

Results of the first pilot run of *Salmonella* spp WGS in Lithuanian public health surveillance laboratory

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Introduction

Salmonella bacteria are one of the main causes of food and waterborne diseases (FWD) in Lithuania. Phenotypic serotyping has been used in suspected outbreaks of *Salmonella* spp. in current time. The implementation and development of sequencing research can be used for deeper outbreak investigations and may enable the detection of cross-border *Salmonella* spp. spread. The pilot study was conducted with European funding.

Objectives

The aim of the research was to suspect possible outbreaks in different geographical locations and different time periods after performing the sequencing. *Salmonella* strains were selected by epidemiologists from sent to NPHSL for serotyping.

Materials and methods

- Archived cultures were recovered using the Tryptic Soy media (TSA).
- DNA extraction was performed using the EZ1&2 Virus Mini Kit v2.0 (Qiagen).
- Sequencing library preparation was performed with the Nextera XT Library Prep Kit (Illumina).
- Sequencing was performed using a MiSeqDx sequencer with the MiSeq Reagent Kit v2 (500 cycles) (Illumina).
- Sequencing results were evaluated using the SeqSphere program and with *S. enterica* cgMLST v2 scheme from Enterobase (3002 loci).
- Clusters were determined using core-genome multi-locus sequence typing. Isolates with fewer than 5 allelic differences (AD) were considered to belong to the same cluster.

Selection of isolates for the first pilot run

- 48 *Salmonella* spp. clinical isolates were selected;
- Most of the selected isolates for sequencing were *S. Typhimurium* and *S. enteritidis* serotypes to search for the presumptive clusters;
- The objective was to detect AMR genes and to compare them with phenotypic results.

Salmonella serotype	Total	Fully sensitive	Resistance ≥1 antimicrobial	Source
Chartres II	2	0	2	Urine
Derby	2	0	2	Feaces
Nakure	1	0	1	Blood
Paratyphi C1	1	0	1	Feaces
Senftenberg	2	1	1	Feaces
Typhimurium	10	0	10	Feaces
monophasic Typhimurium	9	0	9	Feaces
Enteritidis	21	21	0	Feaces
TOTAL:	48			

