National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

The 23rd EURL-*Salmonella* workshop

29 and 30 May 2018, Uppsala, Sweden

RIVM Report 2018-0024
K.A. Mooijman

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RIVM Report 2018-0024

Colophon

DOI 10.21945/RIVM-2018-0024

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This investigation was performed by order, and for the account, of European commission, Directorate-General for Health and Food Safety (DG SANTE), within the framework of RIVM project E/114506/18/WO European Union Reference Laboratory for *Salmonella* (2018)

This is a publication of:
National Institute for Public Health and the Environment
P.O. Box1 | 3720 BA Bilthoven
The Netherlands
www.rivm.nl/en

Synopsis

The 23rd EURL-*Salmonella* workshop
29 and 30 May 2018, Uppsala, Sweden

This report gives a summary of the presentations held at the 23rd annual workshop for the European National Reference Laboratories (NRLs) for *Salmonella* (29-30 May 2018). The aim of the workshop was to facilitate the exchange of information on the activities of the NRLs and the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*).

Annual ring trials
A recurring item at the workshops is the presentation of the results of the annual ring trials organised by the EURL. These provide information on the quality of the NRL laboratories tested. The NRLs had high scores in the 2017 studies; detailed information on the results per ring trial is available in separate RIVM-reports.

***Salmonella* in food and animals**
Salmonella should not be present in food and animals. However, *Salmonella* can occasionally be found in different products. Examples were given about *Salmonella* found in poultry and cattle. Other information concerned the (unwanted) presence of *Salmonella* in infant formula and birds and cats.

The annual workshop was organised by the EURL-*Salmonella*, part of the Dutch National Institute for Public Health and the Environment. The main task of the EURL-*Salmonella* is to evaluate the performance of the European NRLs in detecting and typing *Salmonella* in different products.

Keywords: EURL-*Salmonella*, NRL-*Salmonella*, *Salmonella*, workshop 2018

Publiekssamenvatting

De 23e EURL-*Salmonella* workshop

29 en 30 mei 2018, Uppsala, Zweden

Het RIVM heeft de verslagen gebundeld van de presentaties van de 23e jaarlijkse workshop voor de Europese Nationale Referentie Laboratoria (NRL's) voor *Salmonella* (29-30 mei 2018). Het doel van de workshop is dat het overkoepelende orgaan, het Europese Referentie Laboratorium (EURL) voor *Salmonella*, en de NRL's informatie uitwisselen.

Een terugkerend onderwerp zijn de ringonderzoeken die het EURL jaarlijks organiseert om de kwaliteit van de NRL-laboratoria te controleren. De NRL's scoorden goed in de studies van 2017. In dit rapport staan de ringonderzoeken kort beschreven. Een uitgebreidere weergave van de resultaten wordt apart per ringonderzoek gepubliceerd.

Salmonella mag niet in voedsel en dieren zitten. Toch kan *Salmonella* soms gevonden worden in verschillende producten. Voorbeelden werden gegeven van *Salmonella* die in pluimvee en runderen was aangetroffen. Andere informatie betrof de (ongewenste) aanwezigheid van *Salmonella* in babyvoeding en in vogels en katten.

De organisatie van de jaarlijkse workshop is in handen van het EURL voor *Salmonella*, dat onderdeel is van het RIVM. De hoofdtaak van het EURL-*Salmonella* is toezien op de kwaliteit van de nationale referentielaboratoria voor deze bacterie in Europa.

Kernwoorden: EURL-*Salmonella*, NRL-*Salmonella*, *Salmonella*, workshop 2018

Contents

Summary — 9

1 Introduction — 11

2 Tuesday 29 May 2018: day 1 of the workshop — 13
2.1 Opening and introduction — 13
2.2 EURL-*Campylobacter* — 13
2.3 No decrease of human *Salmonella* Enteritidis despite *Salmonella* control programmes in poultry in the European Union, 2013-2016 — 15
2.4 Multi-country outbreak of *Salmonella* Agona infections linked to infant formula — 16
2.5 Investigating *Salmonella* in the cattle production in France — 18
2.6 Results combined Food-PPS interlaboratory comparison study on detection of *Salmonella* in hygiene swabs (2017) — 18
2.7 Preliminary results of the 4th interlaboratory comparison study on detection of *Salmonella* in chicken feed (2018) — 20
2.8 Results 22nd interlaboratory comparison study on typing of *Salmonella* (2017) - serotyping and PFGE — 23
2.9 Update on activities in ISO and CEN — 24
2.10 Birds, cats, humans and host adaptation in *Salmonella* Typhimurium — 27
2.11 Pilot validation study for confirmation of *Salmonella* following ISO/DIS 16140-6 — 28

3 Wednesday 30 May 2018: day 2 of the workshop — 31
3.1 Activities of the NRL-*Salmonella* to fulfil tasks and duties in Finland — 31
3.2 Activities of the NRL-*Salmonella* to fulfil tasks and duties in Hungary — 32
3.3 Activities of the NRL-*Salmonella* to fulfil tasks and duties in Iceland — 34
3.4 Activities of the NRL-*Salmonella* to fulfil tasks and duties in Estonia — 35
3.5 Activities of the NRL-*Salmonella* to fulfil tasks and duties in Former Yugoslav Republic of Macedonia (FYROM) — 36
3.6 EFSA's molecular typing activities for food-borne pathogens — 37
3.7 EURL working group on Whole Genome Sequencing (WGS) — 38
3.8 Work programme EURL-*Salmonella* second half 2018, first half 2019, discussion on general items and closure — 39

4 Evaluation of the workshop — 43
4.1 Introduction — 43
4.2 Evaluation form — 43
4.3 Discussion and conclusions of the evaluation — 50

Acknowledgements — 51

List of abbreviations — 53

References — 55

Annex 1 Participants — 59

Annex 2 Workshop Programme — 61

Annex 3 Workshop evaluation form — 64

Summary

On 29 and 30 May 2018, the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*) organised its annual workshop in Uppsala, Sweden. Participants of the workshop were representatives of the National Reference Laboratories (NRLs) for *Salmonella* from 27 EU Member States, three European Free Trade Association (EFTA) countries, and two (potential) EU candidate countries. Also present were representatives of the European Commission Directorate General for Health and Food Safety (DG-SANTE), and of the European Food Safety Authority (EFSA). In total, 3 participants of NRLs from one EU Member State, and two (potential) candidate countries, were unable to join the workshop. A total of 46 participants attended.

During the workshop, presentations were given on several items. The results of the interlaboratory comparison studies organised by the EURL-*Salmonella* in the past year were presented: the detection of *Salmonella* in hygiene swabs (October 2017), in chicken feed samples (February 2018), and the study on *Salmonella* typing (November 2017).
As the workshop was organised at the institute where the EURL-*Campylobacter* is situated, it was a good opportunity to get information from the coordinator of this colleague EURL about their activities.
An EFSA representative gave a presentation on the fact that human *Salmonella* Enteritidis infection has not decreased, despite EU *Salmonella* control programmes in poultry. Additionally, the EFSA representative gave an update on EFSA activities concerning molecular typing of food-borne pathogens.
A representative of EC DG-SANTE gave a presentation on an outbreak of *Salmonella* Agona related to infant formula. Furthermore, the DG-SANTE representative informed the participants about the EURL's working group on Whole Genome Sequencing (WGS).
A summary was given of the standardisation of methods in ISO and CEN. Additionally, a presentation was given on a pilot validation study for alternative confirmation of *Salmonella* following prEN ISO/DIS 16140-6:2017.
A representative of the French NRL gave a presentation on the investigation of *Salmonella* in cattle production in France, and a representative of the Swedish NRL gave a presentation on host adaptation of *Salmonella* Typhimurium in birds, cats and humans.
Five representatives gave a summary of their activities as NRL to fulfil the prescribed tasks and duties (Finland, Hungary, Iceland, Estonia and FYROM).

The workshop concluded with a presentation on the EURL-*Salmonella* work programme for the current and coming year.

All workshop presentations can be found at:
https://www.eurlsalmonella.eu/workshop-2018

1 Introduction

This report includes the abstracts of the presentations given at the 2018 EURL-*Salmonella* workshop, as well as a summary of the discussion that followed the presentations. The full presentations are not included in this report, but are available on the EURL-Salmonella website: https://www.eurlsalmonella.eu/workshop-2018

The layout of the report is consistent with the workshop programme. Chapter 2 includes the abstracts of presentations held on the first day. Chapter 3 includes the abstracts of presentations held on the second day. The workshop is evaluated in chapter 4; the evaluation form template can be found in Annex 3.
The list of participants is given in Annex 1.
The workshop programme is given in Annex 2.

2 Tuesday 29 May 2018: day 1 of the workshop

2.1 Opening and introduction

Kirsten Mooijman, head EURL-Salmonella, Bilthoven, the Netherlands

Kirsten Mooijman, head of the EURL-*Salmonella*, opened the 23rd workshop of the EURL-*Salmonella*, welcoming all participants to Uppsala, Sweden.

At this workshop, 46 participants were present, including representatives of the National Reference Laboratories (NRLs) for *Salmonella* from 27 EU Member States, two (potential) candidate EU countries, and three member countries of the European Free Trade Association (EFTA). Furthermore, representatives from the EC Directorate General for Health and Food Safety (DG-SANTE) and the European Food Safety Authority (EFSA) attended the workshop. Apologies were received from representatives of NRLs from Malta, Bosnia and Herzegovina, and Turkey.

The evaluations of the last seven workshops (2011-2017) were then compared. The opinion on the scientific programme was the same in all workshops: very good to excellent.

The workshop started after presenting the programme and general information.
The workshop programme can be found in Annex 2.

2.2 EURL-*Campylobacter*

Hanna Skarin, EURL-Campylobacter, National Veterinary Institute (SVA), Uppsala, Sweden

Campylobacter is a Gram-negative spiral and rod-shaped bacterium that grows in micro-aerobic conditions. It is mainly found in the gut of healthy animals and primarily that of birds. Therefore, handling and consumption of chicken meat may have a significant risk for humans. *Campylobacter* infection causes acute gastroenteritis with diarrhoea, fever, vomiting and abdominal pain. Thermotolerant *Campylobacter* spp. represents the *Campylobacter* mainly associated with disease in humans. The most common thermotolerant spp. is *C. jejuni*, followed by *C. coli*. From 2005 onwards, *Campylobacter* has been the most frequently reported foodborne pathogen in the EU.
The EURL-*Campylobacter* was established in 2006 and, since the beginning, has been located at the National Veterinary Institute (SVA) in Uppsala, Sweden. The current team includes one veterinarian, one epidemiologist, lab technicians, molecular biologists, one administrator, and one financial officer. The EURL-*Campylobacter* network includes 35 NRLs in the EU Member States and 7 laboratories with similar functions in third countries. The current work programme follows Regulation (EC) 2017/625 (EC, 2017) on official controls, and The Zoonosis Directive 2003/99/EC (EC, 2003) on the monitoring of zoonoses and zoonotic agents. In 2017, a process hygiene criterion for

Campylobacter in broiler carcasses was added to Regulation (EC) No 2073/2005 (EC, 2005) for microbiological criteria in foodstuffs.
The key activities of the EURL-*Campylobacter* are to organise proficiency tests (PTs), to validate (and improve or produce) analytical methods, to maintain reference collections of *Campylobacter* strains, and to provide scientific and technical assistance to NRLs, the European Commission, and other organisations. The scope of the PTs and number of different tests produced has varied between years, but generally two tests have been provided, each representing one part of EN ISO 10272:2017 'Microbiology of the food chain – Horizontal method for detection and enumeration of *Campylobacter* spp.' In the current programme, molecular methods for detection, species identification, and genotyping are being evaluated or topics for validation studies. There is also an ongoing project on *Campylobacter* survival in different matrices with the purpose of making recommendations for sampling and transporting different samples. The EURL-*Campylobacter* maintains collections of well-characterised *Campylobacter* strains that can be used in validation studies, for PTs, or as control strains. The annual workshop is usually held in Uppsala in September or October. The EURL-*Campylobacter* organises at least one training course each year, either on request of an NRL or initiated by the EURL. One course on enumeration, detection and species identification of *Campylobacter* is usually organised in the weeks before the onset of the PTs. If laboratories underperformed in previous PTs, they are invited to participate in the course. Courses have also been given on different molecular methods such as PCR, PFGE and MLST for *Campylobacter*. Learning materials and the list of the NRL network can be found on the EURL-*Campylobacter* website (http://www.sva.se/en/service-and-products/eurl-campylobacter). The EURL-*Campylobacter* also provides assistance to the European Commission, EFSA, ECDC and other organisations. This usually occurs on an ad hoc basis and can relate to consolidation of reports, regulations, or any other business. Different members of the EURL-*Campylobacter* are part of different ISO/CEN working groups.

Discussion
Q: Is *Campylobacter upsaliensis* related to Uppsala?
A: Yes, this *Campylobacter* was found for the first time in Uppsala.
Q: In Denmark, cattle are also a source of *Campylobacter*. Is this also the case in Sweden?
A: Yes, in Sweden, cattle are also an important reservoir of *Campylobacter*. Attribution studies have shown that cattle are often a source from which *Campylobacter* may be spread to chicken.
Q: Which molecular method is more discriminatory for *Campylobacter:* PFGE or MLST?
A: PFGE is considered more discriminatory. However, MLST typing can be made more discriminatory by testing for flagellar genes in addition to the seven housekeeping genes.
Q: Is every flock checked for *Campylobacter* in Sweden? What is done if a flock is tested positive?
A: It is a prevalence programme, not a control programme. If an increase of *Campylobacter* is seen, further investigations are done at the farm. The results of the monitoring programme are available one week after slaughter so that the findings come too late for actions at the

slaughterhouse. Hence, many precautions have to be taken to prevent the presence of *Campylobacter.*
Q: In general, the numbers of *Campylobacter* are low in Sweden. What has been done to get it so low?
A: The flocks that never had *Campylobacter* are those that supply slaughter houses that do not perform thinning. Moreover, there is a range of biosecurity levels between farms; some have other livestock and these have a range of *Campylobacter* species.

