



Listeria monocytogenes

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2. *Listeria monocytogenes*

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2.1 *Listeria monocytogenes* in humans in Denmark in 2022

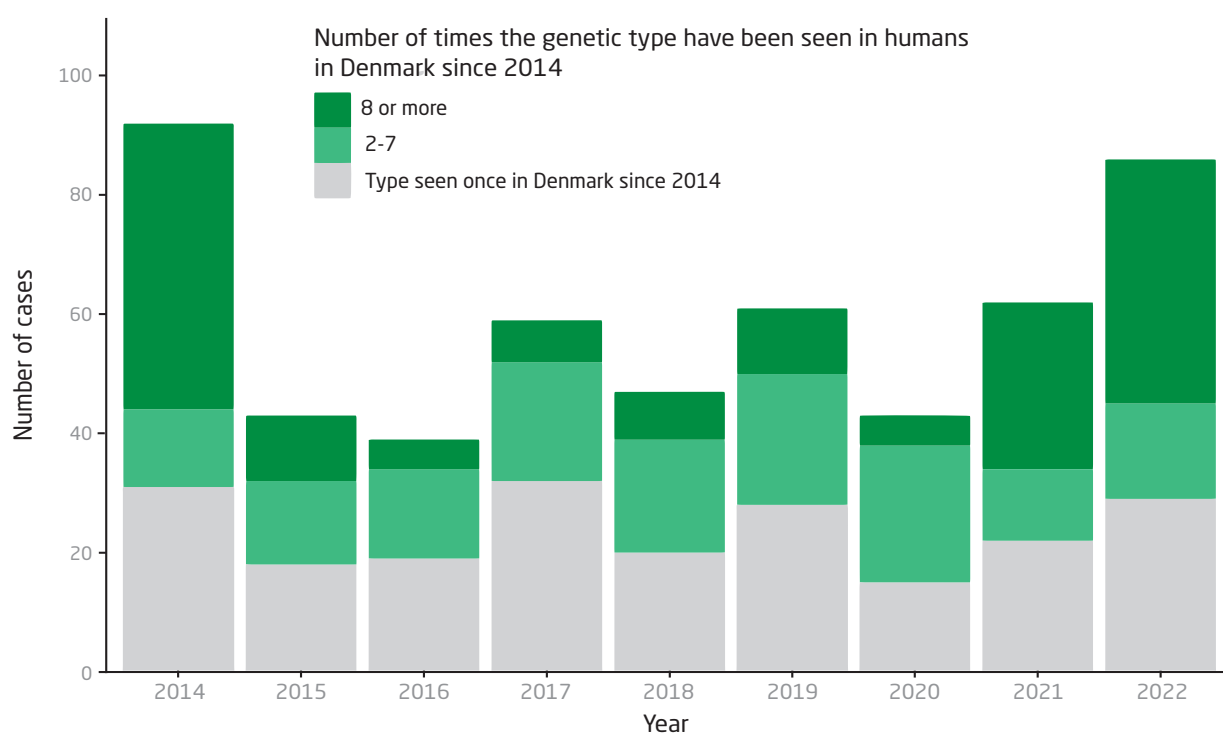
Listeriosis is a foodborne infection caused by *Listeria monocytogenes*. The clinical spectrum of listeriosis ranges from mild to invasive disease including bacteraemia or meningitis. *Listeria monocytogenes* can also be transmitted from mother to fetus in utero or to the neonate during birth. Risk factors for invasive disease include immunosuppression, advanced age, and pregnancy. The incubation is longer than for most foodborne pathogens, usually a few weeks. In Denmark, invasive forms of listeriosis in humans are monitored in the Danish surveillance system. The 30-day mortality of registered cases of listeriosis is about 25% [1].

As part of the national surveillance, whole genome sequencing (WGS) is routinely used at Statens Serum Institut to characterise all *L. monocytogenes*. A genetic related cluster is defined based on the population structure in the

relevant sequence type (ST), usually ≤ 7 allelic differences applying single linkage. All patients are interviewed using a hypothesis-generating questionnaire in regards to their consumption of risk food products (e.g. ready-to-eat (RTE) products including sliced cold-cut meat, smoked fish, and salad) in the four week period before falling ill. The introduction of WGS increased the discrimination of isolates, allowing for more targeted outbreak investigations as well as direct comparison between *L. monocytogenes* isolates from human and food or the production environment [2].

