered from an EU country to Switzerland or has been produced by a Swiss company.

PFGE genotyping and analysis

Basically, a previously described protocol was used [10] with the following minor modifications: use of SeaPlaque agarose instead of SeaKem agarose Gold; use of an additional 200 units (U) of achromopeptidase in the lysis mix; overnight lysis and DNA cleavage with 200 U of ApaI, 50 U of AscI or 50 U of SmaI. The PFGE was run at 14 °C for 20 hours with 6 V/cm under a linear ramp from 4 to 40 seconds using an angle of 120°.

For pattern comparison, BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium) was used. Pairwise similarities between the ApaI and AscI PFGE patterns were calculated using the JACCARD similarity coefficient. Clustering was based on the unweighted pair-group method with averages (UPGMA), setting tolerance and optimisation at 1% each. We used *Xba*I-digested DNA from *Salmonella* serovar Braenderup strain H9812 (ATCC BAA 664) as a fragment-size reference.

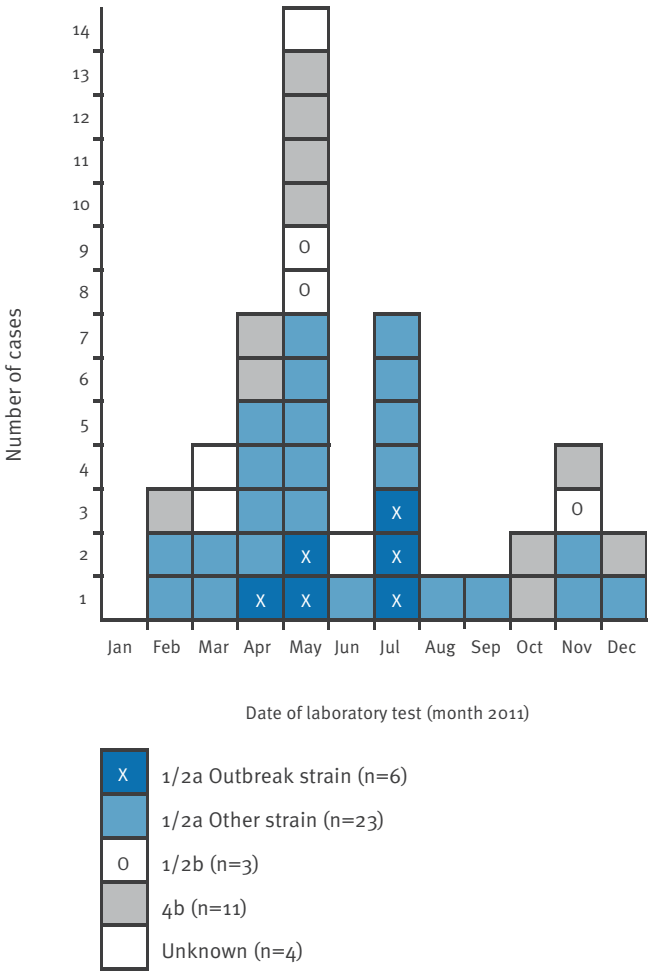
Results

Recognition of the outbreak

In May 2011, the Cantonal Ministry of Health in Zurich reported four laboratory-confirmed cases of listeriosis – occurring between 25 April and 5 May – in whom the *L. monocytogenes* isolate belonged to the serotype

Figure 1

Serotype distribution of listeriosis cases registered at the Federal Office of Public Health by date of laboratory test, Switzerland, 1 January–31 December 2011 (n=47)



1/2a. This represented a clear increase in the number of cases, compared with the normal epidemiological situation in the canton, where a mean of 0.42 listeriosis cases with *L. monocytogenes* serotype 1/2a were observed per month from January 2007 to December 2011 (unpublished data from the mandatory reporting system).

Analysis of all laboratory-confirmed listeriosis cases in Switzerland in 2011 showed that the dominant serotype that year was 1/2a (Figure 1).