2.3 No decrease of human *Salmonella* Enteritidis despite *Salmonella* control programmes in poultry in the European Union, 2013-2016

Frank Boelaert, EFSA, Parma, Italy

In 2016, 94 530 confirmed salmonellosis cases were reported by all EU-MS. The EU notification rate was at the same level compared with the previous five years. A statistically significant decreasing trend of salmonellosis was observed between 2008 and 2016, however in the last five years (2012–2016), the trend has not shown any statistically significant increase or decrease: seven MS reported an increasing trend and four MS a decreasing trend in this period (EFSA and ECDC, 2017). The top five most commonly reported serovars in human cases acquired in EU in 2016 were, in decreasing order: *S*. Enteritidis, *S*. Typhimurium, monophasic *S*. Typhimurium, *S*. Infantis, and *S*. Derby.

The proportion of human salmonellosis illnesses due to *S*. Enteritidis continued to increase in 2016. The data reported for food and animals showed that *S*. Enteritidis was markedly associated with laying hens, broilers and broiler meat. A similar evolution during 2012–2016 was noted between the proportion of *S*. Enteritidis illnesses in humans acquired in EU and the EU flock prevalence of *S*. Enteritidis in laying hens that significantly increased during 2015 and 2016. *S*. Typhimurium cases in humans decreased. *S*. Typhimurium was reported in pigs and cattle and meats from these species and, to a lesser extent, from poultry and their meat. Human cases infected in the EU due to monophasic *S*. Typhimurium remained stable compared with previous years, and this serovar was mostly reported and associated with (contact with) pigs and (consumption of) pig meat.
The proportion of human illnesses due to *S*. Infantis, the fourth most common serovar in humans, also remained stable. *S*. Infantis was mostly reported in the broiler and turkey chains and was able to massively spread along the entire broiler production chain. *S*. Infantis represents an important public health concern because of its high level of multidrug resistance.
Serovar Derby, the fifth most frequently reported serovar among cases in infections in humans in the EU, was most commonly reported from pigs and pig meat and to a lesser extent from poultry and cattle. The 2016 monitoring data related to the compliance of foods with *Salmonella* food safety criteria showed that, as in previous years, the highest level of non-compliance was reported for certain meat categories intended to be eaten cooked (mechanically separated meat, minced meat, and meat preparations from poultry to be eaten cooked and meat products from poultry to be eaten cooked). In contrast, for fresh poultry meat that has

exclusively targeted serovars as a food safety criterion, the percentage of non-compliant samples was negligible. The non-compliance for RTE products was also rare. The overall percentage of non-compliance with the *Salmonella* process hygiene criterion for pig carcass swabs was about 2%.

At primary production level, in the context of the National Control Programmes, the EU-level flock prevalence of target *Salmonella* serovars in breeding hens, broilers, and in breeding and fattening turkeys decreased or stabilised compared with previous years. However, the decreasing EU-level flock prevalence of target *Salmonella* serovars in laying hens reported since the implementation of National Control Programmes in 2008, has reversed into a statistically significant increasing trend in the last two years. Notably, the EU prevalence of *S*. Enteritidis in laying hens increased. This recent increase involved several MS, and it was more pronounced in some of them.

Discussion

Q: We regularly see that the criteria on hygiene for slaughter of pigs are not met. Should the criteria be adjusted?
A: EFSA analyses the data and tries to learn from it, also by comparing the results of the samples taken by the competent authorities with the test results of the samples taken by the food business operators. In case the criteria are exceeded, the competent authority has an important role and responsibility in that member state.

2.4 Multi-country outbreak of *Salmonella* Agona infections linked to infant formula

Ettore Amato, DG-SANTE, Brussels, Belgium

The Commission is working to improve crisis preparedness and management in the food and feed area in order to ultimately ensure a more effective and rapid containment of future food and feed-related emergencies and crises. Threats, which may relate to accidental mismanagement within food production processes or even to intentional acts such as bio-terrorist attacks, may seriously undermine the established high level of consumer protection in the EU single market and thereby reduce their confidence in the safety of the overall system.

An outbreak of *Salmonella* Agona linked to the consumption of infant formula (powdered milk) has been ongoing in France since August 2017. As of 11 January 2018, the outbreak had affected 39 infants (children <1 year of age): 37 in France, one in Spain confirmed by whole genome sequencing (WGS) and one in Greece, considered to be associated with this event based on the presence of a rare biochemical characteristic of the isolate. The date of symptom onset for the most recent case was 2 December 2017. Evidence from epidemiological investigations in humans and traceability investigations in food identified seven different brands of infant formula from a single processing company in France as the vehicles of infection. After receiving the first notification of an unusual number of *S.* Agona cases in France on 2 December 2017, the French authorities carried out investigations at the implicated factory.

On 4 December 2017, they notified the Rapid Alert System for Food and Feed (RASFF) after confirming that some of the affected products had been exported to other countries. Following investigations at the processing company, all products manufactured after 15 February 2017, including products other than infant formula, were recalled and/or withdrawn, as a precautionary measure. The French competent authorities verified that the measures taken by the processing company in response to this event were sufficient and appropriate.
As of 15 January 2018, recalled products had been distributed to 13 European Union (EU) countries (Belgium, Bulgaria, Cyprus, the Czech Republic, France, Greece, Ireland, the Netherlands, Romania, Slovenia, Slovakia, Spain and the United Kingdom) and to 54 third countries. Most of the batches involved in the investigation had not yet passed their expiry date. However, broad withdrawal and/or recall measures, export bans and a suspension of market distribution of these batches, implemented since the beginning of December 2017 by the French competent authority and processing company significantly reduced the risk of human infection. Third countries where the recalled products had been distributed were notified by RASFF through INFOSAN.

This is an example of a good multi-sectorial approach and collaboration between public health authorities (follow-up of human cases), food safety authorities (investigations on source), laboratories, risk assessors and risk managers. The outbreak underlines the importance of cross-sectorial investigations both at national and EU level, which was also possible thanks to the systems and networks in place to manage foodborne outbreaks: notably the RASFF system was effective for coordinating targeted control measures in the food sector.
Molecular typing data together with epidemiological and traceability information were crucial to be able to narrow down the investigations for source identification. The collection of molecular typing data provides valuable support to risk managers to enable them to quickly respond to challenges posed by threats such as multinational foodborne outbreaks.

Discussion
Q: There seem to be a discrepancy between information on the number of suspected and confirmed cases in the rapid outbreak assessment (ROA) and the information in the presentation?
A: This is due to the fact that these totals were for different stages of the outbreak investigation.
Q: Some years ago, the company also struggled with *Salmonella* Agona. Was this not reported? What was the cause?
A: It is not mandatory for food business operators to report *Salmonella* Agona to the competent authority. The same drying tower was contaminated with *S.* Agona as some years ago. All products were withdrawn (also the unaffected products) and the company was closed in order to undertake investigations and to avoid more cases.
Q: Are the batch by batch controls sufficient to detect this contamination?
A: Many of the batches tested negative for *Salmonella* and it was difficult to find positive products. The problem was due to contamination of some batches in the processing plant.

2.5 Investigating *Salmonella* in the cattle production in France

Laetitia Bonifait, NRL-Salmonella, Ploufragan, France

Salmonella is one of the most common and widely distributed foodborne pathogens which may lead to acute gastroenteritis. Poultry (turkeys, broilers, and laying hens) is known to be a potential source of transmission of *Salmonella* to humans, accounting for approximately 30% of the salmonellosis cases. Nonetheless, cattle production is also recognised as an important source of human infection. *Salmonella* transmission from cattle to humans can be achieved through the environment, close contact with sick animals, or with their derived products (meat, dairy products).
The purpose of this study was to investigate the intestinal carriage of *Salmonella* spp. in cattle production in France. A total of 959 intestinal samples from one of the largest slaughterhouses in France were analysed. All isolated strains were analysed by PFGE using two restriction enzymes (XbaI and BlnI). A total of 29 samples were positive for *Salmonella* spp. giving an estimated prevalence of 3% in cattle production. Among these samples nine different *Salmonella* serovars were found with *Salmonella* Montevideo being the most prevalent (34%), followed by *Salmonella* Mbandaka (20%) and *Salmonella* Anatum (13%). Genotyping showed different clusters of isolates by serovars, and within the clusters, 100% of similarity of the strains. Interestingly, associated isolates originated from different French areas and from different animal breeds. This investigation was the first estimation of *Salmonella* prevalence in French cattle production.

Discussion

Q: *Salmonella* serovars Montevideo and Anatum are regularly found in animal feed. As cattle eat more concentrated feed, could this be a cause of infection? Is there monitoring of animal feed in France?
A: I do not know and will need to check.
Remark: If there is a link between *Salmonella* serovars found in poultry and cattle, it is quite likely that animal feed is the common factor.
Q: Do you see different results for different production systems?
A: This is not known, but only 1 sample of 600 silage samples tested positive for *Salmonella*.
Q: Did you consider sampling and analysis of intestinal lymph nodes?
A: This was not considered in the current research study.

2.6 Results combined Food-PPS interlaboratory comparison study on detection of *Salmonella* in hygiene swabs (2017)

Irene Pol, EURL-Salmonella, Bilthoven, the Netherlands

In October 2017, the combined EURL-*Salmonella* interlaboratory comparison study on the detection of *Salmonella* in samples from food origin and primary production stage was organised. Because of recurrence of Avian Influenza caused by migrating birds, it was decided to change the order of the interlaboratory comparison studies on the detection of *Salmonella* in food and in matrices of the primary production stage. The current study was considered as an intermediate study. Hygiene swabs were chosen as matrix as this was suitable for

both the food matrix as well as the primary production stage (PPS) matrix. Participation was obligatory for all EU-MS National Reference Laboratories (NRLs) responsible for the detection of *Salmonella* in food samples, and was voluntary for NRLs responsible for the detection of *Salmonella* in primary production stage samples, as the latter had already participated in the compulsory EURL study for the detection of *Salmonella* in PPS organised in March 2017.

A total of 56 NRLs participated in this study: 33 NRLs for *Salmonella* in Food matrices and 23 NRLs for *Salmonella* in Primary Production Stage matrices (PPS). The participants originated from 28 EU-MS, 4 NRLs from third countries within Europe (EU candidate or potential EU candidate MSs and members of the European Free Trade Association (EFTA)), and one NRL from a non-European country.

Hygiene swabs were used in this study. They were artificially contaminated with background flora as well as with a diluted culture of *Salmonella* Typhimurium at the EURL laboratory.

Each NRL received 20 blindly coded samples consisting of 12 hygiene swab samples artificially contaminated with background flora and two different levels of *Salmonella* Typhimurium (6x low (5 cfu) and 6x high (107 cfu)), 6 blank hygiene swab samples, and 2 control samples consisting of a procedure control blank and a control sample to be inoculated with the participants' own positive control strain. The samples were stored at 5 °C until the day of transport. On Monday 2 October 2017, the contaminated hygiene swab samples were packed and sent to the NRLs. On arrival, the NRLs were asked to store the samples at 5 °C until the start of the analysis.

Method

All laboratories were asked to use EN ISO 6579-1:2017 and select the appropriate enrichment media in accordance with the samples being considered as food matrix or PPS matrix.

Results control samples

All laboratories scored well, analysing both the procedure control as well as their own positive control sample. One laboratory made a mistake in reporting a negative result for the positive control, while their raw data indicated a positive result. As a result, this laboratory scored a moderate performance.

Results artificially contaminated hygiene swab samples

All laboratories detected *Salmonella* in the hygiene swab samples contaminated with a high level of *Salmonella*.
In addition, almost all laboratories detected *Salmonella* in all 6 low level samples. One laboratory scored 1 of the 6 low level samples negative. This is well within the criteria for good performance, which allows for 3 negative samples. The sensitivity score was 99.9% for these samples. The specificity for this study was 99% as given by the correctly scored blank samples. Only 1 laboratory did not score all 6 blank samples negative. This laboratory reported 2 of the 6 blank samples positive for *Salmonella* and scored a poor performance.

Overall, the laboratories scored well in this interlaboratory study. The accuracy was 99.7%. Fifty-four laboratories fulfilled the criteria of good performance, one laboratory scored moderate performance, and one laboratory scored poor performance. This latter laboratory participated in a follow-up study and achieved 100% correct scores.

More details can be found in the interim summary report and full report (Pol-Hofstad and Mooijman, 2017 and 2018).

Discussion

Q: The study shows very good results. Would private laboratories find similar results?
A: We do not know. All NRLs organise PTs for the official laboratories in their countries, they might have that information. It could also be the case that the matrix of the current study was very easy.
Remark by an NRL: we organise PTs in which half of the participants are private laboratories and half are public laboratories. Very few laboratories fail in the studies, but if any laboratory fails it is more often a private laboratory rather than a public laboratory.
Q: Why did you choose *E. coli* and *Citrobacter* as background flora?
A: We checked the literature for bacteria possibly present in this type of sample. Additionally, we tried to find a balance in mimicking real samples with background flora disturbing the detection of *Salmonella* and a background flora that did not interfere too much. Perhaps next time we will use more interesting background flora.
Q: Did all participants use the same method?
A: We asked them to follow EN ISO 6579:2002, or EN ISO 6579-1:2017, or EN ISO 6579:2002/Amd.1:2007. Nine participants used a PCR method in addition to the ISO method.
Q: What reason would qualify for assigning a moderate performance?
A: For example, if a mistake is made in reporting results (e.g. exchange of samples), while the raw data show that the results are correct. As the results are in fact correct, but the reporting is wrong, this is scored as moderate performance and not as poor performance.

