

Listeria monocytogenes: Italian experiences during Listeriosis outbreaks

Marina Torresi

WOAH CC FH, IZS-Teramo

National Reference Laboratory for Listeria monocytogenes

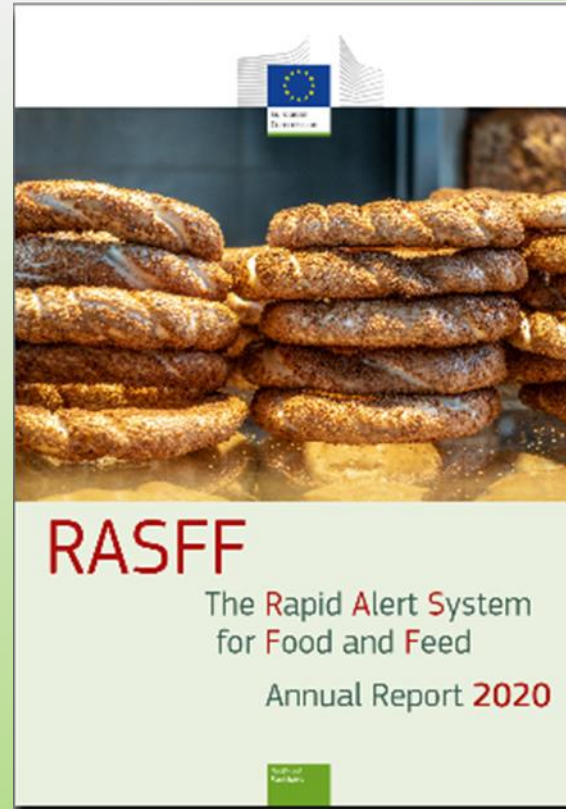
ERFAN Laboratory training course 17-21 October 2022

Legal framework in Europe

- At **EU level**: Foodborne Disease and Food Contamination events are recorded and communicated in different systems, for different purposes; there are legally binding warning systems, such as:
 - **Rapid Alert System for Food and Feed / RASFF** (http://ec.europa.eu/food/food/rapidalert/index_en.htm) for reporting the presence of known food-related hazards to public health and feed on the market.
 - **Early Warning and Response System/ EWRS** (<https://ewrs.ecdc.europa.eu>) for the communication of known diseases that may require the implementation of control measures to improve the coordination of public health measures in the EU (human side).
- At **Global level**: EU Member States are legally obliged to report any outbreak of international concern to WHO and this includes foodborne disease outbreaks (Infosan Network).

Legal framework in EU:RASFF

- **Rapid Alert System for Food and Feed (RASFF)** is a system for the notification of a direct or indirect human health risk deriving from food, feed, food contact materials
- RASFF is hosted and managed by the EU Commission
- Regulation (EC) No 178/2002 is the legal basis of the RASFF
- The system involves a network consisting of the Member Countries, the European Food Safety Authority (EFSA) and the Commission
- This quick exchange of information allows all Member States to verify immediately whether they are also affected by the problem



Legal framework in Europe:EWRS

- EWRS - Early Warning and Response System Official notification
- EWRS is exchanging risk management information in relation to communicable disease events, is owned by the EU Commission and is hosted by ECDC since November 2007
- Each Member State designates competent public health authorities that have to notify the emergence or development of serious cross-border threats to health and control measures
- A web based informatics tool has been developed to allow information to be shared between relevant public health authorities
- The public health authorities receive a login and a password from the Commission to access the system, and full authorization to write and read messages
- When a message is posted on the system, it is automatically circulated to all EWRS competent authorities, and the network (Commission, Member States, acceding and the EEA countries, and ECDC) is informed at the same time

Central level

- The **Italian Ministry of Health (MOH)** is the central body of the National Health Services' dealing with orientation and planning on issues of public health, veterinary health, food hygiene and safety.
- **Directorate-General for hygiene, food safety and nutrition (DGISAN)** safety of food production; control plans of the food chain and operational guidelines for official controls on imported food; management of RASFF system and unit crisis for food, feed, animal by-products.

Regional

- The 19 Regions and 2 Autonomous Province are organized into **Regional Public Health Services (RPHS)**, most of which are divided in two sections: the **Regional Veterinary Service (RVS)**, which is responsible for food of animal origin, animal health and welfare and feed safety; the **Regional Food Hygiene and Nutrition Service (RFHNS-SIAN)**, which is responsible for products of non animal origin, food supplements and food contact materials.

Local

- **Local Health Units (LHU-AUSL)** are present in each Region and act as public bodies responsible for the organization and management of all public health facilities at local level.

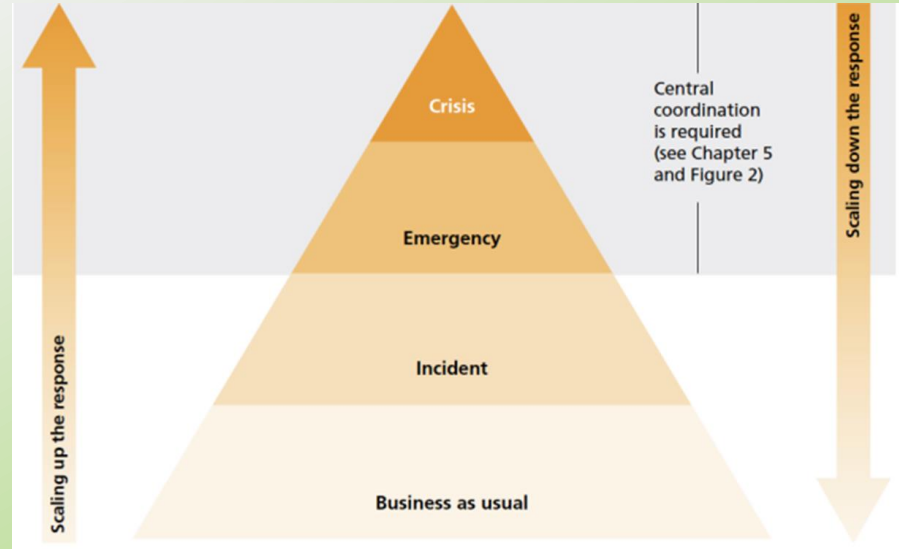
Organization of Competent Authorities in Italy

