



FWD AMR·
RefLabCap

STATENS
SERUM
INSTITUT



Added value of WGS for surveillance and outbreak investigations

Eva Litrup

Senior Scientist, Foodborne infections

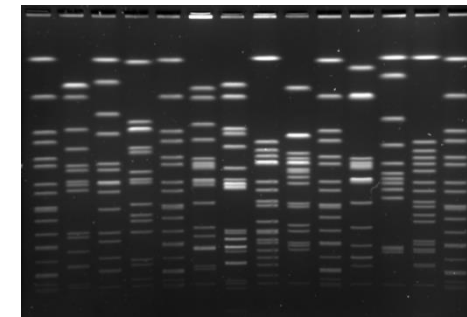
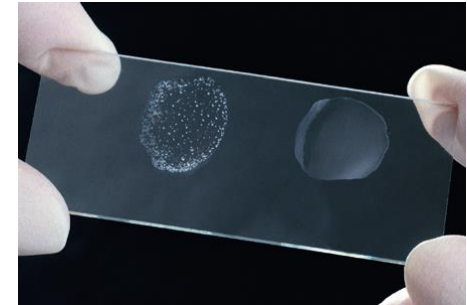
Statens Serum Institut

Copenhagen, Denmark

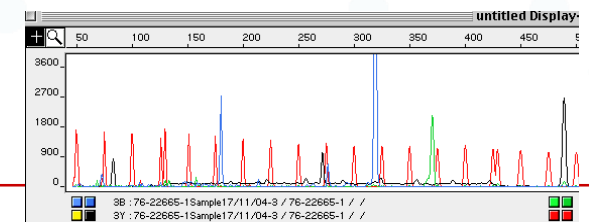
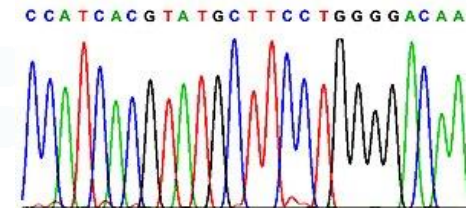
• Real-time typing/characterisation of isolates from patients:

- Antimicrobial resistance
- Subtyping (subspecies, serovars)
- Determine virulence potential
- Detect genetic clusters
- Outbreak investigations
- Linking to sources/reservoirs

- Often many methods used for each isolate, e.g.:
 - Serotyping
 - Virulence factors
 - Antimicrobial resistance
 - High-discriminatory molecular typing methods
 - Highly species/type specific



- Next-generation sequencing technology – why now?
 - Less expensive equipment, easy to use
 - Accessible for more laboratories
 - WGS of pathogens: costs getting competitive to traditional typing
 - Different typing outputs possible by the development of bioinformatic analyses based on WGS



Mix of lab-techniques

*serotyping, antimicrobial-resistance, PCR, hybridization, PFGE, MLVA,
MLST, CRISPR, sequencing...*



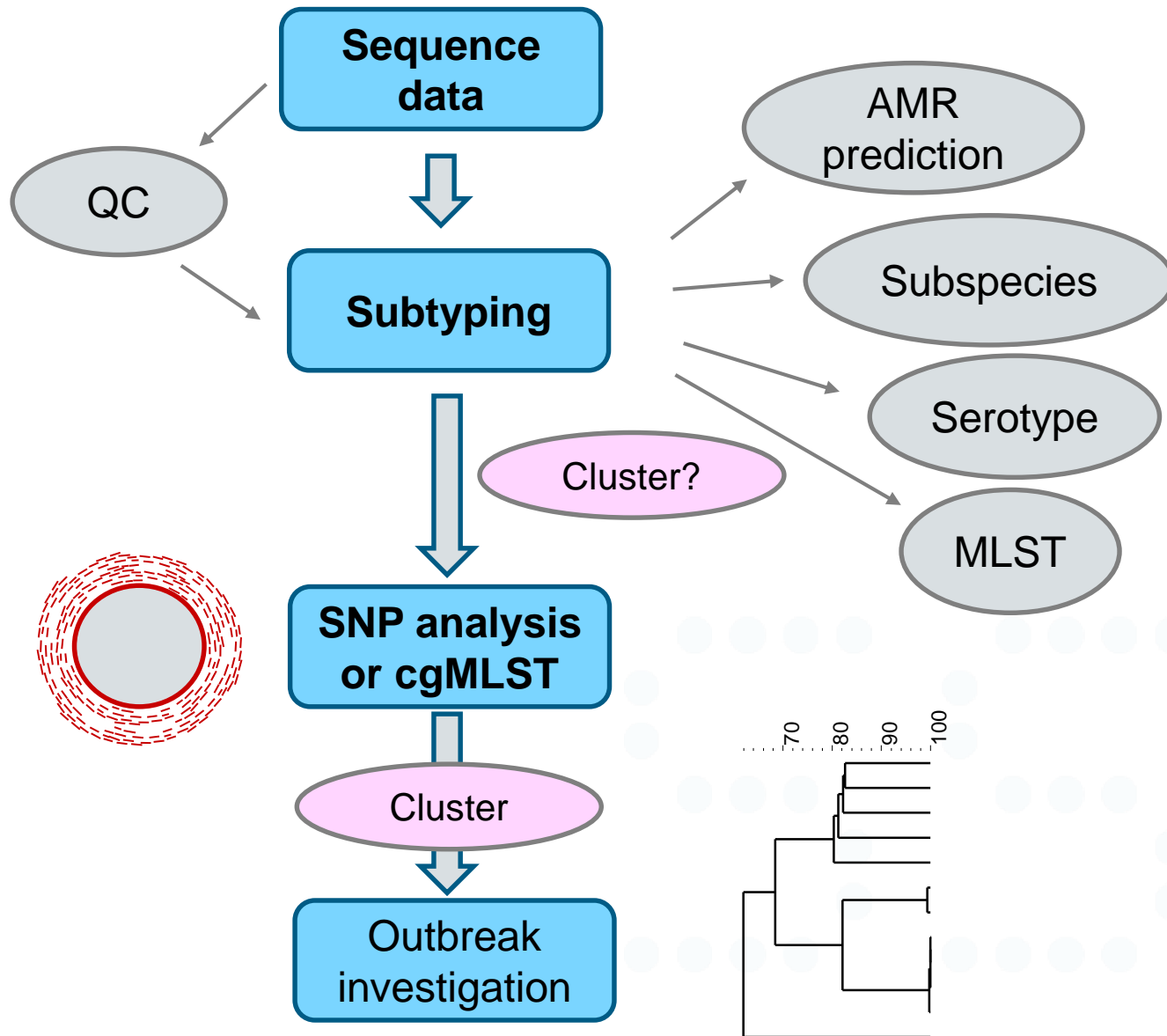
Whole-genome-sequencing

Analysis of sequence data giving many of the same answers

One method to rule them all!

