

Rapid detection of the source of a *Listeria monocytogenes* outbreak in Switzerland through routine interviewing of patients and whole-genome sequencing

Cornelia Speich^{ab}, Roger Stephan^c, Nicola Dhima^d, Florian Hollenstein^e, Jule Horlbog^c, Giulia Delvento^{ab}, Ekkehardt Altpeter^d, Meike Zuske^{ab}, Michelle Raess^d, Helena Greter^{ab}

^a Swiss Tropical and Public Health Institute, Allschwil, Switzerland

^b University of Basel, Basel, Switzerland

^c Institute for Food Safety and Hygiene, National Reference Laboratory for Enteropathogenic Bacteria and Listeria (NENT), Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

^d Federal Office of Public Health, Liebefeld, Bern, Switzerland

^e Kantonales Laboratorium Thurgau, Frauenfeld, Switzerland

Summary

AIMS OF THE STUDY: Listeriosis is a notifiable disease in Switzerland. In summer 2022, the Swiss Federal Office of Public Health noticed an increase in reports of listeriosis cases, indicating a possible ongoing outbreak. Here we present the approaches applied for rapidly confirming the outbreak, detecting the underlying source of infection and the measures put in place to eliminate it and contain the outbreak.

METHODS: For close surveillance and early detection of outbreak situations with their possible sources, listeriosis patients in Switzerland are systematically interviewed about risk behaviours and foods consumed prior to the infection. *Listeria monocytogenes* isolates derived from patients in medical laboratories are sent to the National Reference Laboratory for Enteropathogenic Bacteria and Listeria, where they routinely undergo whole-genome sequencing. Interview and whole-genome sequencing data are continuously linked for comparison and analysis.

RESULTS: In summer 2022, 20 patient-derived *L. monocytogenes* serotype 4b sequence type 388 strains were found to belong to an outbreak cluster (≤ 10 different alleles between neighbouring isolates) based on core genome multilocus sequence typing analysis. Geographically, 18 of 20 outbreak cases occurred in northeastern Switzerland. The median age of patients was 77.4 years (range: 58.1–89.7), with both sexes equally affected. Rolling analysis of the interview data revealed smoked trout from a local producer as a suspected infection source, triggering an on-site investigation of the production facility and sampling of the suspected products by the responsible cantonal food inspection team on 15 July 2022. Seven of ten samples tested positive for *L. monocytogenes* and the respective cantonal authority ordered a ban on production and distribution as well as a product recall. The Federal Food Safety and Veterinary Office released a nationwide

public alert covering the smoked fish products concerned. Whole-genome sequencing analysis confirmed the inter-relatedness of the *L. monocytogenes* smoked trout product isolates and the patient-derived isolates. Following the ban on production and distribution and the product recall, reporting of new outbreak-related cases rapidly dropped to zero.

CONCLUSIONS: This listeriosis outbreak could be contained within a relatively short time thanks to identification of the source of contamination through the established combined approach of timely interviewing of every listeriosis patient or a representative and continuous molecular analysis of the patient- and food-derived *L. monocytogenes* isolates. These findings highlight the effectiveness of this well-established, joint approach involving the federal and cantonal authorities and the research institutions mandated to contain listeriosis outbreaks in Switzerland.

Introduction

Listeriosis is a rare disease in Switzerland with 40 to 60 confirmed cases every year in a population of about 8.7 million inhabitants [1]. However, the disease may be severe and life-threatening, especially for immunocompromised and elderly people [1–4]. Due to its high case fatality rate, listeriosis ranks among the leading causes of death due to food-borne illness and consequently has a significant negative impact on public health and the economy in most European countries [3, 4]. Symptoms of listeriosis may be mild such as diarrhoea and low-grade fever, but the disease can also lead to severe symptoms including sepsis and organ damage [2, 5]. Furthermore, *Listeria monocytogenes* has the ability to cross the blood-brain barrier leading to meningitis and brain infection [5]. In pregnant women, *L. monocytogenes* may cross the placental barrier leading to diaplacental infections of the foetus with high risk of subsequent abortion [6]. The long incubation period

Helena Greter
Swiss TPH
Kreuzstrasse 2
CH-4123 Allschwil
[helena.greter\[at\]swisstph.ch](mailto:helena.greter[at]swisstph.ch)

of approximately 11 days (10% of cases showing incubation periods >4 weeks) [7] is a major challenge when searching for the source of infection with listeriosis patients.