■ Laboratory and Investigations Services:

- National Health Institute (ISS)
- Istituti Zooprofilattici Sperimentali - National Reference Centers (IZS)
- Human side laboratories (hospitals)
- Environment Protection Agencies (ARPA)
- NAS : a special unit of the Italian Corps of Carabinieri. It carries out investigations and controls on illegal adulteration of foodstuffs, fraud, and illegal trafficking of medicines, both on its own initiative or upon request of MOH offices.

Crisis organizational plan

- Emergency planning is an essential component of the work of Competent Authorities, it's essential to use "peacetime" to build structures and procedures, to monitor the situation
- Consists of established structures, procedures and coordination mechanisms between national agencies that routinely function and which can be scaled up during emergencies
- A documented control plan and the establishment of an outbreak control team (OCT) is essential for the management of foodborne disease outbreaks, specifying well roles and responsibilities



The ECDC Toolkit

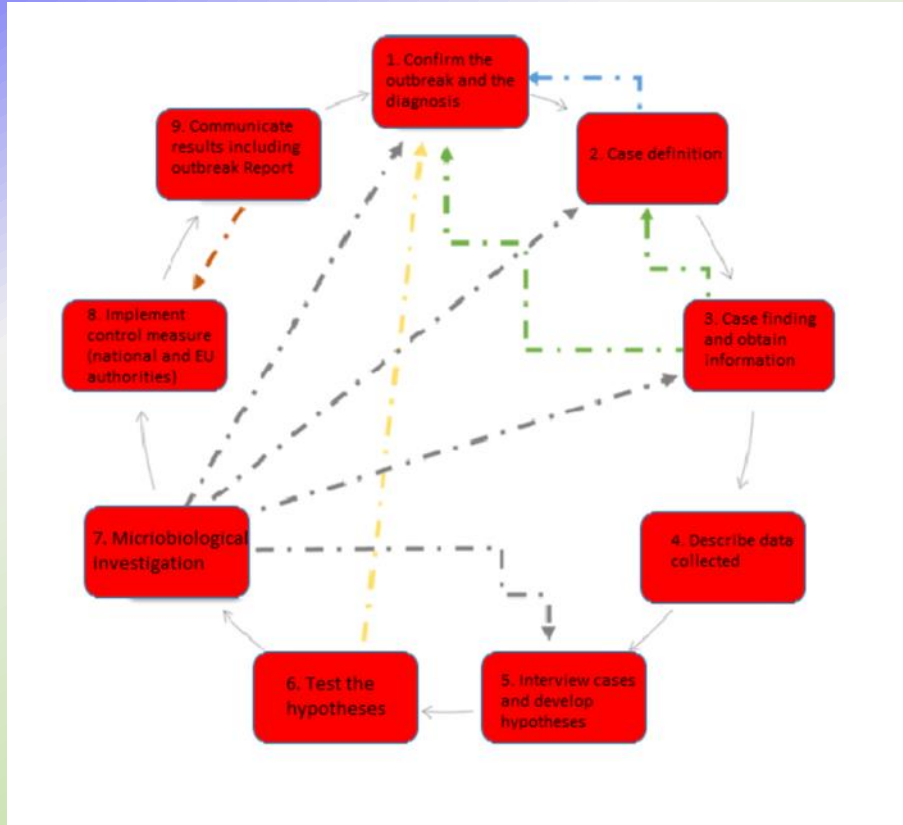
- The “Toolkit for investigating food- and waterborne disease outbreaks with an EU dimension” is composed of a set of documents, templates, guidelines and explanatory texts. These materials follow the **10 steps** of an outbreak investigation scheme, from confirming outbreak and diagnosis to evaluating and updating procedures, including case finding, characterizing the outbreak by epidemic curve and implementing control measures.
- <https://www.ecdc.europa.eu/en/publications-data/toolkit-investigation-and-response-food-and-waterborne-disease-outbreaks-eu>



The 10 steps of the outbreak investigation

1. Confirm the existence of the outbreak and the diagnosis (or make one);
2. Develop a definition of case;
3. Perform case finding;
4. Describe data (time, place, personal data ecc.) – descriptive epidemiology
5. Interview cases and develop hypotheses about source, transmission
6. Test the hypothesis – analytical epidemiology
7. Microbiological investigation and perform additional (targeted) studies (path. typing, food tracing etc.)
8. Implement control measure (by national and EU authorities)
9. Disseminate results – Outbreak Report
10. Evaluate an update procedure and toolbox - Learn the lesson (e.g. review HACCP, official control procedures)