WGS

WGS workflow – routine surveillance





Hospitals: Diagnostics and culturing of isolates



STATENS
SERUM
INSTITUT

Laboratory:

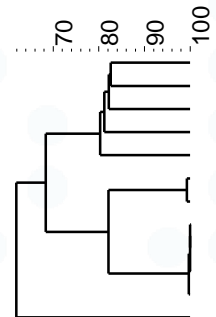
Sequencing of isolates



Linking isolate information and patient metadata
Detection of clusters done using cgMLST

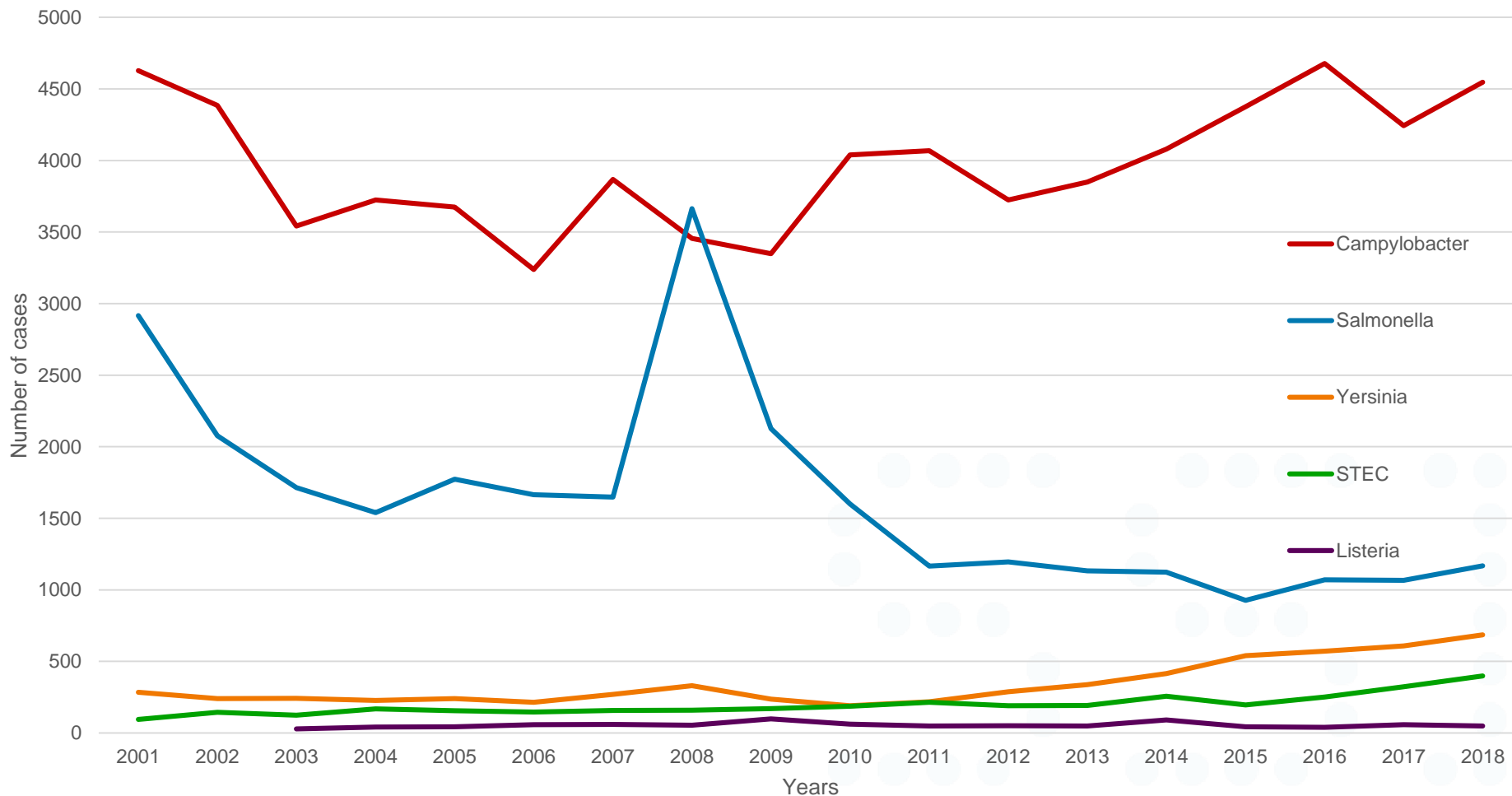
Registration:

Transfer (and cleaning) of cases from the nationwide, automatically updated database of microbiological test results to SSI database



FOODBORNE INFECTIONS

• Population in Denmark 5.8 million



2013

First MiSeq

Hired
Bioinformatician

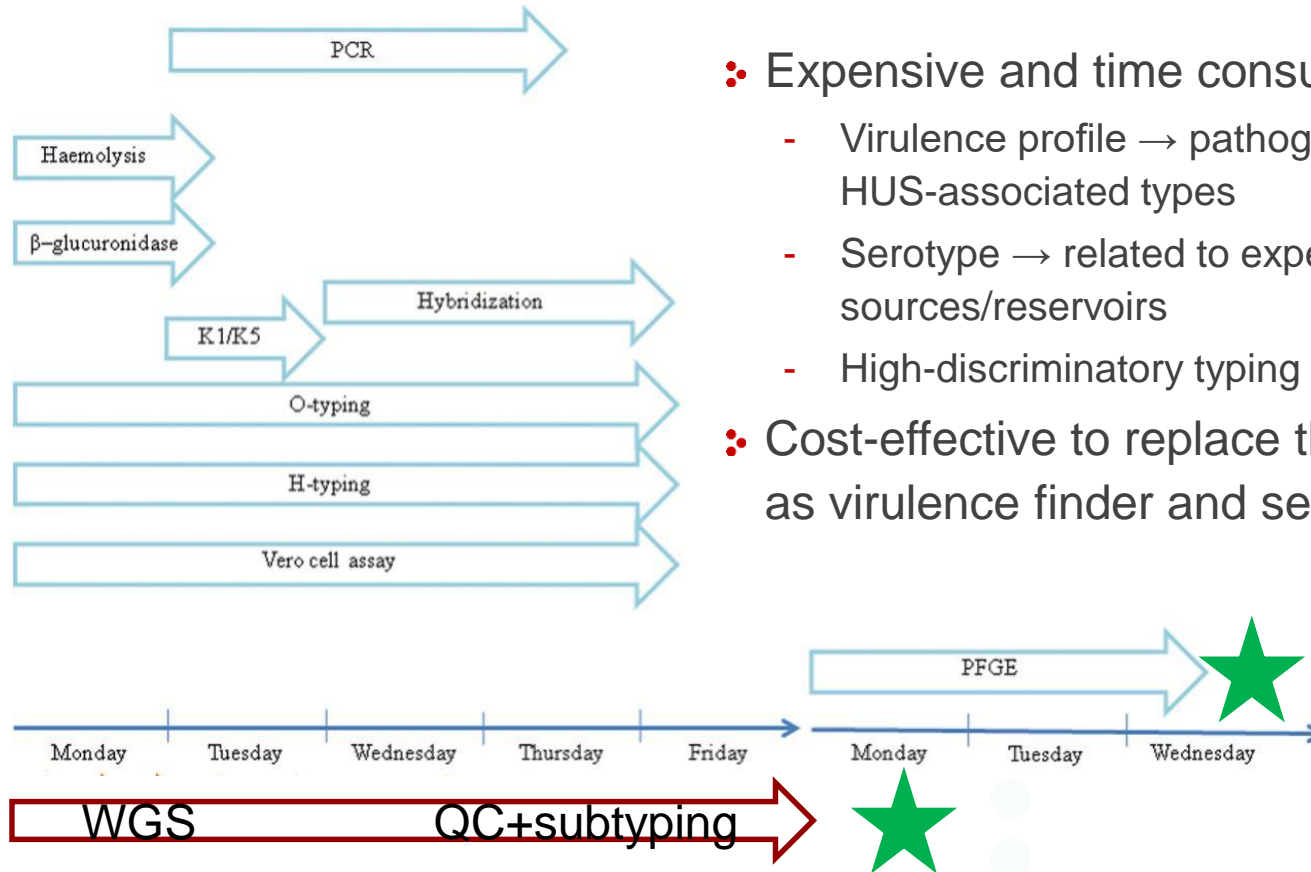
All human
Listeria

Outbreaks
WGS


2015

All human
STEC





- ❖ Expensive and time consuming characterisation:
 - Virulence profile → pathogroup, virulence potential, HUS-associated types
 - Serotype → related to expected epidemiology, sources/reservoirs
 - High-discriminatory typing needed for outbreaks
- ❖ Cost-effective to replace this by WGS – tools such as virulence finder and serotype finder developed

