

VtL



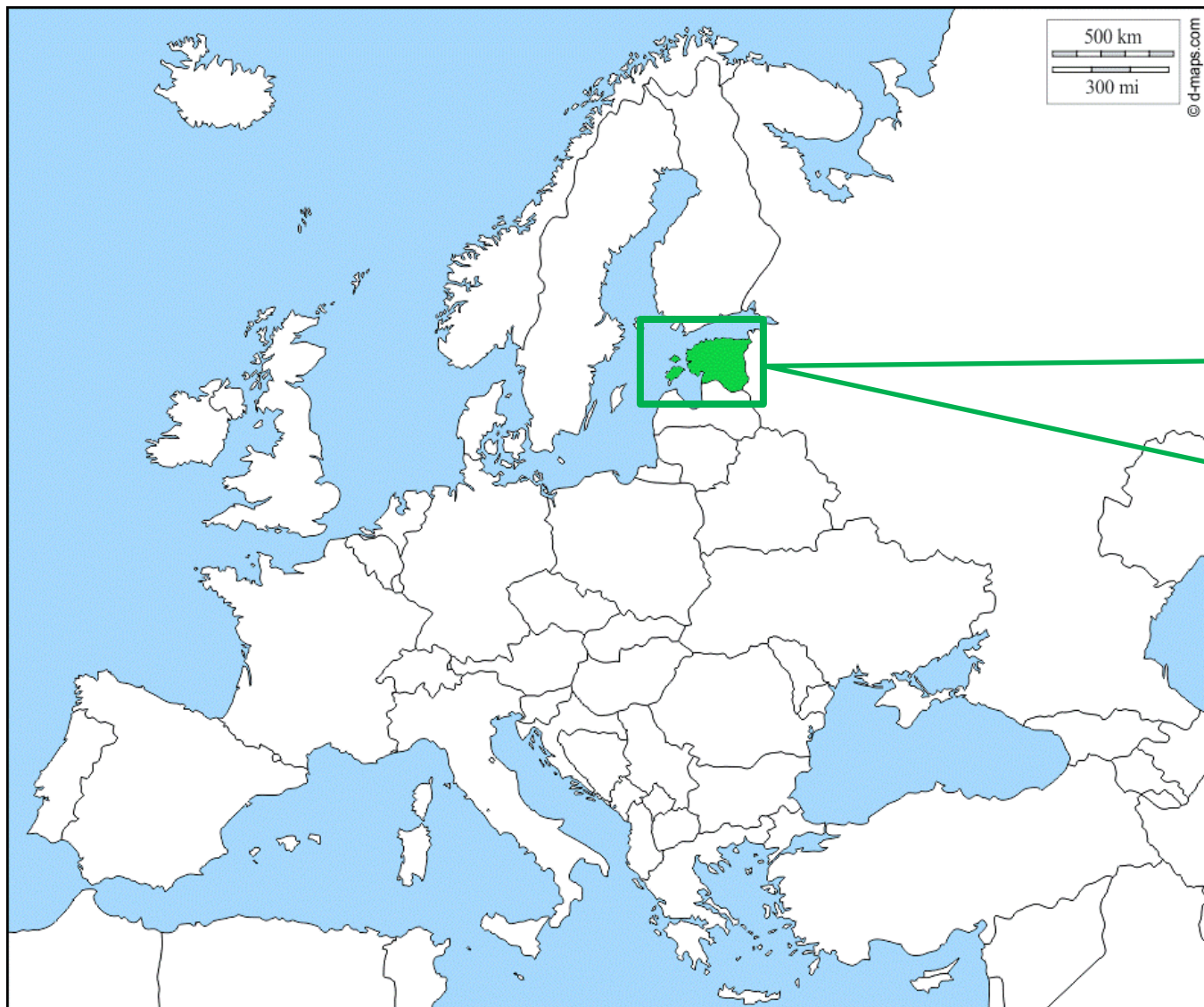
# **NRL *Salmonella* Estonia**

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**Age Kärssin**





**Veterinary and Food Laboratory, Kreutzwaldi 30, Tartu, Estonia**

# Some facts about Estonia



Capital - Tallinn  
Area - 45,227 km<sup>2</sup>  
Population - 1.3 M  
15 counties

