NRL Salmonella Sweden

Erik Eriksson

2022-05-23

National Veterinary Institute, SVA

Situated in Uppsala approximately 60 km north of Stockholm

Photo SVA

National Veterinary Institute, SVA

Total numbers employee 380

Sections innvolved in salmonellaanalyses

Sektion for bacteriology 24

Section for serology 13

Section for molecularbiology 9

NRL Salmonella

mainly handled by the two of us

Erik Eriksson

Laboratory veterinarian

Jenny Eriksson

Laboratory technician

Cultivation Salmonella ISO, 6579, MSRV

Samples from animals

Lifestock production animals	22 319
Poultry	2 221
Horses	418
Pet animals	784
Wild animals/Zoo animals	881
Species not noted	66
	26 689

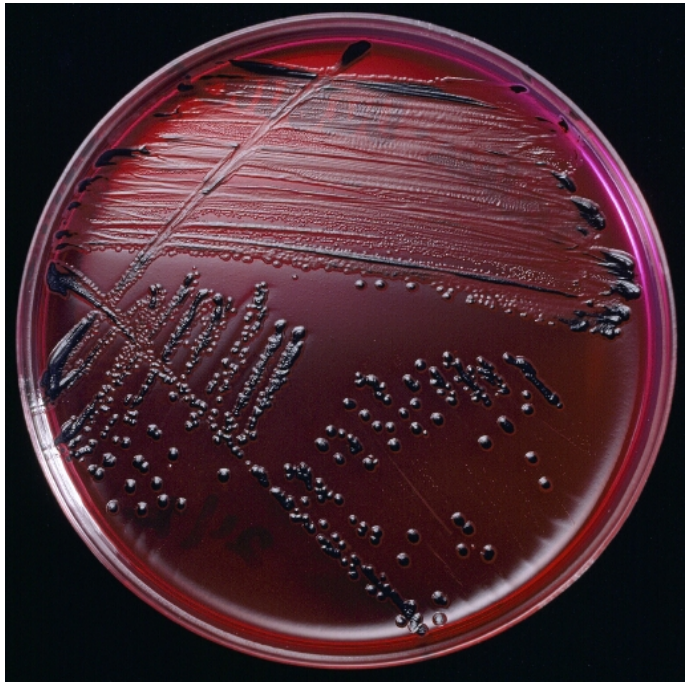


Photo Bengt Ekberg SVA

Feedstuff related samples **9 132**

Serotyping salmonella 2021

Types isolates from Veterinary medicine, Feedstuffs and Food From our own lab and from other Swedish

Samples from animals	
Lifestockproduction	531
Poultry	29
Horses	10
Pet animals	46
Wild animals/Zoo animals	64
Other species	1
	682
Food/ Feedstuff related isolates	480
Total	**1 162**

Photo Bengt Ekberg SVA

Human isolates typed at Public Health agency of Sweden

Serology and molecularbiology 2021

Poultry serology

S. Gallinarum/pullorum ab	3 835

Cattle serology

Salmonella O4 O9 ab	3 750
Salmonella O9 ab	792

Swine serology (performed at DTU)