 Cluster and outbreak detection is faster for STEC

2013

First MiSeq

Hired
Bioinformatician

All human
Listeria

Outbreaks
WGS

2015

All human
STEC

2016

cgMLST

2017

All human
Salmonella

Central unit -> NextSeq
and Bifrost QC

Hired 2nd
Bioinformatician



Three Danish *Salmonella* outbreaks revisited



S. TM. OUTBREAKS IN DENMARK AND THE NETHERLANDS, 2005

- ❖ S. Typhimurium DT104, MDR
- ❖ 32 lab confirmed cases
- ❖ Associated with single restaurant
- ❖ Carpaccio, beef eaten raw
- ❖ Isolate from border control, match
- ❖ Traced-back to European country

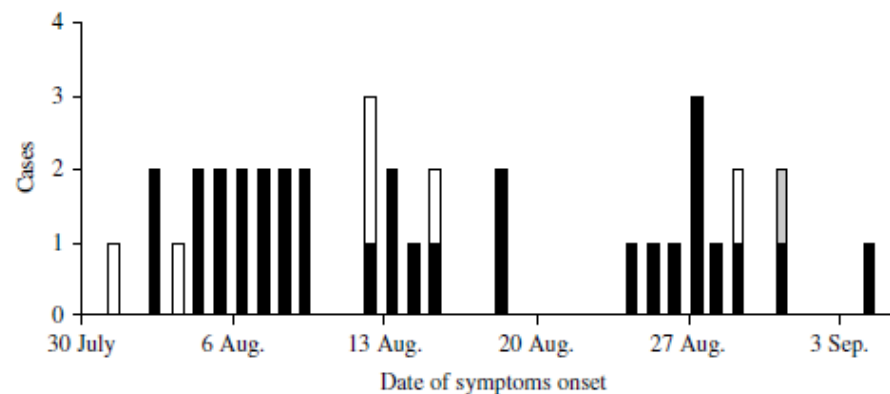
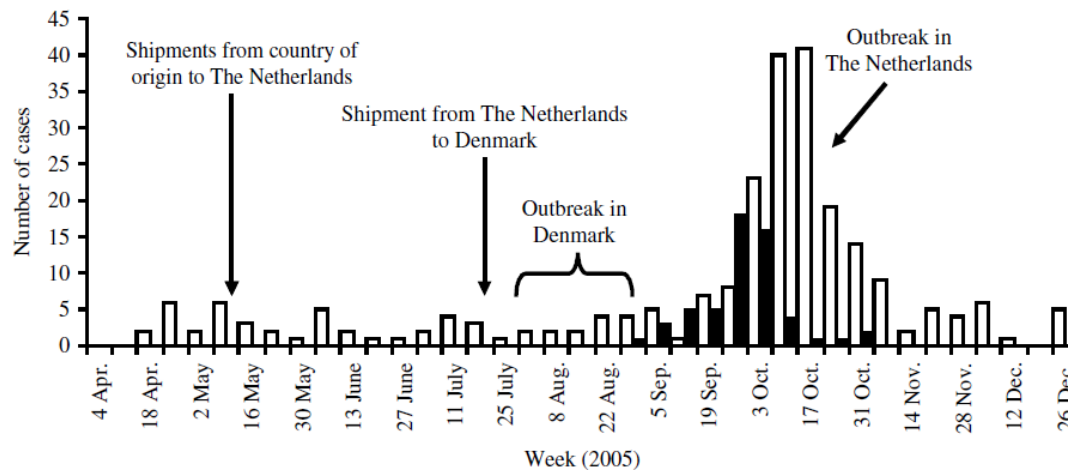


Fig. 1. Date of symptoms onset for 38 restaurant-associated cases. □, Norwegian; ◻, probable; ■, confirmed.

- Same source, but connection not made
- Long epi investigation, successful
- CaCo points to beef; filet américain



- ❖ Trace-back slow and complicated
- ❖ Shared typing schemes needed
- ❖ Difficult with food produced in one country and cases in others...