2.7 Preliminary results of the 4th interlaboratory comparison study on detection of *Salmonella* in chicken feed (2018)

Angelina Kuijpers, EURL-Salmonella, Bilthoven, the Netherlands

In February 2018, the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*) organised the fourth interlaboratory comparison study on detection of *Salmonella* in animal feed samples. The matrix used in this study was chicken feed.
The participants were 35 National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*): 30 NRLs from the 28 EU Member States (EU-MS) and 5 NRLs from third countries (EU candidate MS or potential EU candidate MS, countries of the European Free Trade Association (EFTA) and one NRL from a non-European country).

The most important objective was to test the performance of the participating laboratories for the detection of different concentrations of *Salmonella* in an animal feed matrix. Each laboratory received 18 chicken feed samples (25 g each) artificially contaminated with a

diluted culture of *Salmonella* Mbandaka (SMb) at a low level (approximately 10-15 cfu/25 g of feed), at a high level (approximately 50-100 cfu/25 g of feed), and with no *Salmonella* (blank samples). The participants were asked to follow EN ISO 6579-1:2017 for sample analysis which prescribes selective enrichment in Mueller Kauffmann Tetrathionate novobiocin (MKTTn) broth and in either Rappaport Vassiliadis Soya (RVS) broth or on Modified Semi-solid Rappaport-Vassiliadis (MSRV) agar.
The participants were asked to report 'positive' (1) or 'negative' (0) for each sample (after confirmation), independent of the combination of selective enrichment media and isolation media (as done for routine samples).

Prior to the study, several experiments were conducted to make sure that the samples were fit for use in an interlaboratory comparison study (e.g. choice of *Salmonella* serovar, stability at different storage temperatures, and level of background flora). For this, different types of chicken feed were tested, and it was decided to use flour with 4 grains for laying hens for the interlaboratory comparison study. The artificially contaminated samples were stored at 5 °C and 10 °C to test both the stability of *Salmonella* and the background flora in the chicken feed. From the results of the pre-tests, it was decided to store the chicken feed samples at 5 °C to keep the background flora low and to stabilise *Salmonella*.

Eighteen individually numbered blind chicken feed samples had to be tested by the participants for the presence or absence of *Salmonella*. These consisted of six blank samples, six samples with a low level of SMb (inoculum 8 cfu/sample) and six samples with a high level of SMb (inoculum 91 cfu/sample). Participants also had to test two controls: one blank control sample (procedure control (BPW)) and one own (NRL) positive control sample (with *Salmonella*).

Thirteen participants used all three selective enrichment media (MKTTn broth, MSRV agar, and RVS broth) as indicated in EN ISO 6579-1:2017. Twelve laboratories used MKTTn broth and MSRV agar, 9 laboratories used MKTTn and RVS broth, and one laboratory used only MSRV agar. PCR was used as an own method by 13 participants, and all found similar results to the bacteriological culture method.

This study showed an unexpectedly high number of negative results for the artificially contaminated chicken feed samples. Therefore, it was decided not to set criteria for these samples, but only to compare the number of positive samples found per laboratory with the mean number of positive samples found by all participants. Overall, the laboratories found *Salmonella* in 52% of the high level and in only 5% of the low-level contaminated samples. The MPN (Most Probable Number) analysis of the chicken feed samples showed a very low level of *Salmonella* even in the high-contaminated samples on the day of performance. The high-contaminated samples could have been evaluated as low-contaminated samples, as the sensitivity rate was approximately 50%, indicating a final level in the feed samples close to the detection limit.

The number of positive samples found by all participants was evenly distributed across both the high and low-level contaminated samples.

This indicates that the detection of *Salmonella* in the chicken feed was influenced evenly across all samples. These results were unexpected when compared to the results of the pre-tests, for which the same type of chicken feed and *Salmonella* Mbandaka strain were used. The batch chicken feed used in the interlaboratory comparison study contained a one log higher number of *Enterobacteriaceae* compared to the batch chicken feed used in the pre-test. This high level of background flora may have negatively influenced the detection of *Salmonella*, however this is unlikely to be the only clarification for the high number of negative feed samples found in the study. After the interlaboratory comparison study, the EURL-*Salmonella* repeated the inoculation of animal feed samples using the same batch of chicken feed, the same *Salmonella* Mbandaka strain, and the same inoculation levels. Similar results were observed to those found with the interlaboratory comparison study. In addition to the inoculation levels of 10 cfu/25 g and 100 cfu/25 g, feed samples were inoculated with 1000 cfu/25 g. Only these latter samples all tested positive for *Salmonella*. This 'confirms' that a reduction of almost 2 log cfu of *Salmonella* Mbandaka occurred after addition to the chicken feed samples which explains the high number of negative samples in the interlaboratory comparison study. The cause of this reduction remains unclear, but it is most likely due to the presence of inhibitory substances in the batch of chicken feed used in the main study.

Due to the problems with the chicken feed samples, it was not possible to evaluate the NRLs' performance for *Salmonella* in this study.

More details can be found in the interim summary report and full report (Kuijpers and Mooijman, 2018 and 2019).

Discussion

Participants were invited to suggest possible experiences that might help in finding the cause of the unexpected low isolation rates from the spiked samples.

- Too low or too high temperatures during transport of the parcel? In the pre-test, the stability of the samples at different temperatures was tested, but this large decrease in *Salmonella* was not seen.
- Perhaps organic acid was added to the feed or to the raw ingredients of the feed? The pH of the feed was not tested. It could be a suggestion to compare the pH of the batch of feed of the study with the pH of the batch of the pre-test in which the large decrease in *Salmonella* was not seen.
- Addition of antibacterial to the feed? Formic acid? Aromatic oils? Antibiotics? Vitamins? These ingredients might also affect the background flora, but this was not seen. The number of *Enterobacteriaceae* and total flora was relatively high in the feed.

Q: Some participants also performed PCR. Was this PCR performed after pre-enrichment in BPW?
A: Yes, most PCRs were performed after pre-enrichment in BPW, indicating that *Salmonella* was not present in the feed.
Q: Will the next interlaboratory comparison study also include animal feed?
A: We do not know yet.
Q: Do you normally expect negatives for the low-level samples?
A: Yes, in this study we aimed to test approximately 60% of the low-level samples positive for *Salmonella*. The negative results are then not caused by poor performance of the NRLs, but due to the fact that some samples are negative for *Salmonella*.

2.8 Results 22nd interlaboratory comparison study on typing of *Salmonella* (2017) - serotyping and PFGE

Wilma Jacobs, EURL-Salmonella, Bilthoven, the Netherlands

In November 2017, the 22nd interlaboratory comparison study on serotyping and PFGE typing of *Salmonella* was organised by the EURL-*Salmonella*. A total of 35 laboratories participated in this study. These included 29 NRLs-*Salmonella* of the 28 EU-MS, 2 NRLs of EU-candidate countries, 3 NRLs of EFTA countries, and 1 non-European NRL. The main objective of the study was to evaluate whether typing of *Salmonella* strains by the NRLs-*Salmonella* in the EU was carried out uniformly, and whether comparable results were obtained.

All 35 laboratories performed serotyping. A total of 20 obligatory *Salmonella* strains plus one additional (optional) *Salmonella* strain from an uncommon type were selected for the study by the EURL-*Salmonella*. The strains had to be typed with the method routinely used in each laboratory, following the White-Kauffmann-Le Minor scheme (Grimont and Weill, 2007).
The individual laboratory results on serotyping, as well as an interim summary report on the general outcome, were emailed to the participants in February 2018. The O-antigens were typed correctly by 31 of the 35 participants (89%), corresponding to 99% of the total number of strains. The H-antigens were typed correctly by 28 of the 35 participants (80%), corresponding to 98% of the total number of strains. As a result, 28 participants (80%) also gave the correct serovar names to the full set of strains, corresponding to 98% of all strains evaluated.
Apart from some spelling errors, a completely correct identification was obtained for ten *Salmonella* serovars: Hadar (S2), Durban (S5), Kaapstad (S6), Typhimurium (S7), Virchow (S10), Jerusalem (S13), Infantis (S14), Abony (S16), Enteritidis (S17), and 1,4,[5],12:i:- (S19). Most problems were seen in strains showing a non-typeable or only a partly typeable result, e.g. due to being ‘rough’ or due to a lack of antisera required. Only four strains were incorrectly identified.
All but four participants tried to serotype the additional strain S21, being a *Salmonella enterica* subsp. *diarizonae* (IIIb). However, not all laboratories had access to the required antisera to finalise the serotyping of *S*IIIb 50:k:z.

At the EURL-*Salmonella* workshop in 2007, criteria for 'good performance' of the NRLs regarding the serotyping have been defined (Mooijman, 2007). All participants met the level of good performance at the first stage of the 2017-study, so a follow-up study was not needed.

Fifteen NRLs participated in the PFGE typing part of the study. They were asked to test 11 *Salmonella* strains using their own routine PFGE method for digestion with XbaI. The PulseNet Guidelines were used for the quality grading of the PFGE gel images, based on scoring 7 parameters with 1 point (poor) to 4 points (excellent). Some variation in the quality of the gel images was observed, and four participants scored a 'Poor' for the parameter 'Image acquisition/Running conditions'. However, for 3 of these laboratories, this was due to a mistake in the required use of the Reference strain *S*. Braenderup, which could be easily improved in the future.
The evaluation of the analysis of a gel in BioNumerics was optionally included. New to this study was the use of a common gel for all participants. A total of 10 participants sent in their analysed gel data for evaluation conducted according to the guidelines used in the PTs for the ECDC network of Food and Waterborne (FWD) laboratories. These guidelines use 5 parameters scored with 1 (poor), 2 (fair/good) or 3 (excellent) points. Several participants tended to assign bands of test strains also below 33 kb which is incorrect according to the protocol. Except for this minor deviation, 8 strains were correctly analysed by all participants. The main deviations for the remaining 3 strains were in the assignment of double bands as single bands, a well-known difficulty in the analysis of PFGE images.

PFGE typing regarding the quality of PFGE gel image and optional gel analysis in BioNumerics, will be offered again in the 2018 interlaboratory comparison study on typing of *Salmonella*. Multi-Locus Variable number of tandem repeats Analysis (MLVA) on *S*. Typhimurium and/or *S*. Enteritidis will be offered as a pilot in 2018, but will only be run if more than 7 laboratories are willing to participate.

More details on the typing study of 2017 can be found in the (interim) summary reports and full report (Jacobs et al., 2018a,b,c).

Discussion
Q: Why does the pilot study only include MLVA typing of *Salmonella* Typhimurium and not MLVA typing of *Salmonella* Enteritidis?
A: An EFSA SOP is available for MLVA typing of *Salmonella* Typhimurium, but we may also consider including MLVA typing of *Salmonella* Enteritidis.

2.9 Update on activities in ISO and CEN

Kirsten Mooijman, EURL-Salmonella, Bilthoven, the Netherlands

Kirsten Mooijman of the EURL-*Salmonella* presented an overview of activities in ISO and CEN in relation to *Salmonella*.
The relevant groups in ISO and CEN are:

- ISO/TC34/SC9: International Standardisation Organisation, Technical Committee 34 on Food Products, Subcommittee 9 – Microbiology;

- CEN/TC275/WG6: European Committee for Standardisation, Technical Committee 275 for Food Analysis – Horizontal methods, Working Group 6 Microbiology of the Food Chain.

The last annual meeting of both groups was organised from 19 to 23 June 2017, and the next meeting will be held from 18 to 22 June 2018.

EN ISO 6579-1 'Detection of *Salmonella*'

After publication of EN ISO 6579-1:2017, a mistake was detected in the composition of Selenite cystine medium (broth) in Annex D.3. The EN ISO document indicates that 100 ml L-cystine solution should be added to 1000 ml base medium. However, this should be 10 ml L-cystine solution. Earlier in 2018, the members of ISO/TC34/SC9 were consulted to ask for agreement to publish a correction of or amendment to EN ISO 6579-1 (Detection of *Salmonella*). During the consultation it was also possible to indicate other mistakes. The outcome of this consultation was positive, and a few more remarks were received which need further discussion.
In addition to the comments by SC9 members, questions and remarks of users of EN ISO 6579-1:2017 can also be taken into account when drafting a correction or amendment. At the workshop, several questions were addressed.

- Is verification of EN ISO 6579-1:2017 for introduction in a laboratory needed if this laboratory already works with EN ISO 6579:2002?
  - In principle no. The main changes, compared to EN ISO 6579:2002, are considered as minor, so little to no effect on the performance characteristics is expected.
- Is it necessary to use two incubators for 34-38 °C and 37 °C, and is it necessary to report the exact temperature of the 34-38 °C incubator?
  - No, for both. This range of 34-38 °C was introduced to give more flexibility in the incubation temperature of non-selective media and to harmonise the incubation temperature with USA. Any temperature between 34 °C and 38 °C is fine and it is not necessary to report this specifically. It is easiest to use a 37 °C incubator for all media.
- Is a 1 µl loop required for transfer of material from MSRV agar to XLD agar, or can another size loop be used?
  - Most important is to obtain single colonies. With a 1 µl loop, single colonies on a normal size (ca 9 cm) XLD plate can be obtained. With a larger loop it may be necessary to use two normal size plates or one large plate (ca 14 cm) to obtain single colonies.
- Is it obligatory to apply Annex D (for detection of *S.* Typhi and *S.* Paratyphi) when testing routine samples?
  - No. The intention of this annex is to give (extra) guidance when *S*. Typhi or *S*. Paratyphi are specifically sought e.g. in case of outbreaks. For the general analysis of samples from the food chain for the detection of *Salmonella* spp. like 'normal' routine samples and PT samples, only the procedure described in the main document need to be followed.