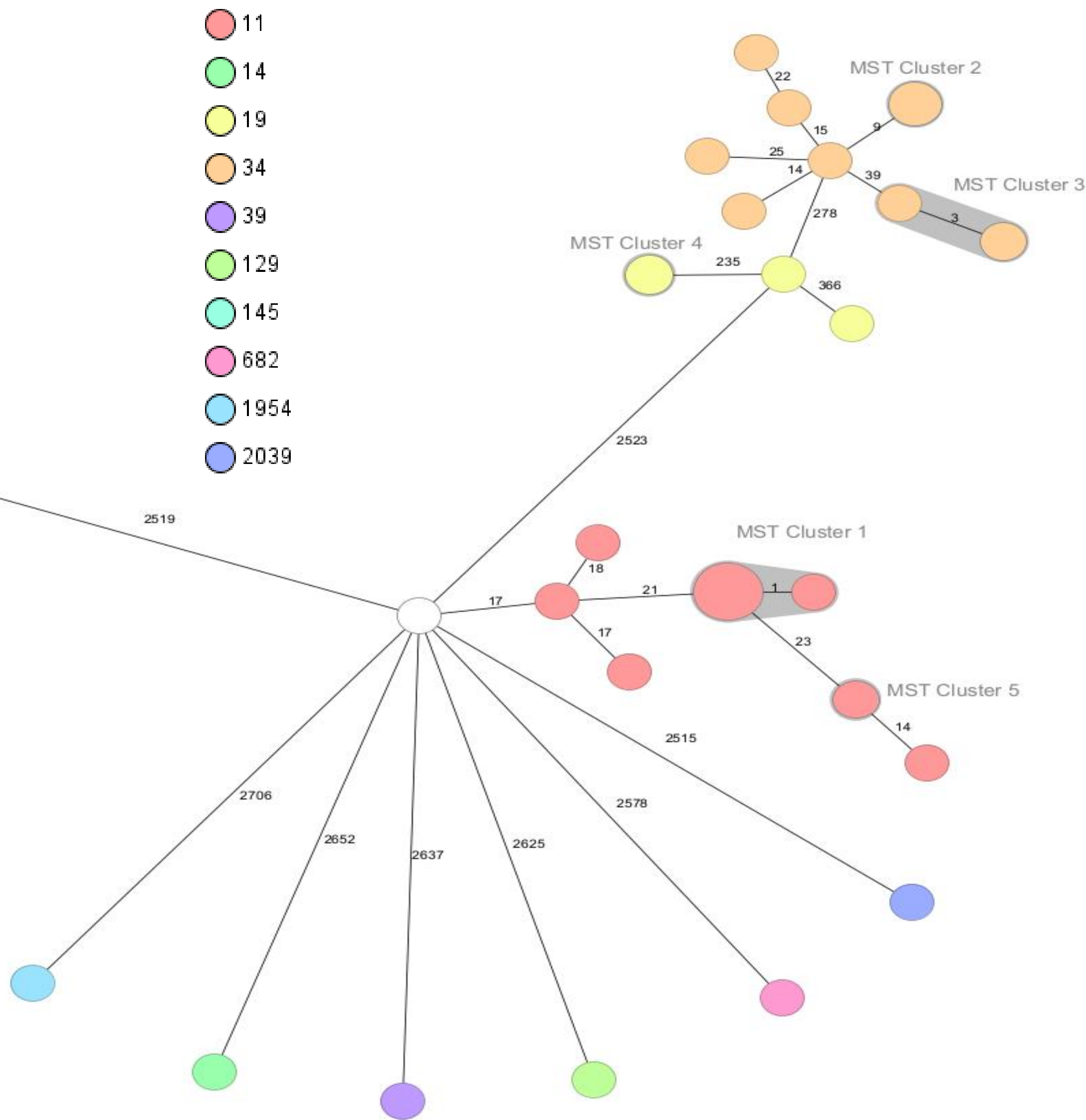
Cluster analysis

Cluster analysis was performed using *S. enterica* cgMLST v2 scheme from Enterobase (3002 loci), with a cluster threshold of 5 AD.

5 clusters were detected:

- 14 - Enteritidis isolates (ST 11);
- 4 - I 1,4,[5],12:i:- isolates (ST 34);
- 3 - I 1,4,[5],12:i:- isolates (ST 34);
- 3 - Typhimurium isolates (ST 19);
- 2 - Enteritidis isolates (ST 11).

The biggest one was *Salmonella enterica enteritidis* (ST 11, cgMLST 468).



ST11 associated with travel to Turkey

- Sequences of Lithuanian ST11 isolates were shared on EpiPulse;
- Four isolates matched clusters that were related to travel to Turkey;
- Three of the four cases were confirmed to have travelled to Turkey in March 2023.

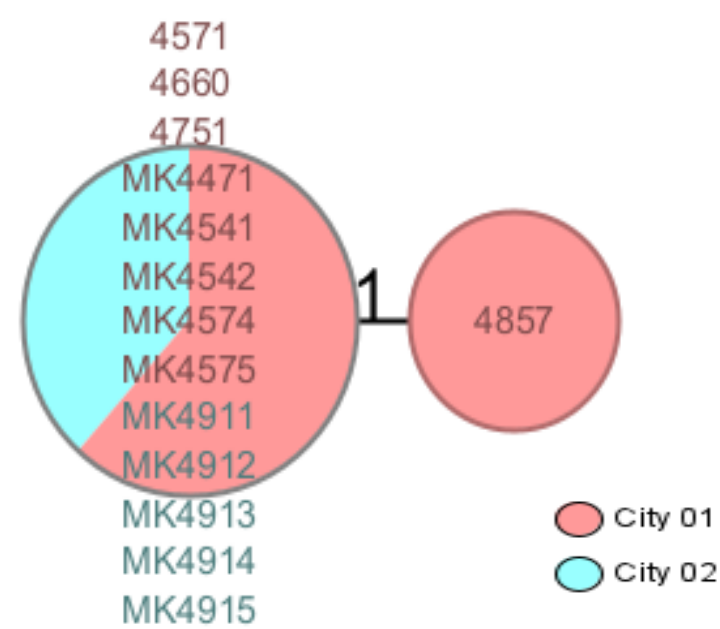
Results

Serovar genotyping results

- Analysis was performed using Ridom Seqsphere+;
- *Salmonella Enteritidis* was the most prevalent serovar from selected isolates.

