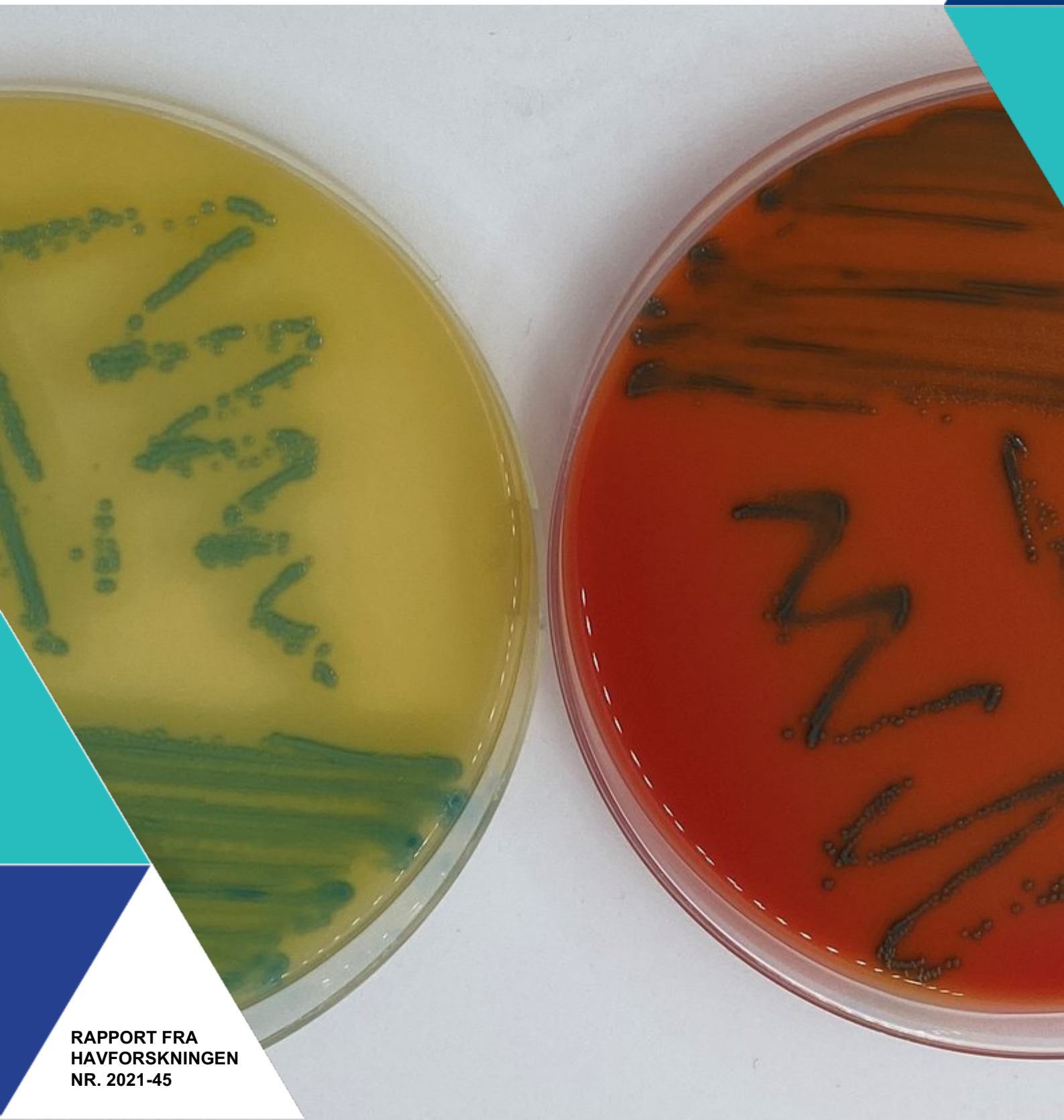
LISTERIA MONOCYTOGENES IN SALMONID SLAUGHTER FACILITIES

Screening program for the Norwegian Food Safety Authority

Cecilie Smith Svanevik, Bjørn Tore Lunestad and Julia Storesund (IMR)