# Animal population in Estonia, 2016

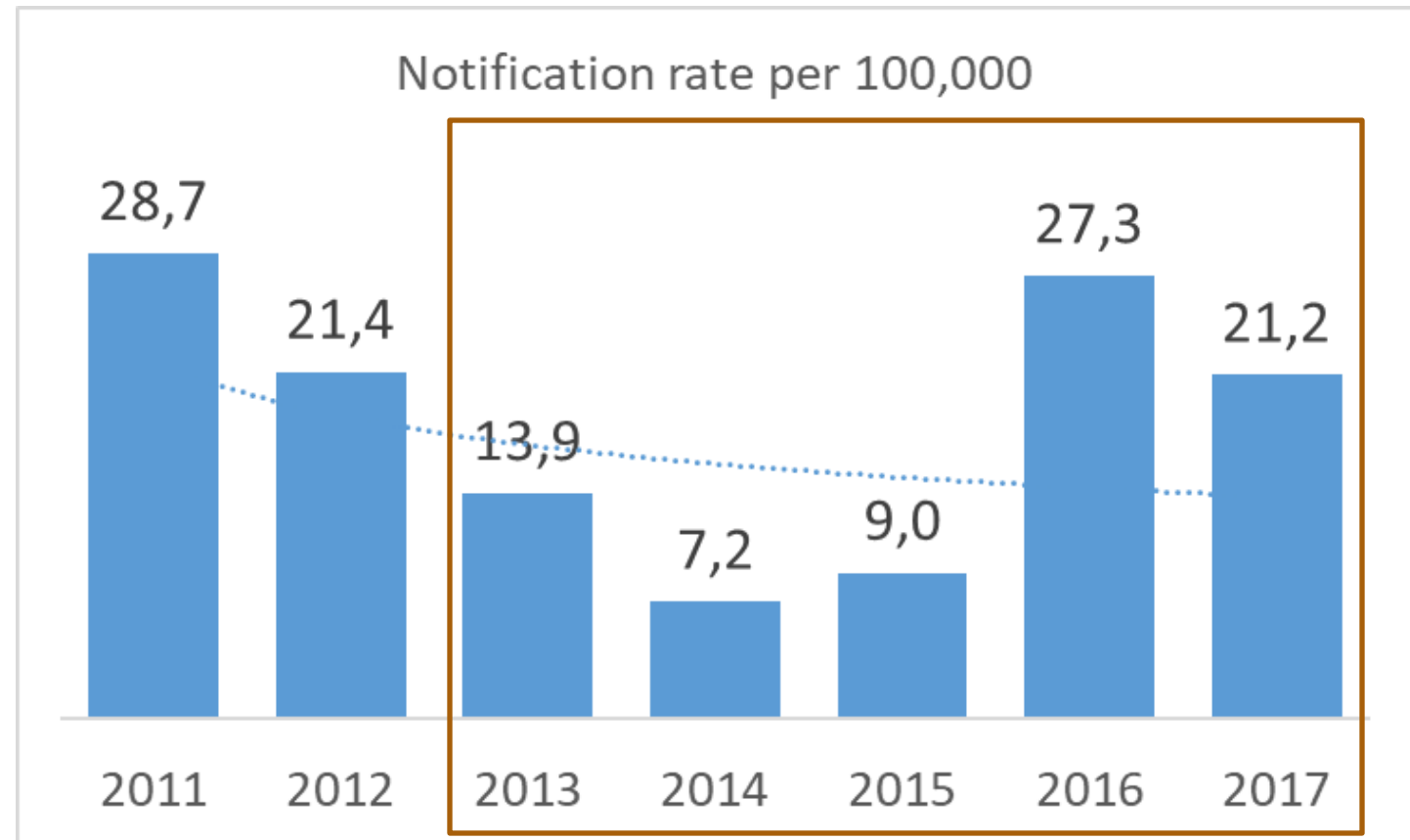
	Animal species		Herds	Animals	Slaughtered
	<b>Cattle</b>	Dairy cattle	2046	126,459	22,716
		Beef cattle	2002	49,295	10,220
		Cattle total	3458	248,236	43,433
	<b>Pigs</b>	Fattening pigs	178	141,602	
		Breeding pigs	91	24,932	
		Pigs total	217	286,790	524,227
	<b>Sheep</b>	Sheep total	1902	83,434	18,354
	<b>Goats</b>	Goats total	590	5011	698
 kana	<b><i>Gallus gallus</i></b>	Laying hens	134		10,769
		Broilers	100		11,237,775
	<b>Quail</b> ( <i>Coturnix coturnix</i> )		52		87,559