The 10 steps of the outbreak investigation

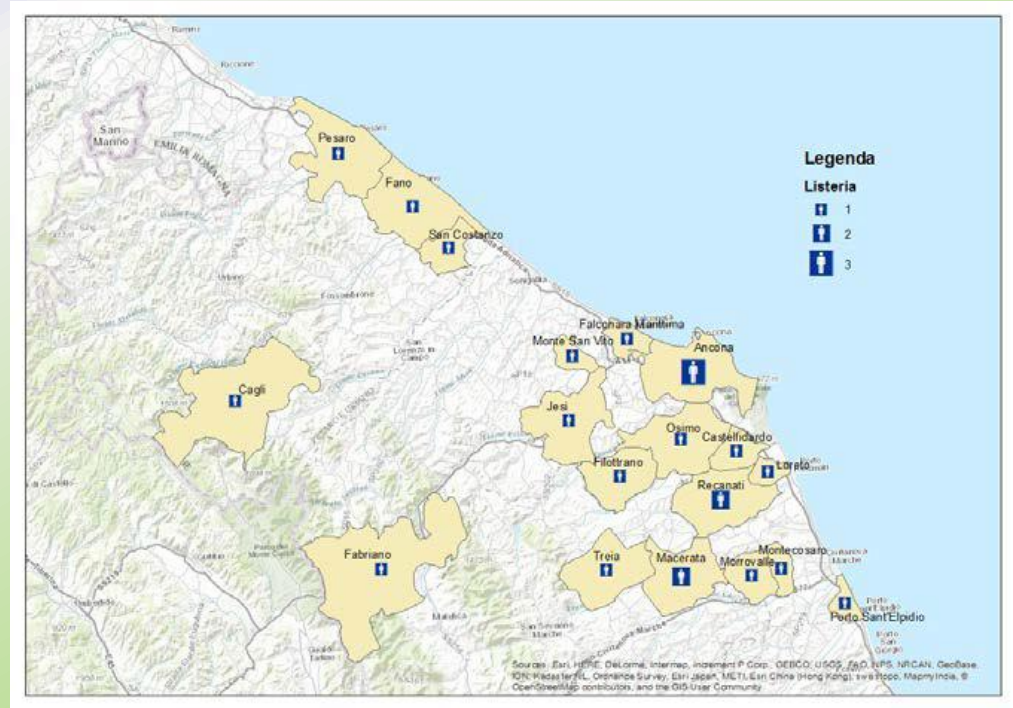


Italian experiences:

Listeria ST7 outbreak in Marche Region 2015-2016:

The onset of the outbreak: An increase in the occurrence of listeriosis was observed in the Marche region between January and May 2015, with eight reported cases compared to two and four cases in 2014 and 2015, respectively.

The Outbreak confirmation was done on the basis of clinical evidence (more than one person has the same clinical symptoms) and of analytical results of the laboratory on samples taken from independent patients (with no known epidemiological relationship at the moment).



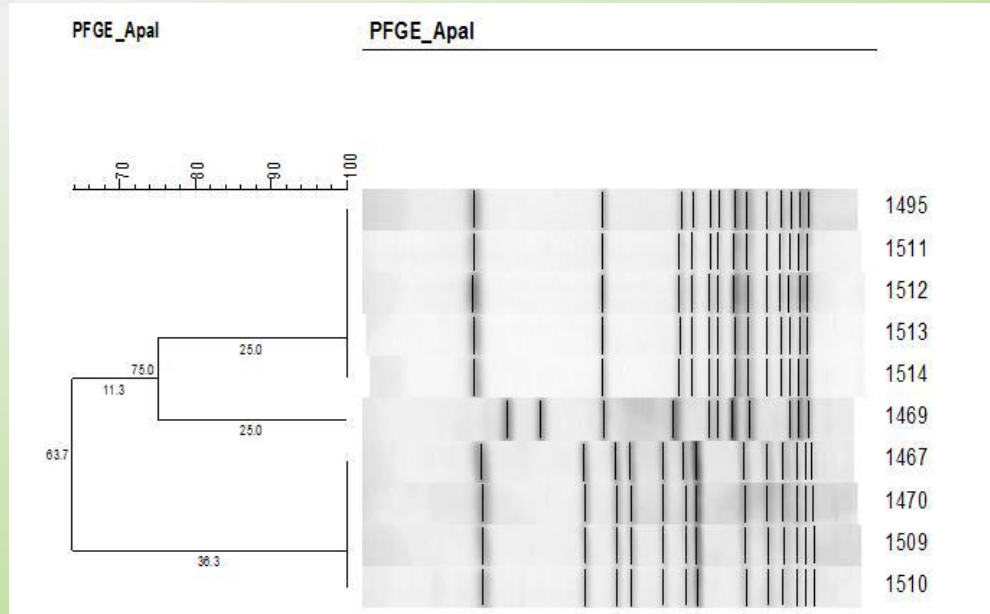
Italian experiences:

Listeria ST7 outbreak in Marche Region 2015-2016:

The Outbreak confirmation:

Outbreak Strain's characteristics

- Serogroup IIa
- PFGE profile Apal 0246 Ascl 0356



Listeria ST7 outbreak in Marche Region 2015-2016:

An **outbreak control team** was established, made up of Public Health Authorities, Food Safety Inspector, Microbiologist, Epidemiologist, Bioinformatician, Communications experts)

The following **Case definition** was adopted:

possible outbreak cases: patients in the Marche region showing clinical symptoms compatible with listeriosis

probable outbreak cases: patients in the Marche region showing clinical symptoms compatible with listeriosis and disease onset from January 2015, and isolation of *L. monocytogenes* serotype 1/2a from normal sterile body fluids.