On 6 June 2011, the national reference laboratory reported three of the four serotype 1/2a isolates to be indistinguishable by PFGE. This finding indicated a possible ongoing outbreak. Subsequent PFGE typing of all 1/2a isolates received by the reference laboratory allowed the detection of three more confirmed cases belonging to the outbreak.

Characteristics of patients

The infection of the six listeriosis patients was laboratory confirmed as *L. monocytogenes* 1/2a in April, May and July (Figure 1). The spouses of three of the confirmed cases (Cases 1–3) developed simultaneously the same or very similar symptoms as their partners. Case 1 experienced vomiting, diarrhoea and syncope (partner: vomiting and diarrhoea), Case 2 and partner had vomiting, diarrhoea, fever and shivering, and Case 3 had abdominal pain, diarrhoea, fever, headache and pneumonia (partner: nausea, abdominal pain, diarrhoea, fever, headache and vertigo). The partners of Cases 1–3 were suspected cases since *L. monocytogenes* was not isolated.

Five of the six laboratory-confirmed cases were aged 65 years or older; two of these elderly patients had underlying conditions (type-2 diabetes, asthma, heart disease, macrocythemia).

Outcome of patient interviews

Due to the unusual increase in the number of cases with *L. monocytogenes* 1/2a in April to May 2011 in Zurich, patients were interviewed. These interviews, with three confirmed cases and their spouses (suspected cases) and a single person, identified the following common behaviour: they purchased foods in shops of a particular retailer and consumed soft cheeses and meat products such as salami. All seven reported having eaten salami of a well-known brand that has a large presence on the Swiss market.