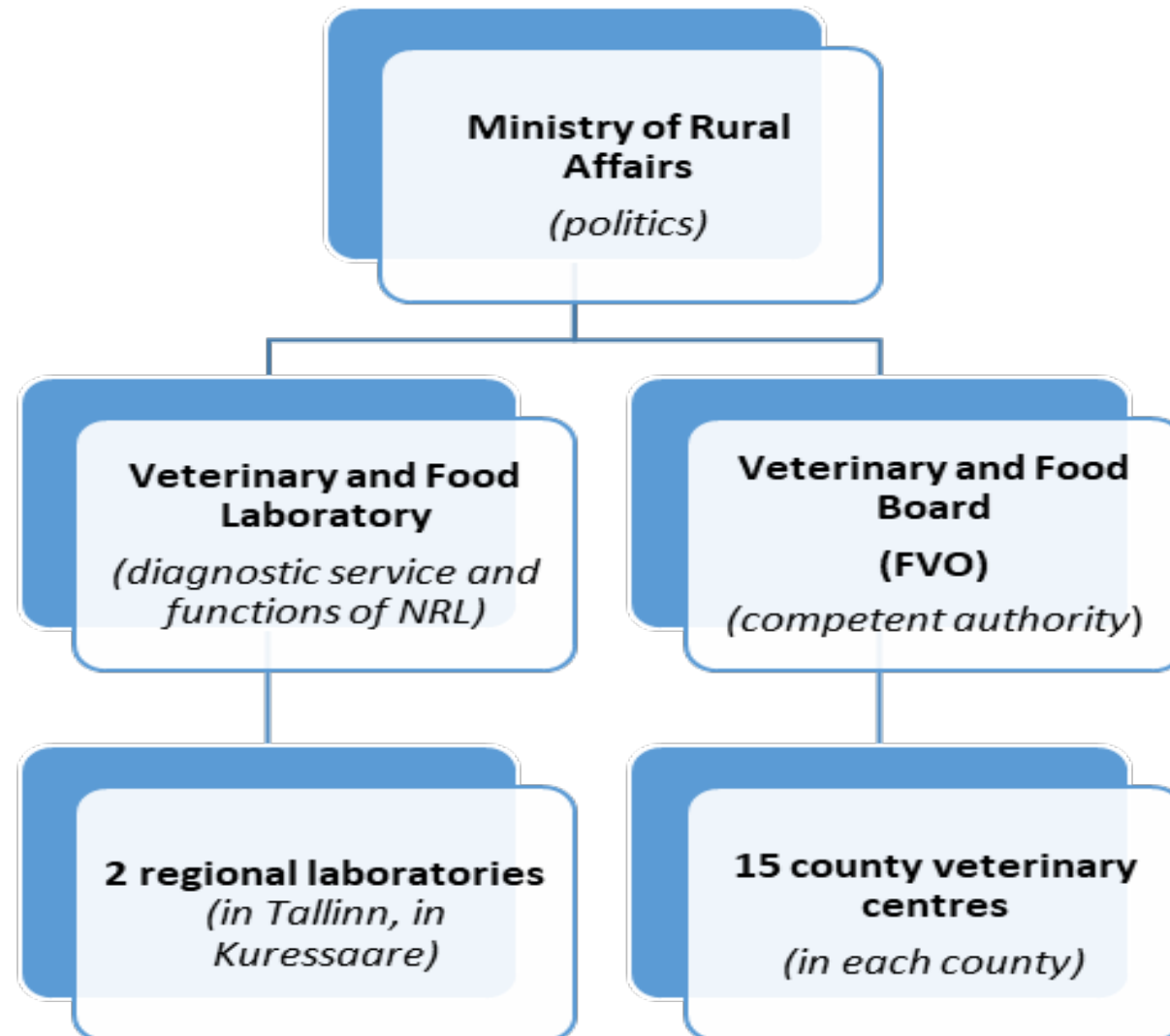
# *Salmonella* cases in human in Estonia (data from Estonian Health Board)

