



# Identification of the source of a *Listeria monocytogenes* outbreak by investigational tracing

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## Abstract

The number of identified listeriosis outbreaks has increased since the sequence typing of *Listeria monocytogenes* isolates was established in Germany. Due to the nature of the disease, listeriosis outbreaks are difficult to solve. We present investigational tracing as a simple and rapid method to conduct outbreak investigations. The method was applied in 2019 to stop a prolonged listeriosis outbreak in Germany. The starting point for the investigational tracing was nine health care facilities (HCF). Single cases developed listeriosis while they were staying at the nine facilities. Data were collected from companies that delivered foods to HCF and from ready-to-eat (RTE) foods that were consumed there. Following a step-wise approach, data analysis identified similarities in the food supply of the HCF. Food data were heterogeneous and needed to be standardised. Own brands and changing article numbers were challenging aspects during the identification of manufacturers. The analysis of the delivering companies revealed no similarities. Detailed information about the consumed risk foods for *Listeria* contamination became available for six HCF. All facilities served a wide variety of cold cut meat products to their in-patients. Investigational tracing revealed that only meat products from one out of 29 food business operators had been consumed in all six HCF. Further activities of the authorities enabled the identification of the outbreak strain on food products and in the processing environment of this company. A product recall and the measures taken stopped the listeriosis outbreak. Thus, investigational tracing can be crucial for the clarification of listeriosis outbreaks.

**Keywords** *Listeria monocytogenes* · Foodborne outbreak · Epidemiology · Evidence · Trace back · Meat products

## 1 Introduction

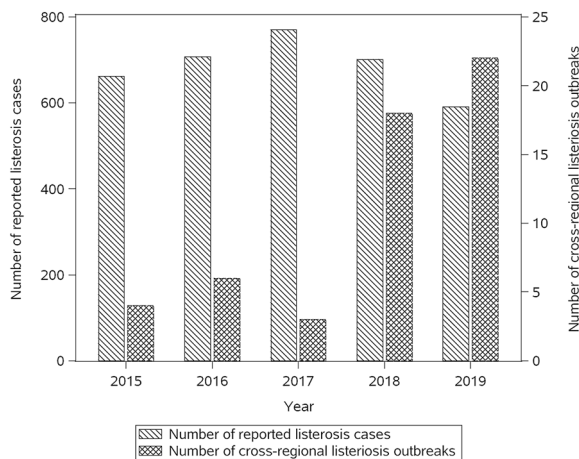
*Listeria (L.) monocytogenes* is a ubiquitous pathogenic bacterium able to cause invasive listeriosis in immunocompromised humans, newborns, the elderly and in pregnant women. Compared to other communicable foodborne diseases, the total annual case numbers in Europe are low, but steadily increasing. In the European Union, the annual number of invasive listeriosis cases increased from 1883 in 2013 to 2621 in 2019 (ECDC 2020; EFSA and ECDC 2021). Listeriosis is associated with a large disease burden due to the severity of its clinical manifestations and its high case fatality rate (EFSA and ECDC 2021). The majority of human listeriosis cases is caused by consumption of contaminated

ready-to-eat (RTE) foods. RTE foods that enable pathogen growth, such as cold smoked fish products, soft cheeses and cold cut meat products, are specifically associated with a high risk of listeriosis (EFSA Panel on Biological Hazards 2018).

In the last five years, about 600 to 700 listeriosis cases have been notified annually in Germany (Robert Koch-Institute 2016–2020). Both sporadic human listeriosis cases and disease outbreaks occurred. Since 2018, the increased use of whole genome sequencing (WGS) methods for characterisation of human *Listeria* isolates in Germany have revealed a larger number of outbreak clusters. These had not been detected with other typing methods. Several outbreaks affected more than one of the 16 German Federal States (Halbedel et al. 2020; Lüth et al. 2020). If outbreaks affect two or more Federal States in Germany, national authorities, namely the national public health institute (Robert Koch Institute, RKI), the national food safety authority (Federal Office of Consumer Protection and Food Safety, BVL) and

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**Fig. 1** Number of reported listeriosis cases in Germany 2015–2019 vs. the number of identified cross-regional listeriosis outbreaks that affected more than one German Federal State

the national reference laboratories responsible for typing isolates from the food chain (at the Federal Institute for Risk Assessment, BfR) become active. Although the number of annually reported listeriosis cases remained stable, the number of identified listeriosis outbreaks that affected more than one German Federal State increased significantly in 2018 and 2019 once WGS-typing had started (Fig. 1).