Following a large *Listeria* outbreak in 2014 [3], there has been reported about 40-60 cases annually in Denmark. Approximately 60% of all the reported cases with listeriosis between 2014 and 2022 were part of genetic clusters (two or more cases with the same type). Of these 37% were part of larger genetic clusters (outbreaks) involving eight or more cases (Figure 2.1).

Figure 2.1. Number of listeriosis cases in Denmark (N=532) per year, categorised into three groups according to the number of times the types of *Listeria monocytogenes* have been reported in human cases during the period from 2014-2022



In 2022, a marked increase was seen, with three large outbreaks that accounted for a third of all registered cases in 2022: sequence type (ST) 8 (FUD2074), ST37 (FUD2080), and ST7 (FUD2127). Below we describe these three major outbreaks.

Following a rise in the number of listeriosis cases in the spring of 2022 (Figure 2.2), communication to the public and to food processing companies was intensified. The food business operators were encouraged to intensify their own-checks of the production lines. Relevant international authorities were informed about the rise through the Rapid Alert System for Food and Feed News run by the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control's (ECDC) surveillance portal for infectious diseases. No other countries reported having seen cases with the same genetic types.

Based on information from the interviews of cases belonging to the FUD2074 cluster, it was noted that all (nine) interviewed cases reported consumption of RTE cold-cut meat products. All the nine cases reported eating spiced sliced meat roll ('rullepølse') and eight of the nine reported that they had eaten sliced ham ('hamburgerryg'). Moreover eight reported shopping in the same supermarket chain. A general traceback on cold-cut meat identified four suppliers with production on six locations in two countries. Sampling was done on the three production sites in Denmark and no *L. monocytogenes* matching the ST seen in patients was identified. The traceback for 'rullepølse' identified one common production site outside of Denmark. On this site, *L. monocytogenes* was identified in the production environment and in a 'rullepølse' sample from the own-check. The company recalled multiple brands of 'rullepølse' on the 5 and 10 July and voluntarily shared the isolates with the Danish Veterinary and Food Administration (DVFA) for analysis by WGS. The WGS of *L. monocytogenes* from the 'rullepølse' matched the *L. monocytogenes* identified in patients. After the recall, two additional cases were reported. This was not unexpected due to the long incubation period often seen for *L. monocytogenes*. This investigation underlines the importance and value of involvement of the food processing companies at an early stage during an outbreak investigation and the power of using WGS for surveillance. Even though interviews showed a strong indication of a cold-cut product, more specifically 'rullepølse', the microbiological evidence could not easily have been established without collaboration from the company and the outbreak would likely have continued.

