

State Veterinary Institute Prague

NRL for Salmonella

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NRL for Salmonella

- NRL for Salmonella is located in the State Veterinary Institute Prague
- NRL for Salmonella was established in 2003 by the State Administration
- Establishing was based on EU legislation
- Close collaboration with EURL for Salmonella
- Participation in projects organized by this EURL
- Diagnostic standards are checked by the PT organized by the EURL three times a year

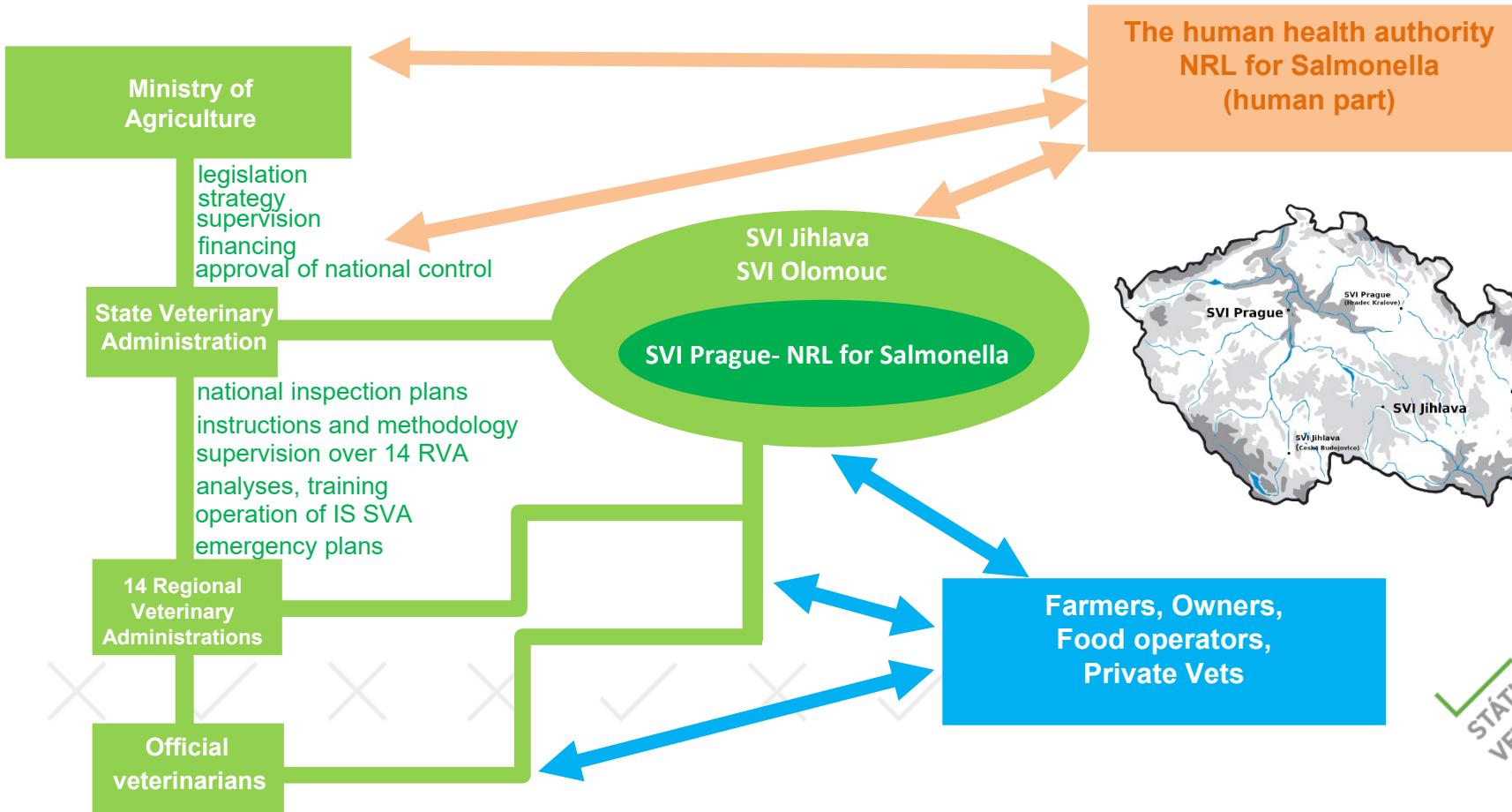


State Veterinary Institute Prague

- Laboratory diagnostics of infectious and non-infectious animal diseases
- Monitoring of residues of veterinary drugs and contaminants in the food chain of humans, animals and the environment
- Analysis of quality of food, animal feed and water, adulteration of food
- Training, educational and expert activities (Training center)
- Foreign Development Cooperation Projects
- 146 employees
- Branch in Hradec Králové (provide full range of diagnostic services)
- Collection lines (10 routes per day, approx. 3000-4000 km daily)



Salmonella – network



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NRL for *Salmonella* in the Czech Republic - task and duties

- Experience transfer - implementing of new knowledges
- Organization of training of other SVI laboratory staff
- Organization of proficiency testing for SVI laboratories
- Coordination of SVI laboratories
- Consulting activity for public and state sides
- Collection and analysis of data from diagnostic activities