- Is it obligatory to confirm *Salmonella* with poly H antiserum in addition to confirmation with poly O antiserum?
  - Yes. The number of biochemical tests has been reduced and therefore polyvalent H-antisera was introduced, to be 'sure' that *Salmonella* is present. Some *Enterobacteriaceae* can give a positive reaction with polyvalent anti-O sera, but will give a negative reaction with polyvalent anti-H sera.
- How can we interpret results in case of a positive reaction with polyvalent anti-O sera but a negative reaction with polyvalent anti-H sera and typical biochemical reactions?
  - According to EN ISO 6579-1:2017 this is presumptive *Salmonella*. Additional testing may be needed as it may also be another *Enterobacteriaceae*, or the polyvalent anti-H serum does not contain the H-factor(s) for the specific strain, or there may also be a small chance that the isolate concerns a (bi-)monophasic variant.

PCR identification of monophasic *S*. Typhimurium (ISO/TS 6579-4)

In May 2016, SC9 agreed to register the Preliminary Work Item (PWI) of ISO 6579-4 to become a Technical Specification (TS).
In 2018, the EURL-*Salmonella* made a selection of 172 of 400 test strains (target and non-target strains) to test the three PCR protocols of draft ISO/TS 6579-4 by the NRL-*Salmonella* in Germany (Burkhard Malorny, project leader in TAG3) and by the EURL-*Salmonella*. Once completed, the draft document may need further amendments. When the technical work is finished, the work will be moved to ISO-WG10. After the New Work Item Proposal (NWIP) has been launched and a final draft version of ISO/TS 6579-4 is available, the interlaboratory study (ILS) will be planned to determine the performance characteristics. The timing of this ILS is not yet definite.

Harmonisation of incubation temperature

In 2014, at an annual meeting of ISO/TC34/SC9 and CEN/TC275/WG6, it was agreed to use a broader temperature range for incubation of non-selective media (34-38 °C instead of 37 °C ± 1 °C). To accept a broader temperature range for the incubation of selective media, data were needed showing no effects on the results when incubating at this broader temperature range. In 2014-2015, the Adria laboratory in France performed experiments to test the influence of incubation temperature (35 °C or 37 °C) on the growth of *Salmonella* and on the growth of several *Enterobacteriaceae* species. These experiments showed no difference in growth of *Salmonella* spp. at either temperature, but some impact on the growth of some other *Enterobacteriaceae* species. Therefore, it was proposed to set up a protocol to test the influence of the incubation temperature with a larger group of laboratories (members of ISO and CEN), especially to test the influence on the growth of *Enterobacteriaceae*. In 2016, a protocol was prepared for comparing incubation of MKTTn broth for detection of *Salmonella* at 35 °C and 37 °C. The members of ISO and CEN were invited to perform experiments using the protocol. By June 2017, results were received from 9 laboratories representing 6 countries, resulting in a total of 855 tests. In total, 10 different product categories were tested. The results were analysed

according to the information of EN ISO 16140-2:2016. The following conclusions were drawn:

- The overall results showed similar sensitivity results: 97.5% for incubation of the selective media at 37 °C, and 98.3% for incubation at 35 °C.
- The data interpretation in relation to the deviating results fulfilled the proposed 'amended' acceptability limits.
- The reported level of background flora after incubation of the selective media at 35 °C or 37 °C was comparable.

These results indicate that comparable results are obtained when incubating selective media for detection of *Salmonella* at 35 °C and 37 °C. It can therefore be concluded that for incubation of these selective media, a temperature range of 34-38 °C can be used, as has been agreed for the incubation of non-selective media.

Note: After the workshop, this information was presented at the annual meeting of ISO/TC 34/SC9 and it was agreed that the information on extension of the temperature range for incubation of selective media would be added to the amendment to be drafted for EN ISO 6579-1.

Discussion
Q: Due to the fact that Annex D of EN ISO 6579-1:2017 (detection of *S.* Typhi and *S.* Paratyphi) is normative, our accreditation body considered this to be part of the full procedure. For that reason, our scope of accreditation indicates 'EN ISO 6579-1, excluding Annex D'.
A: Annex D is not intended to be part of the full procedure, but is intended to be followed in case a specific search is conducted for *S.* Typhi and/or *S.* Paratyphi, e.g. in case of outbreaks. This information will be summarised in a letter to the NRLs and/or in the Newsletter. (*Note: at the annual ISO and CEN meetings in June 2018 it was agreed to draft an Amendment to EN ISO 6579-1 including, amongst others, the change of status of Annex D from normative to informative*).

2.10 Birds, cats, humans and host adaptation in *Salmonella* Typhimurium

Robert Söderlund, National Veterinary Institute (SVA), Uppsala, Sweden

Background: Host-biased lineages of *Salmonella* preferentially infect one or more host species, but spill over to other animal species and may cause zoonotic infections. In the spring of 2016, simultaneous outbreaks of *Salmonella* Typhimurium (STm) with multilocus variable-number tandem repeat analysis (MLVA) profiles historically associated with passerine birds occurred among passerines, cats and humans in Sweden.
Aims: To further investigate the outbreak and revisit historical data to investigate the seasonality, phylogeography, and other characteristics of this STm variant.
Methods: Outbreak isolates were analysed by whole-genome SNP typing. The number of cases among passerines, cats and humans of this type of STm per month and county in Sweden as well as MLVA profiles for the period 2009-2016 was compared and related to passerines counted by birdwatchers in an annual spring survey. Seasonal trend decomposition and correlation analysis was performed.

Results: Outbreak isolates were non-clonal and did not cluster by host. Passerine type STm was seasonal for birds, cats and humans with a peak in March. Observed cases and counts of passerines at bird feeders varied substantially between years. The human incidence of passerine-type STm was higher in the boreal north compared to the south and the capital region, consistent with passerine population densities. **Conclusions:** Short-range mass migration of passerines causes outbreaks of STm among cats on certain years in Sweden, most likely via predation on weakened birds. Outbreaks among humans can follow, presumably caused by contact with cats or environmental contamination.

Discussion
Q: Did you sub-type the *S.* Typhimurium strains?
A: We performed MLVA typing on all STm strains from 2009 onwards, we also performed WGS.
Q: Did you compare this STm type with isolates from other animals?
A: Yes, but we found this specific MLVA type especially in birds and cats.
Q: Do birds get ill from this STm type?
A: Yes, and most birds die. However, we also found this type in living birds. It is not clear if birds die only because of the STm infection.

2.11 Pilot validation study for confirmation of *Salmonella* following ISO/DIS 16140-6

Wilma Jacobs, EURL-Salmonella, Bilthoven, the Netherlands

Introduction: The EN ISO 16140 standard provides technical and interpretation rules for method validation and verification, and has six different parts. Recently, Part 6 successfully passed the DIS (Draft International Standard) stage. It describes the protocol for the validation of alternative (proprietary) methods for microbiological confirmation and typing procedures. The study design was set up in recent years, and acceptability limits for the data interpretation were defined based on expert opinion, i.e. maximum number of positive or negative deviations between the reference and alternative method.
Purpose: Are the defined technical rules sufficiently described to correctly run the method comparison and inter-laboratory studies? Are the proposed acceptability limits (ALs) fit for purpose? Are these ALs, formulated by some experts in the field, too restrictive?
A pilot study was coordinated by MicroVal Organisation as a proof of concept.
Methods: The MALDI Biotyper was tested as an alternative to confirm *Salmonella* spp. from non-selective and selective agars. A method comparison and interlaboratory studies were conducted.
150 *Salmonella* spp. strains and 100 non-target strains were tested by two expert laboratories in the method comparison study. The collaborative study was run by involving a minimum of 10 organisations to produce 10 valid data sets with 16 target and 8 non-target strains.
Results: The MicroVal reviewers and the expert laboratories encountered no specific difficulties in setting up the project, organising the testing, and interpreting the generated data. The collaborating laboratories were easily able to understand the protocol of the ISO 16140-part 6 and to achieve the required tests. All the *Salmonella* spp. strains were correctly confirmed with the MALDI Biotyper on all tested

media in the method comparison and inter-laboratory studies, passing the thresholds of the currently defined ALs.
Significance: EN ISO/DIS 16140-part 6:2017 provides valuable technical rules and interpretation to validate confirmation methods.

3 Wednesday 30 May 2018: day 2 of the workshop

3.1 Activities of the NRL-*Salmonella* to fulfil tasks and duties in Finland

Henry Kuronen, NRL-Salmonella, Kuopio, Finland

The Finnish Food Safety Authority (Evira) is the NRL for most of the bacteria and viruses in the field of food, feed and animals. Evira's laboratory (Veterinary Bacteriology and Pathology Research Unit) in Kuopio is the NRL-*Salmonella* for sampling and analysing samples from the primary production stage and for typing *Salmonella* strains from non-human origin. Evira's laboratory (Microbiology Research Unit) in Helsinki is the NRL-*Salmonella* for sampling and analysis of food and animal feed. In 2019, Evira will be merged with another Institute and will become part of a new Institute (English name still unknown).

Approximately 600 – 800 *Salmonella* strains are serotyped annually; about 20% from imported food, 10% from food production animals, 15% from other animals (mainly wild birds), 30% from all kind of environmental samples, and 20% from imported feed. For epidemiological purposes Evira performs antimicrobial resistance monitoring of isolates from new production animals and of food, by using the disc diffusion method. Selected strains are sent to Evira in Helsinki for monitoring by the dilution method. PFGE is used mainly for epidemiological investigations, and incidentally for laboratory cross-contamination investigations.
Evira has now received its own sequencing equipment in Helsinki and the implementation of WGS is progressing, in collaboration with the National Institute for Health and Welfare (THL). Evira takes part in the organisation of annual meetings for laboratories and gives presentations at training courses organised by other organisations.
Evira has a working group for reference laboratory activities which prepares Newsletters for official laboratories four times per year. These Newsletters contain information on *Salmonella* as well as on other subjects for which Evira is NRL.
At this moment (2018) there are 22 official approved laboratories (municipal or commercial), and these are accredited according to EN ISO/IEC 17025:2005 (granted by The Finnish Accreditation Services FINAS). The official laboratories are obliged to participate in the Scandinavian interlaboratory studies organised by the Swedish National Veterinary Institute (SVA), at least every third year. These studies are described in the presentation by Lennart Melin (SVA) given at the workshop in St Malo in 2016. Contact persons of the NRLs-*Salmonella* receive the results from SVA, and if a laboratory has not achieved good performance, it will be contacted. Evira has prepared a presentation on the possible problems with the MSRV-method, and laboratories can use this to find possible explanations for their poor performance. After introducing possible corrections, a poor performing laboratory has to analyse extra samples with good performance before the study results are finally accepted. Laboratories which analyse food samples have to participate in other Profiency Tests, at least once in four years. In

practice, almost all laboratories analyse both food samples and samples from the primary production stage, so they participate in both studies. Evira closely collaborates with the national Institute for Health and Welfare (THL), which analyses human samples. This year phage typing will stop, when the phages are finished. Currently, Whole Genome Sequencing is being implemented. Evira also collaborates with the Ministry, EURL-*Salmonella*, Nordic co-operation, and the Animal Health ETT which is doing a lot for the prevention of *Salmonella* in Finland.
The Finnish *Salmonella* Control programme has been carried out for decades, concerning food production animals, including feed control. All *Salmonella* serotypes are notifiable, and vaccination or treating of *Salmonella* by antibiotics is not allowed in Finland. The aim is to maintain the very low prevalence of *Salmonella*.
From 2005 to 2017, 6-18 different *Salmonella* serotypes were found in different production animals. During and after the epidemic with *Salmonella* Tennessee related to a feed factory in 2009, no identical *Salmonella* Tennessee isolates were detected in humans.
In 2010, a new *Salmonella* serotype was found in an environmental sample of a feed factory as well as in a broiler flock. The serotype was named after a part of the city where the feed factory is situated: *Salmonella* Nuorikkala (8:z_4,z_{24}:e,n,x).

Discussion
Q: You mention a document giving information on possible problems (and solutions) with MSRV agar. Can this information be shared with the NRLs-*Salmonella*?
A: No problem, I will send it to the EURL for sharing with the NRLs.
Note: the information was published in the EURL-Salmonella Newsletter of September 2018 (Kuronen, 2018).
Q: Do you have a dedicated team in Finland for farm investigations?
A: For this, a private organisation cooperates with the food authority. The veterinarians in this organisation are involved in the *Salmonella* control program and know which samples to take. They not only perform controls but also give advice, e.g. to animal feed producers to sample dust in order to detect the source(s) of infection.
Q: *Salmonella* Tennessee in primary production was not reflected in human cases, what about *Salmonella* Derby?
A: The number of human cases with *Salmonella* Derby is also low.