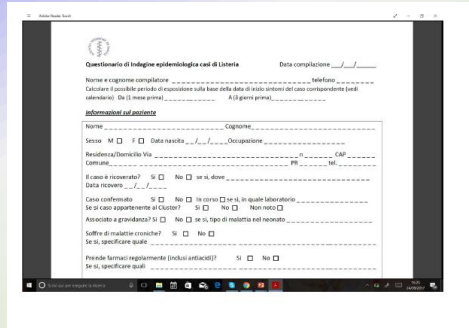
Confirmed outbreak cases were patients matching the above-mentioned criteria and with a *L. monocytogenes* isolate of pulsotype Apal.0246 Ascl.0356

Categories of cases:

- Possible: partial or atypical clinical features
- Probable: typical clinical features without laboratory confirmation
- Confirmed: typical clinical features with laboratory confirmation

Listeria ST7 outbreak in Marche Region 2015-2016:

Case finding: Following patient consent, all cases were interviewed using a standard epidemiological questionnaire until December 2015.



Questionario di indagini epidemiologiche casi di Listeria

Nome e cognome compilatore _____ Telefono _____
 Indirizzo e possibile periodo di esposizione sulla base delle date di inizio sintomi del caso compilatore (vedi calendario). Da (1) mese prima _____ A (2) giorni prima _____

Informazioni sul paziente

Nome _____ Cognome _____
 Sesso M F Data nascita _____/_____/_____, Occupazione _____
 Residenza/Domicilio Via _____, CAP _____
 Comune _____ Prov. _____

Il caso è ricoverato? Sì No se sì, dove _____
 Data ricovero _____/_____/_____

Caso ricoverato? Sì No se sì, in quale laboratorio _____
 Se sì caso ospitato al Cus? Sì No Non noto

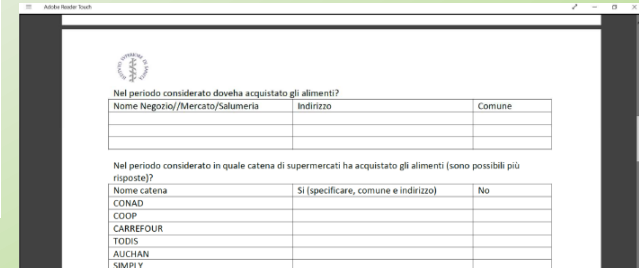
Associato a gravidanza? Sì No se sì, tipo di malattia nel neonato _____

Suflora di malattie croniche? Sì No

Se sì, specificare quale _____

Prende farmaci regolarmente (inclusi antiacidi)? Sì No

Se sì, specificare quali _____



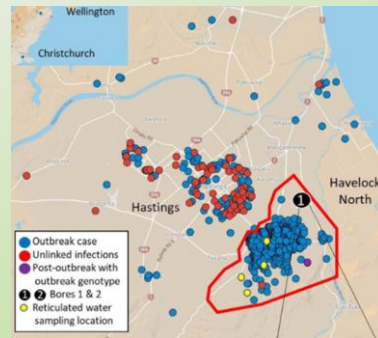
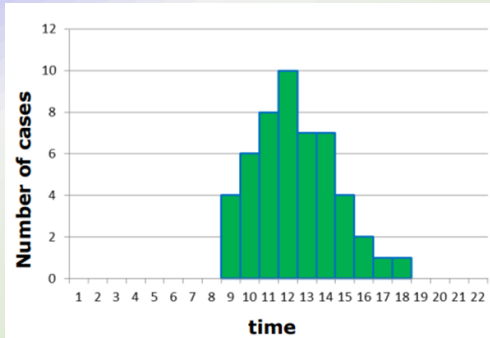
Nel periodo considerato dove ha acquistato gli alimenti?

Nome Negozio/Mercato/Salumeria	Indirizzo	Comune

Nel periodo considerato in quale catena di supermercati ha acquistato gli alimenti (sono possibili più risposte)?

Nome catena	Sì (specificare, comune e indirizzo)	No
CONAD		
COOP		
CARREFOUR		
TODIS		
AUCHAN		
SIMPLY		