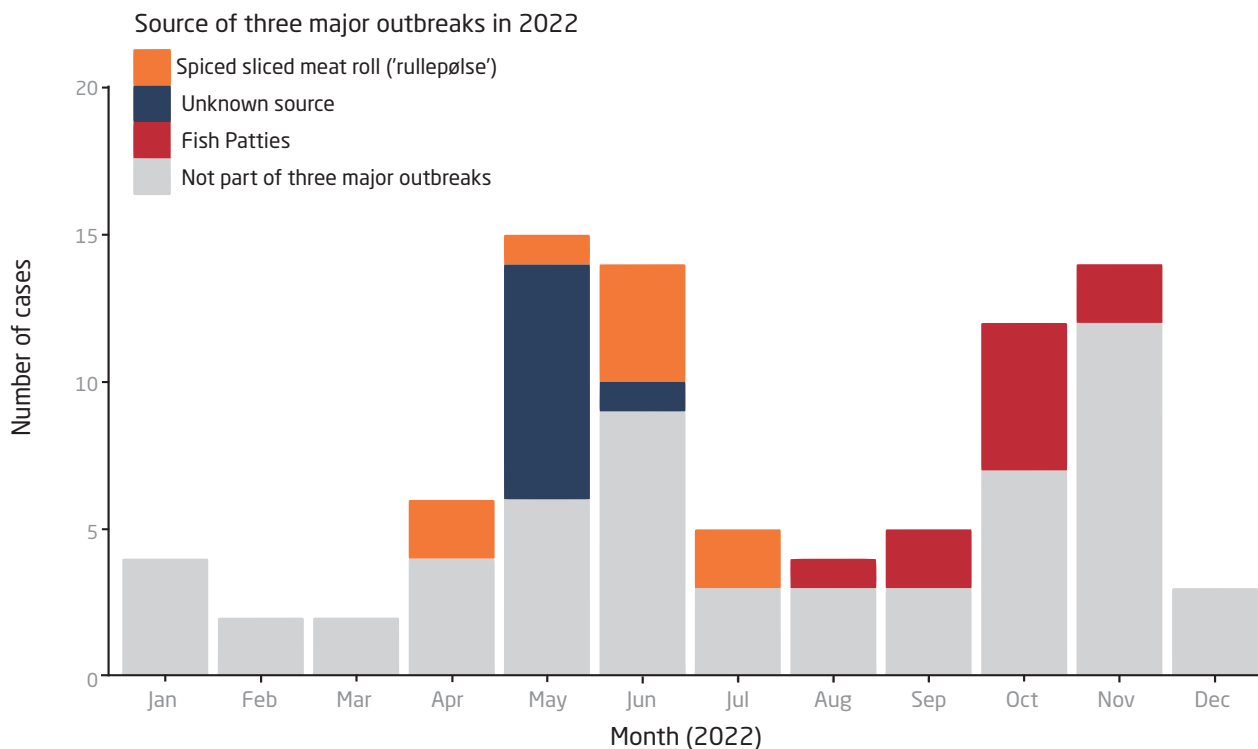
Also, in the spring of 2022, a second large outbreak of listeriosis was investigated (FUD2080). The first cases were diagnosed mid-May and during the following four weeks, a total of nine cases were identified of which four died within 30 days from testing positive. Most cases were

from the Capital region. In the attempt to identify the source of the outbreak, 43 companies were contacted and samples were taken if deemed relevant. In total, 16 types of *L. monocytogenes* were identified in nine companies, 14 in environmental samples. None matched the type identified in patients belonging to the outbreak, and only three types were genetically related to types seen in few Danish non-recent patients since the introduction of WGS in 2014. Despite massive efforts, the source of the outbreak remains unidentified. The abrupt peak with cases falling ill within a short time window, points towards a heavily contaminated product or batch as opposed to inappropriate handling by the consumer.

The third large outbreak occurred in the autumn and was caused by *L. monocytogenes* ST7 (FUD2127). In total, 10 Danish cases and one tourist were identified as being part of the outbreak. The first case occurred in August, week 33. Cases were living in all parts of Denmark. No other countries reported having identified cases belonging to the same cluster. Three patients died within 30 days after testing positive. Based on the first four interviews and investigation of receipts from grocery shopping, it was noted that all cases had eaten and/or bought fish products such as fish patties ('fiskefrikadelle') and smoked salmon. The fish products identified on the receipts obtained from patients were investigated further by the DVFA. No *L. monocytogenes* isolates were identified in the production environment samples from the salmon manufacturer. However, *L. monocytogenes* was identified both in the production environment and in products from the fish patty manufacturer. Whole genome sequencing showed that the *L. monocytogenes* from the fish patties were identical to the type identified in the patients. As a consequence, the fish patty manufacturer was sanctioned on the handling of food, risk of contamination and cleaning by the DVFA. The link between the fish patties and the patients were supported by epidemiological findings, as seven patients in seven completed interviews reported eating or buying fish patties. On the 8 November 2022, the company recalled a batch of fish patties. The following day, the company decided to extend the recall to include all products produced until the 9 November 2022. After the recall only one additional case was identified.

The three major outbreaks in 2022 were unusual in the sense that they accumulated many cases in a relatively short period of time. Outbreaks caused by *L. monocytogenes* typically span over a long period of time, even years, since the bacteria can persist in production facilities as described in the following section. In Figure 2.3, the number of listeriosis cases by year are shown for the six outbreaks for which two or more cases were identified in 2022. Three outbreaks caused by *L. monocytogenes* ST11, ST1607 and ST37, additional to those described in

Figure 2.2. Number of listeriosis cases in Denmark per month in 2022 (N=86). Cases belonging to the three major outbreaks are marked.



detail above, are seen with 8-14 cases accumulating over the last 3-5 years. Despite that many of these cases were interviewed, and several signals were followed up by the DVFA, the sources of these outbreaks remain unknown.