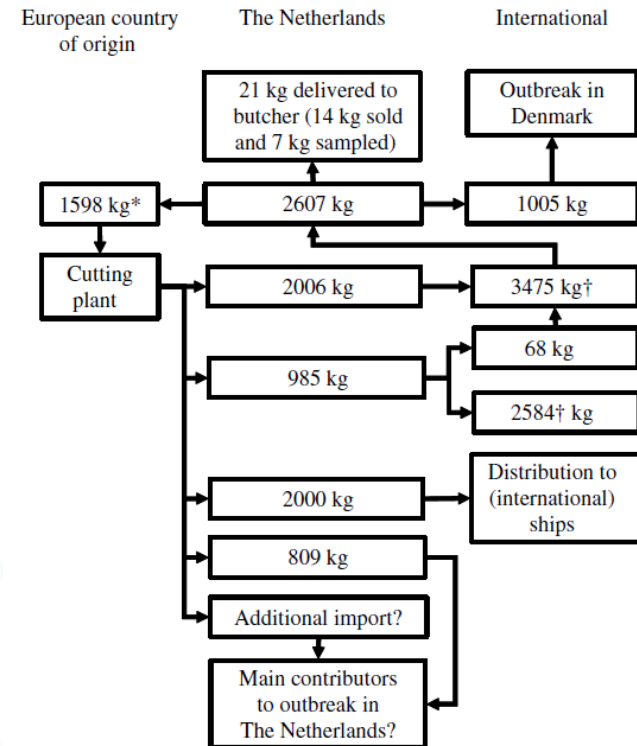
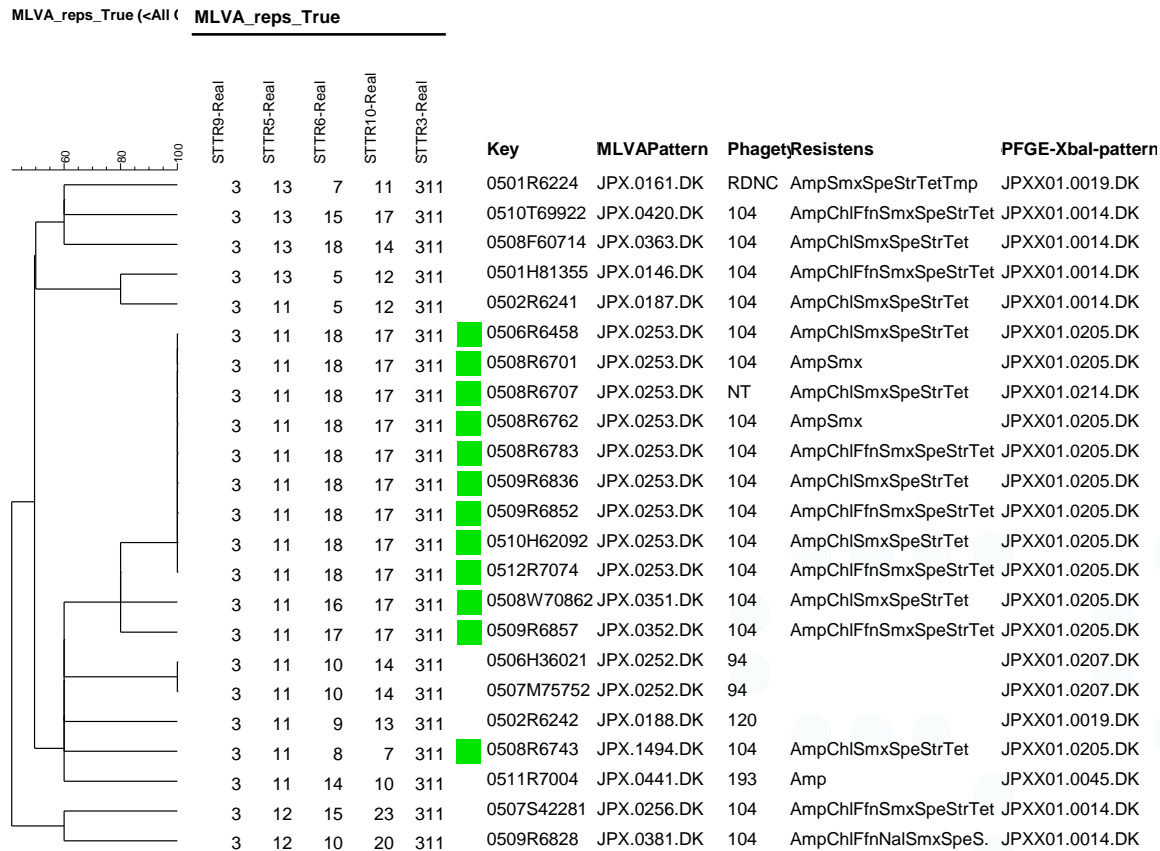
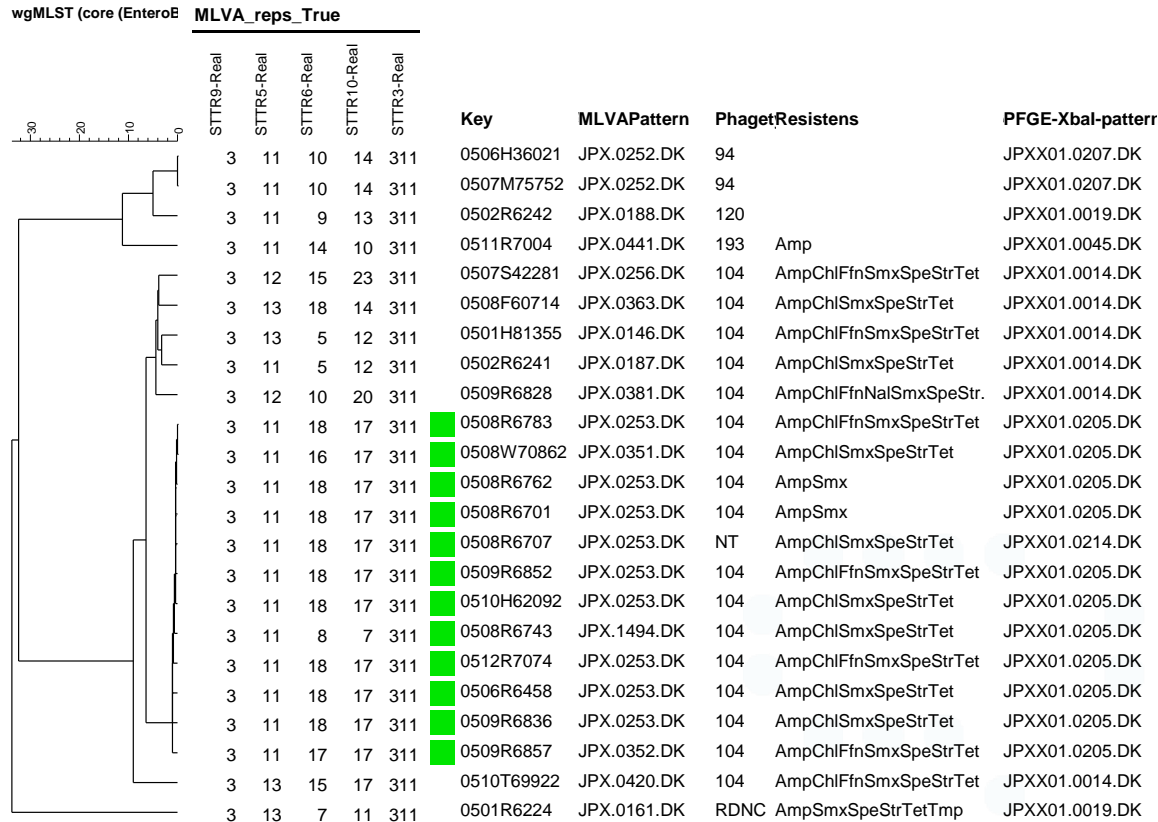


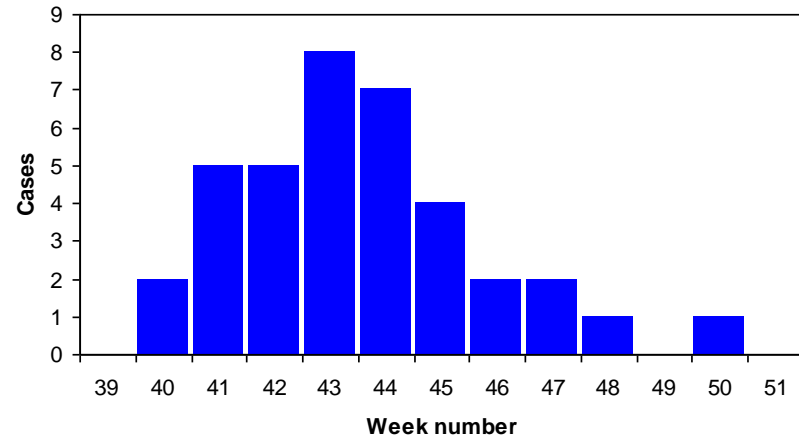
Fig. 2. Distribution routes uncovered by the product tracing, showing the shipment of incriminated beef from the European country of origin to The Netherlands and from there further internationally, May–November 2005.