3.2 Activities of the NRL-*Salmonella* to fulfil tasks and duties in Hungary

Erzsébet Adrián, NRL-Salmonella, Budapest, Hungary

The Hungarian NRL-*Salmonella* is part of the National Food Chain Safety Office, Food and Feed Safety Directorate, Food Microbiological National Reference Laboratory. The Food Microbiological NRL has seven divisions: the laboratory of meat and milk products, other products, *Salmonella* typing, GMO, food poisoning, disinfection agents, mushroom poisoning and entomology. Hungary is divided into 19 counties. The Food Microbiological NRL is a central official laboratory in Budapest and there are six regional official labs distributed around the country.
The Food Microbiological NRL has a number of key activities. One of them is food microbiological diagnosis, as official control laboratory for

Budapest and county Pest. This laboratory is responsible for the investigation of foodborne outbreak associated samples in the whole country, for the EU surveys in previous years, and for testing according to export requirements. Some specific tests that are conducted only in this laboratory in the field of food investigation (STEC, bacterial toxin, GMO, virus detection, species identification etc.). Another main task is to organise the microbiological monitoring sampling system, to test the samples, collect and report the results. The laboratory is National Reference Laboratory for different fields and one of them is *Salmonella*. The Food Microbiological NRL has a central role in the laboratory system. It organises ring tests and supports the other laboratories by sharing information or with training courses.
The NRL-*Salmonella* has been accredited to EN ISO/IEC 17025:2005 since 1995. *Salmonella* detection and serotyping is done according to EN ISO 6579-1:2017. The laboratory examines 6000-7000 samples per year for *Salmonella* detection and 4000-5000 isolates for serotyping. Isolates of *S*. Enteritidis and *S*. Typhimurium are phage-typed according to the PHE, Colindale method. Additionally, differentiation of vaccine strains from wild strains, by using minimal medium inoculation (supplied by the vaccine producing company) and antimicrobial resistance testing (Avipro Plate) are performed. Molecular detection of *Salmonella* is performed using a Real-Time PCR kit (Bio-Rad iQ-Check Salmonella II.). *Salmonella* Enteritidis and *Salmonella* Typhimurium detection can be done by Foodproof *S*. Enteritidis, and *S*. Typhimurium Kit (Biotecon) and Kylt SE DIVA 1 are used for the differentiation of Salmovac (vaccine) *S.* Enteritidis from field strains. Molecular typing is done at other laboratories. The National Food Chain Safety Office plans to build up a high capacity NGS laboratory that serves all the needs of the laboratories from different disciplines (food microbiology, veterinary diagnostics, plant health, identification of breeding animals etc.). Until this is realised, the laboratory has initiated a contract with a commercial laboratory which provides WGS with bioinformatical analyses. This method can be used for outbreak investigation.
The NRL-*Salmonella* organises Proficiency Tests for official and private laboratories. Each year, participants receive a different food or feed matrix and poultry faeces for *Salmonella* detection. The samples are artificially contaminated, inoculated with *Salmonella* cultures. There is also a Proficiency Test for the exclusion of *S*. Enteritidis and *S*. Typhimurium by O-serogrouping. This method is used for broiler flocks before slaughter when the result of the *Salmonella* detection from faeces is positive. In this PT, *Salmonella* isolates are used (no matrix).
The NRL-*Salmonella* coordinates the activities of the official laboratories. There are training courses on new methods or for new colleagues joining the regional labs. At the laboratory meetings (organised regularly), there is a possibility to share information and news concerning the laboratory system, the implementation of monitoring plans, sampling methods, and methods for analysing *Salmonella*. At the beginning of 2018, there was a discussion about EN ISO 6579-1:2017.
The NRL-*Salmonella* remains in contact with the EURL-*Salmonella*: participating in the annual workshops and in Proficiency Tests and in the discussion on methodological problems and outbreak investigations.

3.3 Activities of the NRL-*Salmonella* to fulfil tasks and duties in Iceland

Franklin Georgsson, NRL-Salmonella, Reykjavik, Iceland

Matis ohf. / Icelandic Food and Biotech is an independent R&D company with a leading role in food and biotechnology research in Iceland. It was established in 2007 with the merger of three laboratories – The Fishery, Environmental, and Agricultural laboratories. The analytical service division of Matis provides accredited microbiological and chemical analysis for the official food control authorities and food companies in Iceland. Matis has eight different locations in Iceland with its headquarters in the capital Reykjavik and approximately 110 employees.
Iceland has participated as an NRL for *Salmonella* and bivalve molluscs for 12 years. Recently this has been increased with 9 additional NRLs for both microbial and chemical methods (*Listeria monocytogenes*, *Staphylococcus aureus*, Pathogenic *E. coli*, Animal proteins in feed, Pesticides, Heavy metals, Mycotoxins, PAH, Dioxin and PCB).
In Iceland, it is the Ministry of Industries and Innovation in cooperation with the Icelandic Food and Veterinary Authority that designates NRLs. In addition to Matis, the Institute of Experimental Pathology carries out several NRL tasks, e.g. *Campylobacter*, *Trichinella* and other animal parasites. They are also the NRL for antibiotic resistance.
Two private laboratories in Iceland, Syni ehf and Promat, are also officially appointed laboratories for *Salmonella* analysis in food, animals and environmental samples.
Matis participates in most comparative tests organised by the EURL for *Salmonella.* The other official national laboratories, as well as Matis, also participate in interlaboratory comparison studies on *Salmonella* in food samples, organised by other organisations (e.g. by the Swedish Food Administration).
All *Salmonella* typing is carried out by Landspitali University Hospital that participates regularly in interlaboratory comparison studies on typing organised by the EURL for *Salmonella*.
Matis has a good relationship with the other official national laboratories in Iceland through the Icelandic National Committee for Food Analysis (part of the Nordic Method Committee for Food Analysis, NMKL) and provides the competent authority (MAST) and official national laboratories with information from EURL-*Salmonella.*
Most registered infections caused by *Salmonella* in Iceland are single cases and can be traced to travel abroad. There is an average of 21 confirmed annual domestic human cases from 1990-2017, if two years (1996, 2000) are excluded. In 1996 an outbreak of *Salmonella* in domestic cream buns was registered, and in 2000 an outbreak on *Salmonella* in imported iceberg salad occurred. For the same period, and excluding the two years (1996, 2000), there were 59 average annual confirmed foreign human cases, which are approximately three times more cases than the domestic ones for the same period. From 2009-2017, there was a high reduction in foreign cases to an average of 24 annual cases, most likely because of the high decline in foreign travel due to economic difficulties in Iceland during this period.
In 1994, Iceland started with special control actions to fight *Salmonella* contamination in broiler flocks. At the start of the programme, approximately 15% of the broiler flocks were positive each year, but

from 1997 onwards, the programme's measures have been successful in reducing positive farm groups to below 1% per annum, with few exceptions. The same is true for turkey and pig production, where the annual contamination level is usually very low.

Discussion
Q: What control measures were taken to reduce the prevalence of *Salmonella* in poultry in Iceland?
A: Initial measures in 1997 involved improvement in biosecurity of houses, disinfection, and house access restriction. Additionally, the use of *Salmonella* positive animal feed is not permitted.
Q: What is done with animal feed which tested positive for *Salmonella*?
A: It is no longer used for feeding animals, but I do not know what is, in fact, done with the positive feed.
Q: Do you also have breeding flocks in Iceland? If so, how is the *Salmonella* status of these breeding flocks?
A: Yes, we do have breeding flocks. The flocks are tested within 2 weeks of birth, and if tested positive for *Salmonella,* the flock is culled. In this way, *Salmonella* is well controlled. Only very few breeding flocks have to be culled nowadays.

3.4 Activities of the NRL-*Salmonella* to fulfil tasks and duties in Estonia

Age Kärssin, NRL-Salmonella, Tartu, Estonia

The Estonian National Reference Laboratory for the control of *Salmonella* and other specified foodborne zoonotic agents is part of the Veterinary and Food Laboratory (VFL). The VFL was founded in 1998 from the former State Veterinary Laboratory and regional Veterinary laboratories that used to be part of the local Veterinary Centres, and it reports directly to the Ministry of Rural Affairs.
The VFL has been authorised to execute the function of National Reference Laboratory in different fields of food and feed analyses, and in the diagnostics of animal diseases. The main collaborator is the Veterinary and Food Board, the competent authority organising and coordinating the control of infectious animal disease and zoonotic agents.
The Central Veterinary and Food Laboratory is located in Tartu, except the food microbiology department, which is located in Rakvere. In addition, regional laboratories in Tallinn and Saaremaa are part of the VFL. All these laboratories are accredited according to EN ISO/IEC 17025:2005.
The laboratory uses different techniques, including microbiological culture, biochemical confirmation, MALDI-TOF MS, serotyping, antimicrobial resistance testing, PCR method for *Salmonella* spp. detection from food, feed and primary production stage (Josefsen et al., 2007), and PCR method for differentiation of *Salmonella* Typhimurium and its monophasic variant (Maurischat et al., 2015). All previously mentioned laboratory methods are accredited according to EN ISO/IEC 17025:2005.
The National *Salmonella* Control plan in live animals covers testing of samples from poultry, pigs, cattle, sheep, and goats. VFL is responsible for priority statutory testing including animal disease surveillance and food safety control programmes, and also provides inspection authorities

with relevant analytical support. Any *Salmonella* isolated in the programme, also during passive surveillance irrespective of serovar, is notifiable to the Veterinary and Food Board.
Together with the statutory functions, the VFL offers a laboratory service and advice to private veterinarians and farmers for the diagnosis and control of animal disease, and to the food processing industry for food safety and quality control.
Communication and cooperation with European Union Reference Laboratories (EURL) is also a VFL responsibility: it participates in interlaboratory comparison testing and in annual workshops organised by the EURLs.
Eleven official control laboratories, two food business operator laboratories, and five private laboratories test for *Salmonella* in food and feed. Three official control laboratories, two food business operator laboratories, and two private laboratories test for *Salmonella* in primary production stage samples. VFL has organised workshops for laboratories for *Salmonella* testing in 2008, 2011 and 2017. The most recent interlaboratory comparison study for *Salmonella* detection in a food matrix was organised in 2018, and for *Salmonella* in samples from the primary production stage in 2017. Serotyping and storage of *Salmonella* isolates sent by other laboratories are also the responsibility of VFL.
In 2018, VFL started to perform Next Generation Sequencing for the first time to gain experience in this field. 39 *Salmonella* Derby isolates have already been sequenced, data analysis is ongoing.

Discussion
Q: Do you see trends in certain *Salmonella* serotypes?
A: We find a lot of monophasic *S.* Typhimurium, but no real trends.
Q: Do you have an agreement with the private sector for reporting data?
A: Yes, if *Salmonella* is found, this has to be reported to the competent authority.

3.5 Activities of the NRL-*Salmonella* to fulfil tasks and duties in Former Yugoslav Republic of Macedonia (FYROM)

Dean Jankuloski, NRL-Salmonella, Skopje, FYROM

Since 2007, seven NRLs have been located at the Food institute in Skopje: NRL-*Salmonella*, NRL-*Campylobacter*, NRL-*Listeria monocytogenes*, NRL-*Escherichia coli*, NRL coagulase positive staphylococci (CPS), NRL-milk and NRL-chemical risks. The Food institute includes four laboratories (food and feed microbiology, residues and contaminants, raw milk quality, food quality) and more than 130 methods have been accredited according to EN ISO/IEC 17025:2005.
For the analysis of *Salmonella,* EN ISO methods are followed: EN ISO 6579-1:2017 for detection of *Salmonella*, CEN ISO/TR 6579-3:2014 for serotyping of *Salmonella*, EN ISO 20776-1:2006 and EN ISO 20776-2:2007 for antimicrobial susceptibility testing.
A National control plan and guidelines for the reduction of *Salmonella* in chickens, laying hens, broilers, and incubation stations (*Gallus gallus*) has been set up for the period 2017-2020. For the same period, a programme for antimicrobial resistance testing has also been established.
In 2011, PFGE typing was introduced for *Salmonella, Listeria monocytogenes, Shigella* and STEC. Between 2004-2018, more than

200 *Salmonella* isolates were typed with PFGE. These isolates originated from poultry faeces, environmental samples, food and feed samples, as well as human isolates. For quality control, the laboratory participates in the PTs of the EURL-*Salmonella* for PFGE typing, as well as in the PTs organised by Statens Serum Institut (Denmark).

3.6 EFSA's molecular typing activities for food-borne pathogens

Frank Boelaert, EFSA, Parma, Italy

Molecular typing through microbial DNA fingerprinting has developed rapidly in recent years. Data on the molecular testing of food-borne pathogens such as *Salmonella*, *Listeria monocytogenes,* and shiga toxin-producing *Escherichia coli* (STEC) could substantially contribute to the epidemiological investigations of food-borne outbreaks and to the identification of emerging health threats, as well as to source attribution studies. For the purpose of data collection and subsequent linkage with corresponding data from human isolates, ensuring comparability of typing data from food-borne pathogens isolated from food, feed, animals and the related environment, as well as from human sources, is essential.
A Commission vision paper following the Enterohaemorrhagic *Escherichia coli* (EHEC) crisis was endorsed by the Member States (MS) in December 2012 (EC, 2012). Thereafter, the Commission asked EFSA to provide technical support regarding the collection of molecular typing data of food, feed and animal isolates of *Salmonella*, *Listeria monocytogenes* and STEC, and a similar request was made to ECDC on molecular typing data of human isolates. In addition, the Commission asked EFSA and ECDC to establish a joint database for the molecular typing data of these foodborne pathogens of human and non-human origin. The aim of the joint EFSA-ECDC database is to enhance routine surveillance and outbreak identification by enabling detection of microbiological links between isolates of human and of non-human origin.
Data collection covers molecular typing results obtained through Pulsed Field Gel Electrophoresis (PFGE) for *Listeria monocytogenes*, *Salmonella* and STEC, and Multiple-Locus Variable number tandem repeat Analysis (MLVA) for *Salmonella* Typhimurium only. Molecular typing data production, interpretation and submission is performed according to defined Standard Operating Procedures and technical specifications (Caprioli et al., 2014, EFSA et al., 2014, Jacobs et al., 2014, Roussel et al., 2014). A specific Collaboration Agreement has been signed by the parties involved to address issues with regards to data ownership, availability, access, use, and publication. Data confidentiality is guaranteed by the limited sharing of data in the joint database, and by restricted access to sensitive information. Curation of human isolates is performed by ECDC; curation of non-human isolates is carried out by the European Union Reference Laboratories (EURLs) for the specific pathogen. The joint cluster analysis of both human and non-human isolates is carried out by EFSA, ECDC, and their respective curators in the joint database according to a specific procedure agreed between the parties. The official nomination of MS representatives for this data collection is ongoing and the technical coordination and support from EFSA to laboratories has started.

For more information see Rizzi et al., 2017.

Discussion
Q: Will it be possible to include sequence data in the molecular database?
A: Not yet, but we have it on the agenda.

3.7 EURL working group on Whole Genome Sequencing (WGS)

Ettore Amato, DG-SANTE, Brussels, Belgium

The Commission provided an update on EU activities on Whole Genome Sequencing (WGS).