RAPPORT FRA HAVFORSKNINGEN NR. 2021-45

Title (English and Norwegian):

Listeria monocytogenes in salmonid slaughter facilities

Listeria monocytogenes i laksefisk

Subtitle (English and Norwegian):

Screening program for the Norwegian Food Safety Authority

OK-program for Mattilsynet

Report series:	Year - No.:	Date:
Rapport fra havforskningen	2021-45	30.11.2021

ISSN:1893-4536

Authors:

Cecilie Smith Svanevik, Bjørn Tore Lunestad and Julia Storesund (IMR)

Approved by: Forskningsdirektøre(r) en: Gro-Ingunn Hemre Program leader(s): Livar Frøyland

Distribution:

Open

Project No.:

15600

On request by:

Mattilsynet

Oppgragsgivers referanse en:

OK- program 57408

Program:

Trygg og sunn sjømat

Research group(s):

Fremmed- og smittestoff (FRES)

Number of pages:

11

Partners

Mattilsynet

Summary (English):

Listeria monocytogenes is a bacterium of special concern in the seafood industry beacuse it possesses several properties adapted for the food production environment and may cause severe disease in humans. The Institute of Marine Research has on behalf of the Norwegian Food Safety Authority conducted a screening program for *L. monocytogens* in salmonid slaughter facilities. In total, 358 samples from 60 slaugher facilities (49 slaughtering plants and 11 slaughtering vessels) were examined. Samples were collected from the production environment (n=108), from the surface of fish entering the facilities (n=47), and from the surface of fish (n=59) and raw material (n=144) at end point at the examined facility. None of the samples from the slaughtering vessels were positive for *L. monocytogenes,* whereas 22 positive samples were detected in nine different slaugtering plants. In five of these plants, several positive samples were found. Six of the slaughtering plants had positive samples at the end of the production line, where a higher prevalence was found when swabbing the fish skin and gills compared to the examined raw matierial. All the raw material samples contained low numbers of *L. monocytogenes* below the quatification limit (<10 CFU/g). However, this study found that *L. monocytogenes* can be present in both fish and the production environment, and that in some cases *L. monocytogenes* will be present in fish ready for furhter processing in the salmonid value chain.

Summary (Norwegian):

Listeria monocytogenes kan gi alvorlig sykdom hos mennesker og er i særstilling når det kommer til sjømat på grunn av sin evne til å tilpasse seg produksjonsmiljøet. Havforskningsinstituttet har på oppdrag for Mattilsynet utført et OK-program med fokus på *L. monocytogenes* i norske lakseslakteri. Totalt ble 358 prøver fra 60 ulike lakseslakteri (49 virksomheter og 11 båter) undersøkt. Prøvene ble tatt fra produksjonsmiljøet (n=108), fra overflaten til fisk som kommer inn i slakteriet (n=47) og fra overflaten til fisk (n=59) og råvare (n=144) ved siste ledd i produksjonen. Ingen av slaktebåtene hadde prøver med funn av *L. monocytogenes*. Det ble påvist *L. monocytogenes* i 22 prøver fra ni ulike virksomheter, hvor fem av virksomhetene hadde flere positive prøver. I seks slakterier ble positive prøver funnet på overflaten til fisk og i råvareprøver ved siste ledd i produksjonen. Råvareprøvene inneholdt lave konsentrasjoner av *L. monocytogenes* (<10 CFU/g). Kartleggingen viste at *L. monocytogenes* er tilstede både i produsjonsmiljøet og på fisk i enkelte lakseslakteri og at i noen tilfeller vil *L. monocytogenes* være tilstede i råvarer som er klar for videre foredling.

