

# ECDC State of play on NGS and future perspectives in public health area

Saara Kotila, ECDC

“Science meets Policy” conference: Modern technologies to enable response to crises: Next Generation Sequencing to tackle food-borne diseases in the EU, 25 September 2020

# Whole Genome Sequencing: ECDC Vision 2020

- To establish standards and manage systems
- EU wide use of whole genome sequencing as the method of choice for typing microbial pathogens
- Accurate and effective risk assessment, outbreak investigation, disease surveillance and evaluation of prevention policies

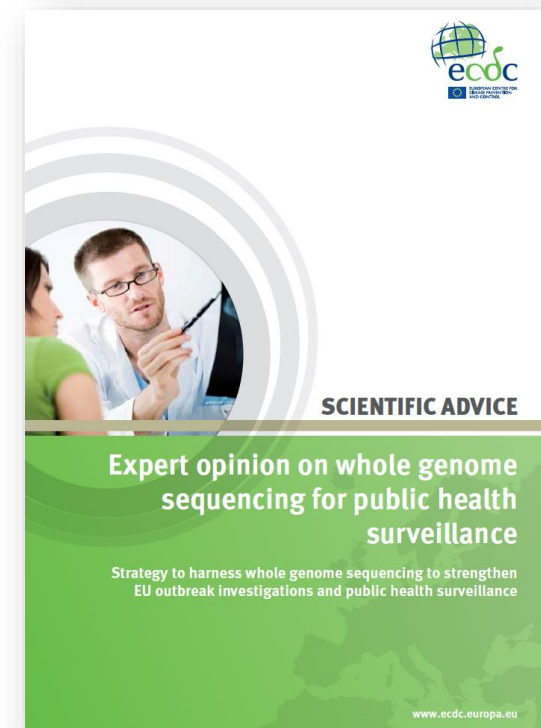
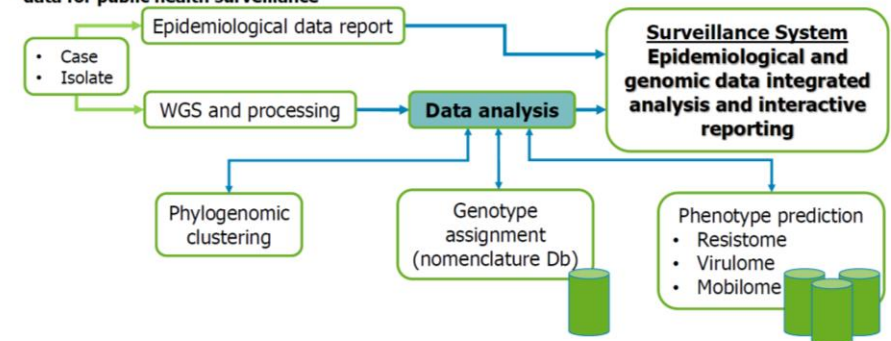
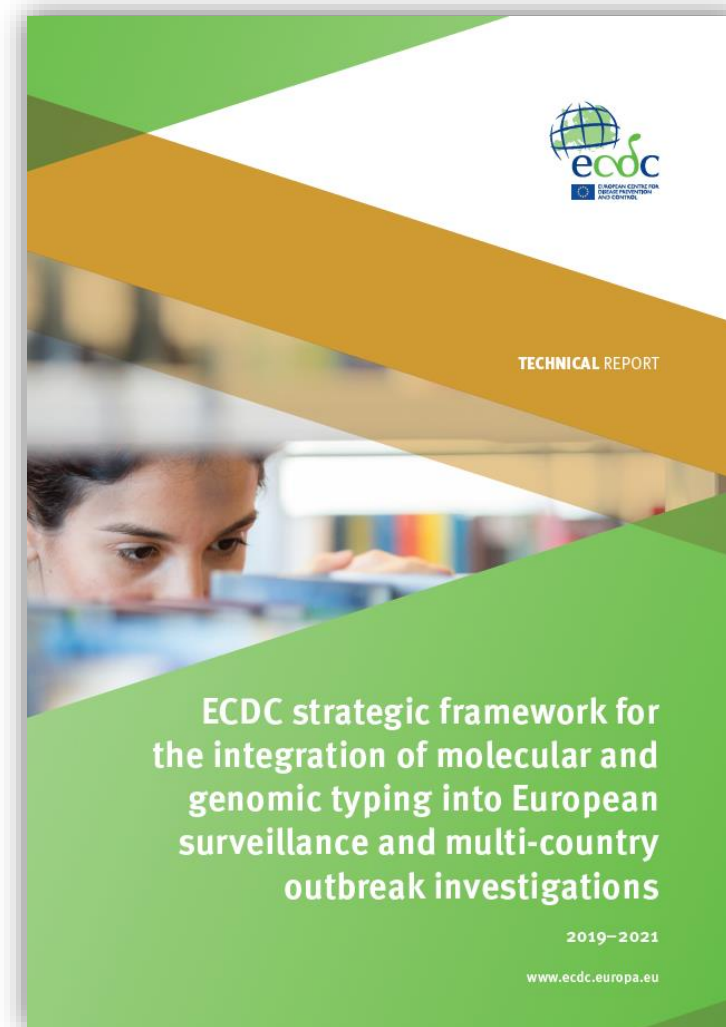