## Most prevalent serotypes in human during last 5 years

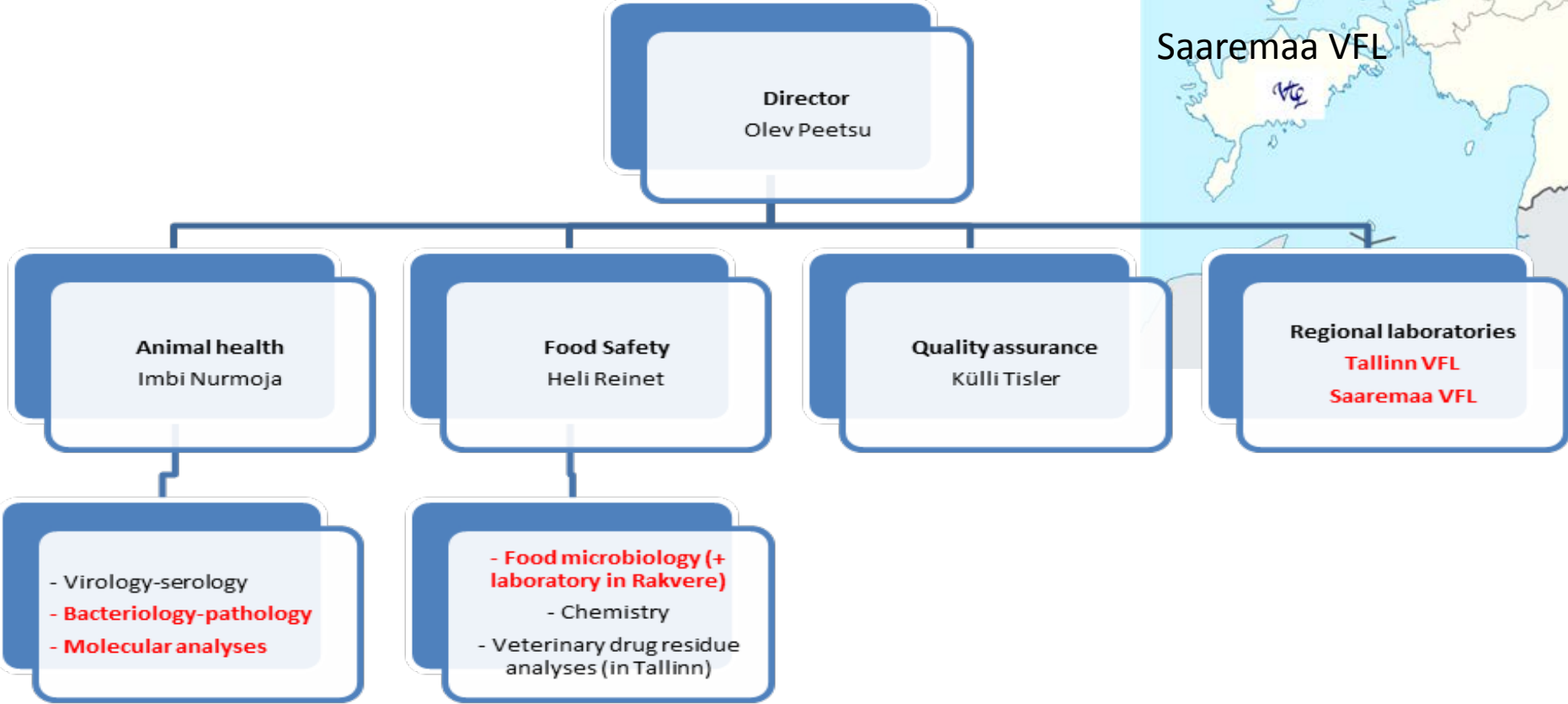
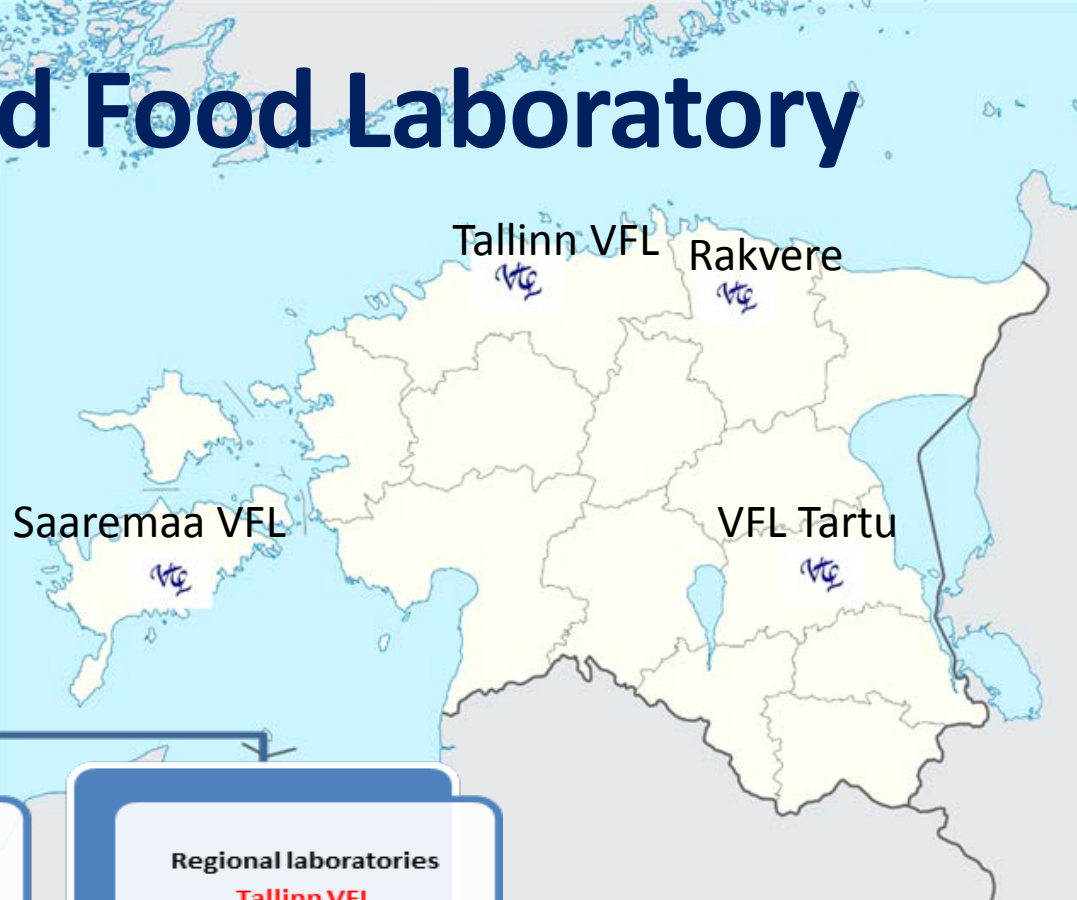
- *S. Enteritidis*
- *S. Typhimurium*
- *S. Infantis*
- *S. Typhimurium monophasic*
- *S. Agona*
- *S. Derby*
- *S. Livingstone*
- *S. Stanley*
- *S. Mbandaka*
- *S. Zanzibar*