1. A mandate on WGS was sent to the European Food Safety Authority (EFSA) and the European Centre for Disease Control and Prevention (ECDC) in order to perform a joint analysis of the EFSA and ECDC surveys on WGS. This included a consultation of the relevant actors and players to assess the state-of-the-art of pipelines for collecting and analysing WGS data in Europe, an assessment of needs/requirements for the analysis of WGS data and their comparability, a description of roles and responsibilities, and to provide a technical report on the identification and the comparison of potential solutions for the set-up and running of a joint EFSA-ECDC pipeline (deadline: April 2019). A second mandate will follow with the aim of creating the EU database on WGS.

2. On May 2017, the Commission decided to create an EURLs Working Group on WGS in order to promote the use of WGS across the EURLs' network, build WGS capacity within the EU, and ensure liaison with the work of the EURLs and the work of EFSA and ECDC on the WGS mandate sent by the Commission, while ensuring no overlap of activities between the WG and EFSA-ECDC work. The EURL *E.coli* was appointed coordinator of the WG. All EURL members will contribute to all the tasks by bringing in the specific needs of their networks as well as their experience. The role of the task leaders will be to facilitate the discussion and to be active in coordinating and drafting the documents related to the task they will lead. The following activities have been agreed by the Working Group: proficiency testing (EURL AR), WGS laboratories procedures - SOPs (EURL Parasites), bioinformatics tools (EURL VTEC), WGS cluster analysis (EURL *Campylobacter*), bench marking (EURL *Listeria*), training on WGS (EURL CPS), reference and confirmatory testing using WGS (EURL *Salmonella*), and follow-up of ISO activities on WGS. The 1st meeting of the EURL Working group was held in Brussels in November 2017, and the 2nd meeting is expected on 11th June 2018.

Discussion
Q: Will it be possible to also upload data of viruses in this molecular database in the future?
A: Yes, this is the intention.
Q: Can we learn from other EU projects concerning WGS?
A: Indeed, we should avoid double work and learn from ongoing projects (e.g. EFSA project ENGAGE).
Q: Will this database for WGS be added to the existing EFSA/ECDC joint database, or will there be a need for another signed agreement (which may take a long time)?
A: This is part of the ongoing discussion. Hopefully, what is in place can be used and expanded.

Q: What about harmonisation of WGS analysis (human and non-human)?
A: Indeed, this is an important issue. In ISO, a working group is preparing an ISO standard for WGS analysis. However, this ISO working group focuses on microbiology of the food chain only and not on human data. EFSA and ECDC are also aware of this need for harmonisation.
Q: Why was the name of the working group changed from WGS (whole genome sequencing) to NGS (next generation sequencing)?
A: This was preferred by several EURLs of the working group and is more accurate.

3.8 Work programme EURL-*Salmonella* second half 2018, first half 2019, discussion on general items and closure

Kirsten Mooijman, head EURL-Salmonella, Bilthoven, the Netherlands

Kirsten Mooijman summarised the information on the work programme of the EURL-*Salmonella* for the rest of 2018 and for early 2019.
For the 2018 work programme, a new template had to be used following Regulation (EU) 625/2017 (EC, 2017), Article 92 (2). This resulted in some sub-activities being described under new headings when compared to former work programmes.

Activity 1 To ensure availability and use of high-quality methods and to ensure high quality performance by NRLs

Sub-activity 1.1 Analytical methods
Objectives:

- Standardisation of methods (ISO and CEN).
- Keep track of developments in (alternative) methods.
- Provide NRLs with information on developments of relevant (standardised/new) analytical methods.

This concerns the activities for ISO and CEN as project leader or as member:

- ISO-WG3 Method validation, especially ISO 16140-6 (validation of alternative confirmation and typing procedures).
- ISO-ad hoc group on harmonisation of ISO/CEN standards for microbiology of the food chain: drafting guidance document.
- ISO-ad hoc group on harmonisation of incubation temperatures: reporting and discussion of results comparison studies.
- CEN-TAG3/ISO-WG10 - drafting ISO/TS 6579-4 PCR identification of monophasic *Salmonella* Typhimurium: testing protocols with set of strains; amendment of document; organisation of validation study.
- CEN-TAG9 Improvement of the pre-enrichment step (member): help with drafting test protocol, performing practical experiments.
- ISO-WG4 Revision of ISO/TS 22117 on Proficiency Tests (member).
- ISO-WG25 Whole genome sequencing (member).

Sub-activity 1.2 EURLs working group on NGS
Objectives:

- Promote the use of NGS across the EURLs' networks.
- Build capacity on producing and using NGS data within the EU.
- Ensure liaison with the work of the EURLs and the work of EFSA and ECDC on NGS.

The working group includes eight EURLs, and eight activities have been defined in relation to NGS. Guidance documents will be prepared for each activity.

Sub-activity 1.3 Interlaboratory comparison studies
Objective:
Organisation of interlaboratory comparison studies to gain information on the performance of the NRLs-*Salmonella* for detection and typing of *Salmonella.*

In 2018, three interlaboratory comparison studies are foreseen:
1. One on detection of *Salmonella* in samples from the primary production stage. This study will be organised in September/October 2018 and the matrix of choice is likely to be boot socks.
2. One on detection of *Salmonella* in food or animal feed samples. This study is foreseen for February/March 2019 and the matrix has not yet been decided.
3. One on typing of *Salmonella* (serotyping, PFGE, MLVA). This study is foreseen for November/December 2018, and will concern serotyping of *Salmonella* (obligatory), PFGE typing (optional), and a pilot for MLVA typing of *Salmonella* Typhimurium (in case of sufficient interest).

Activity 2 To provide scientific and technical assistance to NRLs

Sub-activity 2.1 Workshop
Objective:
Exchange of information on the activities of the NRLs-*Salmonella* and the EURL-*Salmonella*. Exchange of information on (new) developments in the relevant work field.

The 2019 workshop is likely to be organised in the Netherlands.

Sub-activity 2.2 Training courses
Objective:
To train NRLs-*Salmonella* in a specific work field.

The following training courses are foreseen:

1. Training on request of an NRL.
2. Training following advice from the EURL (e.g. in case of repeated poor performance in interlaboratory comparison studies).
3. Training on the use of BioNumerics for PFGE profile analysis. Organised in cooperation with EURL-STEC and EURL-*Listeria monocytogenes*; four NRLs per network (in total 12 participants). This training course will be organised at the premises of EURL-*Salmonella* (Bilthoven, the Netherlands) on 26-27 June 2018.

Sub-activity 2.3 Scientific advice and support of NRLs

Objectives and description:

- Provide scientific and technical assistance to the NRLs-*Salmonella* for the relevant work field;
- Perform confirmatory testing (samples/isolates) for NRLs when needed;
- Maintaining the EURL-*Salmonella* website and keeping the information on the website up to date;
- Informing NRLs on the activities of the EURL and other parties in the relevant work field, as well as on developments in this field;
- Publication of 4 newsletters per year, through the website.

Activity 3 To provide scientific and technical assistance to the European Commission and other organisations

Sub-activity 3.1 Scientific advice and support of EC and other organisations

Objectives:

- Provide scientific and technical assistance to EC DG SANTE for the relevant work field;
- Provide assistance to DG SANTE, EFSA and (NRLs of) Member States in case of (international) Salmonella outbreaks;
- Collaborate with EFSA and ECDC for the relevant work field;
- Cooperation with other biological EURLs.

Description:

- Ad hoc scientific and technical assistance of DG SANTE.
- Participation in working groups/scientific committees of DG SANTE, EFSA, e.g. EFSA-ECDC Steering Committee of molecular database;
- Curation of PFGE data in EFSA molecular database;
- Assistance of DG SANTE, EFSA, NRLs and ECDC in case of outbreaks, e.g. consultation of NRL network for specific information (like recent *S.* Bareilly), (sub)typing of suspect isolates (MLVA, NGS);
- Discussions with DG SANTE and relevant EURLs on distribution of tasks of EURL-Bivalve molluscs after Brexit (from 1/1/2019).

Activity 4 Reagents and reference collections

Sub-activity 4.1 Reference strains and reference materials

Objective:

Supply information on available culture collections and suppliers of microbiological reference materials.

Description*:*

- Provide link to WKLM scheme, keep contacts with WHO reference centre;
- Reference to culture collections and reference materials at website;
- Maintenance of in-house culture collection;
- Subactivity 4.1 is merged with 2.3 (support NRLs; keeping information on website up to date).

Summary results survey NGS

The EURLs' working group on Next Generation Sequencing (NGS) prepared a survey to gain knowledge on capacity and needs regarding NGS at the NRLs. By the end of March 2018, each EURL sent the same survey to its own network of NRLs, with a deadline to respond by 20 April 2018. Institutes with more than one NRL would receive multiple requests for completing the survey, however were requested to complete the relevant survey for each NRL.
The survey was sent to 54 NRLs-*Salmonella* in 36 countries and consisted of 32 (sub)questions. By the end of April 2018, completed surveys had been received from 23 NRLs-*Salmonella* in 20 countries. This is a response rate of 42.6% at the level of NRLs, and a response rate of 55.5% at the level of countries.
The responses to the surveys of the NRLs of the 8 networks will be collected and summarised by the EURLs' NGS working group.

Closure

The workshop closed with many thanks to the NRL-*Salmonella* in Sweden for making the workshop in Sweden possible and for their excellent help with its organisation.

4 Evaluation of the workshop

4.1 Introduction

At the end of the workshop, an evaluation form was given to the participants to ask for their opinions (see Annex 3).
A total of twelve questions were asked. For ten of these questions, participants were asked to answer using a score ranging from 1 to 5. The scores represent: very poor (1), poor (2), fair (3), good (4) and excellent (5).
In addition, it was possible to add comments. Two questions were 'open' questions, in which the participants were asked to give their opinion.
The evaluation form was handed to 43 workshop participants;
38 completed forms were returned, a response rate of 88%.

In section 4.2, the scores on each question are presented and a summary of the remarks is given.

4.2 Evaluation form

1. What is your opinion on the information given in advance of the workshop?
Figure 1 shows that the respondents considered the information given in advance of the workshop as good or excellent (scores 4-5).

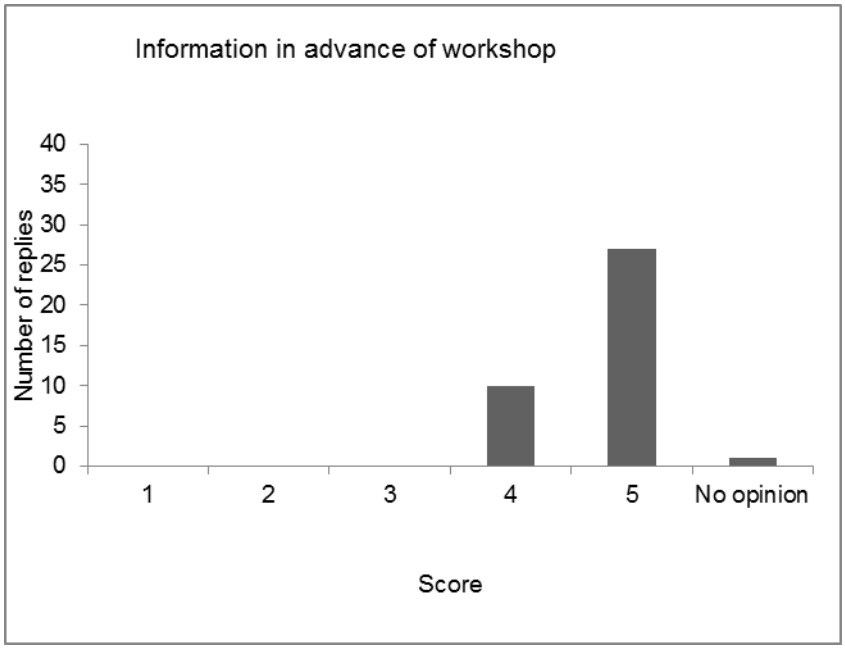

Figure 1 Scores given to question 1 'Opinion on information given in advance of the workshop'

2. What is your opinion on the booking of the tickets by the EURL-Salmonella?
The majority of the participants for whom tickets were arranged by the EURL were very satisfied. Participants who booked their own ticket indicated 'no opinion' (see Figure 2).

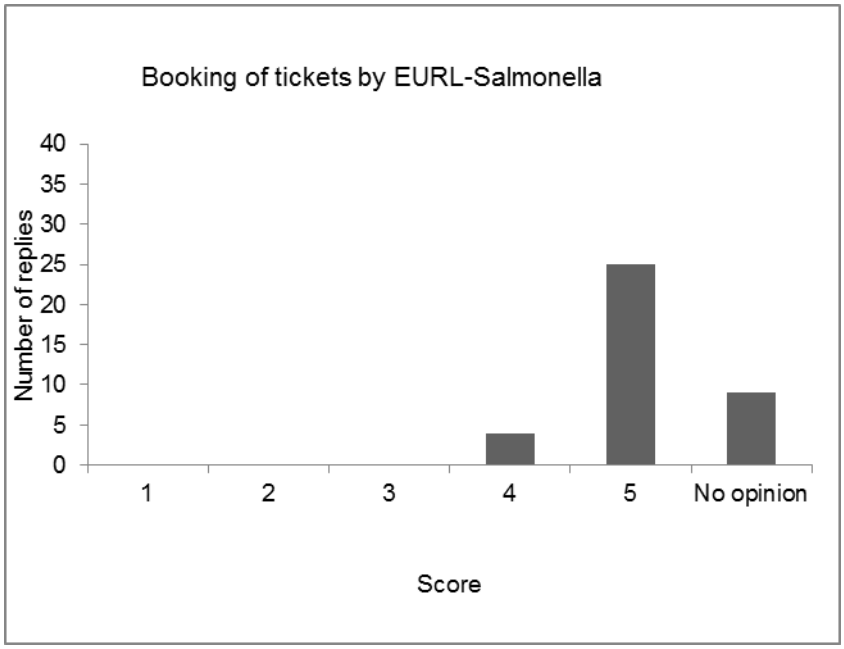

Figure 2 Scores given to question 2 'Opinion on the booking of the tickets by the EURL-Salmonella'

3. What is your opinion on how easy (high score) or difficult (low score) it was to reach the meeting venue?
This year's meeting venue was good to reach, which can be seen in the scores given to this question: either good (score 4) or excellent (score 5), see Figure 3.