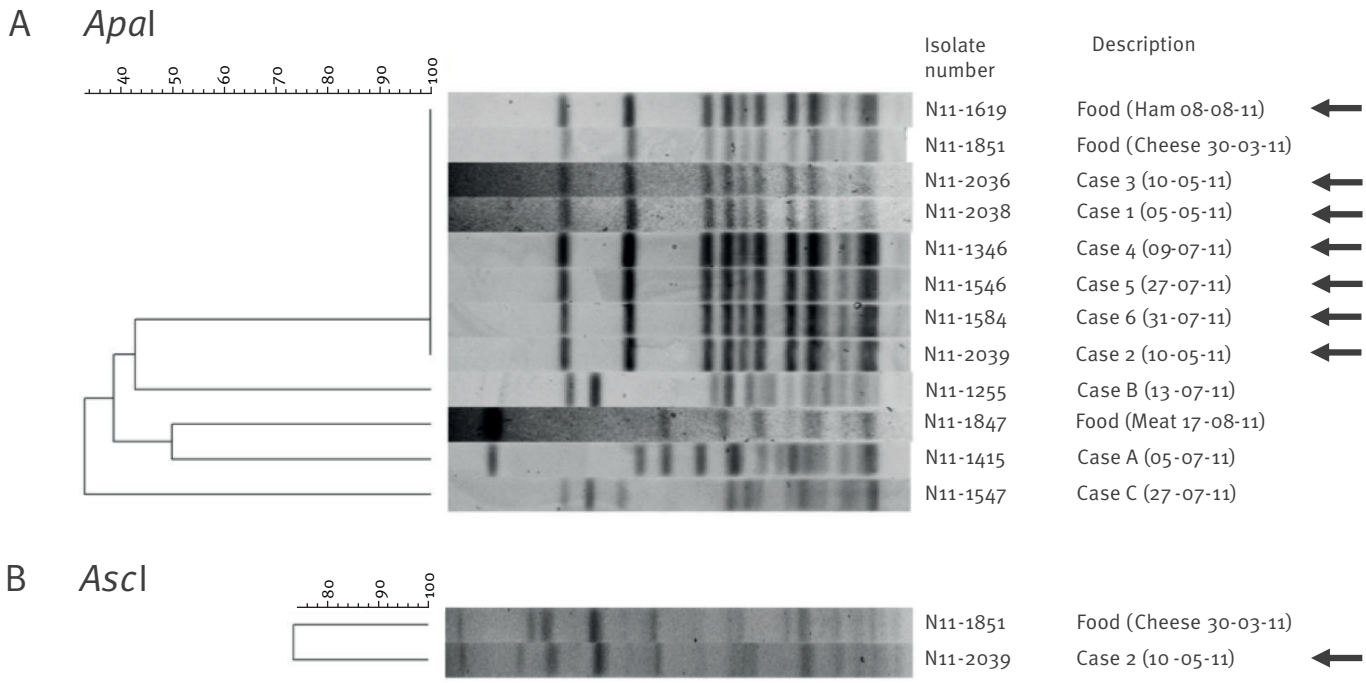
Outcome of bacteriological tests

Given the results of the interviews of the patients, 11 different salami varieties of the suspected producer were sampled at retail and tested by enrichment for the presence of *L. monocytogenes* in 25 gram of salami. None of these analyses revealed a positive result.

On 2 August, a retail company reported to the relevant local authority the finding of an *L. monocytogenes* isolate from a sample of cooked ham imported from Italy (Type A). The laboratory analysis, which revealed 4,800 colony-forming units (CFU) of *L. monocytogenes* per gram, was conducted as part of the retailer's routine quality control practices. On 3 August, the cantonal laboratory of food control took official samples of type A ham at retail. On 4 August, *L. monocytogenes* was demonstrated by rapid detection, and subsequent quantitative analysis revealed 470 CFU/g. On the basis of the analytical evidence, on the same day, recall action was undertaken and information for the public was issued by the retailer on the Internet and by press release. Also on the same day, the food control authority of the Ticino – the canton where the importer of the contaminated product was based – was informed and investigations were immediately undertaken. They ascertained the types and amounts of products that had been imported and also identified the distribution

Figure 2

Relationship of PFGE patterns generated with *Apa*I (panel A) or *Asc*I (panel B) of *Listeria monocytogenes* isolates from clinical (n=9) and food (n=3) samples, Switzerland, 30 March–17 August 2011



PFGE: pulsed-field gel electrophoresis.

Isolates belonging to the outbreak strain are marked with an arrow. The dates in parentheses are the dates of strain isolation.

network. It was found that the contaminated ham was produced exclusively for the Swiss retailer in question.

As part of the inspection of the importing company, samples of the suspected ham (Type A), of 'mortadella' (scalded sausage from pork originally produced in the region around Bologna, Italy) and another type of cooked ham (Type B) of the same producer were also taken and analysed for the presence of *L. monocytogenes*. These tests confirmed the known contamination of the type A ham and also demonstrated *L. monocytogenes* in Type B cooked ham (no quantitative data available). As a result of these findings, legal measures were enacted in order to stop the importation of the relevant products.

Evaluation of shopper cards

Since all the interviewed patients (n=7) reported being regular customers of the particular retailer, an evaluation of shopper cards (client cards) was carried out. This action required legal clarification and the agreement of the patients and the retail company. On 10 August, the data of two such cards became available, showing that two couples had purchased cooked ham of type A. Case 1 and partner had purchased the product on 21 April (onset of symptoms: 25 April) and Case 3 and partner on 28 April (onset of symptoms: 2 May). This added further evidence that cooked ham of Type A was the vehicle of the infection.

Reporting to the Rapid Alert System for Food and Feed (RASFF)

On 4 August, the Federal Office of Public Health reported the isolation of *L. monocytogenes* from the Type A ham to RASFF, which subsequently sent out an alert on 5 August. Further bacteriological findings were also reported to RASFF and initiated a follow-up alert (9 August) and a notification (18 August).