- Reference testing - determination of serotype
- Cooperation with the SVA CR - information transfer
- Issuing an annual report
- Storage of isolates - management of isolates of *Salmonella* spp.



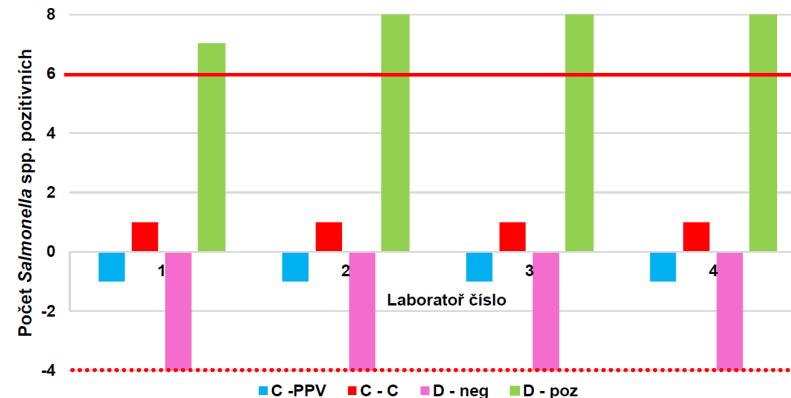
Organizing of proficiency testing for SVI laboratories I

- Scheme of organization of PT is similar as in EURL
- Usually one type of PT annually
- Rotation of PT of serotyping and detection of *Salmonella* spp.
- Only „good performance“ scored laboratories can take a part in examination of official samples
- PT have been organized by the NRL regularly since 2012



Graf č.1

Výsledky testování jednotlivých zúčastněných laboratoří



Organizing of proficiency testing for SVI laboratories II

PT detection of *Salmonella* spp. usual schedule:

- 12 samples + 2 control samples
- 8 *Salmonella* contaminated (30-60 CFU / 25 g)
- 4 blank samples

Matrix: poultry feces, boot swabs, feed

Minimal criteria necessary for good laboratory practice of the participating laboratory:

Sample type	Required number of correctly identified in category	
Negative control sample	100 %	1/1
Positive control sample	100 %	1/1
Blank samples	100 %	4/4
Contaminated samples	>75 %	>6/8



Organizing of proficiency testing for SVI laboratories III

PT serotyping of *Salmonella* spp. usual schedule:

- Total 10 samples of serotypes which were detected in the Czech Republic
- 4 main *Salmonella* serotypes (Enteritidis, STM, Monophasic STM, Infantis)
- 3 usual serotypes which were detected in previous years
- 3 unusual serotypes which were detected in previous years

