



Confirmatory testing and false positive *Salmonella* results in flocks of layers and breeding hens

Whole genome sequencing

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FOOD HYGIENE, FRAUD and FEED

Outline

- Confirmatory testing – false positive results?
- Whole genome sequencing in foodborne outbreak investigations

Confirmatory testing – false
positive results?



European
Commission

Background confirmatory testing in flocks of laying and breeding hens

- Control of *Salmonella* is based on sampling by food business operator
- If positive, confirmatory testing by the competent authorities is only allowed exceptionally e.g. in case of suspicion of “false positive results”. The European Commission has opposed routine confirmatory testing
- Strict trade restrictions apply in case of detection of *Salmonella* Enteritidis or *S. Typhimurium* (after initial positive sampling by operator)
- Court cases occurred in the Netherlands where the competent authority refused to carry out confirmatory testing and applied strict measures although the initial positive results were not confirmed (lost by the authorities)



How to explain “false positive results”?

- Cross-contamination at sampling or in laboratory?
- Transient infection in flocks?
- Detection while not in sample?
- Confirmatory testing not sufficiently sensitive?

Way out?

- Field studies accepted in Belgium and the Netherlands where repeated confirmatory testing will be carried out by the competent authorities after initial positive testing.
 1. Additional samples
(faecal and dust; dedicated sampler)
 2. Repeated sampling (max 3)
(2 successive samplings with interval of 14 days)
 3. Sampling all flocks present on the farm
 4. Testing before slaughter
(caeca samples 300 chickens)
 5. Additional extra random sampling negative farms

Some preliminary results from the Netherlands (9 months)

- 52 houses tested initially positive (SE or STM), 19 agreed to participate in trial
- Reasons not to participate: first detection by official sampling, sent to slaughter, eggs for industry, already second positive sample
- From the 19 houses, in 5 *Salmonella* was never confirmed

SE outbreak in Sweden in 2023

Barn 3A

Sample Date	Samples Taken	Samples Positive	Analysis
23-08-17	4 sock + 2 dust	None	Culture
23-08-18	3 sock + 3 manure conveyor	3 sock + 2 manure conveyor	Culture
23-08-21	4 sock + 2 dust	None	Culture
23-08-24	4 sock + 2 dust	4 sock	Culture

Barn 6B

Sample Date	Samples Taken	Samples Positive	Analysis
23-08-05	1 sock + 1 dust	1 sock	Culture
23-08-08	1 sock + 1 dust	None	Culture
23-08-10	1 sock + 1 dust	None	Culture
23-08-17	3 sock + 3 manure conveyor	1 sock	Culture

Barn 1B

Sample Date	Samples Taken	Samples Positive	Analysis
23-08-31	4 sock + 2 dust	1 sock	Culture
23-09-01	4 sock + 2 dust	None	PCR
23-09-02	4 sock + 2 dust	None	PCR
23-09-03	4 sock + 2 dust	1 dust	PCR, confirmed by culture
23-09-04	4 sock + 2 dust	None	PCR
23-09-05	4 sock + 2 dust	None	Culture
23-09-06	4 sock + 2 dust	None	Culture
23-09-07	4 sock + 2 dust	None	Culture
23-09-08	4 sock + 2 dust	None	Culture

Next steps

- Expected that the study will take until end of 2025
- EFSA will be requested to evaluate the outcome (2026?)
- Possible changes in legislation only considered after EFSA opinion