Figure 2. Process overview – WGS data production, analysis and integration with epidemiological data for public health surveillance



# Whole Genome Sequencing: Strategic framework 2021

- **Priorities for integration into EU surveillance and response support systems, 2019-2021**



# Public health applications of integrated epidemiological and WGS data collection and analysis



- 1. Outbreak investigations:** real-time information sharing and analysis for *rapid risk assessment*, targeted public health response and transmission control
- 2. Control-oriented surveillance:** real-time, continuous surveillance with maximal disease sampling frame for *early outbreak detection*
- 3. Strategy-oriented surveillance:** either by *sentinel continuous surveillance* or *periodic surveys*, with representative sampling frame for programme evaluation and trend monitoring

# Priority criteria for integrating genomic typing data into EU epidemiological investigations



- Disease **public health priority** and added-value of WGS data for infection control
- **Feasibility** of standardised typing schemes and data sharing
- **Capacity** for WGS typing at Member State and ECDC level
- **Interoperability** with information systems of public health partners at EU and international levels

# WGS typing: EU Strategic priorities 2021\*, by objective



<b>Outbreak investigation</b>
Any epidemic pathogen/MDR outbreak
<b>Continuous real-time surveillance</b>
<ul style="list-style-type: none"><li>• <i>Listeria monocytogenes</i></li><li>• <i>Neisseria meningitidis</i></li><li>• MDR tuberculosis</li><li>• <i>Salmonella enterica</i></li><li>• Shiga toxin-producing <i>E.coli</i></li><li>• influenza virus</li></ul>
<b>Sentinel surveys</b>
<ul style="list-style-type: none"><li>• Carbapenem/ colistin-resistant <i>Enterobacteriaceae</i></li><li>• Antibiotic-resistant <i>Neisseria gonorrhoeae</i></li><li>• Carbapenem-resistant <i>Acinetobacter baumannii</i></li><li>• <i>Bordetella pertussis</i></li><li>• HIV-transmitted drug resistance</li><li>• <i>Streptococcus pneumoniae</i></li></ul>

**Some postponements  
due to COVID-19  
pandemic**

27 October 2016

### Multi-country Salmonella outbreak



Seven countries have reported human cases of *Salmonella* Enteritidis and 12 October 2016 (112 confirmed and 148 probable).

Cases have been reported by Belgium, Denmark, Luxembourg, France, Germany, Italy, Netherlands, Poland, Portugal, Spain, Sweden, Switzerland, and the United Kingdom.

Print

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# EU Multi-country Foodborne outbreaks

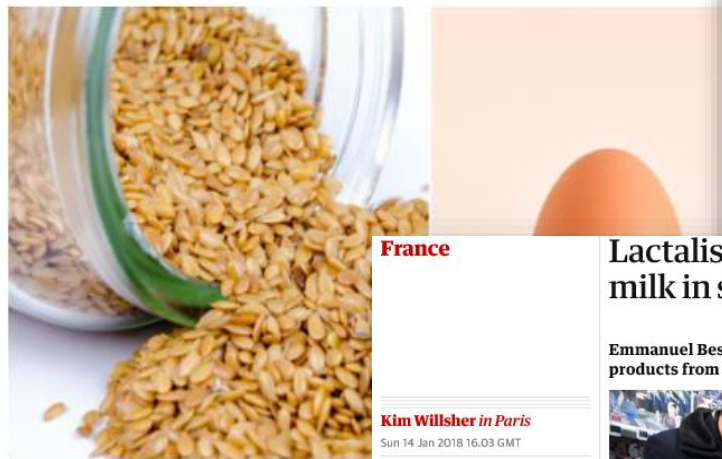


## E. coli cucumber scare: Spain angry at German claims

31 May 2011



## EHEC O104:H4 Outbreak in Germany, 2011



France

## Lactalis to withdraw 12m boxes of baby milk in salmonella scandal

Emmanuel Besnier, chief executive of French dairy giant Lactalis, says products from contaminated factory will be recalled