SERIOUS INTERNATIONAL OUTBREAK SOLVED BY MOLECULAR TYPING

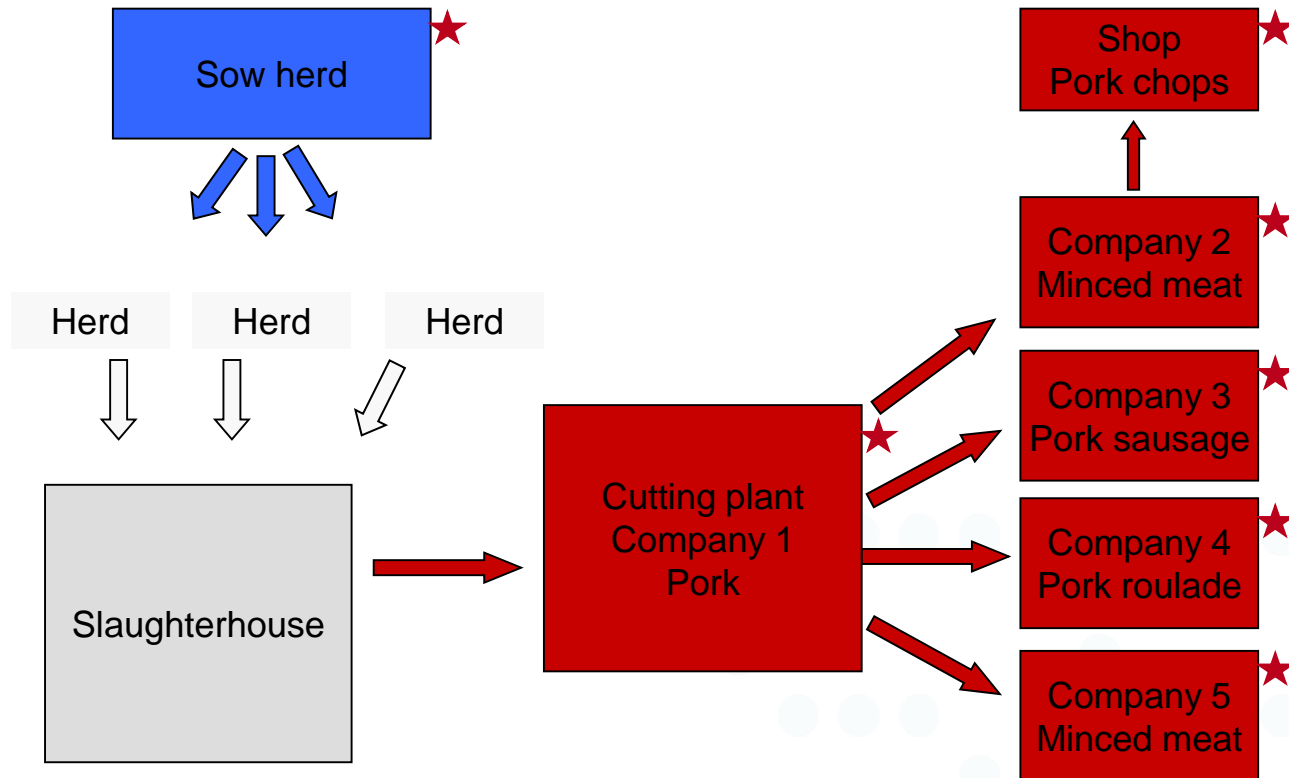
S. Typhimurium U288, 2008



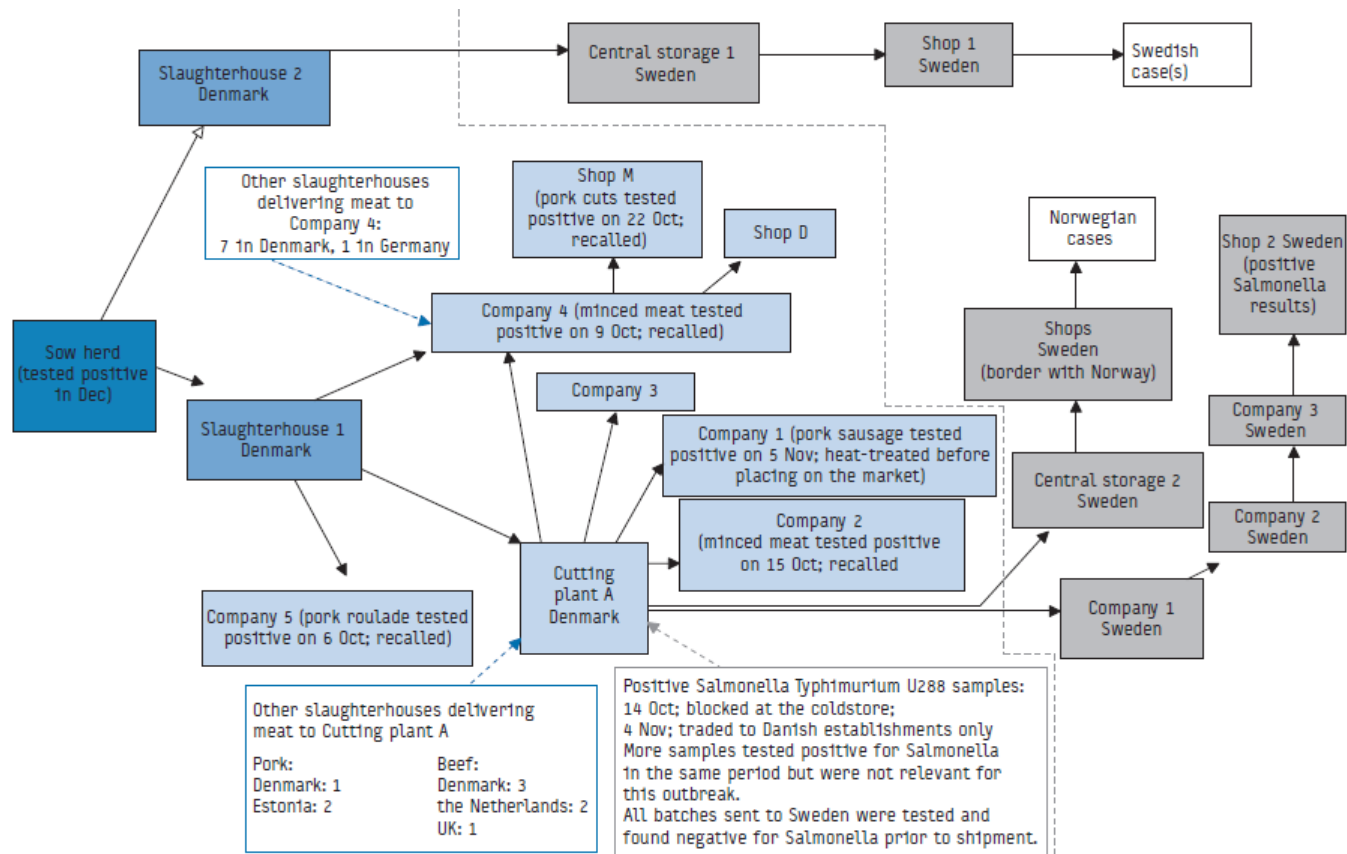
37 patients

4 died

TYPING REVEALED...



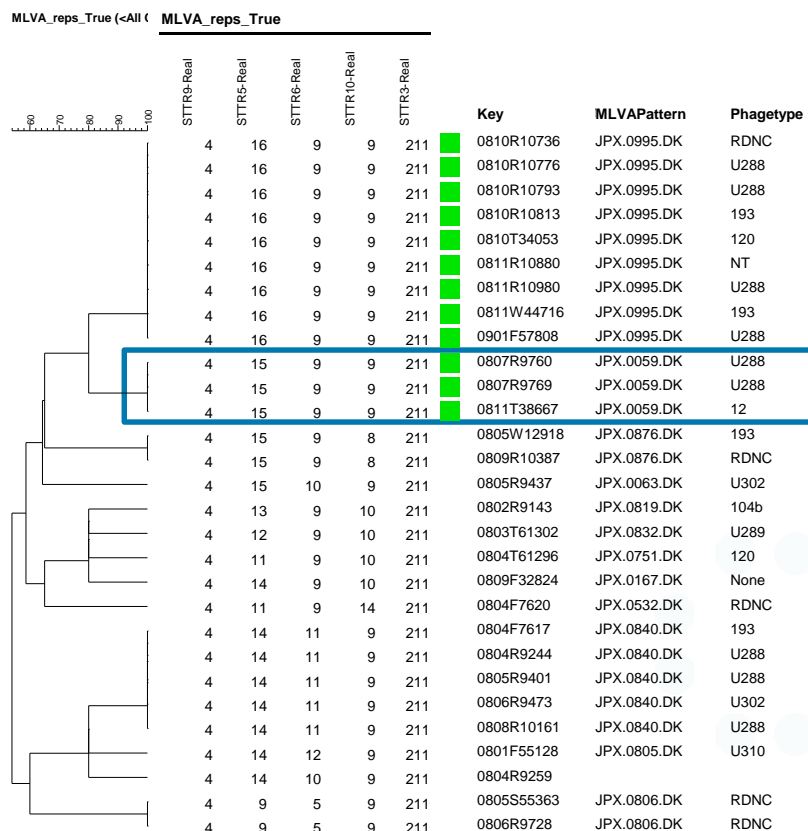
FULL TRACE-BACK DIAGRAM



- ❖ Also patients in Sweden and Norway
- ❖ Could be linked by molecular typing (MLVA)
- ❖ Could all be traced back to the cutting plant in Denmark