As a result of reporting the outbreak investigation data to RASFF, the producer in Italy carried out extensive investigations to find the source of the contamination. It was shown that the production processes and facilities conformed to legal requirements and that *L. monocytogenes* was not detectable in food or environmental samples. Further investigations finally traced the *L. monocytogenes* source to a company that, as an outsourced service, sliced and packed the meat products.

Typing of *L. monocytogenes* isolates

PFGE patterns of the L. monocoytogenes 1/2a strains isolated from six laboratory-confirmed cases of the outbreak (Cases 1–6) and from cooked ham are shown (Figure 2, panel A). For comparison, we also analysed several strains of *L. monocytogenes* 1/2a that were isolated between April and August and sent to the national reference laboratory for typing. The PFGE patterns of these strains, isolated from patients not involved in the outbreak (Cases A, B and C) and from a meat sample (N11-1847) were clearly different from that of the outbreak strain, showing relative relationships of less than 50%.

One strain, however, isolated from cheese (N11-1851) showed an ApaI profile indistinguishable from that of the outbreak strain. Therefore, the isolate from the cheese together with all isolates (clinical and from the ham) that belonged to the outbreak strain were subjected to additional PFGE analyses using the restriction endonucleases AscI and SmaI. All patterns generated by SmaI, including that of the cheese isolate, were indistinguishable (data not shown). However, the AscI pattern of the cheese isolate turned out to be different from the outbreak strain (Figure 2, panel B). The pattern for the isolate from Case 2 is shown as an example, but all isolates belonging to the outbreak strain were indistinguishable using AscI (data not shown).

The seventh patient interviewed was shown by PFGE not to be part of the outbreak (data not shown).

Discussion

Not surprisingly, the patients could not remember precisely where all their food products had been purchased several weeks before symptom onset. However, several food types known to be a risk in the context of listeriosis could be excluded and some common behaviours were identified. Notably, those interviewed purchased food mostly from one particular retail company and all stated having consumed meat products such as salami of a particular brand. As cooked ham was not considered a major risk for infection with *L. monocytogenes*, a question relating to ham consumption was not included in the questionnaire. This was a limiting factor and in future investigations, a questionnaire of higher discriminatory power should be used. Other limiting factors were that not all cases of the outbreak were interviewed and it is also not known whether those who bought the ham actually ate it.

In the case of food-borne outbreaks, the main objective is always rapid identification of the infectious source, thereby allowing the responsible authorities to re-establish food safety for consumers, often using a combination of epidemiological and microbiological techniques. In this outbreak, laboratory techniques played a key role as the isolation of *L. monocytogenes* from a quality-control sample of ham gave the crucial information. If a food company identifies contaminated products that may pose a risk for the consumers, the finding has to be reported to the authorities in charge, as decreed in Switzerland by the Ordinance on Foods and Utility Articles [11]. Analysis of the isolate from a cooked ham sample enabled the identification of the source of infection and origin of contamination. PFGE typing of an initial series of *L. monocytogenes* strains indicated that there was an ongoing outbreak. Use of three restriction enzymes increased the discriminative power of PFGE permitting the identification of small differences.

Evaluation of information from shopper cards has already been used in outbreak investigations [12]. In the outbreak reported here, the cooked ham that was suspected to be contaminated with *L. monocytogenes* could be identified on the cards of two couples. However, this information was only available when the source of the outbreak had already been identified. The reason for this delay was that this was the first time such cards had been used in a Swiss outbreak investigation and several aspects, such as legal questions, had to be clarified. The experience gained should help to speed up the procedure in future outbreak investigations in the country. We consider that evaluation of shopper cards is a powerful instrument, which should be a basic element of all outbreak investigations where commercial products are suspected to be the source of infections.