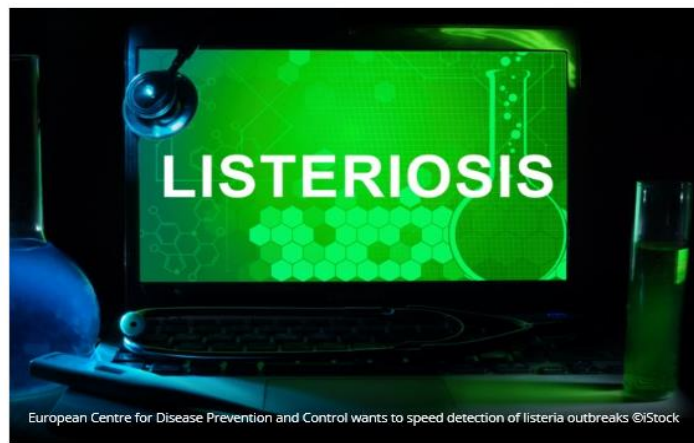


A French government official checks baby milk products in a pharmacy in Orléans. Photo: AFP/Getty Images

The head of a French dairy giant at the centre of an international salmonella scandal has promised to withdraw 12m boxes of powdered milk from the supermarket shelves of 83 countries.

## 'Most outbreaks remain undetected': How the EU is strengthening listeriosis surveillance

By Katy Askew 22-Aug-2018 - Last updated on 22-Aug-2018 at 14:16 GMT



European Centre for Disease Prevention and Control wants to speed detection of listeria outbreaks ©iStock

## Swedes get hepatitis A from eating infected frozen strawberries



File photo of frozen strawberries. Photo: Bertil Enevåg Ericson/TT

Swedes have been reminded not to eat frozen strawberries without properly heating them up first, after 13 people contracted hepatitis A from imported fruit.

## EFSA and ECDC report on salmonella outbreak in egg, sesame seed

By Joe Whitworth on September 26, 2018

More detail has emerged about two parallel outbreaks in Europe.

One was caused by *Salmonella* Enteritidis in Poland and the other by a previously unreported *Salmonella* strain in imported sesame seeds.

Kim Willsher in Paris

Sun 14 Jan 2018 16:03 GMT

1,184 views

This article is over 9 months old

# Joint Molecular Typing for Enhanced Surveillance



STEC O104:H4  
outbreak

EC vision on molecular  
typing support for  
outbreak preparedness

EC request to ECDC and EFSA  
on establishment of molecular  
typing databases

Joint ECDC-EFSA Steering  
Committee for a joint  
molecular typing database

EC request to ECDC and EFSA to  
upgrade joint molecular typing  
database with WGS