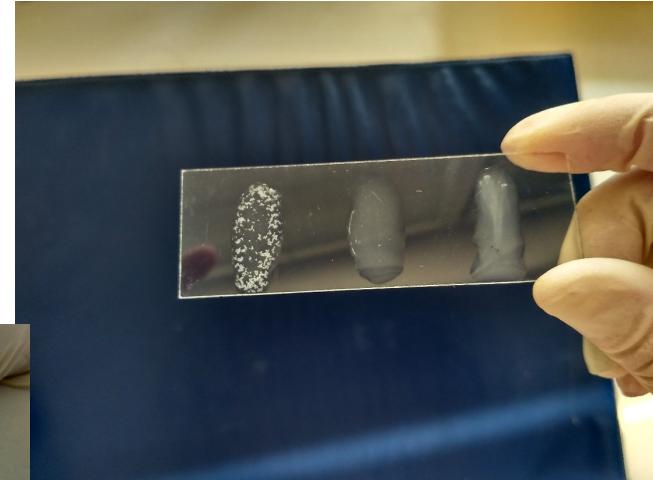
Výsledek	Vyhodnocení	Bodová ztráta	Název sérovaru	O-antigeny	H-antigeny	Bodový zisk
Nesprávný sérovar, nesprávná antigenní determinace sérovaru, smíšená antigenní formule, neodpovídající antigenní determinace a název sérovarů: S. Enteritidis, S. Typhimurium, monofázický kmen S. Typhimurium, S. Infantis	Nesprávný výsledek	4	S. Enteritidis	1,9,12	g,m,-	4
Nesprávný sérovar, nesprávná antigenní determinace sérovaru, smíšená antigenní formule, neodpovídající antigenní determinace a název sérovarů: ostatních testovaných sérovarů	Nesprávný výsledek	1	S. enterica ssp. enterica (4,[5],12:i:-)*	1,4,[5],12	i:-	4
Nekompletní antigenní formule, nekompletní antigenní determinace, neuvedení správného jména sérovarů: S. Enteritidis, S. Typhimurium, monofázický kmen S. Typhimurium, S. Infantis	Částečně správný výsledek	2	S. Derby	1,4,[5],12	f,g:[1,2]	1
Nekompletní antigenní formule, nekompletní antigenní determinace, neuvedení správného jména sérovarů: ostatních testovaných sérovarů	Částečně správný výsledek	0,5	S. Heidelberg	1,4,[5],12	r:1,2	1
Korektní antigenní determinace a uvedení správného názvu sérovaru	Správný výsledek	0	S. Bareilly	6,7,14	y:1,5	1
			S. Agona	1,4,[5],12	f,g,s:[1,2]	1
			S. Typhimurium	1,4,[5],12	i:1,2	4
			S. Minnesota	21	b:e,n,x	1
			S. Kentucky	8, 20	i:z6	1
			S. Infantis	6,7,14	r:1,5	4

Reference testing – serotype determination



Serotyping of strains
in full range of KWL
scheme

Determination of
monophasic Typhimurium
strains by PCR



Subsequent epidemiological
typing of the strains
by MLVA, WGS



Testing – serotype determination and epizootologic analysis

Numbers of analyzes performed annually:

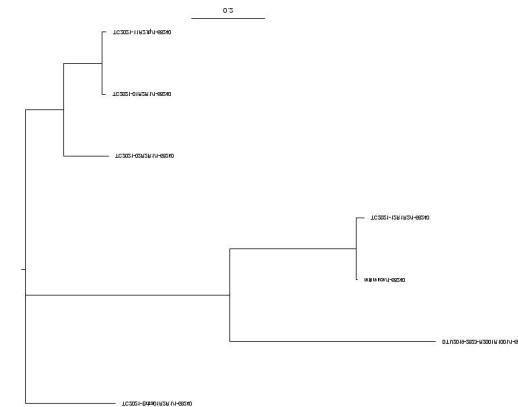
- 700-900 strains are serotyped
- 200-300 strains are MLVA typed (SE, STM, monophasic STM)