Descriptive epidemiology: Time, place, personal data

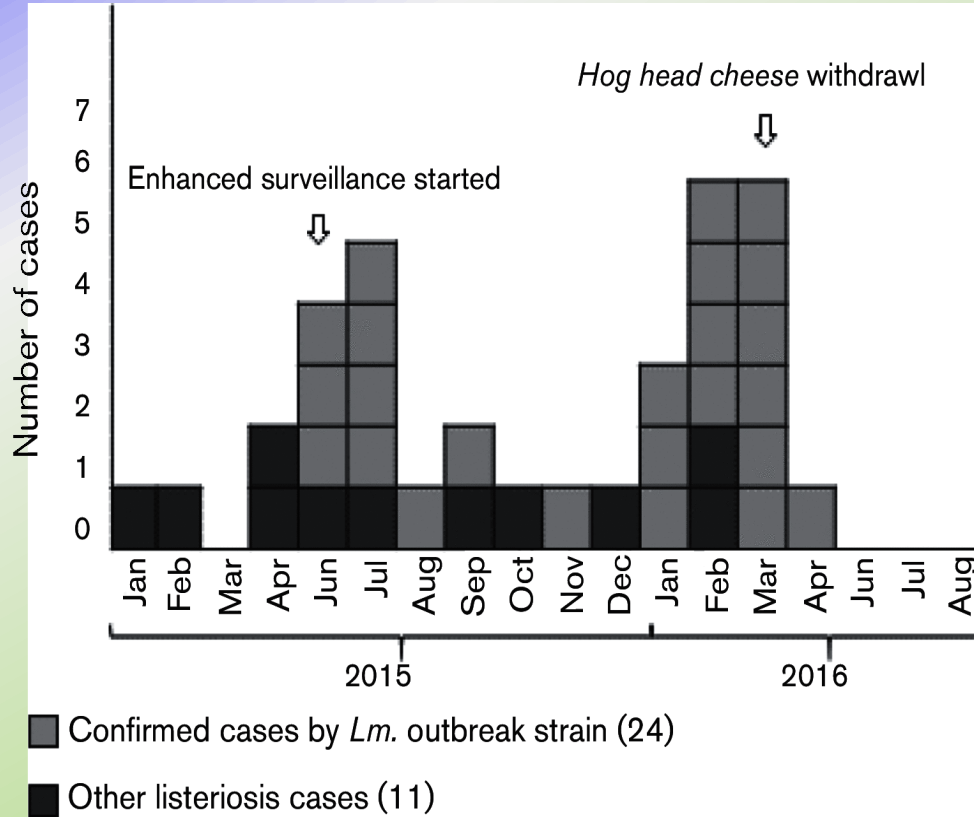


Carne					
Tipo di carne	CONSUMATO	se Sì, specificare se già confezionato		Nome e luogo di acquisto (supermercato, gastronomia, salumeria, ecc.)	Indirizzo luogo di acquisto
Paté (fegato o altro)	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>		
Carpaccio	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>		
Tartare	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>		
Pollo arrosto (già cotto)	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>		
Carne in scatola	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>		
Porchetta	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>		
Vitello tonnato, arrosto e altri piatti pronti a base di carne	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>		
Wurstel	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>		
Altro	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>		
Altro	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>		
Salumi					
Tipo di salume	CONSUMATO	se Sì, specificare se già confezionato		Nome e luogo di acquisto (supermercato, gastronomia, salumeria, ecc.)	Indirizzo luogo di acquisto
Mortadella	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>		
Prosciutto crudo	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>		
Prosciutto cotto	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>		
Salame o salicice stagionate	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>		
Salame o Saliccia fresca	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>		
Bresaola	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>		
Speck	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>		
Coppa	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>	Sì <input type="checkbox"/> No <input type="checkbox"/>		

Listeria ST7 outbreak in Marche Region 2015-2016:

Epidemic curve:

The curve shows the temporal distribution of probable and confirmed outbreak cases reported in the Marche region by symptoms onset. Outbreak cases were reported continuously by month from 21 May 2015 to 11 March 2016. The epicurve showed two peaks. The median age of all cases (35) was 75 years. The proportion of females was higher among confirmed cases than among probable cases (63% vs 36%).

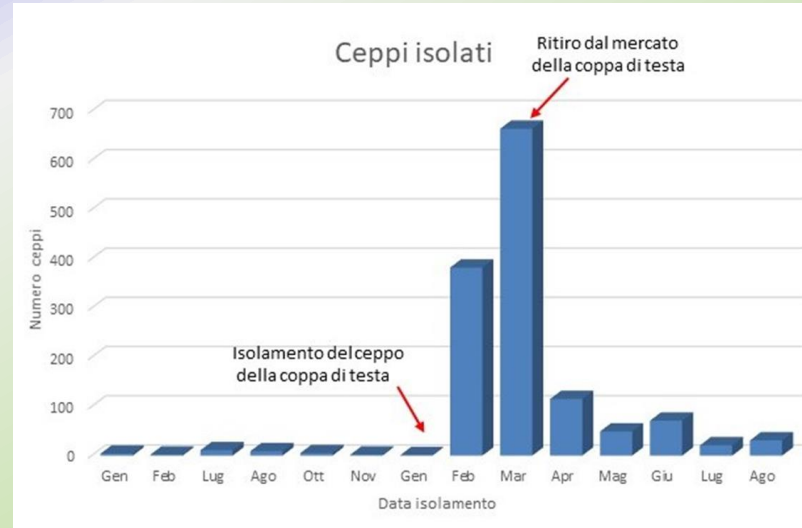


Listeria ST7 outbreak in Marche Region 2015-2016:

Microbiological and genomic analysis

We received more than 1400 strains in two months. Screening and typing (multiplex PCR , Real time PCR, PFGE and WGS) analyses were performed in order to find the same strains isolated in clinical cases.

On the 4th of February, the outbreak strain was found in a hog head cheese (a typical seasoned pork-derived meat jelly product) sample.



Listeria ST7 outbreak in Marche Region 2015-2016:

The field investigation: establishment inspections and traceback and forward activities