Start of WGS-enhanced surveillance  
of listeriosis in EU/EEA

2011 2012 2013 2014 2015 2016 2017 2018 2019 2020

ECDC pilot molecular  
typing data collection  
PFGE and MLVA

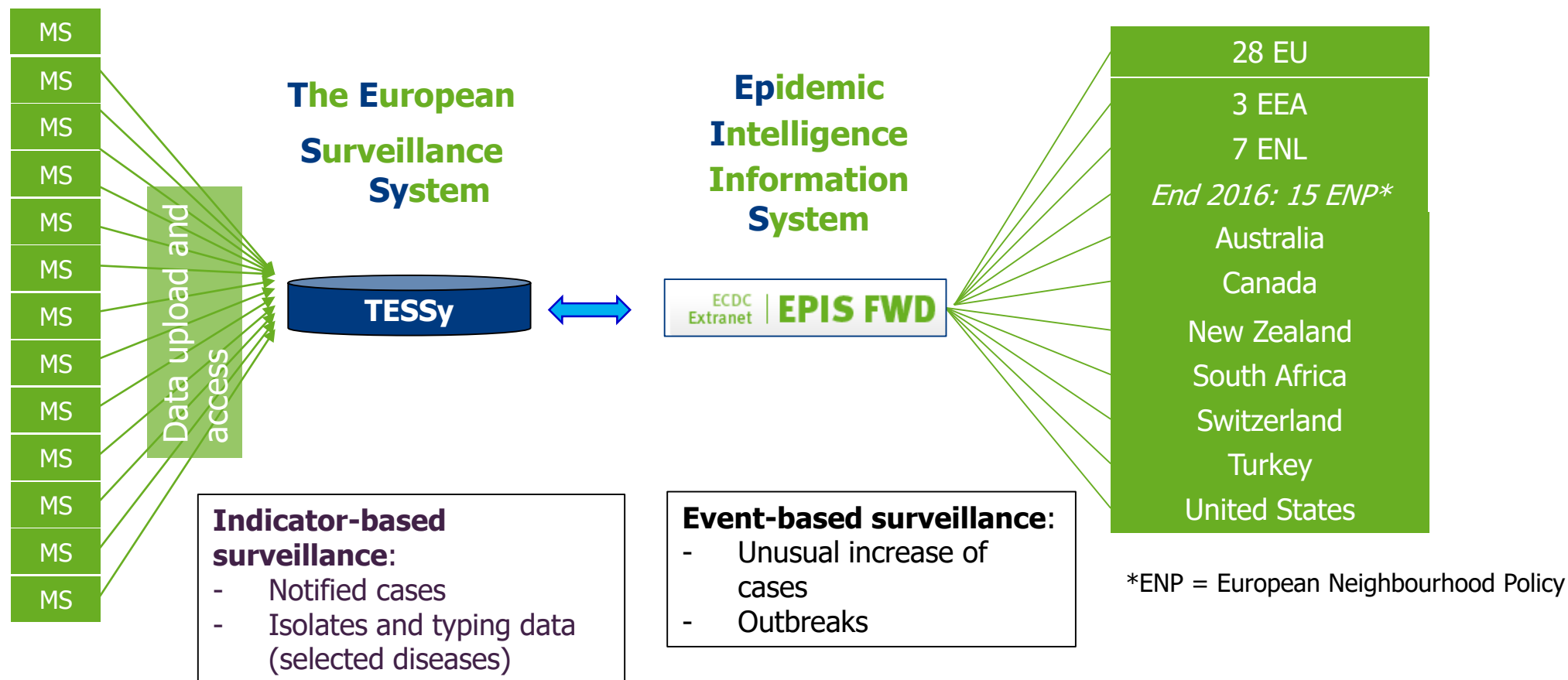
Pilot study WGS *Listeria*

EU-wide validation study  
*Listeria* WGS

1st WGS support to multi-country  
outbreak investigation

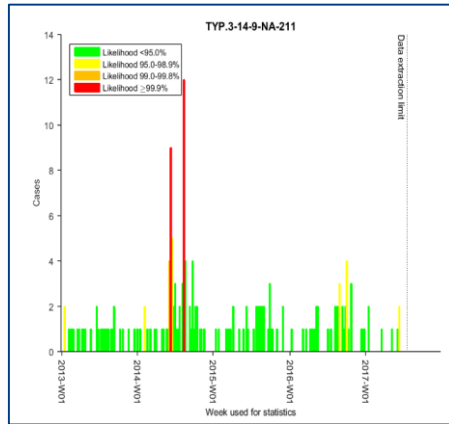


# EU surveillance of foodborne diseases



# WGS Improved Signal Detection and Response to Multi-Country Foodborne Outbreaks

MLVA<sup>1</sup> + PFGE<sup>2</sup> +cgMLST<sup>3</sup>



Weekly cluster reports

Clusters of rare/new genotype <sup>†</sup>					
MLVA	Pathogen	Countries	Size and events	Method	Pattern
0101_021	Salmonella enteritidis	UK, NL	6	PFGE	AGC2015_04620H
0101_041	Salmonella enteritidis	DK, NL, NO, UK	8	MLVA	EW7.3-6-4-1
0101_041	Salmonella enteritidis	DK, NL, NO, UK	20	MLVA	EW7.3-10-9-2
0101_041	Salmonella enteritidis	DK, NO, UK	6	MLVA	EW7.3-10-4-1
0101_041	Salmonella enteritidis	DK, UK	2	MLVA	EW7.3-10-8-0
0101_041	Salmonella enteritidis	DK, NO, UK	10	MLVA	EW7.3-10-8-2
0101_041	Salmonella enteritidis	DK, UK	2	MLVA	EW7.3-10-7-2
0101_041	Salmonella enteritidis	DK, NO	6	MLVA	EW7.3-10-4-0
0101_041	Salmonella enteritidis	NO, UK	2	MLVA	EW7.3-10-7-2
0101_041	Salmonella enteritidis	DK, UK	1	MLVA	EW7.3-10-4-0
0101_041	Salmonella enteritidis	NO, UK	1	MLVA	EW7.3-10-10-1

Clusters of common genotype <sup>††</sup>					
MLVA	Pathogen	Countries	Size and events	Method	Pattern
0101_041	Salmonella Tomivormum	DK, IE, NL, SE	15	MLVA	TRP.2-9-9-16-211
0101_041	Salmonella enteritidis	DK, LU, NL, NO, UK	151	MLVA	EW7.3-10-4-2
0101_041	Salmonella enteritidis	DK, NL, NO, UK	223	MLVA	EW7.3-10-9-7
0101_041	Salmonella enteritidis	DK, LU, NL, NO, UK	102	MLVA	EW7.3-6-4-1
0101_041	Salmonella enteritidis	DK, LU, NL, NO, UK	35	MLVA	EW7.3-10-7-2
0101_041	Salmonella Tomivormum	DK, LU, NL, SE	17	MLVA	TRP.2-9-9-16-211