2.2 *Listeria monocytogenes* in food production environment

Following a large outbreak of *L. monocytogenes* infection in 2014, DVFA, together with the industry, initiated a series of control activities and research projects to improve the control and management of *Listeria* in the food production [4]. Two projects were conducted to obtain a deeper knowledge of the presence and persistence of *L. monocytogenes* in the production environment of RTE food processing companies. Here we describe the summary of results of the projects, which are also published as a scientific article [5].

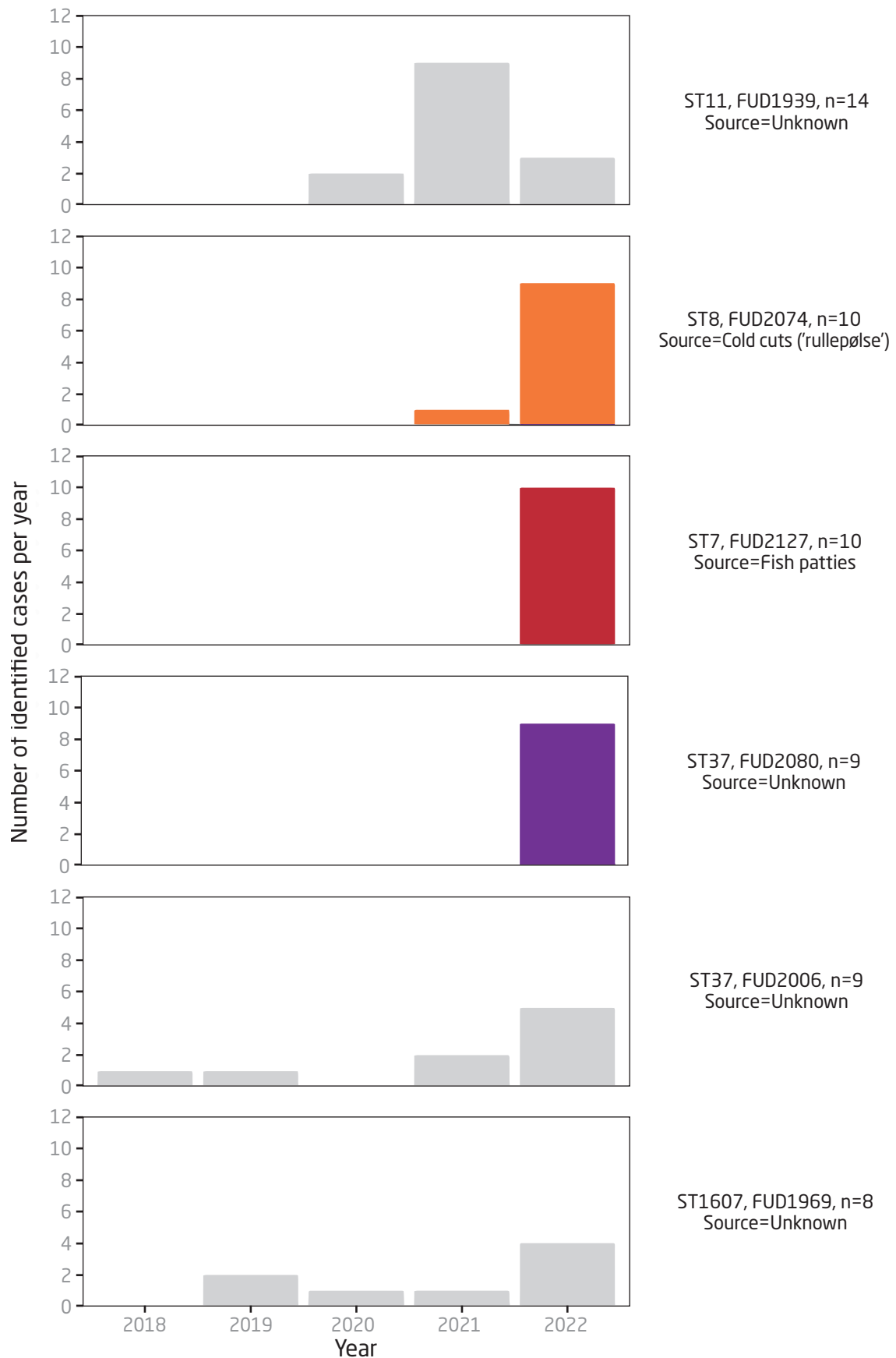
Food processing companies producing Ready-to-eat (RTE) fish and meat products known as risk products with regard to *L. monocytogenes* were selected and visited in 2016 and 2020. A total of 20 food processing companies, nine producing RTE meat and 11 producing RTE fish products, were sampled both in 2016 and 2020. Additionally, 33 companies were sampled only in one of the years. Swab samples were collected in the production environment and