The data on the cards also allowed us to conclude that the incubation period for two confirmed cases and their partners must have been rather short (Case 1: purchase of ham on 21 April and onset of symptoms on 25 April; Case 3: ham purchased on 28 April and symptom onset on 2 May). For listeriosis, the incubation period is in the range of 3 to 70 days, with the median estimated to be 21 days [13]. The short incubation time and the more or less simultaneous onset of symptoms in the cases' partners indicates that the cooked ham may have been heavily contaminated with *L. monocytogenes* at the time of consumption. Cases 1 and 3 purchased cooked ham within a period of seven days. From the information on the shopper cards, it is not possible to say whether the purchased packs of ham belonged to the same lot. However, the short shelf life of cooked ham suggests that a particular lot, or two consecutive lots, were concerned.

In situations such as this, in which an imported meat product was found to be the source of the infectious agent, national authorities can decree certain measures that the importer should carry out, but have no jurisdiction over the producer. However, following the RASFF alert, relevant authorities in the country where the producer was based took certain actions and decreed that risk management measures be carried out. This allowed the identification of a company that sliced and packed the cooked ham as the origin of the contamination. The producer immediately stopped working with the company concerned, awarding the contract to another firm. After this measure, exportation to Switzerland was

possible again. Concerning contaminated foods and feeds, RASFF makes the rapid exchange of information between European countries possible. In the case of certain outbreak investigations, even closer cross-border information sharing (for example, exchange of bacterial isolates) would be useful. Currently, such a form of cooperation is not institutionalised in Europe and depends on the goodwill of the participating institutions and authorities.

In total, nine outbreak cases were detected over a period of around three months. This could indicate that the concerned company had a persistent hygiene problem in their facilities. However, the rather low number of human cases might suggest that the cooked ham was not contaminated at high levels throughout the entire period of the outbreak. This assumption is supported by the quantitative testing, which revealed 4,800 CFU of *L. monocytogenes* per gram in one sample of ham and only 470 CFU/g in another. In an earlier listeriosis outbreak in Switzerland, due to contaminated soft cheese, counts up to 32,000 CFU/g of *L. monocytogenes* per gram were found. This cheese caused 12 human cases of listeriosis in a shorter period, of about two months [6]. There are no dose-response data on *L. monocytogenes* infections in humans; however, a risk assessment showed that the vast majority of cases of listeriosis are associated with the consumption of foods that do not meet current standards for *L. monocytogenes* in foods (maximum 100 CFU/g) [14].

Meat products are known to be an important source of *L. monocytogenes*, leading to human infections [1]. With regards to ham, a retrospective case–control study in England, with people aged over 60 years, identified this product as a risk factor for listeriosis [15]. Furthermore, 'rillettes' – a spread prepared with ham and cooked with grease – was found to be the vehicle of a listeriosis outbreak in France [16]. To the best of our knowledge, the outbreak we describe here is the first in which of the vehicle of a listeriosis outbreak was shown to be sliced and pre-packed ham. In our opinion, the slicer exclusively processed for a large retailer in Switzerland and this was probably the reason why cases were not picked up elsewhere.

The slicing of meat products is a critical step in food production. It had been shown experimentally that *L. monocytogenes* from an inoculated slicer blade could be found on up to 30 slices of un-inoculated products such as turkey breast or salami [17]. *L. monocytogenes* is known to be a psychrotrophic microorganism, and strains particularly adapted to low temperatures are known [18]. In ready-to-eat salads, including smoked ham salad, growth rates of more than 0.5 log10 in 48 hours were demonstrated at storage temperatures of 7 °C [19]. For frankfurters kept at 8 °C, a 2 log10 increase of *L. monocytogenes* counts was demonstrated within 4 to 13 days of storage [20]. These facts illustrate the need for a proper evaluation of storage conditions (time and temperature) for products at risk. In households, eating perishable products within shelf-life dates and having correctly operating refrigerators are essential. Furthermore, persons with a compromised immune system and pregnant women should refrain from eating foods known to be at risk of contamination with *L. monocytogenes*. In hospitals, ready-to-eat meat products should only be served when the absence of *L. monocytogenes* can be guaranteed by an adequate quality control [21].

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