Small clusters of common genotype <sup>†††</sup>					
MLVA	Pathogen	Countries	Size and events	Method	Pattern
0101_041	Salmonella Tomivormum	NL, SE, UK	4	MLVA	TRP.2-10-10-211
0101_041	Salmonella Tomivormum	NL, NO, SE, UK	8	MLVA	TRP.2-10-10-211
0101_041	Salmonella Tomivormum	DK, NL, SE	3	MLVA	TRP.2-10-10-211
0101_041	Salmonella Tomivormum	DK, SE	4	MLVA	TRP.2-10-10-211
0101_041	Salmonella Tomivormum	NL, SE	2	MLVA	TRP.2-9-9-16-211

Endemic clusters <sup>††††</sup>					
MLVA	Pathogen	Countries	Size and events	Method	Pattern
0101_041	Salmonella Tomivormum	DK, FI, IE, LU, NL, NO, SE, UK	75	MLVA	TRP.2-9-9-16-211
0101_041	Salmonella Tomivormum	DK, FI, IE, LU, NL, NO, SE, UK	1079	MLVA	TRP.2-10-10-211
0101_041	Salmonella Tomivormum	DK, FI, IE, LU, NL, NO, SE, UK	393	MLVA	TRP.2-9-9-16-211
0101_041	Salmonella Tomivormum	SE, UK	1	MLVA	TRP.2-10-10-211
0101_041	Salmonella Tomivormum	DK, FI, IE, LU, NL, NO, SE, UK	124	MLVA	TRP.2-10-10-211
0101_041	Salmonella Tomivormum	DK, FI, IE, LU, NL, NO, SE, UK	122	MLVA	TRP.2-10-10-211
0101_041	Salmonella Tomivormum	DK, FI, IE, LU, NL, NO, SE, UK	61	MLVA	TRP.2-10-10-211