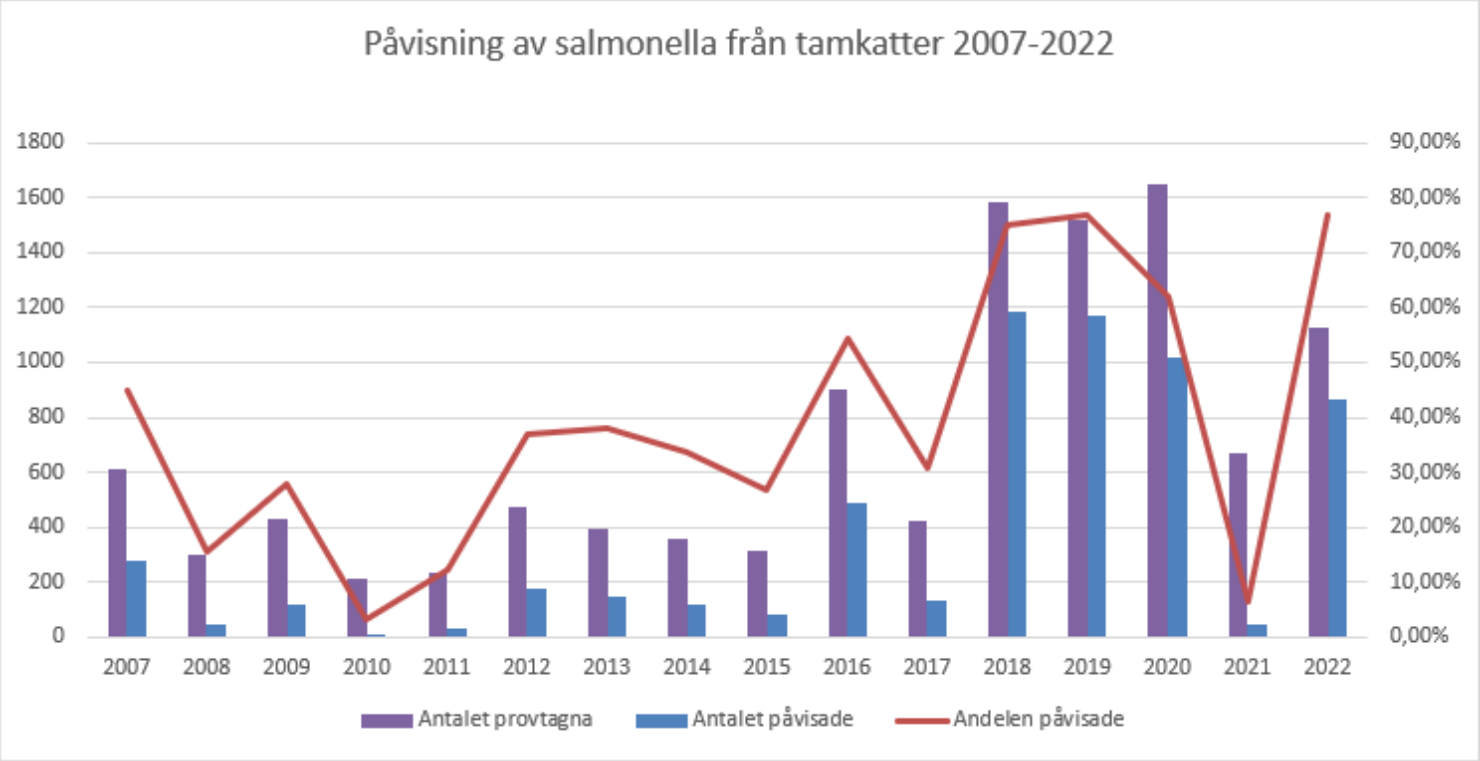
Salmonella O4 an	1 523
Salmonella O6 O7 ab	1 523

PCR from enriched BPW	741
MLVA *S. Typhimurium*	147

Photo SVA

Salmonella in cats, Sweden

- Spreads from passerine birds during late winter/early spring
- *S. Typhimurium* specific types
- Salmonella incoperated in the wildbird population.
  Harsh winters / lack of food
  The birds migrate into the communities and seek food at feeding tables.
- Outdoor cats get infected after they have catched a sick bird and eating it
- Diarrea, vomiting, inappetence fever. Cats can get seriously ill
- Also human cases, mostly children that get infeced by the passerine *S. Typhimurium* types



2019	1179 positive cats
2020	1209 positive cats
2021	42 positive cats
2022	927 positive cats