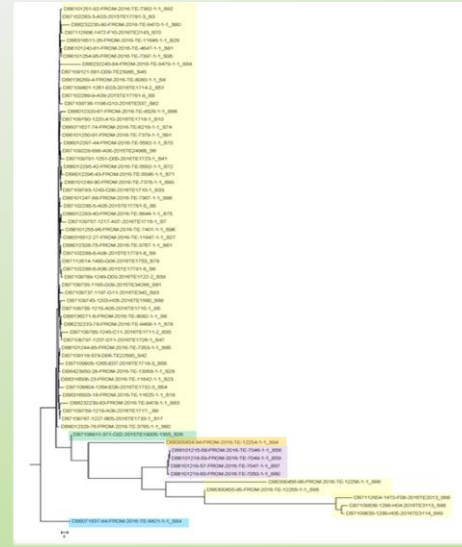
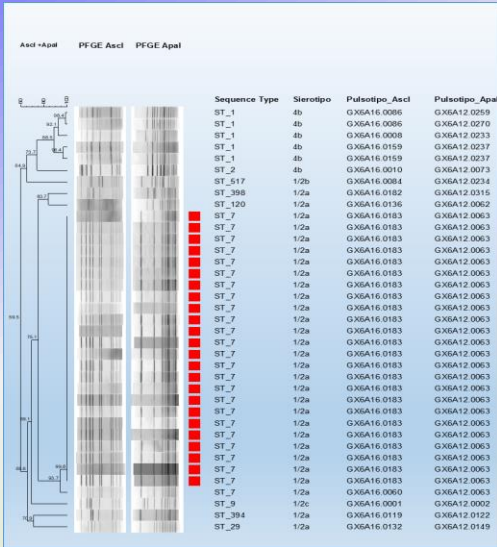
The origin and destination of the implicated foodstuffs and their ingredients were investigated by tracing back and forward their suppliers and customers, with the aim of identifying whether retailers had received food processed by the contaminated food production plants.



Listeria ST7 outbreak in Marche Region 2015-2016:

Typing investigation

PFGE



**WGS:
SNPs
analyses**

Laboratory results, although important, are generally not sufficient to identify all the elements necessary to solve an outbreak. It's fundamental to conduct epidemiological investigation. If during investigation new elements and/or evidence become available or something has not been sufficiently investigated, it is necessary to come back to reconsider all the steps needed.

Listeria ST7 outbreak in Marche Region 2015-2016:

Report and updating Implement control Measures

The adoption of control measures is necessary and aims to mitigate the damage

- Production activities were suspended at plant involved and a Rapid Alert System for Food and Feed (RASFF) notification was sent regarding four hog head cheese batches and all products processed by that plant. The MoH published the alert on their website. Plant involved withdrawn all its products from the market. The production was suspended until disinfection procedures were completed, and screening tests for *L. monocytogenes* contamination of stored products and environmental samples were carried out with a favourable outcome.
- All batches were subjected to high-pressure processing (HPP) treatment before distribution on the market, although they had already tested negative for *L. monocytogenes*.
- The Marche Regional Authority implemented a number of measures including increased number of inspections, revision and control of sanitation procedures.
- Training for inspectors in the Marche region was held with regard to standardizing operational procedures for sampling. Training for food business operators was also conducted to raise awareness on the risk of persistent contaminations by *L. monocytogenes* in food plants

Listeria ST7 outbreak in Marche Region 2015-2016:

A severe outbreak of listeriosis in central Italy with a rare pulsotype associated with processed pork products

Anna Duranti,^{1,†*} Michela Sabbatucci,^{2,3,†} Giuliana Blasi,¹ Vicidalia Aniela Acciari,⁴ Massimo Ancora,⁴ Antonino Bella,² Luca Busani,² Patrizia Centorame,⁴ Cesare Cammà,⁴ Fabrizio Conti,¹ Dario De Medici,² Marco Di Domenico,⁴ Violetta Di Marzio,⁴ Giovanni Filippini,¹ Alfonsina Fiore,² Stefano FisicHELLA,¹ Antonietta Gattuso,² Monica Gianfranceschi,² Caterina Graziani,² Fabrizia Guidi,¹ Maurilia Marcacci,⁴ Cristina Marfoglia,⁴ Diana Neri,⁴ Massimiliano Orsini,⁴ Donatella Ottaviani,¹ Annalisa Petruzelli,¹ Patrizio Pezzotti,² Caterina Rizzo,² Anna Ruola,⁴ Gaia Scavia,² Stefania Scuta,¹ Giuliano Tagliavento,⁴ Alberto Tibaldi,⁴ Francesco Tonucci,¹ Marina Torresi,⁴ Giacomo Migliorati⁴ and Francesco Pomilio⁴

Abstract

Purpose. From May 2015 to March 2016, an outbreak due to *Listeria monocytogenes* serotype 1/2a and clinical pulsotype never previously isolated in Europe occurred in central Italy, involving 24 confirmed clinical cases. The article provides a description of the outbreak and the investigation carried out by a multidisciplinary network.

Methodology. Epidemiological and microbiological surveillance was conducted to confirm the outbreak and to detect the food vehicle of infection. The origin and destination of the implicated food and its ingredients were investigated by tracing-back and -forward investigation.

Results. Next-generation sequencing confirmed the unique outbreak strain. On 4 January 2016, a *L. monocytogenes* strain with pulsotype indistinguishable from that isolated from clinical cases in the outbreak was detected in a sample of hog head cheese purchased from a retail supermarket in one of the patients. The hog head cheese was produced by a small meat processing plant in the Marche region, where microbiological investigation confirmed environmental and food contamination by the outbreak strain. Plant production was suspended and all contaminated batches of the hog head cheese were withdrawn from the market by 19 February by local health authority. We subsequently observed a sharp decline in clinical cases, the last being reported on 11 March 2016.

Conclusion. The key factor in the timely conclusion of this investigation was intersectoral collaboration among epidemiologists, microbiologists, veterinarians, statisticians and health and food safety authorities at national, regional and local levels.

Received 6 February 2018; Accepted 6 June 2018

Author affiliations: ¹Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche 'T. Rosati', Perugia, Italy; ²Istituto Superiore di Sanità, Rome, Italy; ³European Programme for Public Health Microbiology Training (EPHMM), European Centre of Disease Prevention and Control, ECDC, Stockholm, Sweden; ⁴National Reference Laboratory for *Listeria monocytogenes*, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale', Teramo, Italy; ⁵Azienda Sanitaria Unica Regionale Marche – Area Vasta 2, Ancona, Italy; ⁶Agenzia Regionale Sanitaria Marche, Ancona, Italy.