ECDC Extranet | EPIS FWD

**Urgent Inquiry:**  
unusual increase of cases at national level

Joint ECDC-EFSA Rapid Outbreak Assessments

**Whole genome sequencing support since 2015**



**Conclusions and options for response**

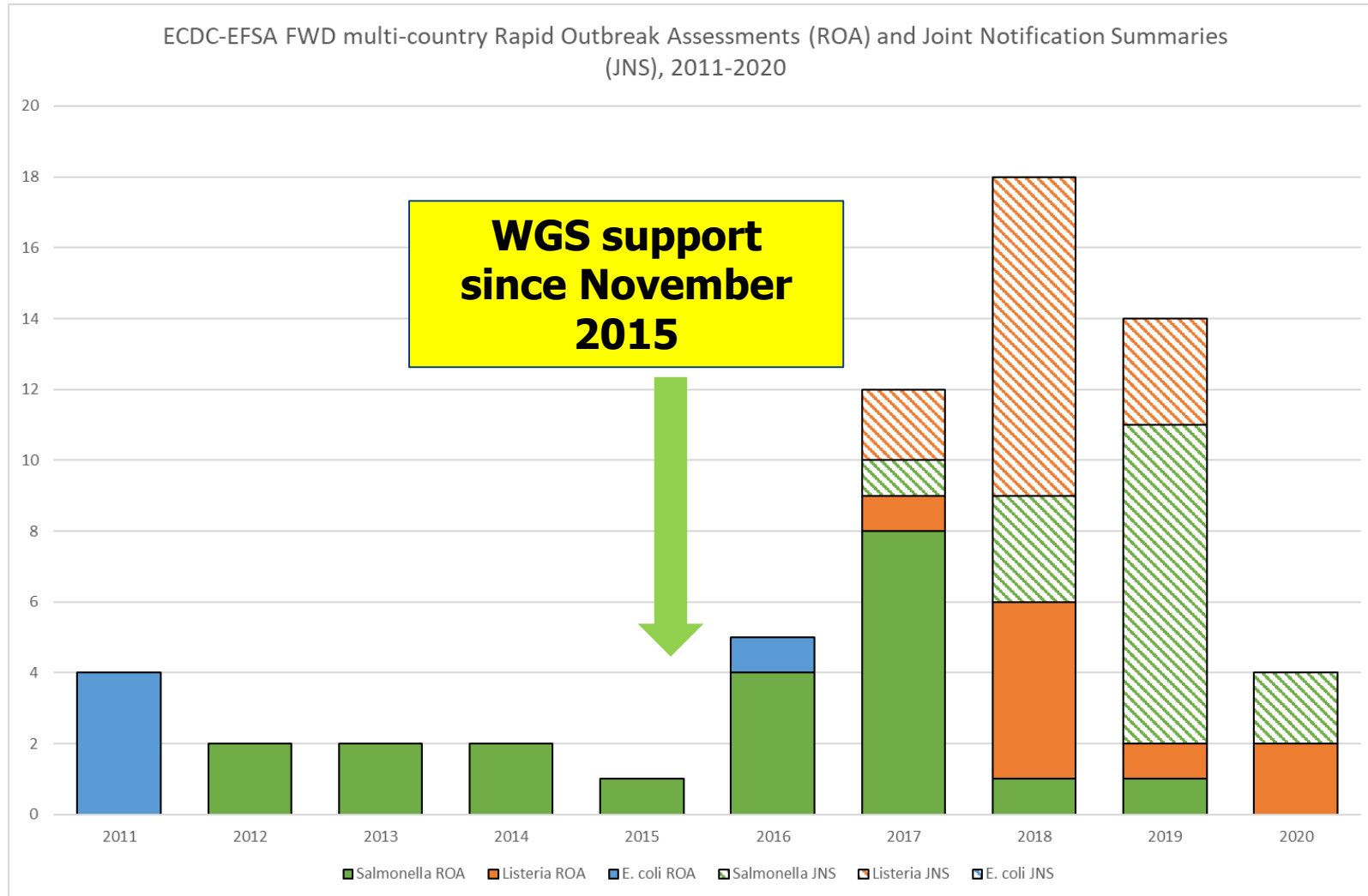
A multi-country outbreak of *Salmonella* Enteritidis phage type (PT) B with multiple human variable-number tandem repeat (VNTR) profiles (2-9-7-3-2 and 2-9-6-3-2) related to cases in Sweden, Denmark, Luxembourg, the Netherlands, Norway, Sweden and the United Kingdom. None of the identified cases are associated with a travel history to Norway or Finland, both of which countries are also considered to be affected by the outbreak. In addition, Croatia reported a cluster of *S. enteritidis* cases, including a fatal case, with an epidemiology fit to the outbreak. The characterisation of the Croatian isolates is currently ongoing.

The food safety authorities in Belgium, Croatia, the Netherlands and Sweden have initiated and implemented national and local investigations to identify the source of the outbreak. Since *S. enteritidis* is closely associated with chicken, the investigation network, across other food items, as the source of meat and poultry, is being investigated. In Belgium, the source of the outbreak, since *S. enteritidis* is closely associated with chicken, the investigation network, across other food items, as the source of meat and poultry, is being investigated. In Denmark, there is a cluster of food establishments and at least a retail case in Denmark involved in supplying fresh poultry carcasses to Finland. Additionally, the cases in the outbreak had consumed eggs originating from poultry contact in Finland. Additional sampling of eggs intended for direct human consumption originating from the implicated poultry contact in Finland was performed on the Netherlands and confirmed eggs originating from poultry contact in Finland. Additional sampling of eggs intended for direct human consumption originating from the implicated poultry contact in Finland was performed on the Netherlands and confirmed eggs originating from poultry contact in Finland. Additional sampling of eggs intended for direct human consumption originating from the implicated poultry contact in Finland was performed on the Netherlands and confirmed eggs originating from poultry contact in Finland.

The available evidence from VNTR, food and environmental investigations, as well as from tracing back investigations of eggs, establishes a link between the multi-country outbreak and the poultry contact in Finland, pointing to eggs as the most likely vehicle of infection for at least part of the outbreak cases. Additional information from epidemiological, food and VNTR investigations might bring further evidence on the possible vehicles and sources of infection associated with this outbreak. The molecular typing of isolates during

<sup>1</sup>MLVA=Multi-Locus Variable number tandem repeat Analysis  
<sup>2</sup>PFGE=Pulsed-field gel electrophoresis  
<sup>3</sup>cgMLST=core genome Multi-Locus Sequence Typing