Passerine birds most common associated with spread of salmonella infection

Spinus spinus

Acanthis flammea

Pyrrhula pyrrhula

Salmonella in Swine. Sweden

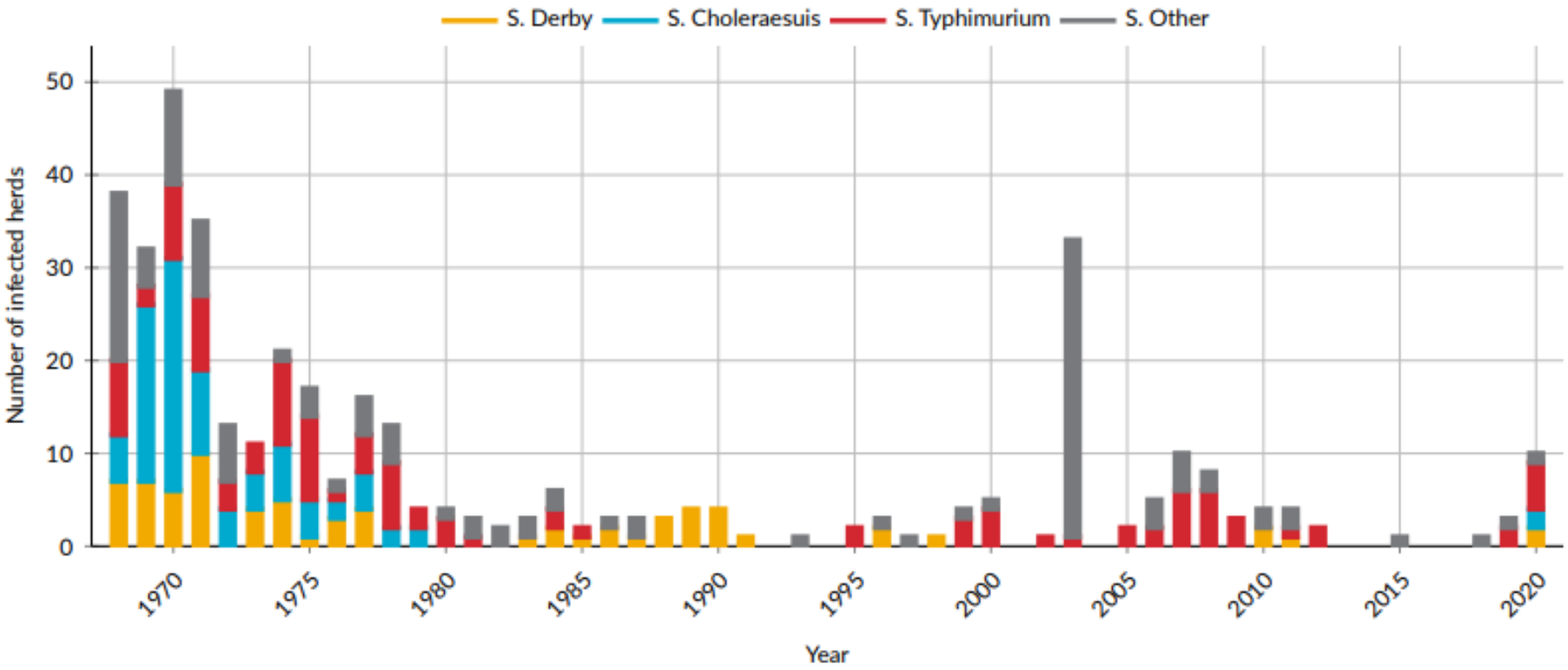


Figure 24: Annual notifications of *Salmonella* in swine herds during 1968–2020. In 2003, a feed borne outbreak of *S.* Cubana occurred in Sweden. In 2016 and 2017, *Salmonella* was not detected in any herd.