Given their ubiquitous occurrence in the environment including the soil and/or surface water, *L. monocytogenes* can be present in raw foods (e.g. meat, fish, poultry, vegetables, raw milk) [8].

Since 1974, human listeriosis is classified as a notifiable disease in Switzerland and hence, every culture- or polymerase chain reaction (PCR)-confirmed human listeriosis case has to be reported to the Swiss Federal Office of Public Health (SFOPH) [9]. Additionally, all isolates are sent to the National Reference Laboratory for Enteropathogenic Bacteria and Listeria (NENT), where all clinical *L. monocytogenes* isolates have been routinely whole-genome sequenced since August 2018. This procedure ensures early detection of *L. monocytogenes* clusters, which indicate that several patients are suffering from an infection with the same strain. Furthermore, for close surveillance and rapid detection of potential sources of contamination to limit outbreaks, SFOPH has commissioned the Competence Centre for Epidemiological Outbreak Investigations (KEA) at the Swiss Tropical and Public Health Institute (Swiss TPH) to routinely interview all patients diagnosed and reported with listeriosis using a standardised questionnaire. Topics covered in these interviews include, among others, exposure risk behaviours and food consumption prior to illness. Here, we describe the processes leading to the detection of a regional listeriosis outbreak in summer 2022 linked to *L. monocytogenes* serovar 4b, sequence type 388, and the intervention measures taken to contain the outbreak.

Materials and methods

Data collection

In accordance with the standard procedure for listeriosis surveillance in Switzerland, isolates of (patient) samples that tested positive for *L. monocytogenes* at different medical laboratories were routinely sent to NENT for molecular analysis (routine whole-genome sequencing). Similarly, the attending physician of each patient reported to SFOPH was routinely contacted by KEA and asked for his/her assessment of the patient's ability to be interviewed. If a direct interview was ruled out due to the patient's health status, the physician was asked to identify a proxy person (relative, partner, caregiver, etc). In rare cases, the physician advised against conducting an interview. Reasons for advising against an interview included language barriers (neither the patient nor the proxy person could speak or understand German, French, Italian or English), non-availability or absence of a proxy person, or consideration for the potential proxy person in light of the patient's serious illness or recent death. Attending physicians were also interrogated regarding their information and/or suspicions concerning the source of infection, if any. Next, an information letter was sent to every eligible patient or his/her representative, announcing the anticipated telephone interview. A trained interviewer then called the person. In this call, the interviewer sought oral informed consent after informing patients that participation is voluntary, confiden-

tiality is guaranteed, and that they do not have to provide answers if they are not willing to do so and this would not have any negative consequences for either themselves or their representative. Also, patients were informed that by sharing detailed information such as the names of a brand, store, producer or restaurant, they were not informing against anyone but might help to identify a problem fast and to prevent harm to both the business and to public health. The telephone interview took approximately 20–30 minutes. The standardised questionnaire contained the following categories and covered a time frame of four weeks prior to symptom onset, a period in which incubation of 90% of the cases can be assumed [7]: (a) national and international travel; (b) out-of-home food consumption in restaurants, canteens or take-aways; (c) special diets, food allergies and intolerances; (d) habitual shopping places for different food categories; and (e) habitual and recent consumption of different food categories (fish and seafood, meat and sausages, poultry, cheese and dairy products, vegetables, fruits, salads and ready-to-eat food items). For grocery shopping and food consumption, habitual practices were taken into account because of the difficulty in remembering food items consumed over a time period that is necessarily long in the case of listeriosis (incubation period is median 11 days and >4 weeks in up to 10% of cases).