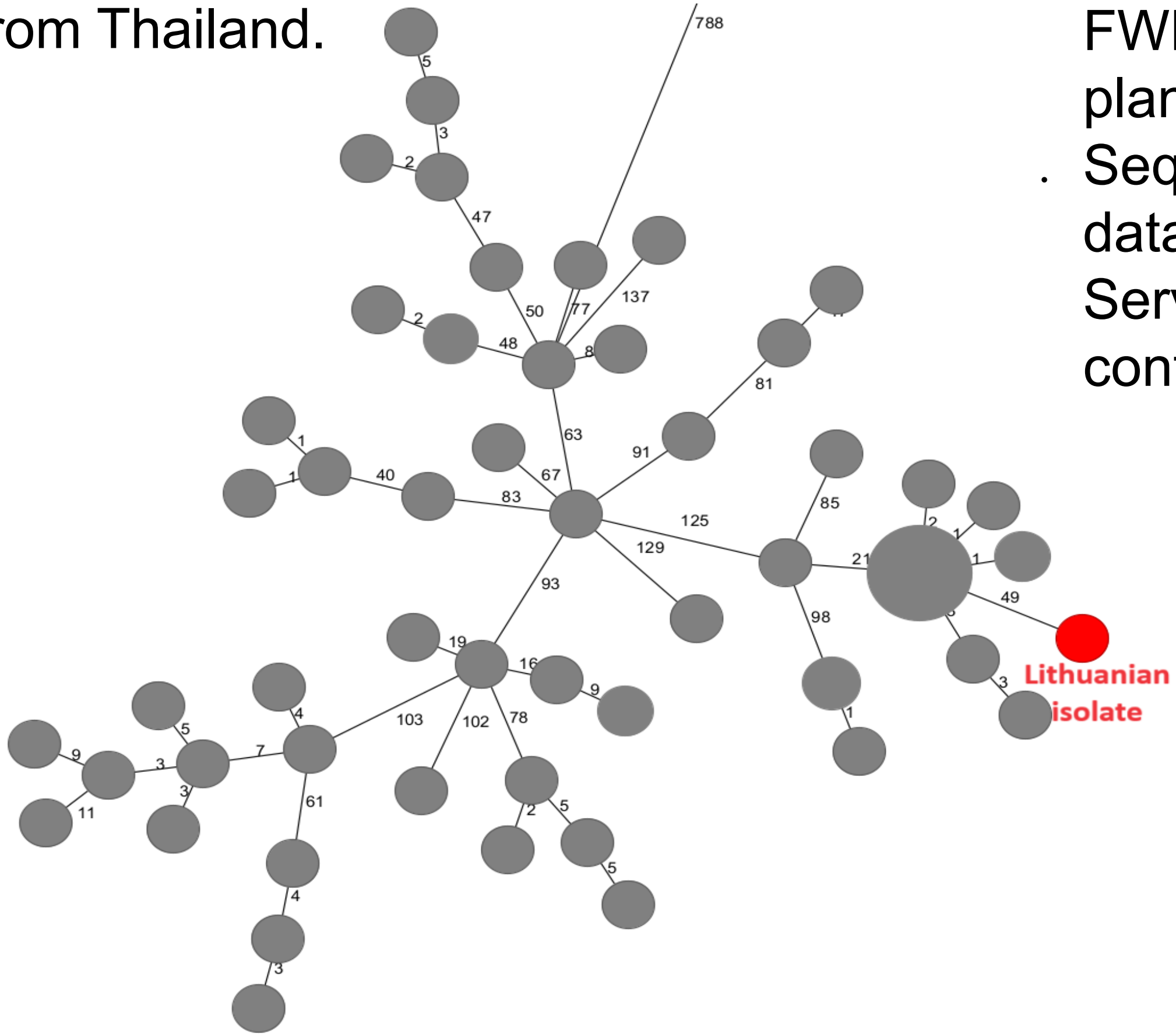
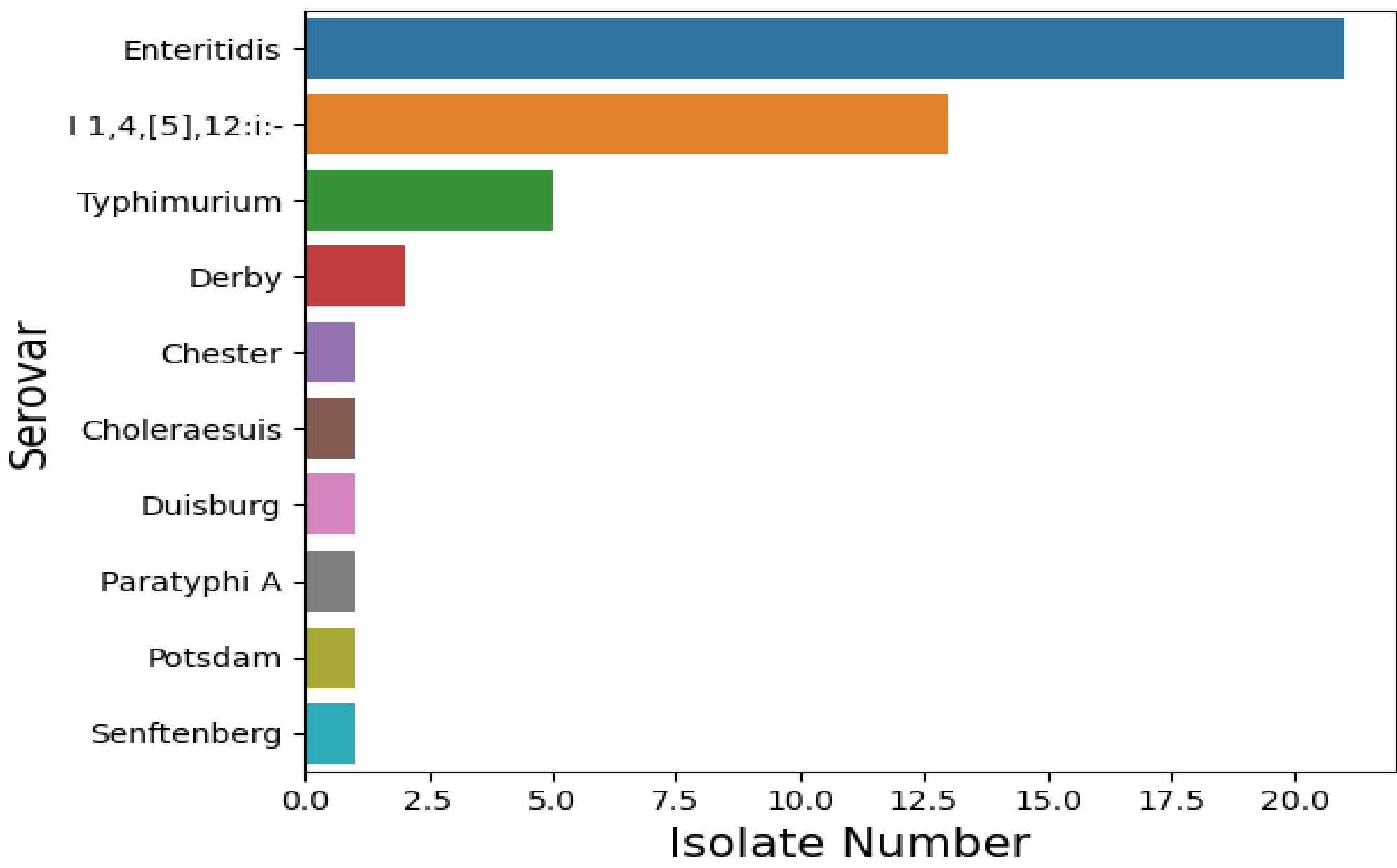
Outbreak investigation

WGS has helped epidemiologists link outbreak isolates from two different cities. The cluster included 13 isolates, with an AD of 0 between different isolates and 1 isolate with an AD of 1.



Choleraesuis isolate

- This is a rare serovar in Europe, known to cause severe disease.
- cgMLST comparison with 90 European *Choleraesuis* sequences retrieved from the European Nucleotide Archive showed a minimum of 49 allelic differences.
- The isolate is believed to be travel-related, possibly from Thailand.



Conclusions

- After implementing the NGS method, an outbreak was identified and confirmed across different geographical locations for the first time, whereas previously it could only be suspected using serotyping methods.
- The resulting data can be published on the EpiPulse platform and used to investigate the cross-border spread of bacteria.
- NGS enabled the detection of a rare serovar in Europe that causes severe disease – *Salmonella Choleraesuis*.
- NGS provides significant knowledge for FWD outbreak investigations and is planned to be performed routinely.
- Sequencing results can be compared with data from the State Food and Veterinary Service to assess potential sources of contamination.