# Veterinary and food control in Estonia



# Structure of the Veterinary and Food Laboratory



# Tasks of the Veterinary and Food Laboratory



Food safety



Functions of NRLs

Animal health



# Number of staff at Veterinary and Food Laboratory 2017

Laboratories	Total	Specialists	Technicians	Supporting staff
VFL (+Rakvere)	72 (+4)	44 (+2)	17 (+1)	11 (+1)
Tallinna VFL	35	17	13	5
Saaremaa VFL	8	4	3	1
<b>Total</b>	<b>119</b>	<b>67</b>	<b>34</b>	<b>18</b>



# Quality system accreditation

All laboratories of the VFL are accredited by the Estonian Accreditation Centre ([www.eak.ee](http://www.eak.ee)) according to EVS-EN ISO/IEC 17025:2006

- ✓ Central Laboratory in Tartu 1997
- ✓ Tallinn VFL 1996
- ✓ (Rakvere VFL 1999)
- ✓ Saaremaa VFL 2000

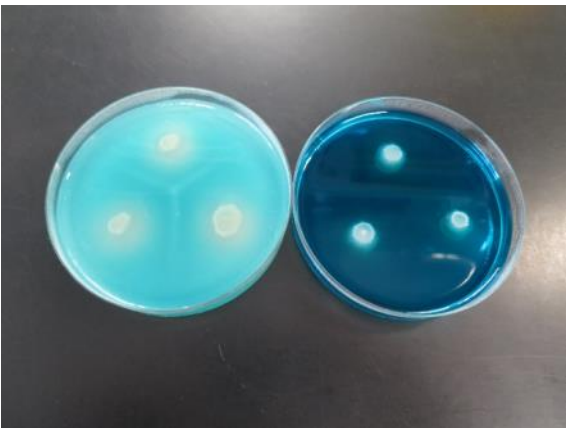
~500 different analytical and diagnostic methods  
(~ 75 % accredited)



# Methods (1)

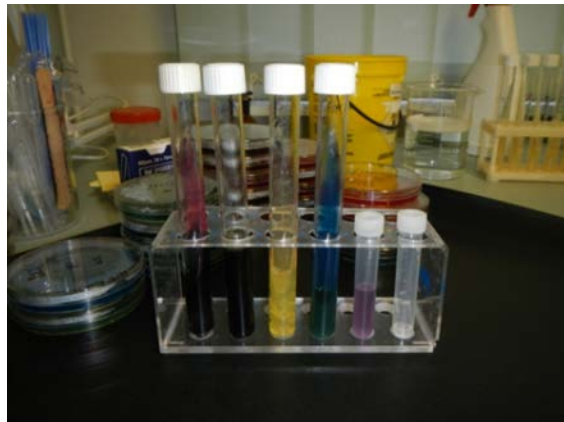
## Detection method

- EVS-EN ISO 6579-1



## Serotyping

- ISO/TR 6579-3
- White-Kauffmann-Le Minor scheme



## Methods (2).

### ***Salmonella* spp. detection by real-time PCR**

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Josefsen, M.H., Krause, M., Hansen, F. & Hoorfar, J. (2007) *Optimization of a 12-Hour TaqMan PCR-Based Method for Detection of Salmonella Bacteria in Meat. Applied and Environmental Microbiology* 73(9): 3040-3048.

