Multinational outbreak of salmonellosis (*Salmonella* Bareilly) confirmed by whole genome sequencing in the Czech Republic
Public Health Authority (PHA) alert

• In spring 2018 human health authorities informed during the regular meeting of the Working Group for Zoonoses other stakeholders about an increased incidence of S. Bareilly:

  • Since 8/2017 number of reported human cases of the S. Bareilly have been increasing in the Czech Republic (CZ)
  • 5-year maximal incidence has been exceeded
  • Increased number of requests for serotyping in the NRL (human) in the CZ and SK

Number of diseases caused by S. Bareilly
by the beginning of the disease week, CZ, EPIDAT 2017
Salmonella enterica serotype Bareilly (SB)

- Enterobacteriales; g: Salmonella; sp: Salmonella enterica
- Bareilly serovar (6.7: y: 1.5), group C1
- In 1928 first identification in India
- Since 2016 has been in the top 20 serotypes in the EU / EEA
- In the CZ usually 5-20 human cases annually
- This serotype occasionally isolated from broiler environment (SCP)
- Described atypical sources of epidemics in the world:
  - Scraped tuna for sushi (2012)
  - Mung bean sprouts (2010)
  - Peeled sesame seeds (2017)
Confirmation of the outbreak by PHA

• NRL (human) and VRI performed PFGE analysis of SB strains originated from human cases in 1/2017-10/2018 period

• PFGE identified occurrence of the identical pulsotype (TESSy reference type XbaI.2667) depicted as SB-Xba-1 from 28th week 2017

• Strains originated from human cases in 7/2017-12/2017 period were sequenced (WGS)

• The existence of the epidemiological cluster was confirmed by these analyzes
Conduction of laboratory survey by PHA

- During the 2017 NRL (human) obtained 174 strains of S.B. for reference serotyping
- In autumn of 2018 NRL (human) distributed the questionnare to another 121 microbiological laboratories

- 87% of laboratories answered
- 71% of laboratories confirmed isolation of S.B. strain during the focused period
- By this survey were 168 primary S.B. isolates detected
- Only 16% (n = 32) of all isolated S.B. strains were not serotyped by the NRL (human)
- NRL (vet) joined this survey
Distribution of probable and confirmed cases of SB by week of symptom onset in CZ from 1/2017 to 12/2018
Distribution of probable and confirmed cases of SB by week of symptom onset in SK from 1/2017 to 12/2018

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Map displaying number of cases and notification rate by region of CZ and SK
Conduction of epidemiological survey by PHA

Reports from routine epidemiological screening from 11 regions of the CZ were analysed for probable source (vehicle)

  • NRL (human) obtained 97 results of investigation from the Regional Public Health Authority

In order to generate hypothesis the trawling questionnaire was distributed during 2018

  • Modification of the Danish trawling questionnaire for the purposes of the CZ investigation in 2018
  • Questionnaire was shared with CZ and SK

Results from survey did not allow to identify common exposure to unique food item

RESULT = WEAK HYPOTHESIS FOR ANALYTICAL STUDY
Reactions of State Veterinary Administration (SVA) to the primary PHA alert

• NRL (vet) was activated by SVA
• The EURL Salmonella network was joined for sharing information about SB
• NRL (human) and NRL (vet) have started with close cooperation and sharing key information
• NRL (vet) searched its databases and tried to find suspect isolates of SB
• All known isolates of SB from 2015 - 2018 period originated in broiler environment with different pulsotypes
The Regional Veterinary Administration was informed about a positive SB finding in September 2018. Is this the primary source of SB?

Dried egg melange
- Industry sampling
- Produced in July 2018
- Lot size 390 kg

Origin of the eggs: CZ
Three eggs producing holdings

SB has never been detected in Czech egg-laying hens during the period of implementation of the SCP (since 2007).

The entire batch was disposed of in July 2018 due to alteration in organoleptic properties (The product was not placed on market).
The Regional Veterinary Administration performed an official sampling in the processing plant. The samples were examined in NRL (vet).

**Dried egg white**
- Produced in August 2018
- Lot size 480 kg

**Origin of the eggs:** CZ
Two eggs producing holdings
But not the same as in the first case

The whole batch was disposed of
(The product was not not placed on market)

September of 2018
Result of SVI Prague, NRL (vet) examination
*Salmonella* Bareilly (1/5) positive

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Is it possible that the primary source of SB has been revealed?

• The RVA during September and October 2018 performed a series of official sampling in this processing plant

• Additional SB positive samples were detected in the following egg products:

  Dried egg melange 1/5 SB
  Dried egg melange 2/5 SB

• In the other sampled products (liquid egg white, mayonnaise catering, hamburger, roasted pork knee, roasted pork hip and pork chop-fried) was not salmonella detected

• In samples (swabs) from the environment of processing plant was not salmonella detected
Measures taken by the RVA

• The producer is under the control of the RVA and each batch produced must be examined for the presence of salmonella and it is suspended until the end of the examination and is only released on the basis of a negative (satisfactory) examination result.

• In September 2018, the production line was completely sanitized. The technology was largely disassembled and properly sanitized according to the established sanitation plan.

But the answers to this basic questions are still missing:

• What is the primary source of SB: eggs or industry technology ???

• Is there any connection between this finding and FBO in human population ???
SVA investigation of possible primary source of SB in the processing plant

The technology in the processing plant was put into operation during June - July 2018. SB FBO started already during 2017.

Technological equipment for drying eggs came from other processing plant, which was closed by its owner in the end of March 2017.

But after the end of production, this plant still served as a warehouse. Dried egg mass was sold from this place until July 2018.
Does there really exist a connection between SB isolated from dried egg mass and SB FBO in the human population???

• PFGE analysis was performed on all SB strains (4) originated in dried egg mass in 8/2018-10/2018 period
• Identical pulsotype (SB-Xba-1) was identified in all these strains
• This pulsotype was the same as that of the SB FBO strains
• First strain of SB originated from the RVA official sampling in the processing plant was sequenced (WGS) in the same batch as the FBO isolates
• Examined strains from human cases and strain from dried eggs clustered within 8 alleles (cgMLST analysis, 3002 alleles)
WGS cgMLST

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WGS – SNP phylogenetic analysis

Epidemic cluster
Summary

• 325 probable cases of SB salmonellosis belonging to the FBO were found in the period from 7/2017 to 10/2018
• 82 cases (26%) were typed by the molecular-genetic methods
• The last case of human SB infection in the CZ was recorded in the 43rd week (October) 2018
• Since September 2018 veterinary measures have been imposed at the processing plant (egg dryer)
• The identical PFGE pulsotype was identified in environmental and FBO isolates of SB
• Environmental and FBO isolates forming cluster (within 8 alleles)
• We are planning the WGS of SB strains, which had been isolated from environment of the processing plant
• Egg matter was identified as highly probable vehicle of infection
• The most probable source of SB appears to be the long-term contaminated egg spray dryer
Conclusion

• The investigation of this FBO proved the functionality of cooperation network of Public Health and Veterinary authorities in CZ

• Close cooperation among stakeholders is essential

• Molecular typing methods (WGS; PFGE) helped to link human cases and probable source of outbreak
Many thanks for all participants
Thanks for your attention