Data management

During the interview, data were recorded on paper and then transcribed to a digital database (Microsoft Access 2016). Anonymisation was achieved through the strict separation of personal identifying data and the interview data, whereas all paper-based or digital documentation of personal identifiers were stored separately and secured. Both access to files and to the database were restricted to the team members of SFOPH and KEA involved in this study by physical locks and digital login protection, respectively, as per Swiss federal data protection laws. The whole-genome sequencing results for each case were shared by NENT in an anonymised form (Microsoft Excel 2016) every week, and matching of surveillance and whole-genome sequencing results was performed on sample identification number and date of birth. Data were inspected case-by-case and compared with other cases of the respective cluster on a rolling basis in order to allow identification of similarities in travel and food consumption habits as well as consumed food items between cluster-associated cases.

Statistical analysis

Descriptive statistical analysis was performed using Microsoft Excel 2016 and R version 4.1.3 and summary statistics are presented as median (interquartile range), counts and percentages.

Laboratory work-up

For the molecular analysis of all *L. monocytogenes* isolates, routine whole-genome sequencing on human, food and factory environmental strains was performed using Illumina MiSeq next-generation sequencing technology (Illumina, San Diego, CA, USA) as described in [10]. Sequencing reads were mapped against a multilocus sequence typing (MLST) scheme based on seven house-

keeping genes and a 1701-locus core genome multilocus sequence typing (cgMLST) scheme using Ridom SeqSphere+ software version 7.7.5. Strain types and cluster types were determined upon submission to the *L. monocytogenes* cgMLST Ridom SeqSphere+ server (<http://www.cgmlst.org/ncs/schema/690488/>). Clusters are defined as a group of isolates with ≤ 10 different alleles between neighbouring isolates [10].

Based on the results of the patient interviews, food products of a local producer were collected by the responsible cantonal authorities (Kantonales Laboratorium Thurgau) in July 2022 and processed according to ISO 11290-1 and ISO 11290-2. Moreover, 60 swabs from the production environment of the respective facility were collected. Swabs were incubated in Half Frazer Broth (HFB, BioRad, Cressier, Switzerland) at 30 °C for 48 hours. *L. monocytogenes* was detected by real-time PCR using the Assurance Genetic Detection System (GDS[®], Endotell, Allschwil, Switzerland) according to the manufacturer's instructions. To obtain strains for whole-genome sequencing, the PCR-positive HFB cultures were streaked on Oxoid chromogenic *Listeria* agar (OCLA) plates (Oxoid, Pratteln, Switzerland) and incubated at 37 °C for 48 hours.

Ethical considerations

The procedures involved in the routine interviewing of all listeriosis patients in Switzerland through standardised questionnaires for surveillance are authorised by the Swiss Epidemics Act (SR818.101, EpG) and hence no additional ethical clearance was needed for this subanalysis. All included patients were asked for their oral informed consent before the interview was conducted. All data were stored, managed and analysed in anonymised form.

Results

Outbreak progression

In the third week of April 2022, four cases of listeriosis were reported to SFOPH, all living in the canton of St. Gallen in northeastern Switzerland. This represented an unusual accumulation of listeriosis cases in the canton as it had reported only one listeriosis case earlier in 2022 (in January). Whole-genome sequencing analyses confirmed the interrelatedness between two of the four reported cases (*L. monocytogenes* serotype 4b sequence type 388 cgMLST 18052) and data from interviewing these two patients in early May 2022 revealed that both patients reported having consumed fish (without yet clear congruency among the mentioned fish species or products) bought at local stores or farmers' markets.