cultured for the presence of *L. monocytogenes*. *Listeria monocytogenes* was found in 6.7% of the total 777 swab samples. Moreover, one positive sample was found in 17/39 (43.5%) of the companies in 2016 and 11/34 (32.4%) in 2020. Four companies had at least one positive sample in both years. The prevalence was slightly lower in 2020 but *L. monocytogenes* was still found in one third of the participating RTE food processing facilities.

Fifty *L. monocytogenes* isolates (30 from 2016 and 20 from 2020) from 24 companies from the abovementioned projects were used for further WGS analyses. In addition, *L. monocytogenes* isolates from samples taken at the 53 sampled companies between 2016 and 2020 through the DVFA's routine surveillance programme for environment and food were identified. This resulted in additional 35 *L. monocytogenes* positive samples. Hence, a total of 85 *L. monocytogenes* isolates were analysed by WGS to assess the strain diversities and persistence of genetically similar strains over the years.

The results of the WGS revealed that there were 16 different STs of either lineage I or II. Sequence types, ST8 and ST121, were the most common. Moreover, ST7, ST8, ST121, ST399 and ST451 were isolated over two or more years from the same company, while the other ST's were

Figure 2.3. Annual number of listeriosis cases belonging to large genetic clusters (outbreaks) consisting of eight or more cases and with at least two cases in 2022



found only on one occasion within a company. Out of 27 companies where *L. monocytogenes* was found in either the production environment (n= 24) or in the products between 2016 and 2020, 10 companies had isolates belonging to more than one ST between 2016 and 2020. There were only a small genetic difference among isolates from the same ST, if they were collected within the same company, regardless of the sampling years and source (environment vs. product). On the other hand, greater genetic differences existed, if isolates belonging to the same ST came from different companies.

Genetic links between isolates from humans and production site/food products are frequently identified during outbreak investigations, which was also observed in 2022. Our results showed a similar link. Here *L. monocytogenes* ST451 isolates from humans from the period 2014-2020 and production sites (2016-2020) could be linked genetically. This suggests that this strain has persisted in the production environment over many years.

2.3 Conclusion

Findings from the two projects on listeriosis in food production environment confirm that *L. monocytogenes* strains are able to colonise the production environment in food processing companies making RTE products, and that some of the strains can persist for many years. The study has also shown that the overall prevalence of *L. monocytogenes* in high risk RTE food processing companies was largely unchanged between 2016 and 2020, despite the DVFA's Listeria awareness campaign launched in 2015. This is also reflected in the fact that the incidence of listeriosis in Denmark has not decreased.

These continuing challenges with *L. monocytogenes* in the production environment, and the two outbreaks described above, also underline the need for increased focus on combining different risk management strategies in the companies including e.g. reassessment of product shelf life, a change in product recipes, and intensified focus on cleaning and disinfection procedures. The large outbreaks in 2022 were, however, caused by *Listeria* types which had not been seen previously. Nevertheless, since *L. monocytogenes* can persist in the food production environment, real time comparison of genetic sequences of *L. monocytogenes* from human cases and from the food industry is valuable for investigating and preventing outbreaks. Also, current risk communication strategies may need to be revised and improved to better reach all target groups to decrease the number of listeriosis cases.

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