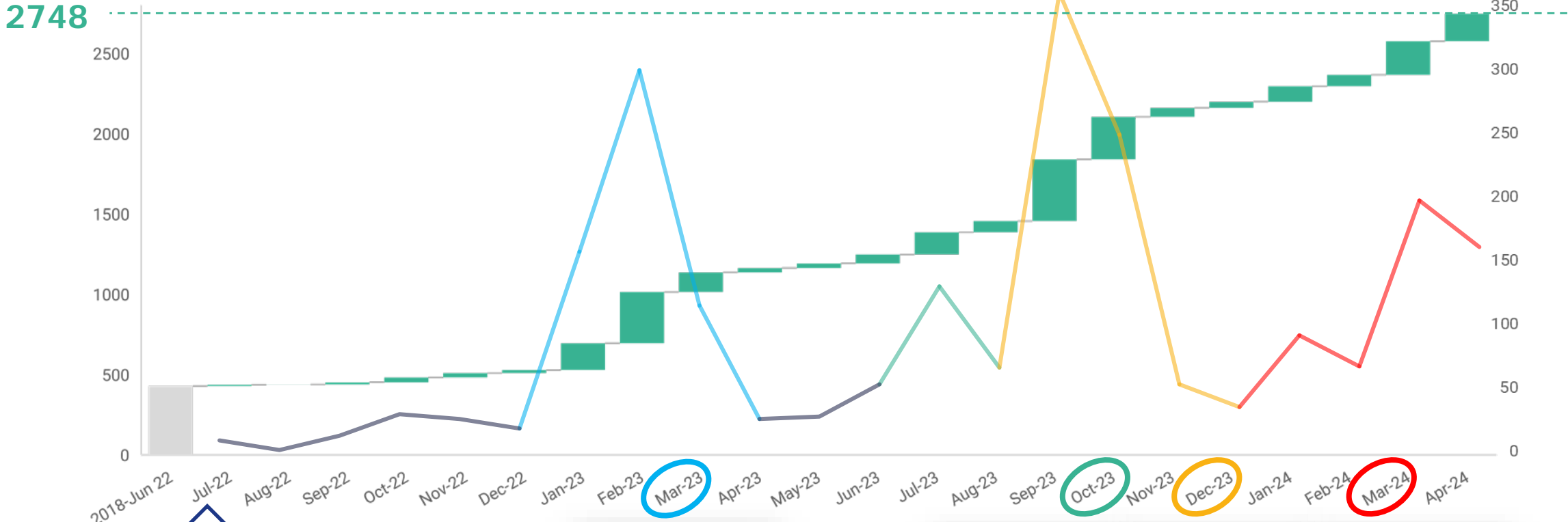


Whole genome sequencing in foodborne outbreak investigations

Background

- Article 8(2) of Directive 2003/99/EC requires (food safety) competent authorities to investigate food-borne outbreaks in cooperation with public health competent authorities;
- Whole genome sequencing (WGS) facilitates greatly the swift identification of causes of an outbreak and the batches, lots or consignments of unsafe food since it enables to establish links between different isolates recovered from humans, food, animals, feed and the related environment
- EFSA has developed a molecular typing system (EFSA One Health WGS System) for the collection of WGS data of isolates of *Salmonella enterica*, *Listeria monocytogenes* and *Escherichia coli* recovered from food, animals, feed and related environment. Addition of *Campylobacter* under development. ECDC has developed and integrated a Molecular Typing system in EpiPulse for the collection of WGS and epidemiological data of isolates recovered from patients.

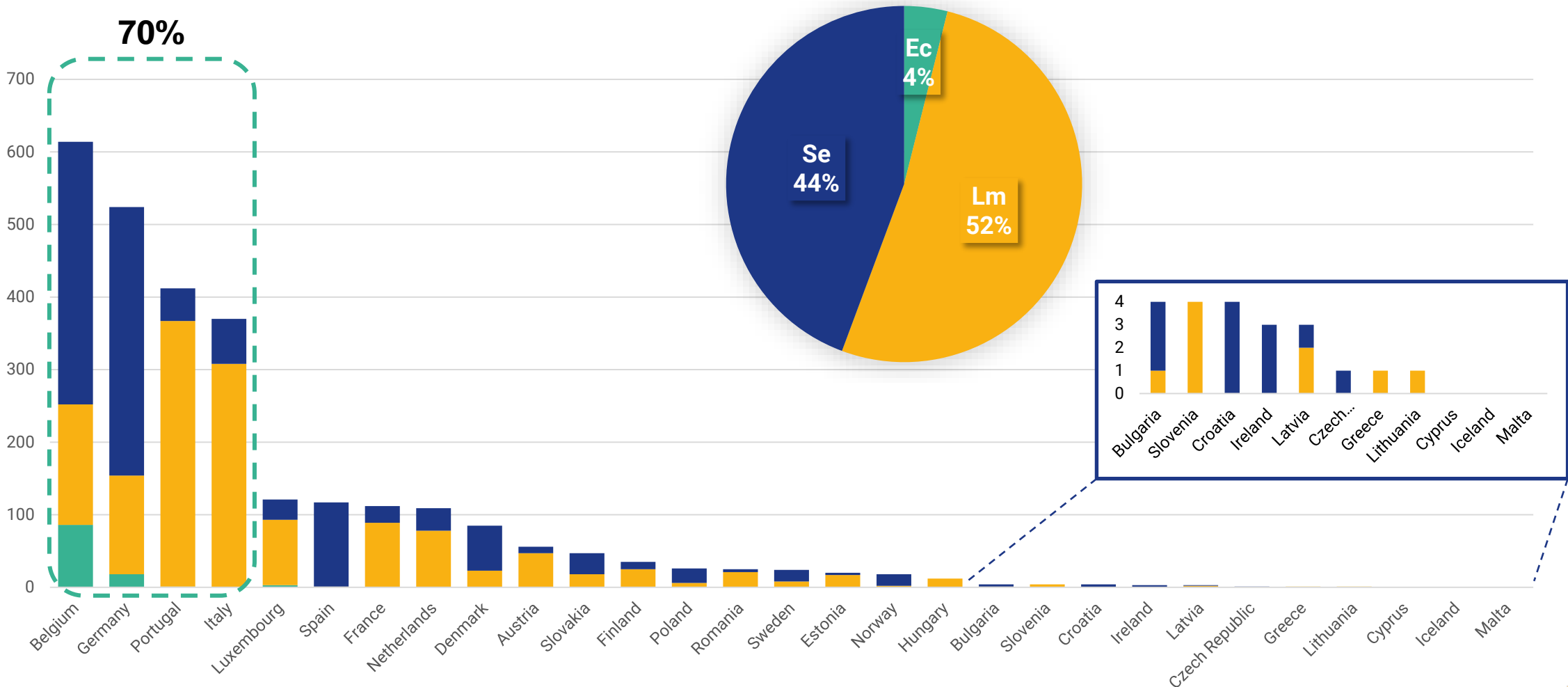
MEMBER STATES CONTRIBUTION TO EFSA DATABASE



Start data collection



MEMBER STATES CONTRIBUTION TO EFSA DATABASE



Proposal from the Commission

- Mandatory WGS analysis and reporting to EFSA of at least one (pre-screened) isolate from sampling of food, animals, feed or related environment within the frame of (a suspicion of) a foodborne outbreak;
- Outbreaks of *Salmonella enterica*, *Campylobacter jejuni/coli*, *Listeria monocytogenes* and *Escherichia coli*;
- Number of reported *Salmonella* outbreaks in 2022:
 - Total: 1014
 - Strong-evidence*: 200
- Questions circulated to NRLs: cost, experience capacity, ...
- Proposed to become applicable from early 2026 on
- Final decision (vote) expected in Autumn 2024

*: see <https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2014.3598>

Thank you



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