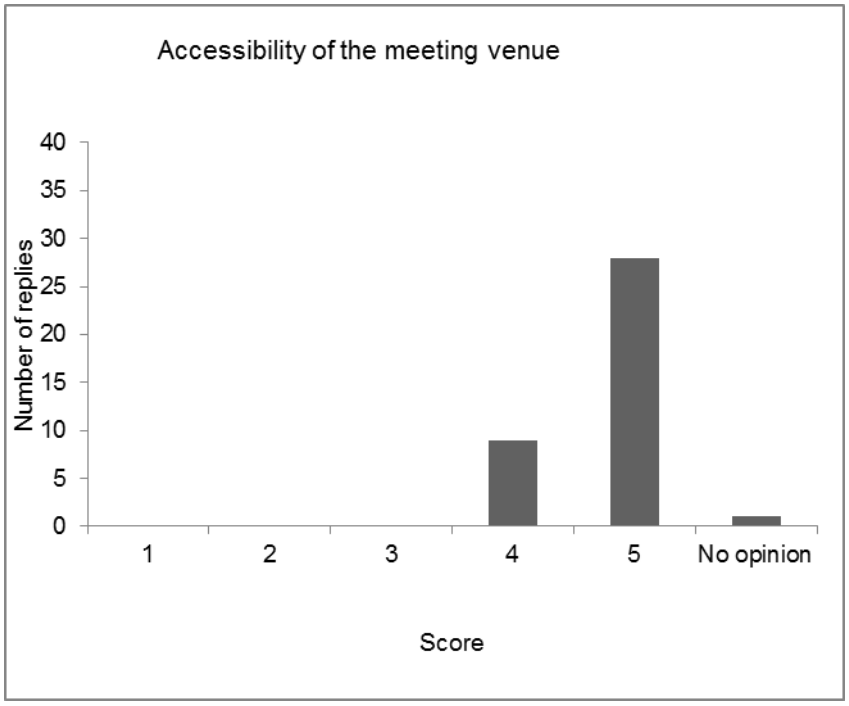

Figure 3 Scores given to question 3 'Opinion on the accessibility of the meeting venue'

4. What is your opinion on the hotel room?
The majority of the participants were satisfied with the hotel rooms (Figure 4).

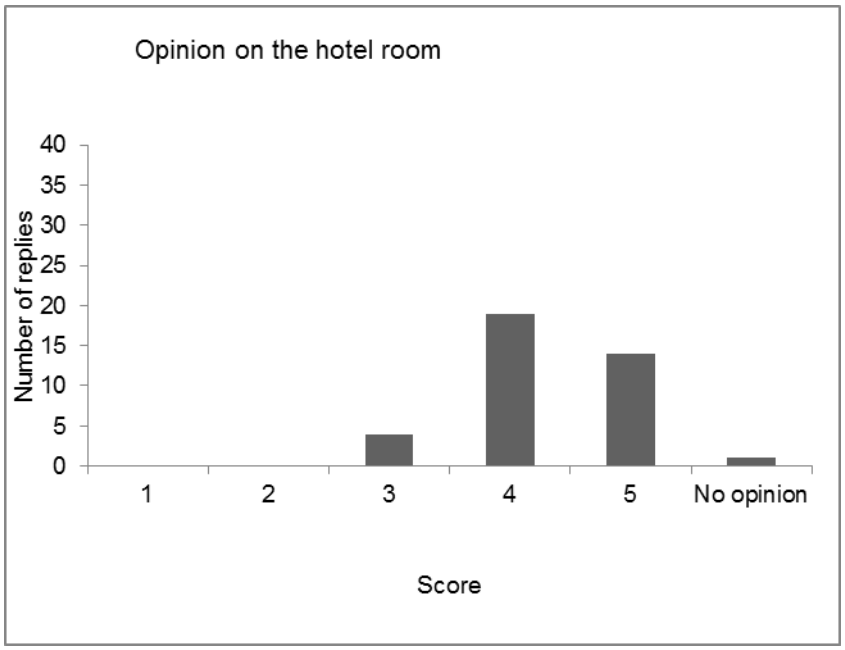

Figure 4 Scores given to question 4 'Opinion on the hotel room'

5. What is your general opinion on the meeting room?
The opinion on the meeting room was, in general, good to excellent (scores 4 and 5; see Figure 5). Some participants made a remark about the microphones which did not work, so some speakers were difficult to hear.

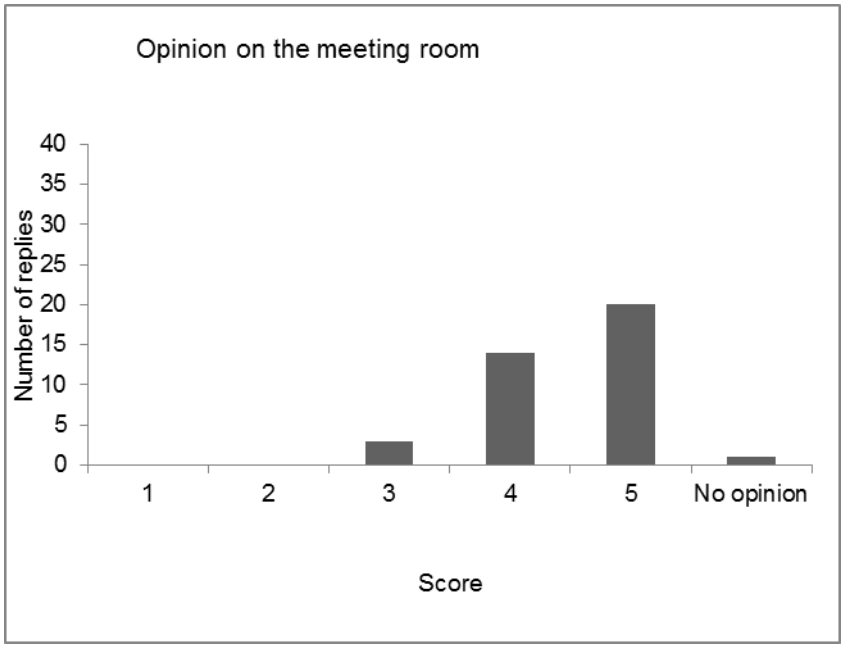

Figure 5 Scores given to question 5 'Opinion on the meeting room'

6. What is your opinion on the readability of the presentations on the screen?
The majority of respondents were satisfied about the readability of the presentations on the screen (see Figure 6).

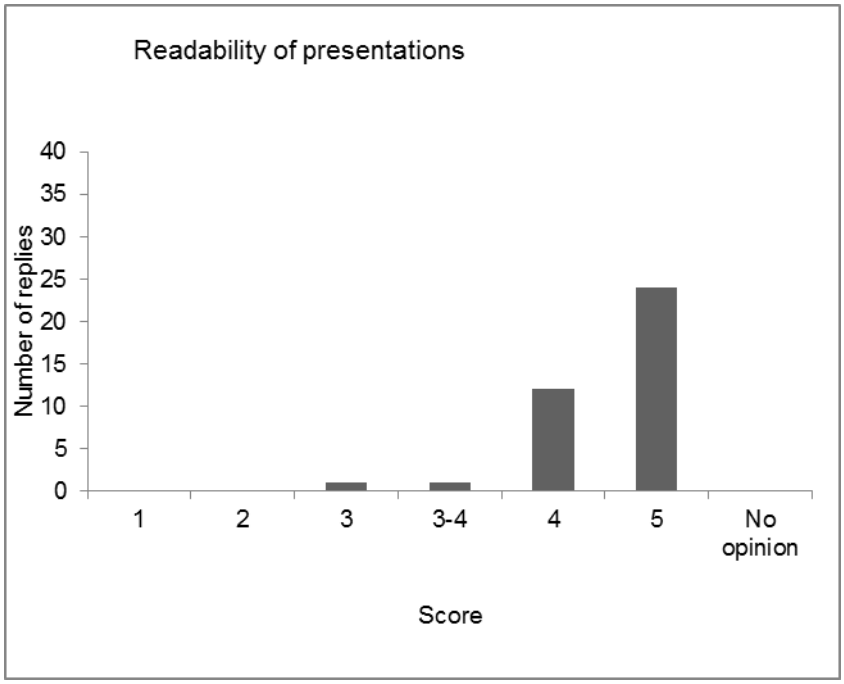

Figure 6 Scores given to question 6 'Opinion on the readability of the presentations on the screen'

7. What is your opinion on the technical equipment in the meeting room (computer, screen, microphones, etc.)?

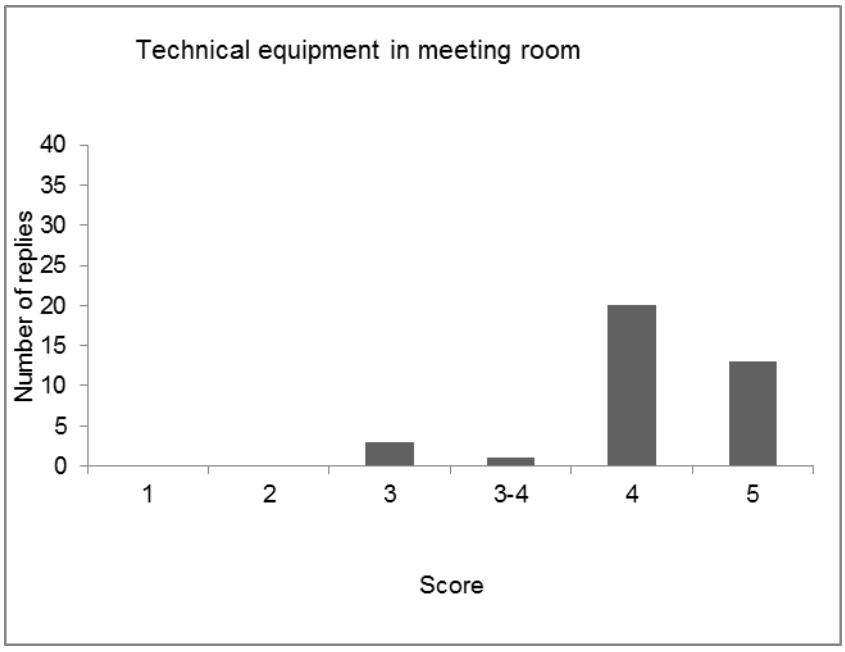

Figure 7 Scores given to question 7 'Opinion on the technical equipment'

The majority of respondents were also satisfied with the technical equipment in the meeting room (Figure 7), although, as for question 5, remarks were made about the lack of microphones.

8. What is your opinion on the catering provided during the workshop (breakfast, coffee, tea, lunch, dinner)?
The respondents found the catering fair (score 3) to excellent (score 5), see Figure 8.

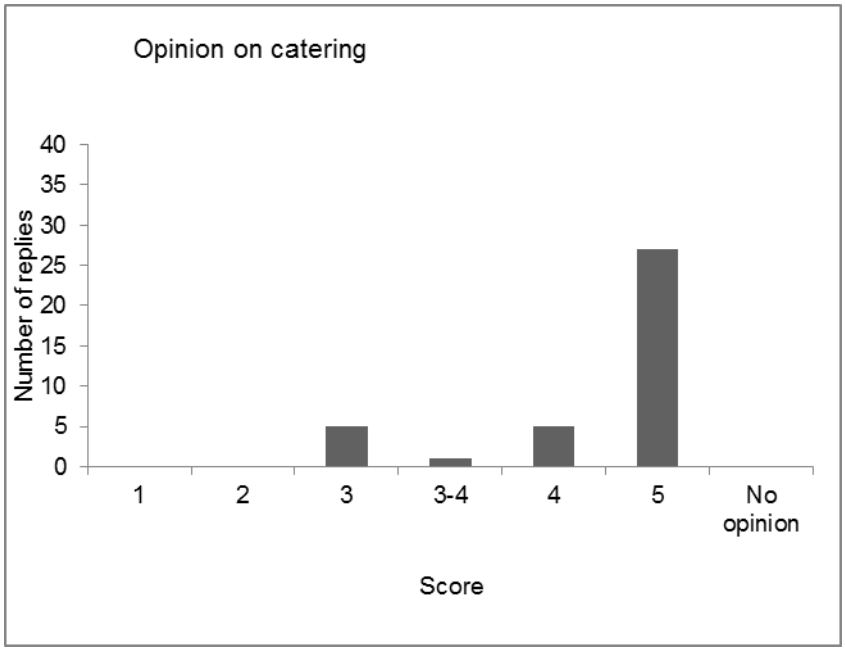

Figure 8 Scores given to question 8 'Opinion on the catering'

9. What is your opinion on the scientific programme of the workshop?

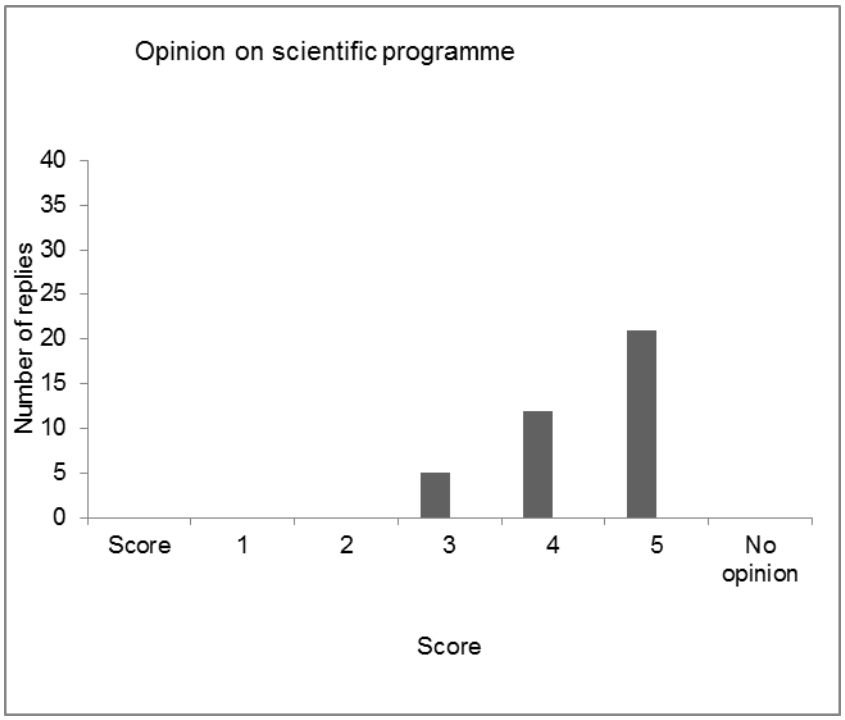

Figure 9 Scores given to question 9 'Opinion on the scientific programme'

The opinion on the scientific programme of the workshop varied from fair (score 3) to excellent (score 5), see Figure 9; the majority of the respondents were satisfied. Additional remarks:
'The timeslot per presentation and the whole workshop were just right (not too long, not to short).'
'I liked the presentation of the EURL-*Campylobacter* and about the French cattle.'