# ECDC-EFSA multi-country outbreak assessments and notifications for foodborne diseases, 2011 – 2020



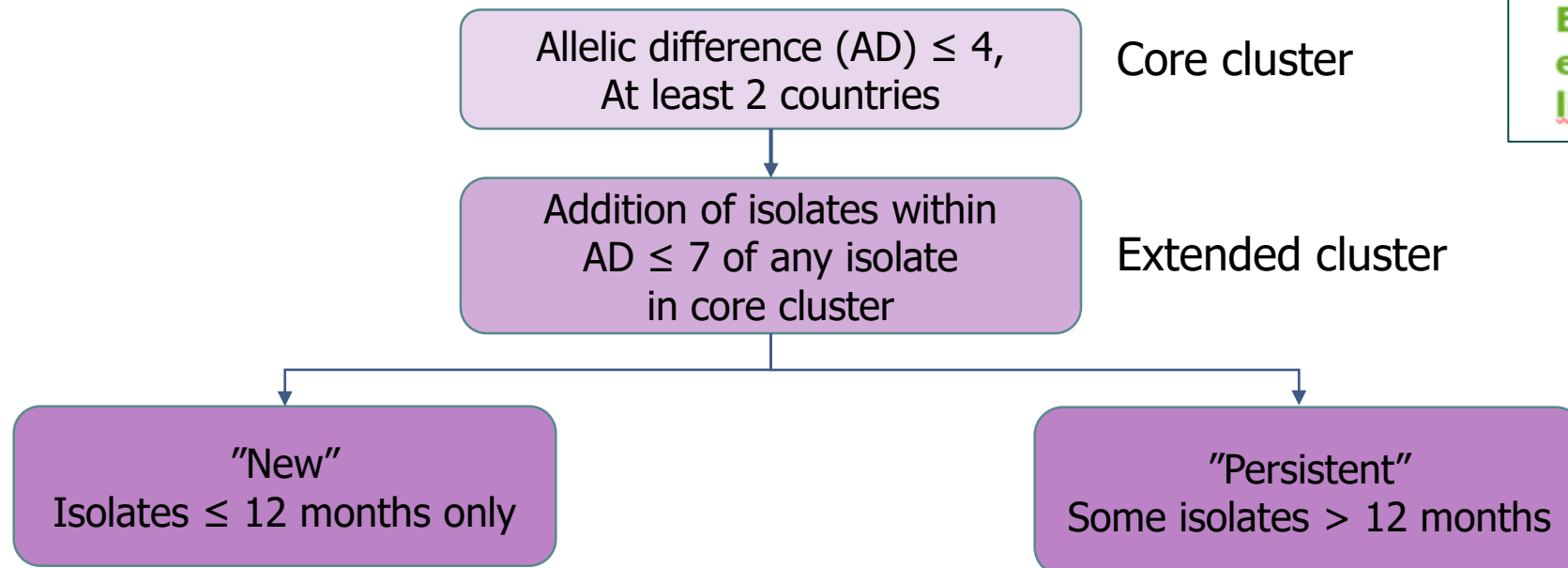
\*As of September 2020

# Implementing WGS for surveillance of listeriosis

## Objective:

**Early detection** and **delineation** of multi-country listeriosis outbreaks and/or **dispersed clusters** to trigger **outbreak investigations** and contribute to **trace back and forward** investigations so that appropriate **control measures** can be implemented in the food chain

- Real-time reporting of human *Listeria monocytogenes* (*Lm*) sequences to ECDC
- Analytical pipeline: core genome MLST (cgMLST)
- Weekly analysis for signals of a multi-country event:



ECDC TECHNICAL DOCUMENT (DRAFT for MSs consultation)

**EU protocol for the use of WGS and exposure data for the surveillance of listeriosis in EU/EEA**



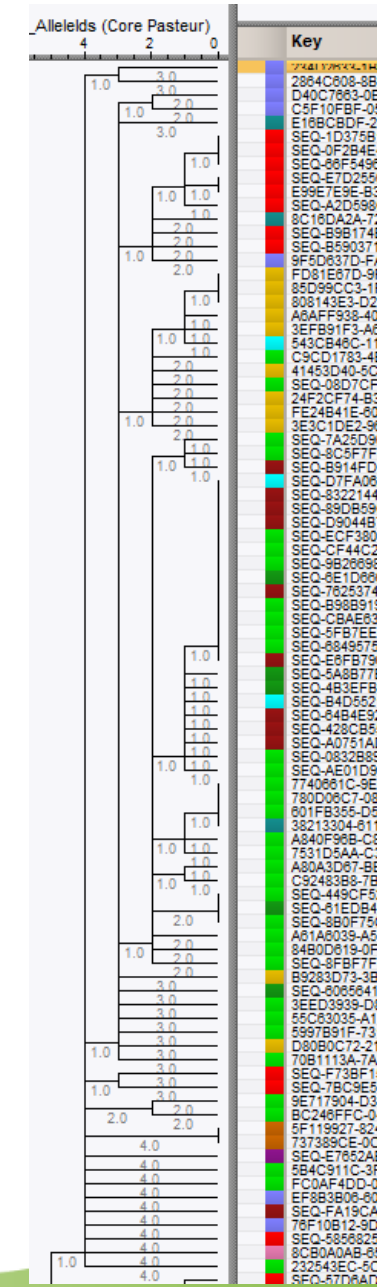
Standard questionnaire to be developed with MSs