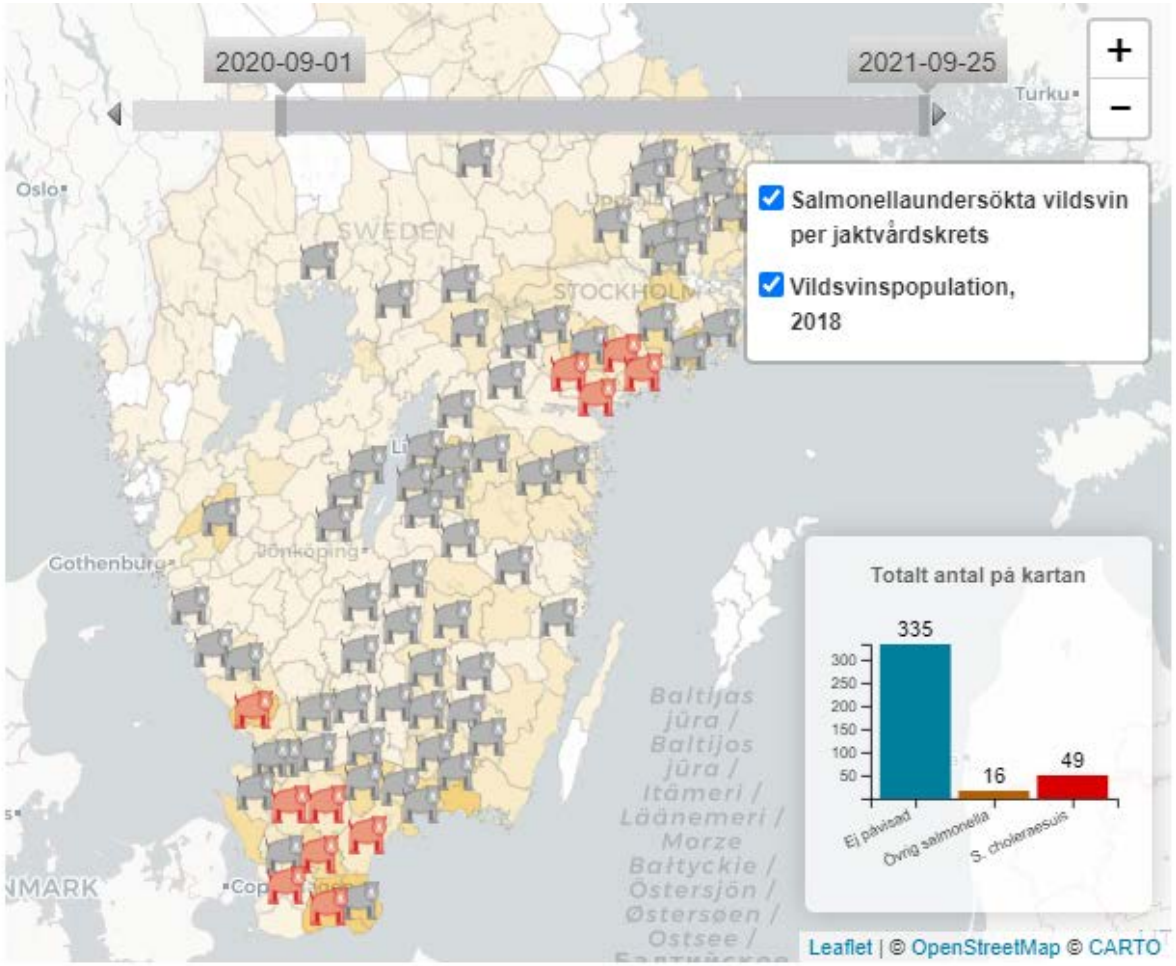
Salmonella Cholerasuis detected in Swine herds 2020-2022

- Detected in 3 swineheards during 2020-2022
- Extensive sampling in contact herds to check for spread of the bacteria
- Whole genome sequencing shows matching strains inbetween farms and strains isolated from wild boars.
- No known source for introduction in the wild boar population
- Screening of samples from killed wild boars/ found dead, approx 12-14% positive for S Cholerasuis
  (Samples from suspected areas)
- Ongoing antibody screening Swedish swine heards.
  Approx. 160 herds. 45 animals/herd

Photo SVA

Surveillence of Salmonella i wild boar 2020-2021

https://www.sva.se/djurhalsa/smittlage/overvakning-av-salmonella-choleraesuis-hos-vildsvin/