Content

1 - Background

Listeria monocytogenes is a gram-positive bacterium that is widespread, and has been detected in food, freshwater, seawater, soil, plant material, sewage sludge, as well as faeces from asymptomatic animals and humans [1]. Disease caused by *L. monocytogenes* is called listeriosis and the most vulnerable groups are immunocompromised, young, elderly, pregnant and their foetus. Worldwide, the incidences of listeriosis appears to be increasing, which may be due to better diagnostics and an increase in the number of people in the risk group [2]. Several food-borne listeriosis outbreaks are known, where particularly ready-to-eat foods with long shelf life, including cold-smoked fish products, have been identified as the source [3]. Estimates from the European Food Safety Agengy indicates as much as 10% prevalence of *L. monocytogenes* in ready-to-eat fish products [4].

L. monocytogenes is a bacterium of special concern in the seafood industry, as it possesses several properties that makes it less affected by disinfectants and food preservation agents and is therefore able to survive and grow in the food production environment. The bacterium grows with and without oxygen, at temperatures from 1 to 45 °C, and survives freezing [1]. It is described to grow at pH between 4.5 and 9.0, and can survive in foods with pH values beyond this range [1], and has been shown to grow in the presence of 10% salt (NaCl) [5]. *L. monocytogenes* can colonise smooth surfaces such as stainless steel by forming biofilms [6]. These biofilms protect the bacterial cells against external stress factors like UV radiation and disinfectants, and can contributes to persistent contamination of *L. monocytogenes* in production environment [7].

The EU set a maximum limit for *L. monocytogenes* in ready-to-eat foods intended for healthy adults at 100 colony forming units/g sample at the end of the expiration date [8]. To achieve this, food producers must design the production line and handle raw materials in such a manner that contamination and growth of *L. monocytogenes* is prevented [9]. Documentation and control of *L. monocytogenes* is the food producer's own responsibility and often conducted by an independent third-party laboratory, and to little extent by governmental control bodies. Consequently, there is currently little public information available about the prevalence of *L. monocytogenes* in Norwegian salmonid slaughter facilities.

On behalf of the Norwegian Food Safety Authority (NFSA), the Institute of Marine Research (IMR) has conducted a screening program for *L. monocytogenes* in salmonids in the period September 2020 to August 2021, focusing on raw materials and the production environment in both slaughtering plants and slaughtering vessels. The aims of this program were to gain data on the prevalence of *L. monocytogenes* at the slaughter step of the Norwegian salmonid value chain and to prepare a *L. monocytogenes* culture collection for future strain assesments and outbreak investigations.

2 - Materials and methods

2.1 – Samples and sampling

The sampling regime included sampling at all active Norwegian facilities, both slaughtering plants and slaughtering vessels, with approval for slaughter of salmonid fish. Samples were collected by the NFSAs own inspectors in the period of September 2020 to August 2021. Due to the Covid-19 pandemic, sampling at some facilities was cancelled.

The sample categories are presented in Table 1. The NFSA inspectors were given the following instructions: 1) All sampling should be conducted aseptically. 2) Sampling of the production environments could be of any equipment that comes or could come in contact with the fish during production, and the inspectors were encouraged to swab areas (30 cm^2) that showed signs of being worn, such as disrupted surfaces. 3) Sampling of the surface of fish entering the facility was conducted with cloths swabbing skin and gills of the fish. One cloth for each fish, five in total. 4) Gills and surfaces of fish at the end point of the production line was sampled as described in pt.3. 5) Samples of raw materia at the end point of the production line comprised > 200 g fish muscle (NQC cut) aseptically withdrawn with sterile knives. 6) All samples were either chilled or frozen after sampling.

All samples were sent to the IMR either chilled or frozen, where it was analysed directly or kept frozen until the start of the analyses.

Table 1. Overview of sample types included from each slaughtering facility.

Sample type	***Slaughtering plants***	***Slaughtering vessel***
Production environment (Cloth swab of area of 30cm^2)	2	1
Fish surface entering (Cloth swab of skin and gills of 5 fish)	1	
Fish surface end point (Cloth swab of skin and gills of 5 fish)	1	
Raw material end point	3	1*
Total	**7**	**2**

* Cloth swab of skin and gills of 5 fish from slaughter vessels that produced whole, round fish.