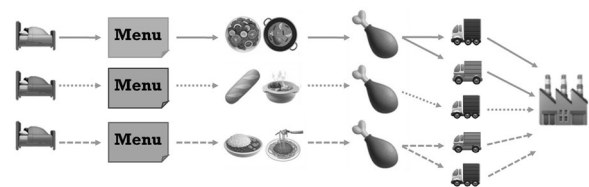
Identifying causative foods and *L. monocytogenes* outbreak sources is hampered by the large number and amount of RTE foods consumed in Germany. Currently, only a small number of *L. monocytogenes* isolates from the food chain can be typed by WGS. Thus, the identification of a contaminated food by typing is comparable to finding a needle in a haystack. Unless typing capacities will significantly increase in future, this situation will not change. The creation of epidemiological evidence from questionnaires and epidemiological studies for outbreak clarification by public health facilities is difficult due to the following reasons:

1. The attack rate is low and incubation periods can be long, especially for pregnancy associated cases (Goulet et al. 2013).
2. Morbidity and mortality of listeriosis cases contribute to the limited success of questionnaires as patients or their relatives cannot remember details about foods consumed during the possible time of exposure (Kiefer et al. 2016).

A simple new method for the identification of contaminated foods is investigational tracing. Compared to tracing back and forward from suspicious foods, the aim of investigational tracing is to find similarities between small outbreak clusters within a large outbreak. For example, similarities could be same consumed foods or same catering company providing dishes to affected establishments. The described

method is a comparative analysis of food distribution in supply chains that displays epidemiological correlations. Figure 2 illustrates the concept by means of an example. Results of investigational tracing are hints for likely involved food vehicles and indications on possibly involved food business operators. Investigational tracing is a tool to inform outbreak investigations. Based on its findings, specific kinds of foods can be prioritised for testing an outbreak strain. Moreover, investigational tracing can help to identify causative food business operators, which, based on the findings, can be further investigated by official food control services. Generally, the method of investigational tracing needs comparable aspects or items as starting points, e.g. in nursing homes, menu plans and consumed foods items can be compared with outbreak cases. The method has been successfully applied in Germany during the investigation of an *E. coli* O104:H4 outbreak in 2011 (Cheung and Lubert 2016).

This manuscript describes in detail how investigational tracing was successfully used in 2019 to identify the causative food business operator of a listeriosis outbreak in Germany. First cases infected with *L. monocytogenes* of the WGS-type ‘Sigma1’ had been observed in 2018 in several Federal States. An epidemiological description of the outbreak has been published by Lachmann et al. (2020). Briefly, it encompassed a molecular cluster of 39 listeriosis patients occurring between 2014 and 2019. Three patients died as a result of listeriosis. In the beginning, questioning of affected individuals revealed no hints to similarities for foods consumed. The outbreak strain had not been identified in samples from foods or environmental sources before. However, in the first months of 2019, the RKI identified that an unusually large number of single cases had been infected while they were staying in a HCF. This information was used as starting point for an investigational tracing analysis of food products that had been catered to patients during their exposure timeframe. Only one food business operator had delivered products to all HCF. Finally, sampling and testing identified the outbreak strain in the premises and on meat products of this German food business operator.



**Fig. 2** Illustration of an example for the concept of investigational tracing. An analysis of menus consumed by patients identifies chicken thighs as common food item. Trace back shows that the chicken meat was delivered by different companies but originated from the same manufacturer