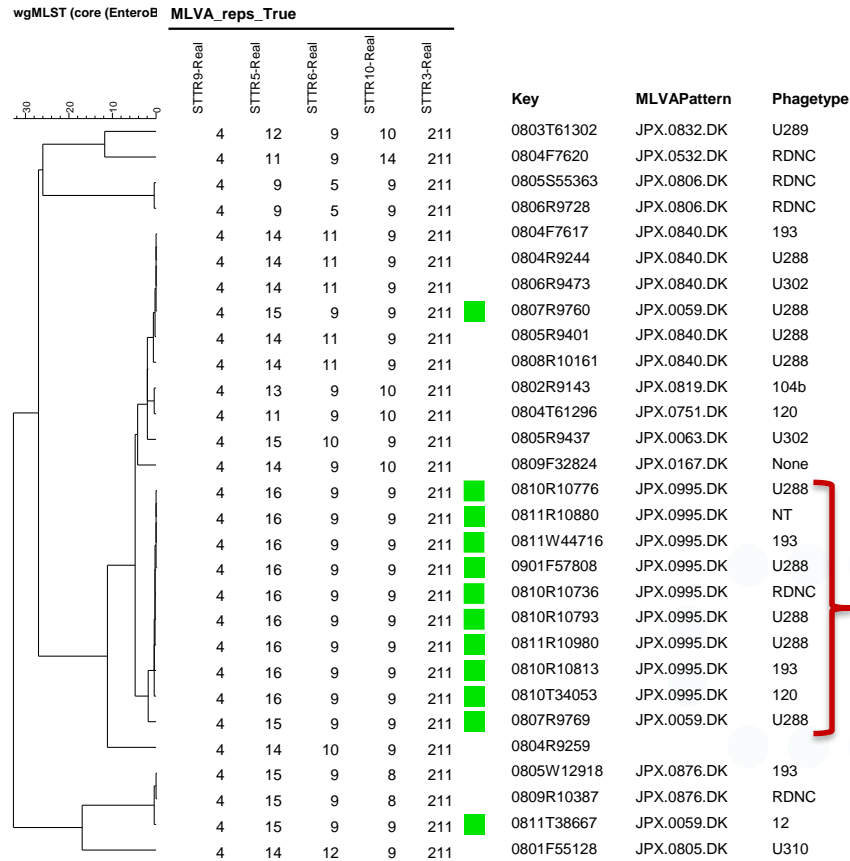


GLUMSØ-1 MLVA TREE



1 repeat/1 allele included

ST19, fully sensitive



9 alleles

S. TYPHIMURIUM DT120, UNUSUAL SOURCE, 2010

- Detected through surveillance and typing (MLVA)
- 20 cases, April-May 2010
- DT120, unusual resistant profile

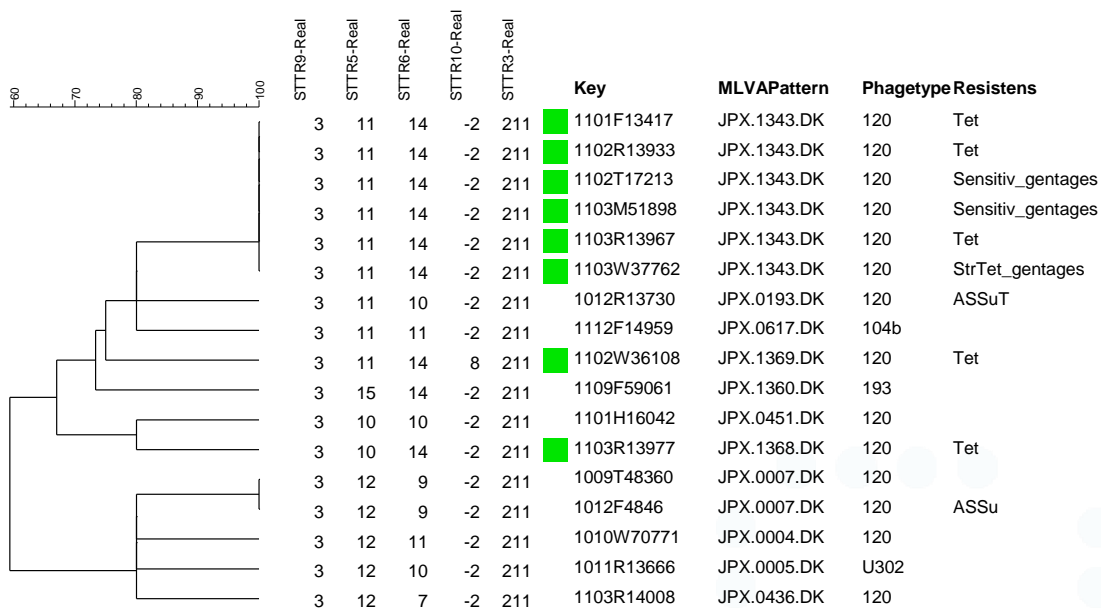


- ❖ Particular sausage
mentioned twice
- ❖ Unusual product
- ❖ One supermarket
- ❖ Produced in Germany
- ❖ Case-control study



MLVA_reps_True (<All Characters>)

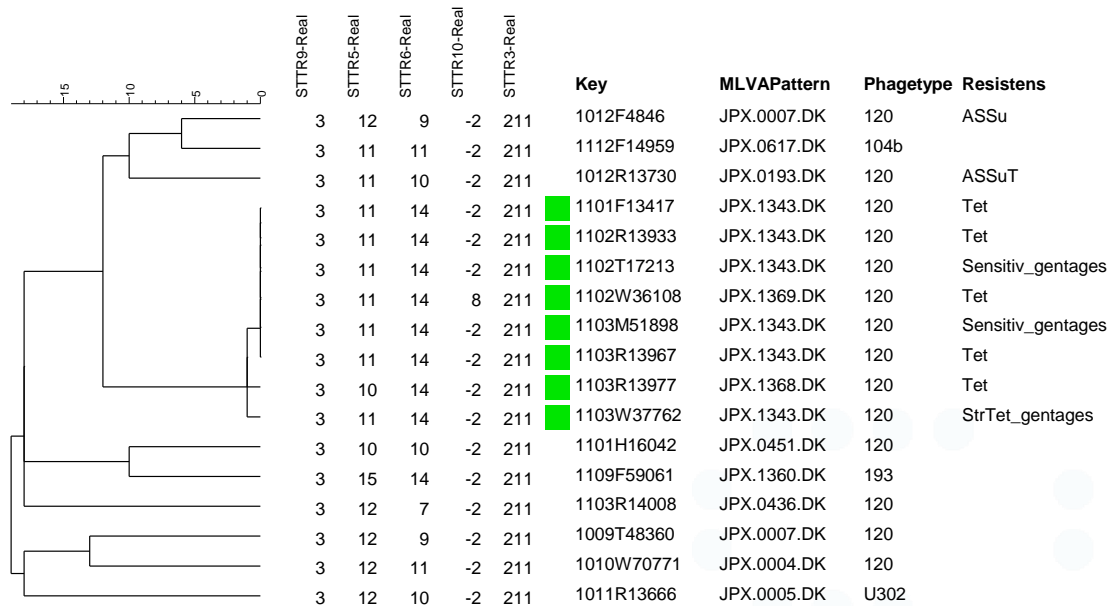
MLVA_reps_True



SAUSAGE CGMLST TREE

wgMLST (core (Enterobase))