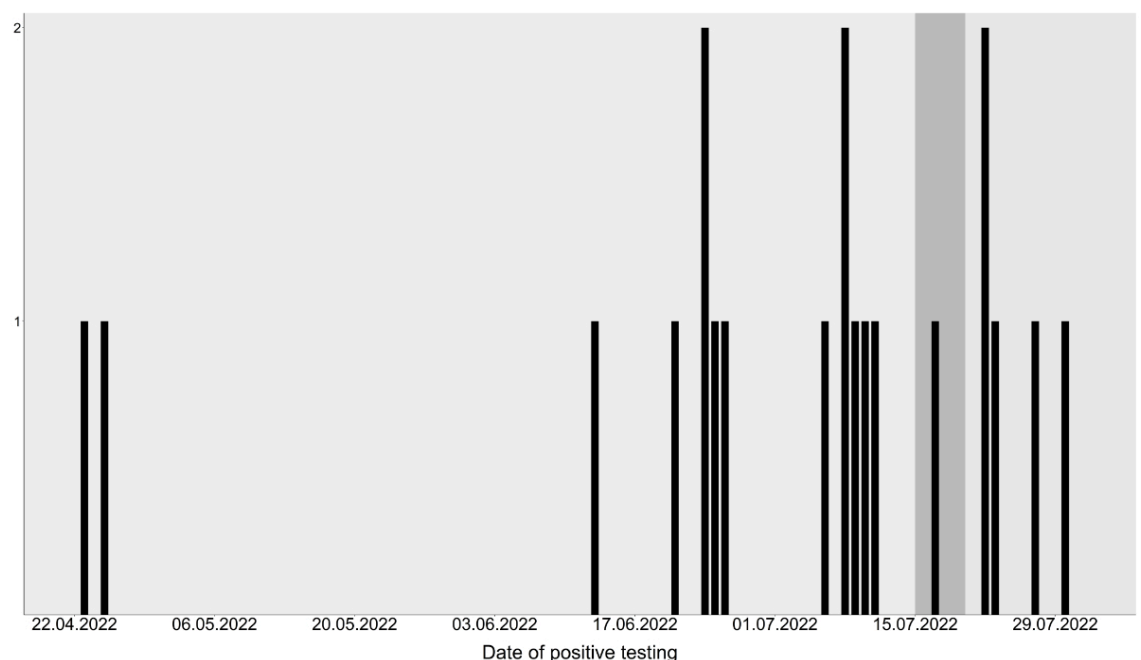
Another listeriosis case in St. Gallen diagnosed seven weeks later was linked to the same cluster based on whole-genome sequencing analysis. In the same period, four more listeriosis cases from other regions in Switzerland had been reported, with no relation to the cluster. However, the third cluster-associated case in St. Gallen marked the starting point of a strong increase in cases reported from northeastern Switzerland for the next month, with 11 additional cases linked to the cluster, resulting in a total of 14 cases to mid-July 2022 when corrective measures could be taken (figure 1).

After implementation of corrective measures on 15 July 2022, an additional six listeriosis patients were identified as belonging to the outbreak cluster, with the last related patient testing positive on 30 July 2022 (figure 1).

Outbreak investigation

Interviewing of the patients took place in a rolling procedure following the standardised questionnaire, but with

Figure 1: Occurrence over time of listeriosis cases related to the smoked trout outbreak in Switzerland. Corrective measures started on 15 July 2022 and the source of contamination identified and respective products recalled on 20 July 2022. Respective dates indicated by changing background colours.



special probing on fish consumption during the four weeks prior to symptoms onset as per the suspicion from the first two interviews. Out of the 14 cluster-associated listeriosis cases diagnosed to mid-July 2022, 8 could be interviewed between 6 May 2022 and 15 July 2022 (table 1). All patients or their respective proxy people interviewed before 15 July 2022 confirmed fish consumption during the critical period (figure 2). Six of the interviewed or proxy-interviewed patients reported having consumed smoked trout during the critical infection period. Among them, four patients named the same producer for the consumed smoked trout, with the first mention of this specific product recorded on 4 July 2022 in two independent interviews, and confirmation of the suspicion by two other patients / proxies by 15 July 2022.

After on-site audit and sampling on 15 July 2022, additional 2 of the listeriosis cases reported before that date, as well as 4 cases reported thereafter could still be interviewed (among which 3 proxy interviews) as per routine

practice, to further allow acting as soon as possible before final whole-genome sequencing confirmation was available.