## 2 Methods

### 2.1 Methodological approach to investigate the *Listeria* Sigma1 outbreak

A starting point for an investigational tracing was identified when the outbreak investigation of public health authorities revealed that nine single cases had been in different HCF each 14 days before the onset of the listeriosis disease. The aim of the analysis was to find similarities between the nine HCF. The theory was that probably a common delivering company existed, such as a canteen kitchen or a catering establishment that had delivered food contaminated with the outbreak strain to each of the nine HCF. A second possibility was that in each HCF, the same contaminated food item was used and offered to the patients. Following a risk-based approach, data collection focussed on RTE foods with a higher risk for being contaminated with *L. monocytogenes*. This included cheese (especially soft cheese), RTE meat products such as cold cuts and spreadable raw sausages, smoked fish such as cold smoked salmon or gravad RTE fish products, pre-packaged sandwiches, fresh pre-cut fruits, vegetables or leaf salads and frozen vegetables, which were served without being heated, e.g. in salads or as salad garnish. The list was provided to guide control personnel through the collection of food data. The two weeks before the onset of the disease at each affected HCF were of interest for the investigation. All nine single cases had been infected in the second half of 2018 so that tracing information was still available in the HCF when the investigation started.

The food safety authorities in six German Federal States collected data from delivering companies serving the affected nine HCF. For three HCF, no further data on food items was available. From six facilities, detailed information on the RTE food catered to the patients in the period of interest was collected, e.g. brands, food product names and batch numbers. A table with pre-defined variables was used to accumulate the data. All data collected in the Federal States was sent to BVL for further analysis. The variables actually used in analysis were name and address of the HCF, a standardised food description, and names and addresses of food business operators involved in the food chain such as caterers, delivering companies, suppliers and manufacturers.

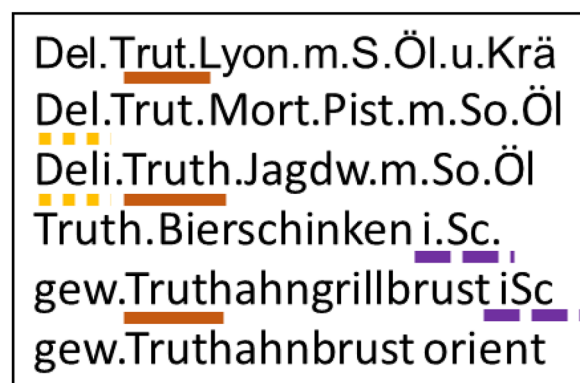
### 2.2 Data processing and analysis

Incoming data was merged and processed at BVL. Data processing included a quality check and grouping data into risk food categories such as meat products or cheeses. The

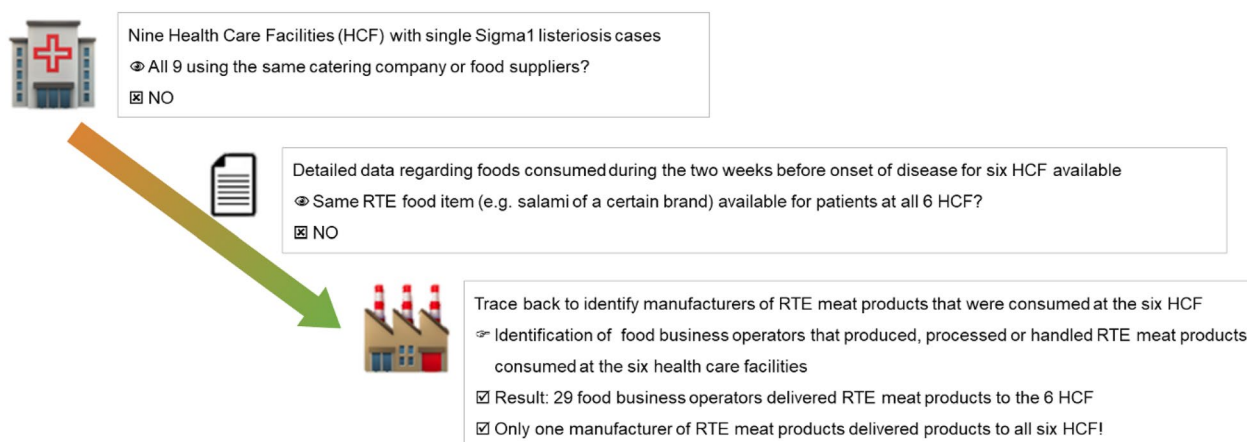
quality check focused on correct and consistent spelling, especially regarding the name of HCF, the description of the food, the manufacturer and name of supplier or the delivering company. These turned out to be key variables in the tracing analysis. Due to the heterogeneity of food product names and variations in article numbers used by suppliers and delivering companies, a standardisation of the food denomination was also necessary. An example shows Fig. 3.