MLVA_reps_True



2013

First MiSeq

Hired
Bioinformatician

All human
Listeria

Outbreaks
WGS

2015

All human
STEC

2016

cgMLST

2017

All human
Salmonella

Central unit -> NextSeq
and Bifrost QC

Hired
Bioinformatician

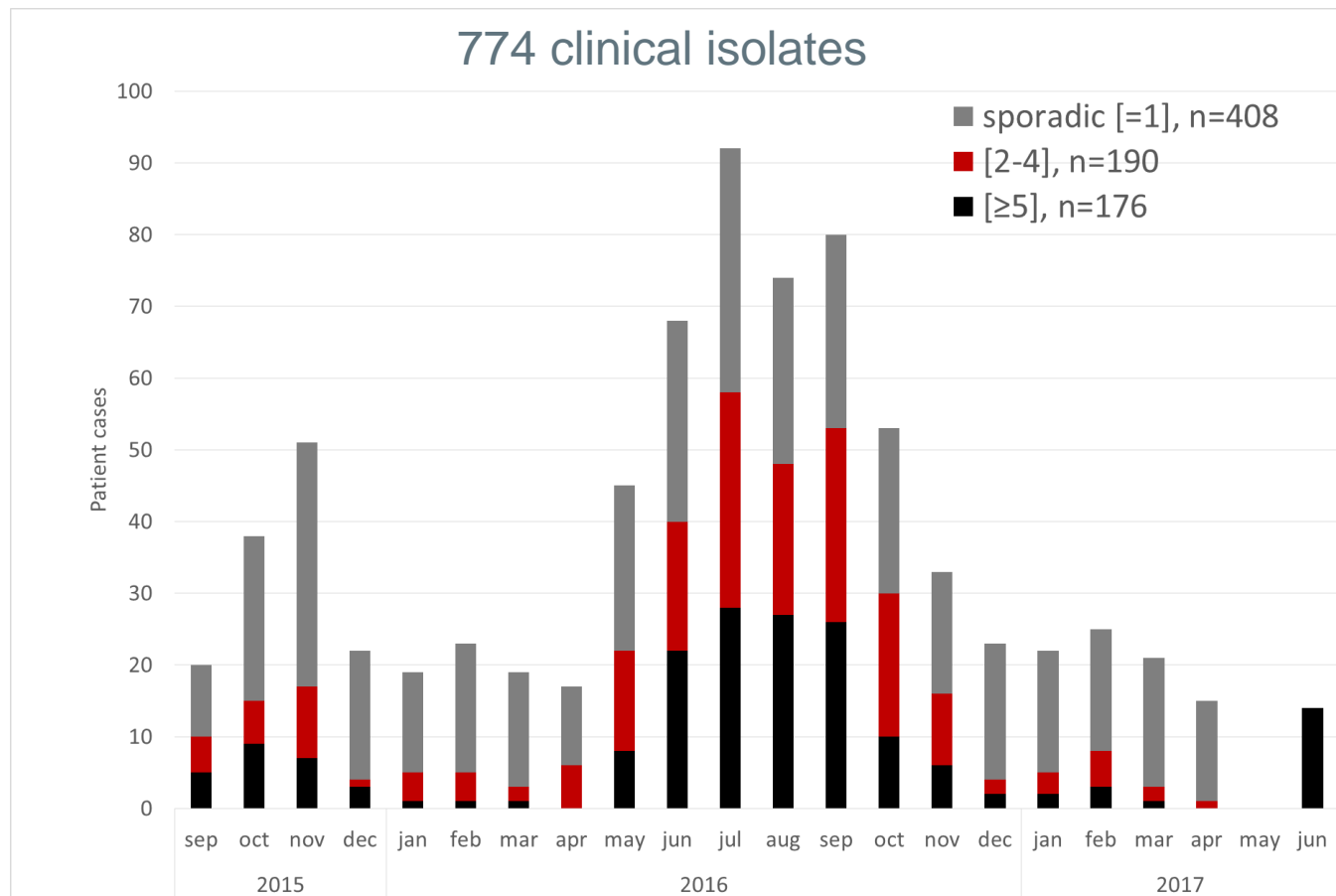
2018

C. difficile
sentinel

2019

Campylobacter
from 4 regions

- Isolates: 774 human (~10% of cases nationwide), 735 food/animal
- 47% of all cases part of a cluster - total of 104 clusters