Validated and accredited in 2009

144 samples analyzed by PCR in 2017

# Methods (3).

## PCR method for specific differentiation of *Salmonella* Typhimurium and its monophasic variant

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### Adoption and validation of PCR method in 2017

Maurischat, S., Baumann, B., Martin, A., Malorny, B. (2015) *Rapid detection and specific differentiation of Salmonella enterica subsp. enterica Enteritidis, Typhimurium and its monophasic variant 4,[5],12:i: - by real-time multiplex PCR. International Journal of Food Microbiology* 193: 8-14.

**Accredited in May 2018**

### Modifications compared to the article:

- S. Enteritidis is analysed in separate single-plex PCR
- RotorGene Q (Qiagen) system instead of BioRad
- PCR kit from Solis BioDyne

# National control programs for *Salmonella* in livestock

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## ACTIVE SURVEILLANCE

- Poultry
- Cattle
- Pigs
- Sheep and goats.

## PASSIVE SURVEILLANCE

NRL reports *Salmonella* positive findings in animals to national authority



# Salmonella serotypes in animals 2013-2017

## Cattle

S. Typhimurium  
S. Typhimurium  
monophasic  
S. Dublin  
S. Mbandaka  
S. Derby  
S. Altona  
S. Lexington  
S. Hindmarsh  
S. Enteritidis  
S. Infantis  
*S. enterica* subsp. *arizonae*

## Pig

S. Agona  
S. Derby  
S. Choleraesuis var.  
Kunzendorf  
S. Worthington  
S. Mbandaka  
S. Typhimurium  
S. Typhimurium  
monophasic  
S. Saintpaul

## Sheep

*S. enterica* subsp.  
*diarizonae*  
*S. enterica* subsp.  
*arizonae*  
S. Typhimurium

## *Gallus gallus*

S. Enteritidis  
S. Typhimurium  
S. Typhimurium  
monophasic  
S. Infantis  
S. Derby  
S. Senftenberg  
S. Dublin  
S. Hessarek

## Quail

S. Enteritidis  
S. Typhimurium  
monophasic  
S. Typhimurium  
S. Anatum

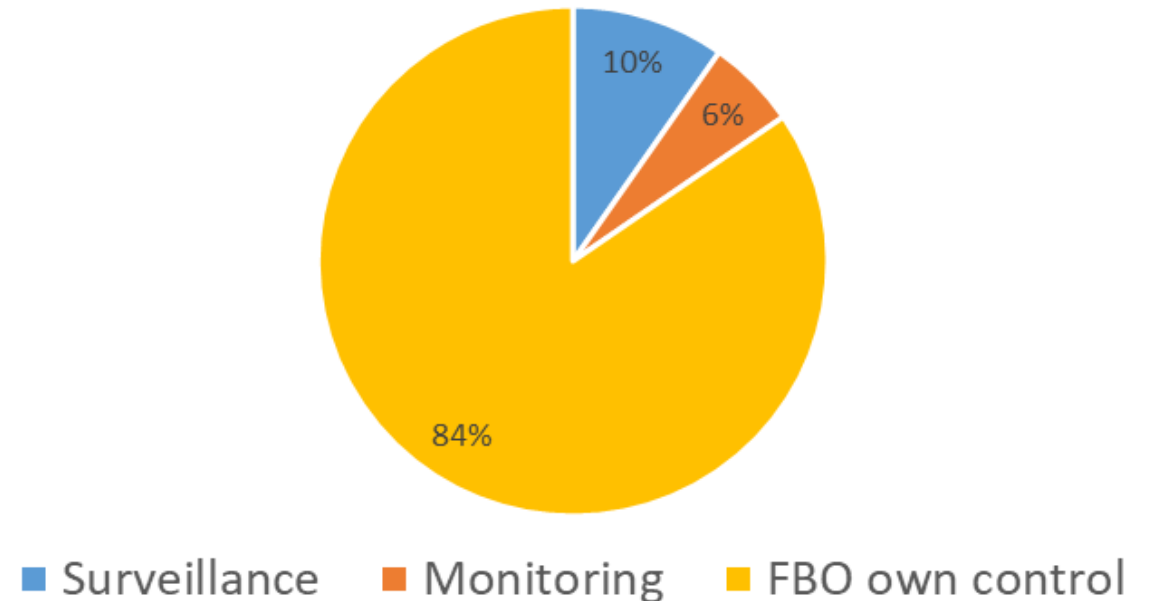
Ca 2 200 analyses annually (control program, health status monitoring by veterinarians, testing for diagnostic purposes)