Outbreak management

Based on the repeated reporting of consumption of smoked trout from the same producer, the responsible cantonal authorities were informed of the suspicion on Friday 15 July 2022. On the same day, a first audit of the production facility and sampling of two of the suspected products was initiated. Smoked trout products sampled during the on-site investigation were analysed in the cantonal laboratory and seven of ten samples tested positive for *L. monocytogenes*. In six samples, *L. monocytogenes* was qualitatively detectable in 25 g, while in one sample 10 colony-forming units/gram were detected.

The producer company supplied several of the main retailers in the respective area with their products, ran a farm shop and sold the products directly to the public at a weekly market and online. After detection of *L. monocytogenes* in the sampled smoked trout, the respective cantonal authority ordered a ban on production and distribution as well as a product recall on 20 July 2022. To alert the public, the Federal Food Safety and Veterinary Office released a nationwide public warning covering the implicated products on 21 July 2022. Finally, following the whole-genome sequencing confirmation of the interrelatedness of the *L. monocytogenes* isolates from the smoked trout samples and the patient-derived isolates on 23 July 2023 (figure 3), the authorities issued comprehensive measures on 26 July 2022. These included extensive sanitation measures at the manufacturing facility informed by the analysis of 60 swabs from the production environment and a revision of the company's self-monitoring policy. After the company took action to restore its legal status and listeria was no longer detectable in environmental samples and ready-to-eat products, the ban on production and distribution was lifted several months later.

Characteristics of the outbreak patients

The 20 outbreak cases were reported from six Swiss cantons. Demographic characteristics of affected patients are shown in table 2. No clinical characteristics are collected in a standardised manner in routine listeriosis surveillance given that rapid identification and elimination of the source of infection are the main objectives in the case of foodborne disease outbreak investigations. Clinical characteristics reported to SFOPH via clinical notifications from attending physicians are not routinely shared with either KEA or NENT and death certifications are not routinely shared with SFOPH by hospitals, except if directly related

Figure 2: Source of infection identification through eight cluster patient interviews leading to corrective measures from 15 July 2022. * Includes one patient mentioning a slightly different name of the smoked trout producer that was assumed to be a wrong re-combination of letters in the patient's memory in the course of the investigation.

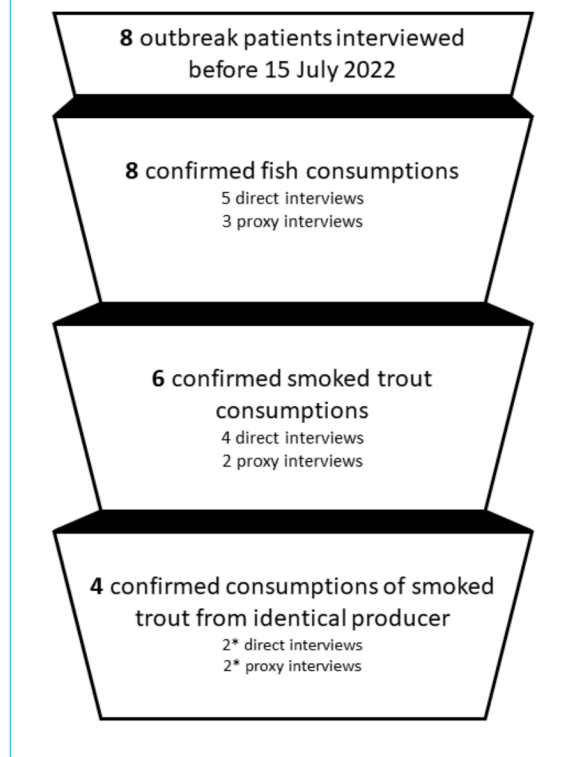


Table 1:

Overview of the outbreak interviewing process. Of 20 patients, six were not interviewed because (a) the patient did not answer the phone despite several attempts (n = 1), (b) the attending physician advised against contacting the patient's proxy person out of consideration for their partner's severe illness following the listeriosis infection (n = 1) and (c) patients diagnosed only after identification of the source of contamination and/or patients already mentioning consumption of smoked trout to their attending physician (n = 4).