∴ 30% of all clinical isolates had match to food/animal isolates

- 25% Danish chicken meat or broilers
- 2% Imported poultry
- 2% Cattle

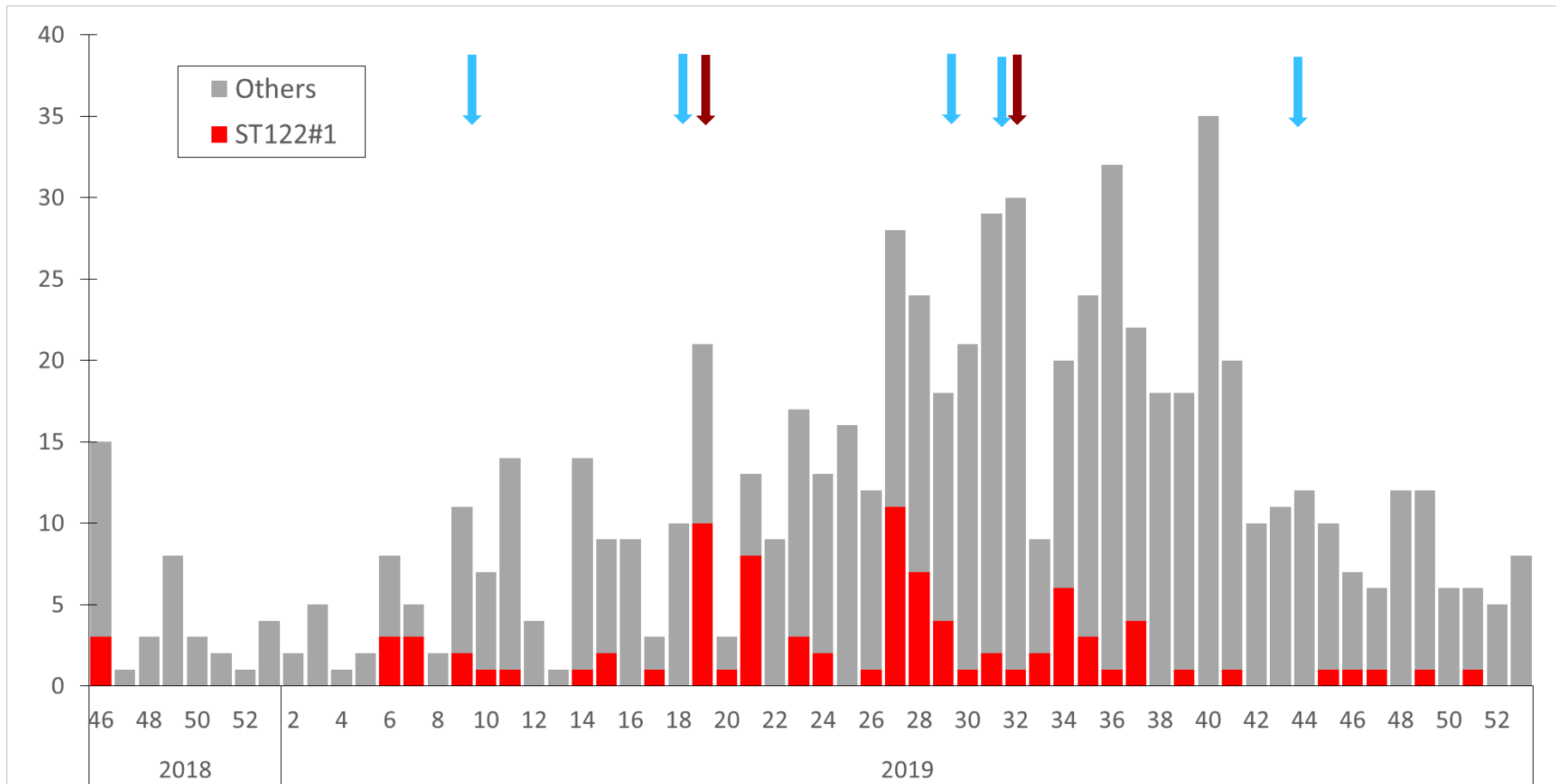
∴ 104 clusters of clinical isolates

- 82 small clusters (2-4 cases)
 - 29% match to food/animals
- 22 large clusters (5-17 cases)
 - 77% (17/22) match to food/animals

- ❖ WGS of isolates from 4 regions (~12% of cases in DK)
- ❖ Analysis of WGS from retail chicken
- ❖ Continuous comparison of human and food isolates
- ❖ Similar results as in 2015-17 project
 - ~50% cases were sporadic
 - ~30% cases in larger clusters
 - 11/14 large clusters match to chicken meat
 - 2 geographically confined (one with milk as suspected source)
 - 1 cluster matched a 2016 cluster of human and chicken isolates

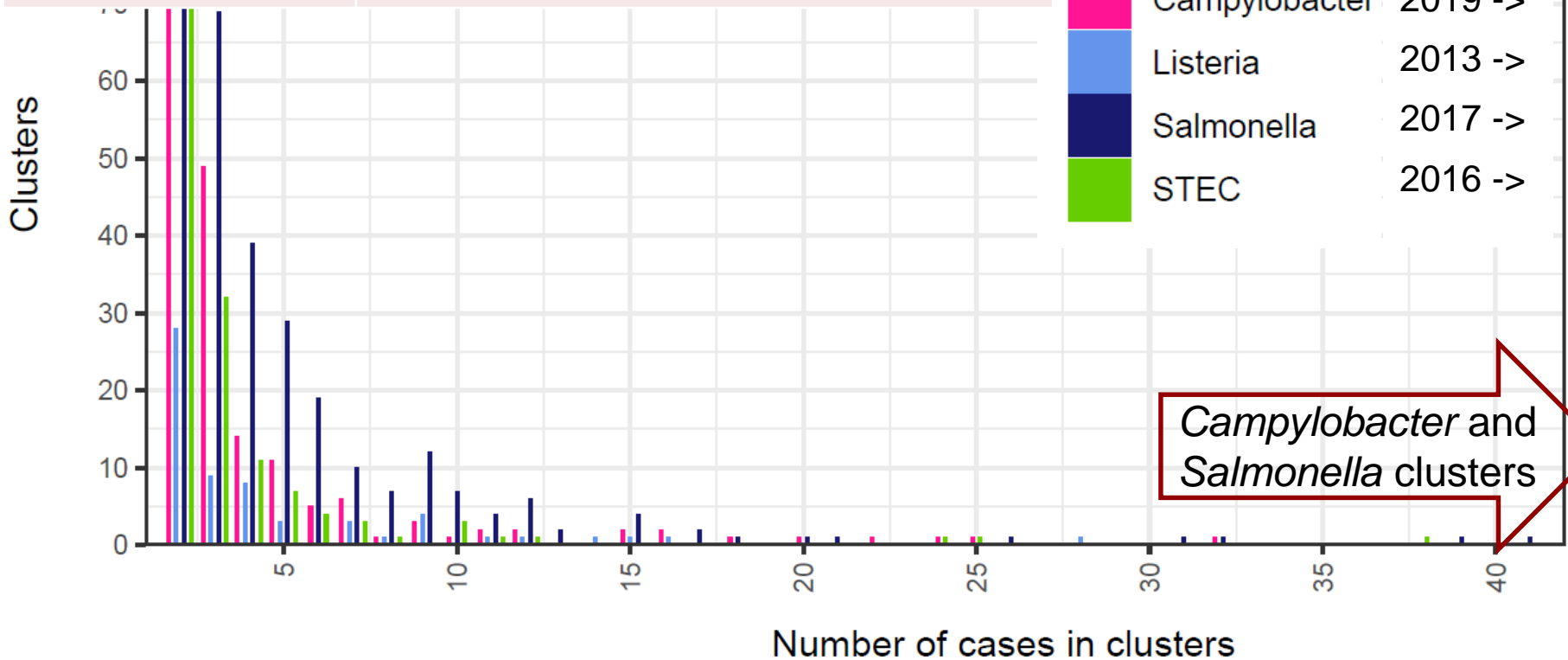
Large continuous cluster

- ST122; 88 cases in 2019 – 13% of all typed clinical isolates
- Match to chicken meat samples at retail ↓ and at slaughter house ↓



Genetic clusters

Species	% isolates assigned to clusters
STEC	30%
<i>Salmonella</i>	47%
<i>Campylobacter</i>	55%
<i>Listeria</i>	62%



- ❖ Building on established and functioning surveillance system
- ❖ Long-standing collaboration with food authorities
- ❖ Implementation for minimal extra resources (no extra funding)
 - Limited parallel use of old and new methods
 - Old methods are also expensive
- ❖ Great collaborators
- ❖ Build on hard work and experience from others

❖ Clinical microbiologists

- Slower initial subtyping for all organisms
- Serotyping and PCR is fast
- Better typing in-silico

❖ Epidemiologists

- Faster cluster detection in most cases
- WGS is more accurate than PFGE and MLVA
- Inclusion or exclusion of cases in outbreaks are more certain
- More clusters and outbreaks are detected

❖ Public Health microbiologists

- WGS is the same method for all species
- Similar workflow in lab, one analysis, many results
- New skills required

❖ Analysis still under development

- Plenty of tools and databases
 - Updates, retirements
- Validation
- Continuing education of staff

❖ Data sharing

- Comparability (sectors, countries)
- GDPR



❖ Interpreting new data

- Case definitions
- Clusters? SNP and AD

❖ Risk of loosing reference laboratory skills

- one-sided lab work
- WGS fails sometimes

❖ Budget

- Investments in equipment
- Expensive reagents
- Education of staff
- Hire bioinformaticians?
- Commercial software?



- ❖ One lab method for all bacteria and all typing
 - Possibility for batching species
- ❖ Defining clusters/outbreaks
 - More confident definition of clusters/outbreaks
 - More certain microbiological evidence for linking to sources
- ❖ More outbreaks solved
 - due to food authorities sequencing
- ❖ New challenges are coming
 - We need new methods due to culture-free diagnostics



Thank you for your attention!

Thanks to all clinical labs at the Danish hospitals for taking part in projects and sending isolates for surveillance!