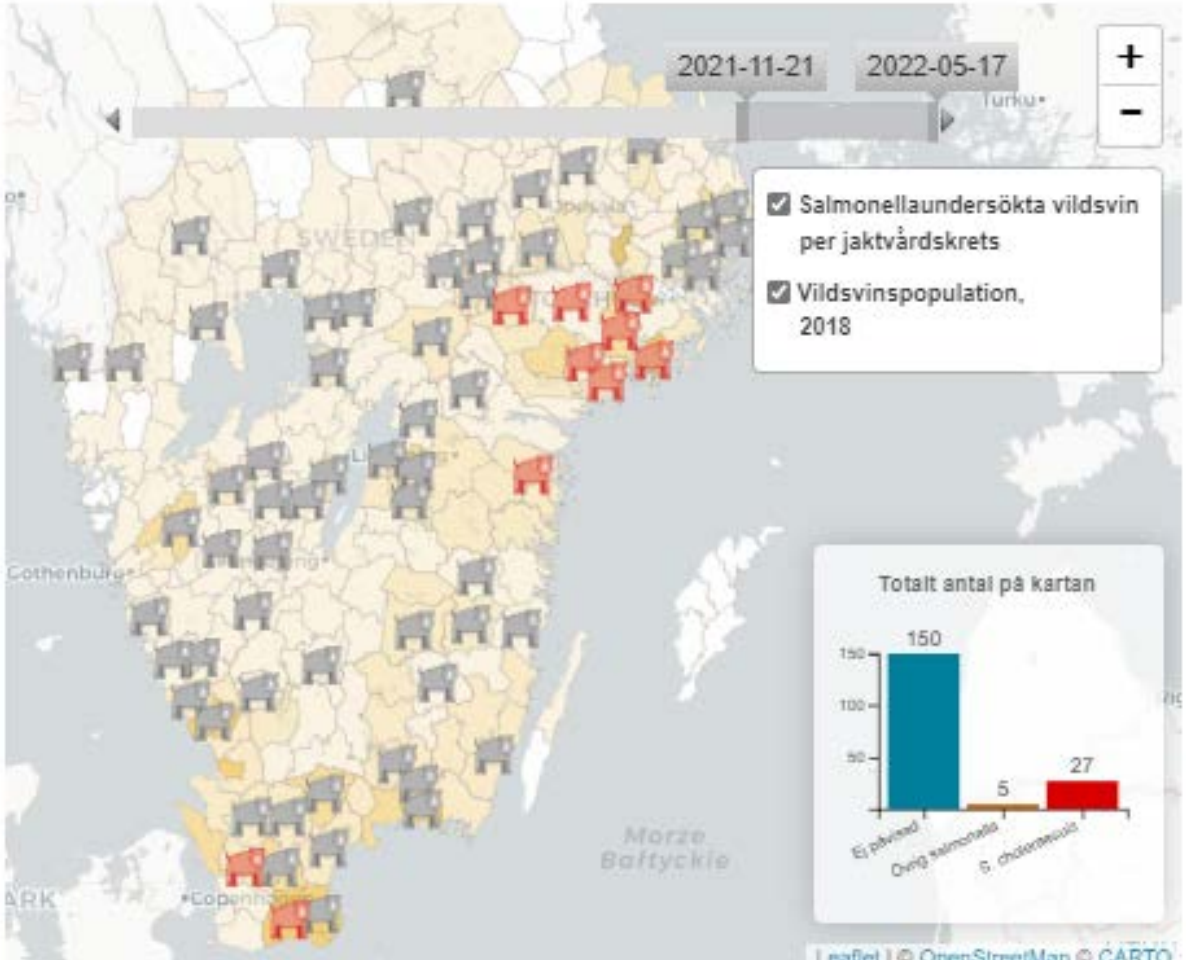
All isolates very similar on sequencing – and very similar to isolate from pig herds.

Surveillance extended to all counties with wildboar - ongoing

400 wildboars
Appox 12 % S. cholerasuis
Aprox 4 % other serovars

Surveillence of Salmonella i wild boar 2021-2022

https://www.sva.se/djurhalsa/smittlage/overvakning-av-salmonella-choleraesuis-hos-vildsvin/