Product catalogues of suppliers were used to annotate article numbers to manufacturers. One HCF provided raw data in form of scanned delivery sheets (PDF files). This data was extracted using Optical Character Recognition and transferred to the data table.

Following a step-wise approach, we started looking for similarities amongst companies providing catering or supplying foods to the nine HCF. When this did not lead to the identification of a common link between the outbreak places, we started to analyse the detailed food data provided by six HCF in a second step. Food data were checked for a common food item, e.g. the same brand of salami, or a batch of soft cheese. When this did not show similarities between the six HCF, a trace back analysis was started in order to identify food business operators that produced, processed or handled food items that were consumed at all six facilities. Following a risk-based approach, food data had been categorized into RTE foods with a known higher risk for contamination with *L. monocytogenes*. Not all categories of RTE food product categories associated with higher *Listeria* contamination probabilities were consumed at all six HCF. The trace back analysis started with meat products, as they were the RTE food group with the largest number of items. Many different RTE meat products had been served at HCF during the presumptive exposure time of cases. The plan was to repeat the analysis for cheese products in case no similarities would be found for meat products (Fig. 4).



**Fig. 3** Example for the heterogeneity of food product names used by suppliers and delivering companies for meat products made of turkey (German: Truthahn)



**Fig. 4** Step-wise approach of investigational tracing that delivered evidence for the causative food business operator

Statistically, cross tables or frequency tables were used in each step to identify the similarities between the variables of question. For example, in the first step, a frequency table between the two variables HCF and supplier was calculated to display their absolute frequency distribution, i.e. the table provided information on which supplier delivered food to which HCF. For example, if a supplier would have been the source of contamination, we would have expected that this supplier would have delivered to all six HCF. In the second step, a frequency table between the variables food product and HCF displayed which HCF obtained which products. And in the third step, a frequency table between food product and HCF, and manufacturer and HCF was able to display a common relation between the HCF. SAS Enterprise Guide (Version 7.1 HF8) was used for programming the data base queries.

### 3 Results

The first step of the investigational tracing revealed no similarities between the nine HCF with regard to the companies that provided catering or supplied foods. In six facilities, the food control services were able to collect detailed information on RTE foods offered to in-patients during the presumed 2-weeks' exposure time-frame before the onset of the disease. In total, 1775 datasets on RTE foods previously described as being contaminated with *Listeria* were submitted for analysis. A first analysis of this data set showed that none of the various food stuffs was supplied to the six facilities and that 24.7% ( $n=438$ ) of the RTE foods were meat products. As a large number of RTE meat products such as cold cuts of ham or salami were consumed at all six HCF, this category was prioritised for the investigational trace back analysis.

Investigational tracing revealed that 438 RTE meat products had been delivered by seven different suppliers to the six HCF. Further trace back identified 29 food business operators that were involved in the production, processing or handling of the meat products. Amongst these, 25 were manufacturers and four were distributing companies that processed or handled the meat products, e.g. repackaging or slicing. Table 1 shows the contingency between the 29 companies and the six facilities. The majority of food business operators ( $n=25$ ) delivered their meat products only to one or two of the HCF. Two companies delivered to three HCF and one company delivered to four HCF. Only one of the 29 food business operators, i.e. a manufacturing company (no. 28), had delivered RTE meat products to all six HCF during the time period of interest.

The authority responsible for food safety controls in the Federal State where this manufacturing company was located was immediately informed about these epidemiological analysis results. Based on the suspicion that this company might have distributed products contaminated with the *Listeria* Sigma1 outbreak strain, the authorities were able to take action.