2.2 – Analyses of *Listeria monocytogenes*

All samples were analysed at the microbiological lab at IMR by accredited analyses for qualitative detection of *Listeria monocytogenes* according to ISO-11290-1:2017. Results were reported as detected/not detected per cloth sample or per 25 grams. Positive samples of raw material were subjected to quantitative analyses according to ISO-11290-2:2017. Results were reported as colony forming units (CFU) per gram and the limit of quantification (LOQ) was 10 CFU/g. Genotyping of isolated *L. monocytogenes* was not performed in this screening program.

3 - Results

In total, 358 samples were examined from 60 slaughter facilities (49 slaughtering plants and 11 slaughtering vessels). Fifty-eight of the facilities produced Atlantic salmon (*Salmo salar*), and one slaughtering plant and one slaughtering vessel produced Atlantic char (*Salvelinus alpinus*) at the time of sampling. The examined samples included 108 samples from the production environment and 250 samples from fish. None of the 21 samples from slaughtering vessels contained *L. monocytogenes* at detectable levels.

L. monocytogenes were detected in 22 samples (Table 2) from nine different slaughtering plants (18.4%).

Table 2. Examined samples (n), positive samples (n) and frequency (%) of samples from slaughtering plants.

Sample type	***Examined (n)***	***Positive (n)***	***Frequency (%)***
Production environment	98	9	9.2
Fish surface entering	47	4	8.5
Fish surface end point	48	5	10.4
Raw material end point	144	4	2.8
Total	**337**	**22**	**6.5**

Seven samples were examined from each of the nine slaughtering plants, where four slaughtering plants had one positive sample, one had two positive samples, two had three positive samples, one had four positive samples, whereas one slaughtering plant had as much as six of seven samples positive for *L. monocytogenes* (Table 3).

Table 3. Overview of positive samples at different slaughtering plants.

	Production environment	***Fish surface entering***	***Fish surface end point***	***Raw material end point***	***Total***
Plant A	2	1	1	2	6
Plant B	2	1	1		4
Plant C		1	1	1	3
Plant D	1	1	1		3
Plant E	1		1		2
Plant F	1				1
Plant G	1				1
Plant H	1				1
Plant I				1	1
Total	**9**	**4**	**5**	**4**	**22**

L. monocytogenes was detected in the production environment within seven slaughtering plants where one positive sample were from either the bleeding area, the fileting area, the packaging area and from an unknown area. Two postive samples were found in the grader area, whereas three positive samples were found in the slaughter area. These nine positive samples were from the following items: a water hose handle, parts of the slaughter machine, manual slaughtering equipment, fileting machine parts, conveyor belts (three samples), knives and cutting boards, and from a sorting table.

Four samples of fish surface samples entering the slaughtering plants were positive for *L. monocytogenes*. All these slaughtering plants also had positive fish surface samples at the end point of production. In addition, one more slaughteing plant had a positive fish surface sample at end point. Four samples of raw material containing *L. monocytogenes* were detected in three different slaughtering plants.

The four positive raw material samples at the end point were obtained from three different slaughtering plants. The quantitative analyses showed that all samples contained a lower number of *L. monocytogenes* than the LOQ at 10 CFU/g.

4 - Discussion and conclusion

This screening program detected *L. monocytogenes* in 9 out of 49 (18.4%) slaughtering plants, and in none of the slaughtering vessels. In six (12.2%) of the slaughtering plants, positive samples were found at the end of the production line from either the surface of the fish or in raw material. All the positive raw material samples contained *L. monocytogenes* below the limit of the quantitative method (<10 CFU/g). This shows that fish with *L. monocytogenes* will in some cases enter the downstream processing chain, however containing low numbers of the bacterium.