Direct interviews, n (%)	8 (40%)
Interviews before 15 July 2023 and thus contributing to identification of the source of contamination, n (%)	5 (25%)
Proxy interviews, n (%); proxy people were the patient's spouse (n = 5) or daughter (n = 1)	6 (30%)
Proxy interviews before 15 July 2023 and thus contributing to identification of the source of contamination, n (%)	3 (15%)
Time from diagnosis to interview in days, median (interquartile range)	10 (9–15)
Time from diagnosis to physician interview in days, median (interquartile range)	7 (5–11)

to the disease case and not to comorbidities as per the attending physician’s judgement.

The outbreak patient samples that tested positive for *L. monocytogenes* at different medical laboratories were blood (17), pleura (1), cerebrospinal fluid (1) and stool (1) samples. The analysed patient isolates did not differ by any allele while the analysed food isolates differed from the patient isolates by one allele (figure 3).

In total, 14 of 20 outbreak-associated patients were interviewed in the context of this listeriosis outbreak investigation, while whole-genome sequencing typing was available for all 20 patients. As per standard procedures, attending physicians could be contacted for 19 of the 20 affected patients (for one patient the attending physician could not be reached, so the interview was conducted directly with the patient without pre-discussion with the attending physician). For 3 of the 6 patients who could not be interviewed personally or through a proxy person by the KEA team, the attending physician confirmed that during questioning the patient remembered having consumed smoked trout from

the affected producer (figure 4). Thus in total, 17 of the 20 *L. monocytogenes* outbreak-associated patients mentioned – personally, through their representative or through their attending physician – consumption of fish. Consumption of smoked trout was confirmed by/for 15 patients and 9 patients mentioned the same producer for the smoked trout that they had consumed (figure 4). One of the early detected cases in this outbreak mentioned a smoked trout producer with a similar but not identical name. Furthermore, two of the later listeriosis cases mentioned having bought their smoked trout at specialist gourmet food distributors that list smoked trout from the affected producer in their product catalogue. Table 1 characterises the conducted interviews in terms of who was interviewed and within what timeframe.

Discussion

Rapid identification and elimination of the source of infection is the main objective in investigations of food-borne disease outbreaks. Epidemiological surveillance combined

Figure 3: Minimum-spanning tree based on cgMLST allelic profiles of isolates from all diagnosed human *Listeria monocytogenes* cases in Switzerland between 1 March 2022 and 31 August 2022 as well as a representative smoked trout sample collected at the production facility on 15 July 2022. Each circle represents an allelic profile based on sequence analysis of 1701 cgMLST target genes. Values on connecting lines indicate number of allelic differences between two strains. The food isolate only differs from the outbreak strain in one allele as highlighted by red shading.