Manufacturer no. 28 had delivered various RTE meat products to the six facilities. Some contained pork, whereas meat from poultry formed the basis of others. The majority of products were pre-sliced cold cuts, a food product typically consumed without further processing as a topping on bread or rolls. For the analysis, the RTE meat products were categorised into 18 different product groups such as cooked ham, ham sausage, blood pudding, or raw sausages. The facilities provided various amounts of RTE meat product groups that originated from manufacturer no. 28 to their in-patients. One HCF had only ham sausage of the manufacturer on the menu. The other HCF purchased RTE meat products of 2, 3, 5, 8 or 10 different product groups from this manufacturer. The ham sausage eaten in one of the facilities

**Table 1** Contingency table of the variable “manufacturer/distributor” and the six different health care facilities (HCF)

Manufacturer/distribution company	HCF 1	HCF 2	HCF 3	HCF 4	HCF 5	HCF 6	Sum
1	1	0	0	1	0	0	2
2	1	0	0	0	0	0	1
3	1	0	0	1	0	0	2
4	1	0	1	0	0	0	2
5	1	0	0	1	0	0	2
6	1	0	0	1	0	0	2
7	1	0	0	0	0	0	1
8	1	0	0	0	0	0	1
9	0	0	0	1	0	0	1
10	1	0	0	0	0	0	1
11	1	1	1	1	0	0	4
12	1	0	0	1	0	0	2
13	1	0	0	1	0	1	3
14	1	0	0	0	0	0	1
15	0	0	0	0	1	0	1
16	0	0	0	1	0	0	1
17	1	0	0	1	0	0	2
18	1	0	1	1	0	0	3
19	1	0	0	0	0	0	1
20	1	0	0	0	0	0	1
21	0	1	0	0	1	0	2
22	0	0	0	1	0	0	1
23	1	0	1	0	0	0	2
24	0	0	0	0	1	0	1
25	1	0	0	0	0	0	1
26	0	0	1	0	0	0	1
27	1	0	0	1	0	0	2
28	1	1	1	1	1	1	6
29	1	0	0	0	0	0	1

could not have been the only contaminated product. Two more HCF dished out the ham sausage, but three of the HCF affected from the outbreak did not. One of the latter HCF had received meat products of the categories blood pudding and cooked ham. The epidemiological data from the investigational tracing suggested that most likely several meat products produced by manufacturer 28 had been contaminated with the outbreak strain *Listeria* Sigma1. This was followed by the outbreak investigation of food control authorities, and the *Listeria* Sigma1 outbreak strain was found in samples of RTE meat products from manufacturer no. 28. Moreover, the outbreak strain was detected during sampling and testing in the premises of the manufacturing company. A product recall and further measures enforced by the authorities finally stopped the outbreak.

In total, 95.9% of the delivered datasets contained sufficient suitable information and were useful in the analysis that indicated that manufacturer no. 28 was the most probable source of the outbreak.

## 4 Discussion

The application of WGS typing methods leads to the increased detection of listeriosis outbreaks in Europe. However, as genomic typing capacities for *L. monocytogenes* isolates from the food chain are still sparse, the identification of causative food sources may take a lot of time. Moreover, finding a match of molecular typing results for a *L. monocytogenes* isolate from food with the human outbreak strain is not enough evidence. Additionally, epidemiological evidence is needed. For example, in an ongoing listeriosis outbreak for twelve months with cases in three different states, the causative food would to be provided in all places during the year. Finding a matching *Listeria* isolate in a sausage coming from a small scale producer in a different state does not mean that the causative food business operator has been identified. As the small scale producer did only produce a small batch which was only locally distributed, he cannot be the source of the outbreak. The match with the human

outbreak strain only provides a hint that a meat product is very likely the causative food. In order to clarify and stop foodborne outbreaks, molecular surveillance data needs to be integrated with epidemiological information (Van Walle et al. 2018). Gathering epidemiological information from questionnaires or epidemiological case–control studies is generally restricted for listeriosis outbreaks. Due to the nature of the disease with long incubation periods, a low attack rate, and high morbidity and mortality, the amount of information that can be gathered from case interviews is sparse. Thus, the generation of epidemiological evidence on the food side of the outbreak investigation can be key to clarify listeriosis outbreaks.