Performed WGS analysis:

- 2020: 30 strains
- 2021: 230 strains
- 2022: > 600-700 strains ??? (plan)

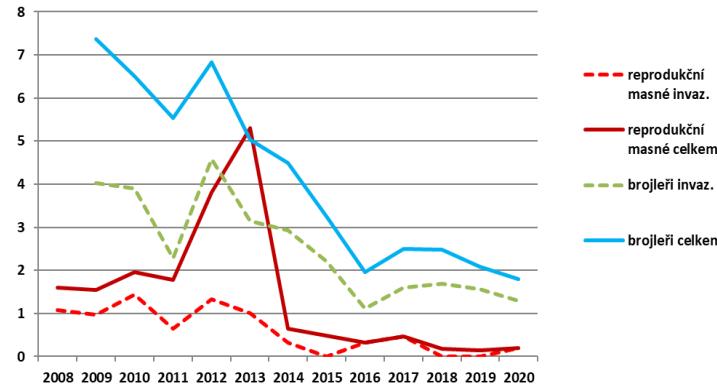
WGS analysis methodology:

- following: EURL- AR SOP (including QC rules)
- technology: NEBNext Ultra II FS DNA Library Prep Kit, NextSeq, Single-end150b
- SW: trimming, QC, assembly (Trimmonatic, FastQC, SPAdes)
- SW: <http://cge.cbs.dtu.dk/services> (MLST, cgMLST, serotyping, KmerFinder, CSIPhylogeny)
<https://blast.ncbi.nlm.nih.gov/Blast.cgi> (serotyping)

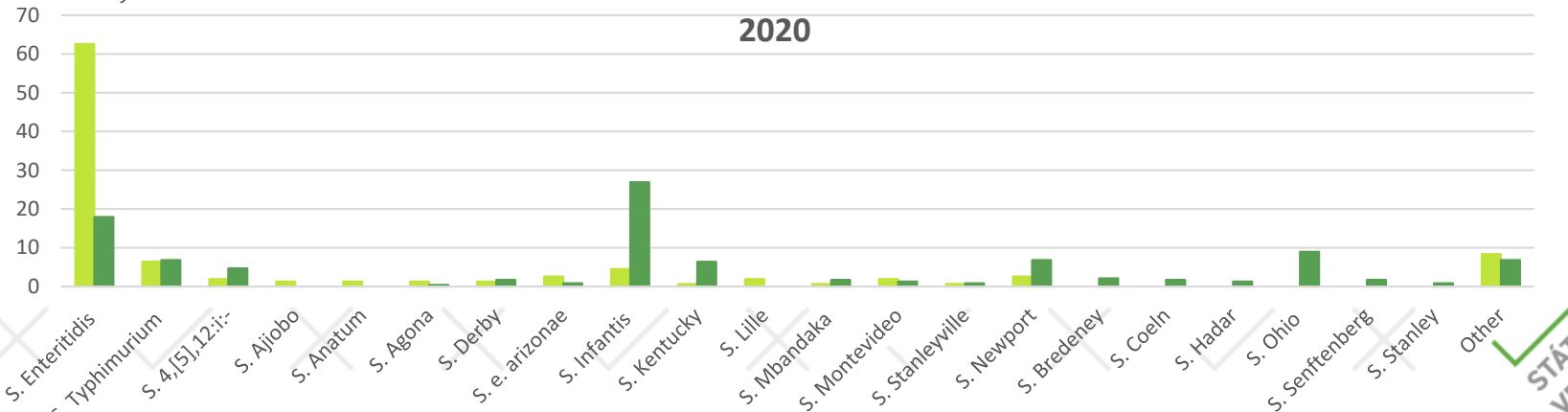
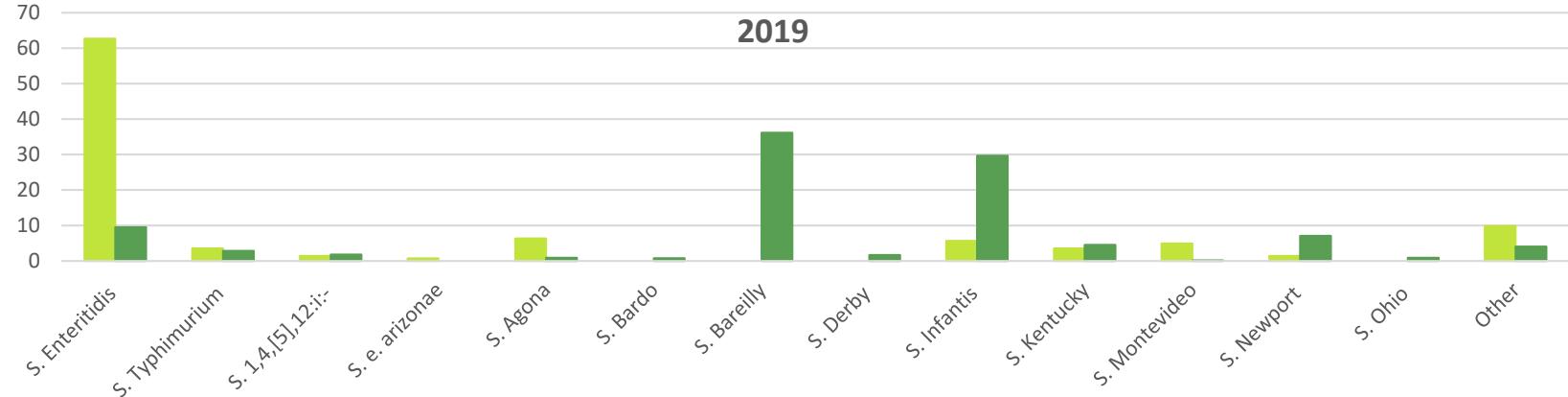