A higher prevalence of *L. monocytogenes* was found when sampling was done with cloth swabbing of the surface and the gills of five fish (10.4%) compared to the sampling of raw material (2.8%). This sampling method allows a large sampling surface, increasing the sensitivity of the method, and provides important information in cases where the end product is whole fish with head and gills. Interestingly, the prevalence of *L. monocytogenes* was just slightly lower for fish entering the slaughtering plants compared to the fish at the end point of the production line, indicating that contamination of gills and skin may originate earlier in the production line before the fish enters the slaughtering plants. The prevalence of *L. monocytogenes* in the production environment were also similar to the surface of fish, at 9.2%.

The relation between the prevalence of *L. monocytogenes* and their genotypic profile is not addressed in this report. It is recommended to include such analyses in the future as this will reveal weather positive samples from different areas in the same slaughtering plant are contaminated with the same strain of *L. monocytogenes* or if multiple strains are present in the slaughtering plant at the same time. Whole-genome sequence (WGS) based typing and core genome Multi Locus Sequence Type (cgMLST) analyses will also indicate the genotypic relation with the strains found in this study with strains found in other studies and in cases of outbreaks, both within and outside of Norway.

In case of any future needs for samples of a larger public culture collection, sampling could be coordinated with samples from already running surveillance programs to provide material for *L. monocytogenes* analyses, such as in the surveillance program for prohibited substances. Given that the fish muscles were sampled with microbiological analyses in mind (sterile techniques which requires only little training), these samples would have no extra sampling cost.

In conclusion, *L. monocytogenes* can be found in low concentrations in salmonid fish in Norwegian slauthering plants and hence could follow along the salmonid value chain. In this study, *L. monocytogenes* was found on the fish surface or in raw material in 6 out of 49 slaughtering plants, indicating an increased risk of transmission for raw material sold as whole slaughtered fish with head and gills still attached. Maintaining the cold chain during transportation and further processing will be crucial to prevent growth of *L. monocytogenes* in the final products. Furthermore, the fate of *L. monocytogenes* will depend on the application of the raw material, and any non-listeriacidal processing techniques such as cold smoking and *graving* increases the risk of sustaining *L. monocytogenes* in products entering the market.

5 - Referanser

1. Khelef, N., et al., *Listeria monocytogenes and the genus Listeria.* The prokaryotes, 2006. **4**: p. 404-476.

2. European Food Safety Authority and European Centre for Disease Prevention Control, *The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017.* EFSa Journal, 2018. **16**(12): p. e05500.

3. Rakhmawati, T., Nysen, R. & Aerts, M. (2013) Statistical analysis of the *Listeria monocytogenes* EU-wide baseline survey in certain ready-to-eat foods Part A: *Listeria monocytogenes* prevalence estimates. EFSA Supporting Publications, 10, 441E.

4. European Centre for Disease Prevention Control and European Food Safety Authority, *Multi-country outbreak of Listeria monocytogenes clonal complex 8 infections linked to consumption of cold-smoked fish products*. 2019, Wiley Online Library.

5. Adams, M.R. and M.O. Moss, *Food microbiology*. 2008, Cambridge: RSC Publ. XIV, 463 s.

6. Carpentier, B. and O. Cerf, *Review — Persistence of Listeria monocytogenes in food industry equipment and premises.* International Journal of Food Microbiology, 2011. **145**(1): p. 1-8.

7. Yin, W., et al., *Biofilms: The Microbial "Protective Clothing" in Extreme Environments.* International Journal of Molecular Sciences, 2019. **20**(14): p. 3423.

8. Commission Regulation (EC) No 2073/2005, *of 15 November 2005 on microbiological criteria for foodstuffs*, in *Official Journal of the European Union*.

9. Skjerdal, T., L.H. Moen, and K.S. Pettersen, *Belastningsstudier-et verktøy for håndtering av Listeriarisiko i spiseferdige produkter.*

HAVFORSKNINGSINSTITUTTET

Postboks 1870 Nordnes
5817 Bergen
E-post: post@hi.no
www.hi.no