*Correspondence: Anna Duranti, aduranti@isum.it

Keywords: listeriosis; food borne; bacterial meningitis; *Listeria monocytogenes*; serotype 1/2a; bacteria.

Abbreviations: AFNR, Association Française de Normalisation; ECDC, European Centre for Disease Prevention and Control; ECDC-OCF, ECDC Operational Contact Point; EPHMM, Epidemic Intelligence Information System Food and Waterborne Diseases and Zoonoses; EU, European Union; EURL, EU Reference Laboratory; FS, food contact; HFF, Haff Fraser; IR, incidence rate ratio; IZS, Istituto Zooprofilattico Sperimentale; ISO, International Organization for Standardization; ISTAT, Italian National Institute for Statistics; ISG, Istituto Superiore di Sanità; IZSUM, Istituto Zooprofilattico Sperimentale Umbria e Marche; UKH, Local Health Authority; MoH, Ministry of Health; NGS, Next-Generation Sequencing; NRL, National Reference Laboratory; NRL, Lm, IZSAM, National Reference Laboratory for *L. monocytogenes*; PFGE, pulsed-field gel electrophoresis; RASFF, Rapid Alert System for Food and Feed; RIVM, Dutch National Institute for Public Health and the Environment; RTE, ready-to-eat; SEAP, epidemiological surveillance of foodborne pathogen systems; SNP, single nucleotide polymorphism; TESSy, The European Surveillance System; US/FDA, United States Food and Drug Administration.
 †These authors contributed equally to this work.

Listeria ST7 outbreak in Marche Region 2015-2016:

Communicate results: Outbreak report

- During investigation it is often necessary to prepare **intermediate reports** in order to update the competent Authorities not directly involved in investigation.
- As soon as possible from the conclusion of the investigation activities (which generally extend even after the end of the epidemic in humans), and regardless of previous communication activities (including the drafting and sharing of intermediate reports), the activities carried out by the Control Team must be summarized in a **final scientific report**.



Italian experiences:

Listeria ST155 outbreak at National level : ongoing

On the 21st of July we were advised by the ministry of Health (alert system) that there were an increase of listeriosis cases matching in time and genetic characteristics (same cgMLST)




Roma, 20 luglio 2022


Oggetto: Cluster genomico ST155 a cui appartengono ceppi di *Listeria monocytogenes* isolati nelle Regioni Lombardia ed Emilia Romagna.

Listeria ST155 outbreak at National level : ongoing

As Italian National Reference Laboratory for *Listeria monocytogenes*, we advised EURLm of the ongoing outbreak.
Consequently EURLm sent an Urgent Inquiry to other European lab to find strains isolated from food or environment genetically similar to our outbreak strain



Laboratory for Food Safety
Maisons-Alfort Location
European Union Reference Laboratory for *Listeria monocytogenes*
EURL management:
Adrien ASSERE,
EURL Manager
Corinne DANIEL,
Deputy EURL Manager
Unit:
Salmonella and Listeria
E-mail:
eurl-listeria@anses.fr
Ref.:
LIS-CI-202218L



EURLm
European Union Reference Laboratory for
Listeria monocytogenes
<http://eurl-listeria.anses.fr>

(1) Circular letter addressed to:

- the Heads of the National Reference Laboratories of the European Union for *Listeria monocytogenes*;

and for information to:

- Marçal PLANTADY and Eric THEVENARD (EC/DG SANTE Unit G4)

Maisons-Alfort, 30/08/2022

Subject: Urgent Inquiry – Ongoing outbreak of *Listeria monocytogenes* CC155 ST155 in Italy

Dear Colleagues,

On demand of Istituto Zooprofilattico Sperimentale dell’Abruzzo e del Molise G. Caporale, the Italian National Reference Laboratory for *Listeria monocytogenes* (NRL Im), DG SANTE requested EURLm to invite NRLs Lm to search for matching Lm strains in their national collection. Please find the reference genomes of the strains of the outbreak: *Listeria monocytogenes* (serogroup IIa, CC155, ST155) - link (valid until 6/9/2022) to download the file GENOMI.zip.


Genomic comparison should be performed by core-genome MLST analysis with an allelic difference less or equal to seven to define matching genomes of stains.

Additionally, the EURLm is interested in any information about related strains of non-human origin from CC155 ST155. Please contact us in case you have such strain and not WGS equipped; the EURLm would organise the sequencing of it.

We would be grateful for your response, as far as possible, by 13 September 2022 to eurl-listeria@anses.fr.