Collection and analysis of data from diagnostic activities

- Providing laboratory results to SVA/RVA electronically
 - Regular monthly data check and cross-checking of SVI and SVA databases
 - Identification and subsequent correction of the errors found
 - Statistical data analysis - quantification of results (prevalence level etc.)
 - Annual submission of data to the EFSA database



Relative serotype distribution in the Czech Republic



Animals Food

Storage of isolates - management of *Salmonella* spp. isolates

- Management of strain database
- Storage of isolates for at least five years
- Preservation of the isolates in a deep-frozen state (-80 °C)
- System is set up to quickly locate and revive isolates (< 48 h)



NRL for *Salmonella* in SCP in poultry - responsibilities

- Provides referential serotyping of *Salmonella* spp. strains which were isolated in other SVI laboratory from SCP samples
- Provides determination and differentiation between wild and vaccination strain of serotype *Salmonella* Enteritidis and *Salmonella* Typhimurium.
- Provides confirmatory tests from the holding environment (faeces, swabs, dust) to the extent determined by the SVA methodology



NRL for Salmonella in SCP in poultry - responsibilities

- Establishing rules for sampling within the SCP
- Development and production of unique sampling kits for each category of poultry
- Distribution of manufactured sampling kits to the end samplers (official or private vets, farmers etc.)



Plans for the future (2022 – 2023)

To organize PT:

- PT serotyping (10 control strains, including differentiation between wild and vaccination strains SE)
- PT detection of *Salmonella* spp. in carcasses swabs
- 14 samples + 2 control
- 12 *Salmonella* spp. contaminated in two levels (low 10-20; high 100-200 CFU / 10 g)
- 2 blank samples

Building of WGS testing capacity (>1000 samples/year in 2023)

Building an online network for data sharing between stakeholders on the veterinary and humane side



Thanks for your attention!



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