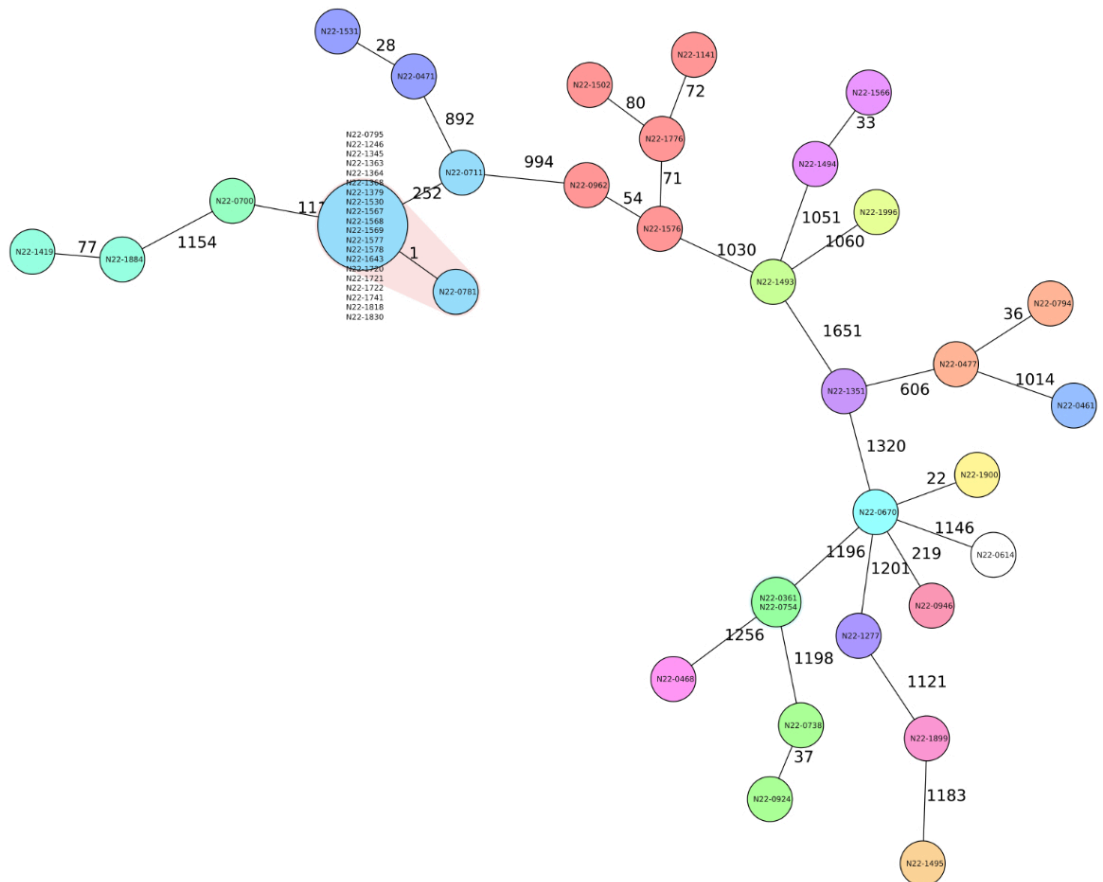


Table 2: Characteristics of the 20 outbreak patients.

Age in years; median (interquartile range): range	77.4 (67.2–82.5) 58.1–89.7
Male, n (%)	10 (50%)

with molecular analysis of the pathogens have proven highly effective for early detection and containment of food-borne disease outbreaks. Epidemiological surveillance through timely routine interviewing of all listeriosis patients in Switzerland played a key role in rapidly identifying the source of contamination and provided the basis for re-establishing food safety for consumers in this outbreak. Through the routine characterisation of all positive human-derived *L. monocytogenes* isolates by the Swiss reference laboratory NENT and routine investigation of food habits of all listeriosis patients through interviews based on a standardised questionnaire, similarities in food habits and food choices could be analysed from early on. Fish was suspected as a potential source of contamination very early in this outbreak based on the reports by the first two patients of the cluster mentioning repeated fish consumption and purchase of fish at markets and farm shops. This observation was taken up and responded to by integrating additional, targeted probing on fish consumption into interviews with all later patients. However, the type of fish or fish product remained obscure for the following weeks with the first three patients mentioning consumption of different fish species and products, and smoked trout consumption only mentioned by one of the first three patients. Subsequent interviews with patients four to eight revealed overlapping information on consumption of smoked trout, the brand, producer and retailer where smoked trout had been purchased, and hence pro-

vided the decisive information that led to the targeted audit of the suspected production facility.