# Salmonella serotypes in food 2013-2017

## Most prevalent serotypes in food during last 5 years

- S. Derby
- S. Infantis
- S. Isangi
- S. Mbandaka
- S. Agona
- S. Typhimurium
- S. Enteritidis
- S. Lexington
- S. Senftenberg
- S. Anatum

Salmonella analyses from food matrix ,  
at VFL 2013-2017



Ca 16 000 *Salmonella* analyses per year

# Coordinate activities of official laboratories for analysis of samples

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- Food - 11 official control laboratories, 2 FBO laboratories, 5 private laboratories
- Primary production stage – 3 official control laboratories, 2 FBO laboratories, 2 private laboratories

Collection of *Salmonella* isolates from VFL regional laboratories and official control laboratories.

Confirmation/ additional typing:

- MALDI-TOF MS
- serotyping of *Salmonella* isolates
- confirmation of isolates typed as 'monophasic *Salmonella* Typhimurium' by official laboratories
- collection and storage of isolates for further testing (AMR - MIC method).



# Inter-laboratory proficiency testing

## Primary production stage

- September 2017
- 8 laboratories
- 10 sock samples per laboratory
- *Salmonella* Enteritidis (2 low & 2 high level)
- *Salmonella* Abaetetuba (2 low & 2 high level)
- 2 blank samples
- Satisfactory results

## Food

- February 2018
- Participants (n = 14):
  - public labs n = 8
  - private labs n = 4
  - FBO labs n = 2
- 4 samples per laboratory (chicken broiler ground meat)
- *Salmonella* Enteritidis (1 low & 1 high level)
- 1 blank sample & 1 lot sample (low or blank)
- Satisfactory results

# Collaboration with EURL in relevant area

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Participating in ring trials organized by EURL Salmonella:

- Food
- Feed
- Primary production stage
- Serotyping

Participation in EURL Salmonella workshops

*Ad hoc* questions

Participation in validation study of *Salmonella* Typhimurim monophasic variant (supply of *S. Typhimurium* and *S. Typimurium* monophasic strains)

# Other activities

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## Workshops

- 2008, 2011 and 2017 for laboratories dealing with *Salmonella* testing in food, feed, animals.

## Publications

- Kramarenko, Toomas; Nurmoja, Imbi; Kärssin, Age; Meremäe, Kadri; Hörman, Ari; Roasto, Mati (2014). The prevalence and serovar diversity of *Salmonella* in various food products in Estonia. Food Control, 42, 43–47. [10.1016/j.foodcont.2014.01.032](https://doi.org/10.1016/j.foodcont.2014.01.032).
- Roasto, Mati; Kramarenko, Toomas (2017). Salmonelladest ja salmonelloosist. Eesti Loomaarstlik Ringvaade, 3, 12–15.

# Next Generation Sequencing

In 2018 VFL started to perform NGS for the first time to gain experience in this field

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79 *S. Derby* were collected for library construction (performed by VFL)

39 samples are already sequenced with ILLUMINA MiSeq platform (ordered in subcontract from Estonian Genome Centre)

Data analysis is ongoing...

- ✓ Genomes assembled with SPADES
- ✓ Sequence type (ST) determined with MLST tool available in Centre for Genomic Epidemiology
- ✓ Resistance genes and mutations determined with ResFinder available in Centre for Genomic Epidemiology

## **Acknowledgements**

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**THANK YOU FOR YOUR ATTENTION!**

Photo: CDC