# Key statistics from listeriosis cluster detection, March 2019 – August 2020

Number of countries submitting sequences	16
Number of prospective* isolates submitted (range per country)	888 (1-452)
Number of multi-country clusters detected	30



	Core cluster (within 4 cg-AD)	Extended cluster (within 7 cg-AD)
Number of countries involved in clusters	2 (2-10)	3 (2-14)
Median number of isolates (range)	4 (2-48)	4.5 (2-149)
Median duration in years (range)	4.4 (0-15.6)	
Clusters escalated to urgent inquiry	2	



\*date used for statistics in March 2019 onwards

# Lessons learnt from start of listeriosis WGS-enhanced surveillance



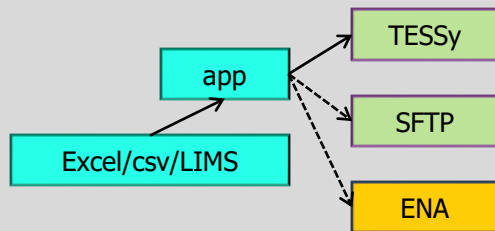
- Only few countries submitting data “in real-time” so far
  - Incentive with better visualisation tools and facilitated submissions?
  - Concerns with data sharing?
- Several WGS-confirmed genetically close strains persisting in the EU for years, even decades
  - Likely multi-source
  - Need to investigate by sub-cluster with more non-human isolate sequence data
- WGS technology has enabled seeing “the bigger picture”, not only single source outbreaks
- More epidemiological data needed
- Good collaboration and timely sharing of data between public health and food safety authorities is crucial

# Options for WGS data upload

## ECDC WGS upload application

### Features

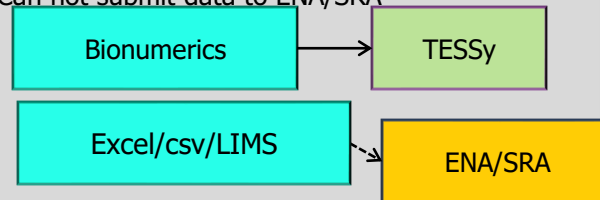
- Can be configured to import data from databases or local files (MySQL, SQL Server, SQLite, Excel, csv)
- Configure only once, single click upload
- Can upload assemblies to TESSy and SFTP, raw reads to SFTP and ENA (configurable)
- Data sharing through SFTP and ENA



## ECDC Bionumerics client plugin

### Features

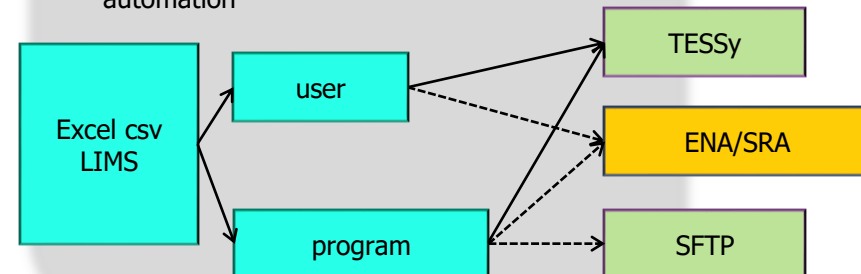
- Requires Bionumerics and that either ENA/SRA run accession or assemblies are stored in the Bionumerics database
- Simple upload process
- Can upload assemblies and ENA/SRA identifiers to TESSy
- Can not share raw reads through SFTP
- Can not submit data to ENA/SRA



## Direct TESSy submission – manual or machine-to-machine

### Features

- Can upload assemblies and ENA/SRA identifiers to TESSy
- Manual upload is easy to set up but involves recurring manual work
- Machine-to-machine upload requires development but enables high levels of automation



# User-defined sharing principles



Molecular  
Typing

Prototype version 0.17  
Powered by [MicroReact](#)

Listeria monocytogenes

Neisseria meningitidis

Visualisation

Molecular clusters/datasets

My data

Settings

Choose which data to share with other users by default:

- Assembly
- Allele identifiers

In addition to these data, epidemiological and descriptive data submitted to TESSy (except your national isolate identifier), and WGS-derived data

Save settings



# Way forward

- Currently main focus on COVID-19 pandemic/SARS-CoV-2 sequencing
- Continue implementation of WGS for diseases previously postponed by pandemic
- Interoperable WGS analyses with EFSA for joint FWD investigations
- Listeriosis data collection and cross-sectoral collaboration



# Thank you