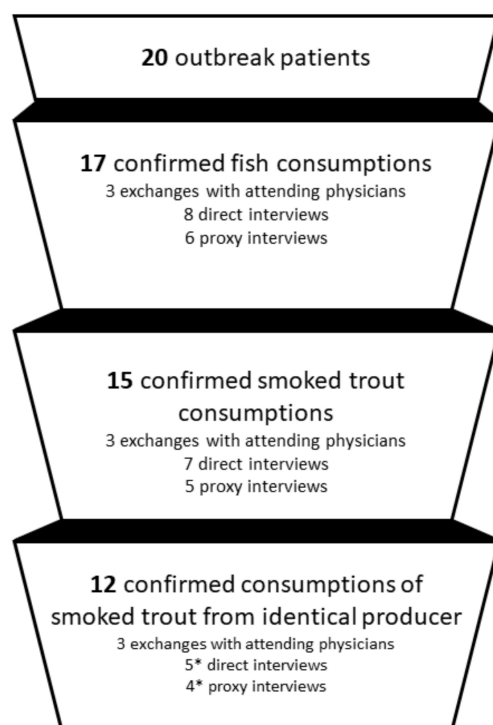
Smoked fish products such as smoked salmon and smoked trout are ranked as high-risk products for *L. monocytogenes* infection given their high pH and a_w value, combined with the fact that they are generally consumed directly as the product does not require any cooking processes and is more of a ready-to-eat item [11, 12]. A recent study from Germany investigated the interrelatedness between clinical *L. monocytogenes* isolates and non-clinical isolates from fish and other food samples and found high correspondences [13]. Reasons for contamination might be either primary contamination of the fish itself or cross-contamination within the manufacturing and retailing process up to consumption [2]. In 2020, of 12 larger *L. monocytogenes* outbreaks reported in 34 European countries, eight outbreaks have been attributed to consumption of ready-to-eat fish products, two to consumption of meat products and two to consumption of milk and dairy products [14–16], emphasising the high risk of listeriosis related to the consumption of ready-to-eat fish products. Also, in a large analysis collecting more than 800 samples from the production environment and fresh fish from two processing facilities producing smoked fish, Hoffman et al. identified drains as the locations carrying the highest load of *L. monocytogenes*, if any, although product contamination can occur at various steps throughout the production line and any following handling of the product [17].

In the present outbreak, related cases were reported over a period of nearly four months despite the short shelf life of the food item identified. This is an indication of a persistent contamination source in the production facility rather than contamination of a specific batch. Trout bred at the same facility and intended for sale as fresh fish was not connected to any listeriosis cases and had tested negative in the analysis carried out.

Clear limitations of this study are the pragmatically decided, long yet potentially still insufficient recall period of four weeks prior to symptom onset used in routine listeriosis surveillance in Switzerland as well as the lack of systematic analysis of clinical symptoms. Similarly, the practice of obtaining information from family representatives or even attending doctors when it could not be obtained directly from patients might mean that important information is missed but it remains the best alternative solution for getting information. However, the combined approach (patient interviews and whole-genome sequencing) and the close collaboration of cantonal and national authorities with mandated research institutions have proven effective and rapid in numerous listeriosis outbreaks in the past.

The parties involved in listeriosis outbreak investigations in Switzerland are in constant communication, allowing for continual revision and improvement of their approaches. In order to further reduce the risk of listeriosis infections for consumers, one important pillar of the current Federal Act on Foodstuffs and Utility Articles (Foodstuff Act, FSA; SR: 817.0) [18] is the existing self-monitoring by, official audits of and awareness-raising among food-producing companies. A second pillar is raising awareness among vulnerable population groups, such as the elderly, immunosuppressed people and pregnant women, about

Figure 4: Source of infection identification by the 20 patients affected by listeriosis from the smoked trout outbreak in Switzerland in summer 2022. * Includes two patients only mentioning smoked trout from a gourmet food reseller that clearly lists smoked trout from the affected producer as a supplier and one patient mentioning a slightly different name of the smoked trout producer that was assumed to be a wrong recombination of letters in the patient's memory in the course of the investigation.



food items with an increased risk for *L. monocytogenes* contamination.

Data sources and availability

The sequence data of a representative strain of the outbreak (N22-1530) has been deposited at the NCBI BioSample database under project number SAMN32990935, accession number JAQQAV000000000.

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Potential competing interests

All authors have completed and submitted the International Committee of Medical Journal Editors form for disclosure of potential conflicts of interest. No potential conflict of interest related to the content of this manuscript was disclosed.

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