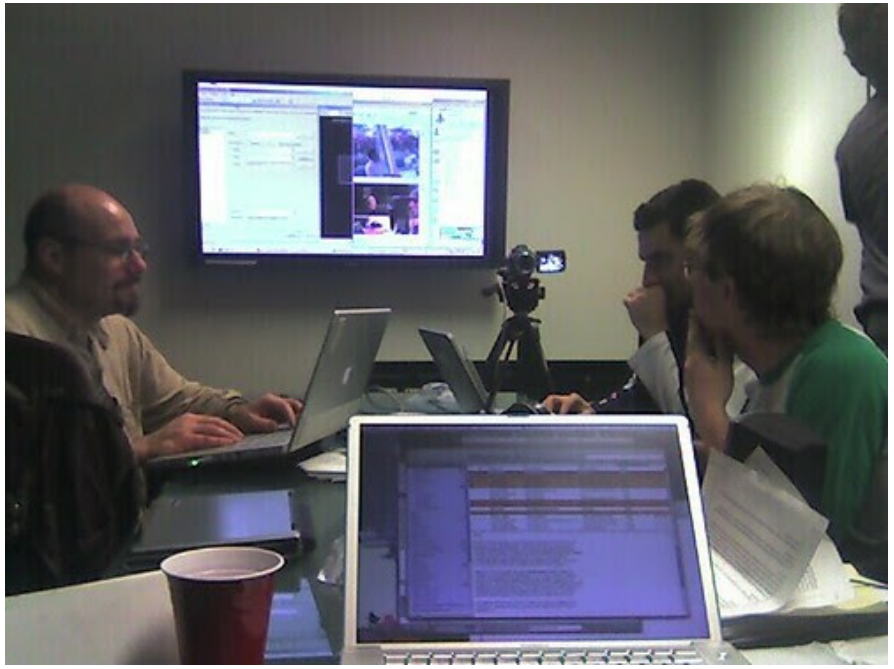
All isolates very similar on sequencing – and very similar to isolate from pig herds.

Surveillance extended to all counties with wildboar - ongoing

182 wildboars
Appox 14 % S. cholerasuis
Aprox 3 % other serovars

Activities as NRL

- Arranges meeting with regional labs and other authorties
- Give advice to regional labs in matters concerning analyses for salmonella
- Provide education for regional labs in salmonella diagnostics
- Cooperation with National food administration in their contact with official labs that analyse salmonella in foodstuffs
- Arranges laboratory comparisons/ profiency testring for regional labs in cooperation with other NRL:s in the Nordic and Estonia



Meetings approx every second year Labs - Authorities

- Regional labs that performs salmonella analyses on feedstuff samples and faecal/ environmental samples from animals in lifestock production

- National Board of Agriculture
- National Veterinary Institute, SVA
- County administrative board

Discussion about:

- Analyses
- Practical things regarding the labs interaction with authorities
- How we the authorities can help labs e.g if they have problems with suspected contamination in their analyses etc

Rewarding discussions

Problems with a regional lab
Analyses in the national controlprogram

- Sweden National guarantees for salmonella (EU)
- Salmonella controll program run by the Swedish Food Agency, SFA, Sampling at slaughter since 1995
  - Lymphnodes from cattle and swine
  - Swabsamples from carcasses cattle and swine
  - ”Necksamples” from slaughtered poultry
  - Samples from cutting plants
- 2013 procument from SFA
  The Lab with the lowest prize was assigned to perform the analyses
- The number of positive samples during 2014-2015 markedly dropped compared to earlier years
- The lab sent SVA many strains for serotyping that were not salmonella Enterobacter, Proteus and Citrobacter etc
- When positive samples were detected by the lab. Typing indicated contamination problems as they at the same time had isolatet the same strain from several samples from different slaugtherhouses

Photo SVA

Problems with a regional lab-
Analyses in the national controlprogram

- Our idea as a NRL: Start with helping the lab to perform better.
- Lennart Melin and I visited the lab and discussed critical working steps during salmonella analyses e.g
  - Substrate control
  - The preenrichment procedure regarding temperature, time intervall etc
  - Handling of samples to avoid risk of contamination
  - Reading of agarplates and importance of training of staff for that etc
- Introduced waterbath for incubation of RVS broth at 41,8-42,2 °C
- Personel from the lab was invitated to our lab at SVA and worked in parallell with our personel to see how we performed salmonella analyses
- We repeadedly sent them samples of lymphnodes spiked with salmonella at different leves both for training and to tests their performance.
- In the end we forced to judge out the lab as we considered them unable to perform analyses at an satifactorally level and the analyses in the controlprogram were transferred to an other lab