This approach to clarify the *Listeria* ‘Sigma1’ outbreak has been used before to identify causative foods and food business operators during an *E. coli* outbreak in 2011 and a norovirus outbreak in 2012 (Cheung and Luber 2016). Investigational tracing is a simple method that looks at food flows in supply chains in order to identify similarities (Fig. 2). It can be used to inform the investigation of foodborne outbreaks when initial starting points for a comparison or trace back exist. Examples are different institutions or hospitals that have received foods from the same catering company (Bernard et al. 2014) or several restaurants as clusters within an outbreak (Buchholz et al. 2011). In case of the *Listeria* ‘Sigma1’ outbreak, several months passed between the detection of the human outbreak and the following identification of the causative food source. The number of patients available for interviews was low and only general information on foods consumed was generated during case interviews.

When the RKI identified several single ‘Sigma1’ cases that had spent the whole possible exposure time frame before the onset of the disease in HCF, a starting point for the investigational tracing by food safety authorities became available. This generated a chain of evidence for the possible involvement of a food business operator. This induced further activities of food safety authorities. Meat products of the manufacturer and samples from the processing environment could be sampled. WGS-typing confirmed a contamination with the outbreak strain. A product recall finally stopped the outbreak. The epidemiological evidence from investigational tracing enabled more targeted use of sparse WGS-typing capacities.

Although several examples of successful outbreak investigations with the detection of isolates within the food chain matching the WGS-types of human *L. monocytogenes* outbreak strains have been described (ECDC and EFSA 2019; Kleta et al. 2017; Kvistholm Jensen et al. 2016; Pietzka et al. 2019), for many listeriosis outbreaks a causative food cannot be identified. Investigational tracing as described here can be used to lower the number of foods that need to be tested in order to find the outbreak strain. RTE foods such as soft

cheeses, cold smoked fish and packed sliced sausages are associated with a higher risk for causing listeriosis (EFSA Panel on Biological Hazards 2018). Following a risk-based approach investigational tracing in case of listeriosis outbreaks can be focussed on this food category. Even though data for many variables were not provided in the end, the delivered data was sufficient to solve the case. Key variables were the HCF names, the different food business operators, and the standardised meat product name. For future applications of investigational tracing, we suggest to pay particular attention to these variables.

The retrospective collection of data on foods that had been served and possibly have been consumed by persons in health care facilities half a year earlier was intensive work. Access to data was limited. If such an investigation would start at a later time, record-keeping requirements for traceability documents might become an issue. For the ‘Sigma1’ outbreak it was only possible to collect detailed data on specific food items for six out of nine HCF. The assignment of food business operators that produced, processed or handled RTE meat products via article numbers and product catalogues of delivering companies done at national level was a time-consuming step. Food business operators in Europe only need to collect and provide traceability data for one step forward and one step back in the food chain. (Regulation (EC) No 178/2002 2019). Thus, currently the collection and compilation of data from the whole food chain of products needs to be done by food safety authorities. Data are often not kept and provided by food business operators in an electronic format. Often data need to be transferred from delivery sheets into databases before they can be analysed. Another obstacle is that data for the identical food item are presented in various formats (Fig. 3). The possible future use of block chain technologies, which are decentralised transaction protocols recording every change transparently, with the integration of already assigned data from the food chain would simplify this process tremendously (Kamath 2018).

## 5 Conclusions

The source of the *L. monocytogenes* ‘Sigma1’ outbreak was identified by means of epidemiological and microbiological evidence. Investigational tracing identified RTE meat products of one manufacturer as the only link between six health care facilities. This chain of evidence enabled us to generate microbiological evidence. The ‘Sigma1’ outbreak strain was detected in meat products of manufacturer no. 28 and in the production area of this manufacturer. Measures taken by the food authority at the manufacturing plant and a product recall terminated the outbreak. Without investigational tracing, the source of this foodborne outbreak probably would not yet have been identified. In general, the use

of investigational tracing represents an important additional tool for generating epidemiological evidence for the investigation of listeriosis outbreaks, especially because questionnaires and epidemiological studies on food consumption histories often fail to deliver results. A focus on RTE foods and the step-wise approach of investigational tracing (Fig. 4) has the potential for reducing the resources and capacities needed for listeriosis outbreak clarifications.

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**Author contributions** PL, NB, TS and MS together designed the investigational tracing research study. NB carried out the research. FS carried out the statistical data analysis and all authors analysed the data. PL and NB wrote the paper.

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## Declarations

**Conflict of interest** All authors declare no conflict of interest.

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