We sincerely thank you for your helpful contribution in this investigation and we look forward to hearing from you,

With best regards,

Laurent LALOUX
Laboratory Director


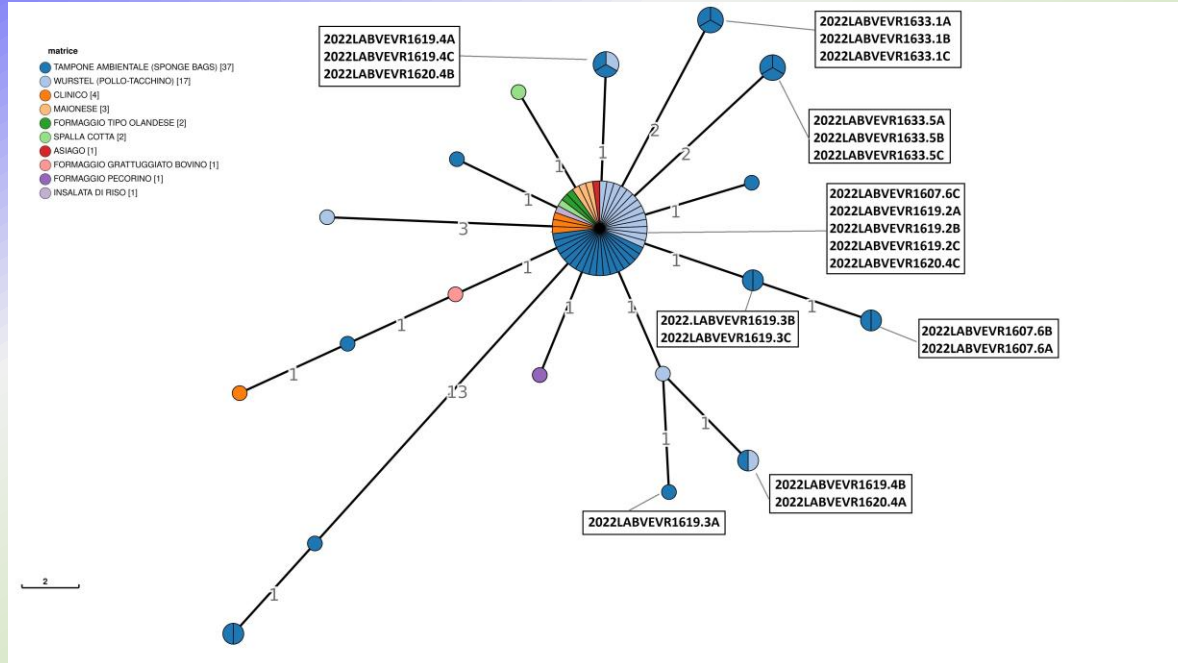
Listeria ST155 outbreak at National level : ongoing

An Outbreak control team has been established with meeting every week in order to update number and characteristics of clinical cases, epidemiological and microbiological/ typing studies and analyses.

At the moment (12nd of October):

- 12 Italian regions involved
- Number of cases: 86
- Females more than males
- The majority of cases are elderly immunocompromised people, few cases are children.

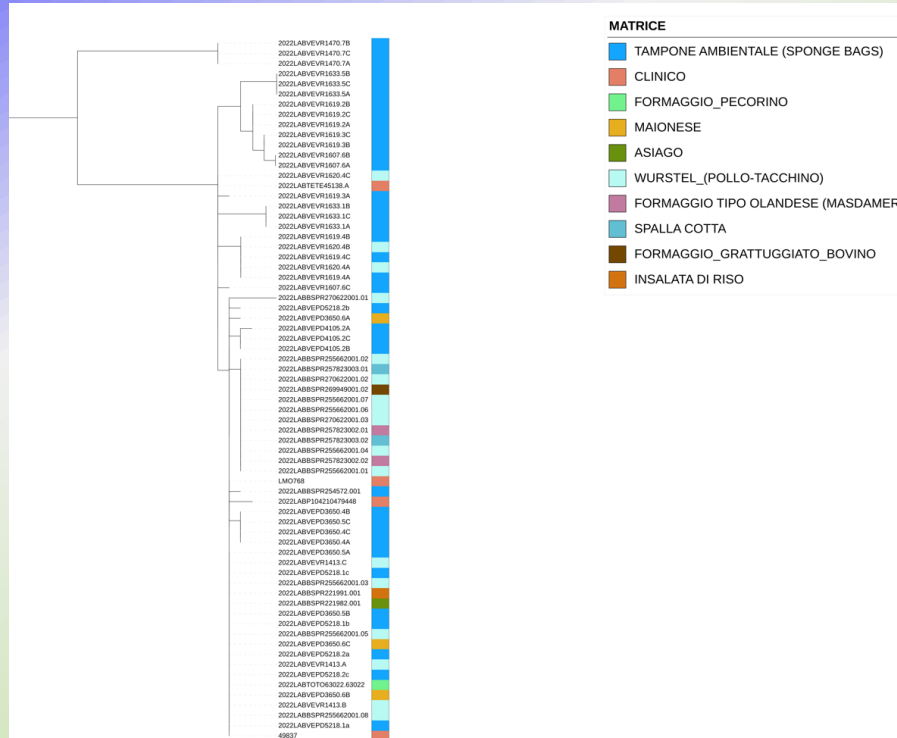
Listeria outbreak at National level : ongoing



Following epidemiological investigations a correlation between some of the clinical cases and the presence of the bacterium in chicken wüstels was detected.

Microbiological and typing analyses detected the presence of *Listeria* strains belonging to sequence type (ST) 155 in clinical cases, wüstels samples and swab carried out at the plant.

Listeria outbreak at National level : ongoing



Confirmation of genetic correspondence was done also through SNPs analysis. It's important to use different typing methods in order to be sure of clustering results.

Listeria outbreak at National level : ongoing

The company has taken all steps to safeguard the public by withdrawing the batches that have come out positive and, applying the principle of maximum precaution, also pulling all those produced before September 12 this year.

Investigations are also continuing into other products that might be linked to the human cases of listeriosis.

There are new cases every week and the OCT suspect wurstel could not be the only source involved.

Outbreak ST155 is still ongoing....





THANK YOU!

m.torresi@izs.it