Photo Bengt Ekberg SVA

Nordic interlaboratory comparison for salmonella sent out from NRL to regional labs

- This a joint venture between the NRL:s in
  - Denmark
  - Finland
  - Norway
  - Sweden
  - Estonia

- Started in 2006
- Primary production Faeces
  - Bovine
  - Pig
  - Poultry

Nordic interlaboratory comparison studies for salmonella sent out from NRL to regional labs

2016-2021

Started out i smaller scale Norway, Denmark and Sweden
Later year approx. 18 lab labs each year

The same layout as EURL had when we started the study

- 25 samples
- Two different Salmonella serovars
- Each serovar 5 samples at two different levels + 5 blanks

Now when the fiery sole Lennart Melin has retired we have discussed if we shoul continue

A lot of work for both the participating labs and for SVA to organize these comprensive studies

Requirement for accreditaion

Nordic intelaboratory comparison for salmonella sent out from NRL to regional labs

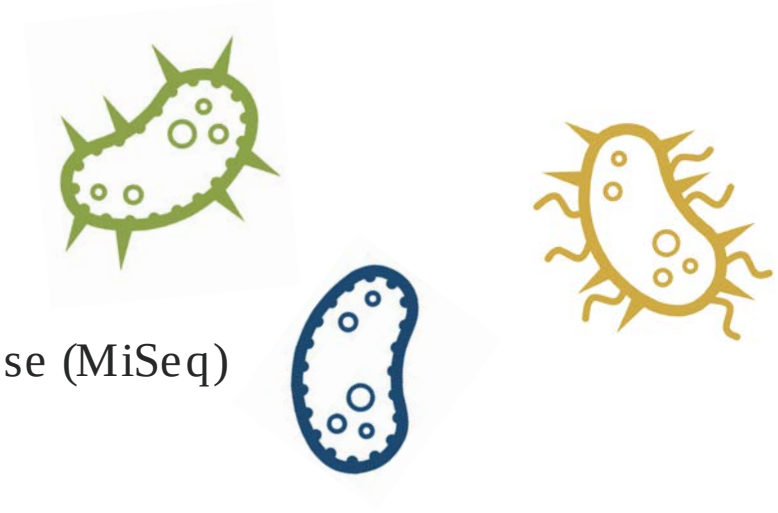
- 2022 decided to continue with the studies but a less comprehensive layout.
- Every third year we will send out 18 samples.
  The two years inbetween we will send10 samples.
- We only use one Salmonella serovar, For instance for 10 samples
  - 4 replicates at a higher level
  - 4 replicates at low level
  - 2 blanks
  - Poultry faeces
- Results registred digitally with a simplified answer form

Guidance Document
for the organisation of Proficiency Tests by NRLs for national networks, including partial outsourcing

Drafted by EURLs *Campylobacter,* Coagulase Positive Staphylococci, *Listeria monocytogenes*, *Salmonella*, STEC

Version 2, 12/07/2019

Routine NGS analysis of Salmonella



Scaled up since 2021 – all ’interesting’ isolates sequenced

Sequencing at national core (SciLifeLab Stockholm, NovaSeq) and in-house (MiSeq)

Data analysed with

- SISTR for serotype
- cgMLST / wgSNP for clusteranalyses
- Resistance gene detection for comparison with resistance phenotype

Data exchanged ad hoc with Public Health Agency and Swedish Food Agency

Accreditation underway 2022

Conventional serotyping still carried out in parallell

MLVA still in use for S. Typhimurium, abandoned for S. Enteritidis

Routine NGS analysis of Salmonella – a few recent applications

Re-emergence of S. Choleraesuis in wild boars

Population structure of introduced clone, matching with domestic pig and human isolates, comparison with isolates from other countries

Photo SVA

Multi-serotype European outbreak associated with imported tahini and halva

Matching of food and human isolates, comparing strains across products, sequence-based identification of rare serotypes

Photo Joshua Wickerham from The Americas, China, CC BY-SA 2.0

S. Dublin in cattle

Monitoring of regionally linked SNP clusters, comparison with isolates from domestic/imported food and human cases

Photo SVA