10. Are there specific presentations you want to comment on, or did you miss information on certain subjects?
This concerned an 'open' question and the following responses were obtained:
'All excellent.'
'Number of samples taken in control programmes for *Salmonella* in different matrices in the Member States.'
'Very good presentation by Wilma Jacobs about the pilot validation study for confirmation of *Salmonella* following ISO/DIS 16140-6.'
'Interesting presentation about the activities of the NRLs-*Salmonella* in different countries and to see the differences in *Salmonella* serovars isolated.'
'Excellent presentation by guest speaker Robert Söderlund.'

11. What is your general opinion of the workshop?

Figure 10 Scores given to question 11 'General opinion of the workshop'

The respondents indicated that the workshop as a whole had been good (score 4) or excellent (score 5), see Figure 10. Additional remarks:
'This is my first EURL-*Salmonella* workshop and I thoroughly enjoyed it and found it all very informative and interesting.'
'I would like to have had the opportunity to visit a laboratory different from mine.'
'The live music band with Lennart was good!'

12. Do you have any remarks or suggestions which we can use for future workshops?
This concerned an 'open' question and the following responses were obtained:
'More information about alternative travel routes would be helpful (bus routes, payment) other than back to the airport.'
'More details (from EURL) about the preparation of samples for PTs, this is interesting for NRLs which organise PTs for their official laboratories.'
'Many thanks for the workshop.'
'It would be better to renew the scientific programme with invited academic speakers or international researchers and present new topics on *Salmonella* science.'
'Focus on PFGE data curation within the molecular typing project (role of EURL and NRLs) and practical information.'
'Budapest next year.'
'Not so strict with the programme in general, e.g. I would have liked to see the laboratory or have some free time.'
'Excellent. The workshop is so short; I would have liked 3 days instead of 1.5 days.'
'I would like to have the contact details of all participants.'
'The presentations of the NRLs should be more specific.'

4.3 Discussion and conclusions of the evaluation

In general, the participants were satisfied with the workshop. For almost all items 'good' (score 4) or 'excellent' (score 5) scores were given. Unfortunately, the microphones gave some extra noise so that they could not be used. This resulted in some problems with hearing some of the speakers and hearing questions from the audience.
The participants made several interesting remarks in the evaluation forms. Where one remarked that the timing of the workshop was perfect, another remarked that it would have been nice to have a longer workshop. Some remarks were made about the programme, varying from very satisfied to a request to totally renew the scientific programme.
The EURL-*Salmonella* will try to take all remarks into account for next year's workshop, although inviting many external speakers is not likely to be possible due to restricted budget.

Acknowledgements

The author would like to greatly thank Noël Peters (RIVM) for her help with the organisation of the workshop, and Jeanette van Essen (RIVM) for booking the flights for the participants.

In addition, the author would like to thank the colleagues from the United Kingdom, Amisha Vibhakar, and from Ireland, William Byrne for making notes during the workshop. This was of great value when drafting the discussions in this report.

List of abbreviations

A	Answer
AL	Acceptability Limit
BPW	Buffered Peptone Water
CEN	European Committee for Standardization
cfu	colony forming units
DG-SANTE	Directorate-General for Health and Food Safety
DIS	Draft International Standard
EC	European Commission
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
EFTA	European Free Trade Association
EU	European Union
EURL	European Union Reference Laboratory
FYROM	Former Yugoslav Republic of Macedonia
GMO	Genetically Modified Organisms
INFOSAN	International Network of Food Safety Authorities
ISO	International Organization for Standardization
MKTTn	Mueller Kauffmann Tetrathionate broth with novobiocin
MLST	Multilocus Sequence Typing
MLVA	Multi-Locus Variable number of tandem repeats Analysis
MPN	Most Probable Number
MS	Member State
MSRV	Modified Semi-solid Rappaport Vassiliadis
NGS	Next Generation Sequencing
NRL	National Reference Laboratory
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
PHE	Public Health England
PPS	Primary Production Stage
PT	Proficiency Test
Q	Question
RASFF	Rapid Alert System for Food and Feed
RIVM	National Institute for Public Health and the Environment
ROA	Rapid Outbreak Assessment
RVS	Rappaport Vassiliadis broth with Soya
SC	Sub Committee
SMb	*Salmonella* Mbandaka
SOP	Standard Operating Procedure
STEC	Shiga toxin-producing *Escherichia coli*
STm	*Salmonella* Typhimurium
TAG	Technical Advisory Group
TC	Technical Committee
TS	Technical Specification
UK	United Kingdom
WG	Working Group
WGS	Whole Genome Sequencing
XLD	Xylose Lysine Deoxycholate

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Annex 1 Participants

European Food Safety Authority (EFSA)	Frank Boelaert
EC DG-SANTE	Ettore Amato
EURL-*Salmonella*	Kirsten Mooijman Angelina Kuijpers Wilma Jacobs
Guest speakers (Sweden)	Robert Söderlund (SVA, Uppsala) Hanna Skarin (EURL-*Campylobacter*, SVA, Uppsala)

National Reference Laboratories for *Salmonella*

AUSTRIA	Christian Kornschober
BELGIUM	Nadine Botteldoorn
BOSNIA HERZEGOVINA	-
BULGARIA	Gergana Mateva
CROATIA	Borka Simpraga
CYPRUS	Konstantinos Arsenoglou
CZECH REPUBLIC	Tomas Cerny
DENMARK	Søren Aabo AnneMette Seyfarth
ESTONIA	Age Kärssin
FINLAND	Henry Kuronen
FRANCE	Frederique Moury Laetitia Bonifait
FYROM	Dean Jankuloski
GERMANY	Istvan Szabo
GREECE	Aphrodite Smpiraki
HUNGARY	Erzsébet Adrián
ICELAND	Franklin Georgsson
IRELAND	William Byrne
ITALY	Veronica Cibin
LATVIA	Madara Streikisa
LITHUANIA	Aista Darata Brazdilyte
LUXEMBOURG	Gilbert Moris
MALTA	-
NETHERLANDS	Irene Pol Anjo Verbruggen
NORTHERN IRELAND	Angela Lahuerta Marin
NORWAY	Bjarne Bergsjø
POLAND	Elzbieta Kukier Renata Kwit Elzbieta Mackiw
PORTUGAL	Patricia Themudo
ROMANIA	Carmen Manea
SERBIA	Jasna Kureljusic

SLOVAK REPUBLIC	Lubos Mikula
SLOVENIA	Jasna Micunovic
SPAIN	Mauel Durán
SWEDEN	Lennart Melin
	Jenny Kantz
SWITZERLAND	Denise Althaus
TURKEY	-
UNITED KINGDOM	Gail Wise
	Amisha Vibhakar

Annex 2 Workshop Programme

Programme of the 23rd EURL-*Salmonella* workshop
29 and 30 May 2017, Uppsala, Sweden

General information

Place of accommodation

Best Western Hotel Svava
Bangårdsgatan 24
SE-753 20 Uppsala
Sweden
https://www.hotelsvava.se/

Meeting venue

National Veterinary Institute (SVA)
Ulls väg 2B
SE-751 89 Uppsala
Sweden
http://www.sva.se/en

Information for the ones giving a presentation:
Presentations: Send your presentation to Kirsten Mooijman (kirsten.mooijman@rivm.nl), preferably one week before the workshop.

Abstract: For the preparation of the workshop report, it is necessary to also receive an abstract of your presentation (approximately 0,5-1 page). Please hand this over to Kirsten during the workshop or send it to Kirsten.mooijman@rivm.nl **preferably before 1 June 2018**

Monday 28 May 2018

Dinner information

For participants for whom the costs of travel and stay are paid from the budget of EURL-*Salmonella,* the EURL will also cover the expenses of a dinner on Monday 28 May, with a maximum of € 40,- per person. We will need a receipt in order to reimburse you for this meal.
Remark*: alcoholic drinks are very expensive in Sweden and can therefore not be reimbursed.*

Tuesday 29 May 2018

08:15 - 08:45 Transport by bus from hotel to SVA

Morning Chair: Wilma Jacobs

09:00 - 09:30	Opening and introduction	Kirsten Mooijman, EURL-*Salmonella*
09:30 - 10:00	EURL-*Campylobacter*	Hanna Skarin, Sweden
10:00 - 10:30	No decrease of human *Salmonella* Enteritidis despite *Salmonella* control programmes in poultry in the European Union, 2013-2016	Frank Boelaert, EFSA
10:30 - 11:00	*Coffee/tea*	
11:00 - 11:30	Multi-country outbreak of *Salmonella* Agona infections linked to infant formula	Ettore Amato, DG-Sante
11:30 - 12:00	Investigating *Salmonella* in the cattle production in France	Laetitia Bonifait, France
12:00 - 12:30	Results combined Food-PPS interlaboratory comparison study on detection of *Salmonella* in hygiene swabs (2017)	Irene Pol, EURL-*Salmonella*
12:30 - 13:45	*Lunch*	

Afternoon Chair: Kirsten Mooijman

13:45 - 14:15	Preliminary results 4th interlaboratory comparison study on detection of *Salmonella* in chicken feed (2018)	Angelina Kuijpers, EURL-*Salmonella*
14:15 - 14:45	Results 22nd interlaboratory comparison study on typing of *Salmonella* (2017) – serotyping and PFGE	Wilma Jacobs, EURL-*Salmonella*
14:45 - 15:15	Update on activities in ISO and CEN	Kirsten Mooijman, EURL-*Salmonella*
15:15 - 15:45	*Coffee/tea*	
15:45 - 16:15	Birds, cats, humans and host adaptation in *Salmonella* Typhimurium	Robert Söderlund, Sweden
16:15 - 16:45	Pilot validation study for confirmation of *Salmonella* following ISO/DIS 16140-6	Wilma Jacobs, EURL-*Salmonella*
16:45 - 17:30	*Drinks and music at SVA*	
17:30 - 18:30	*Transport by bus to hotel and short break*	
18:30 - 19:30	*Guided walk in Uppsala*	
19:30 - ….	*Dinner at Dryck och Mat, Gamla Stationshuset, Olof Palmes plats 2, Uppsala, Sweden*	

Wednesday 30 May 2018

08:15 - 08:45 Transport by bus from hotel to SVA

Morning Chair: Kirsten Mooijman

09:00 - 10:40	Activities NRLs to fulfil tasks and duties, and information on national *Salmonella* control programs	
09:00 - 09:20	NRL-*Salmonella* Finland	Henry Kuronen
09:20 - 09:40	NRL-*Salmonella* Hungary	Erzsébet Adrián
09:40 - 10:00	NRL-*Salmonella* Iceland	Franklin Georgsson
10:00 - 10:20	NRL-*Salmonella* Estonia	Age Kärssin
10:20 - 10:40	NRL-*Salmonella* Former Yugoslav Republic of Macedonia (FYROM)	Dean Jankuloski
10:40 - 11:15	*Coffee/tea*	
11:15 - 11:45	EFSA's molecular typing activities for food-borne pathogens	Frank Boelaert, EFSA
11:45 - 12:00	EURL working group on Whole Genome Sequencing (WGS)	Ettore Amato, DG-Sante
12:00 - 12:30	Work programme EURL-*Salmonella* second half 2018, first half 2019 Discussion on general items Closure	Kirsten Mooijman, EURL-*Salmonella*
12:30 - 13:30	*Lunch*	
13:30	*Transport by bus to Arlanda airport, Stockholm*	

---------- End workshop----------

Annex 3 Workshop evaluation form

Evaluation of the 23rd EURL-*Salmonella* workshop
29 and 30 May 2018, Uppsala, Sweden

We would highly appreciate if you could give us your opinion on the 23rd EURL-*Salmonella* workshop, organised in Uppsala, Sweden on 29 and 30 May 2018. Thank you very much in advance for completing this questionnaire and returning it to the EURL-*Salmonella* team by the end of the workshop.

Please give your opinion by indicating a score from 1 to 5, where 1 is the lowest score and 5 is the highest score representing the following:
1 = very poor; 2 = poor; 3 = fair; 4 = good; 5 = excellent

1. What is your opinion on the information given in advance of the workshop?

1 (very poor)	2	3	4	5 (Excellent)	No opinion

Remarks:_____

2. What is your opinion on the booking of the tickets by the EURL-*Salmonella* (if relevant)?

1 (Very poor)	2	3	4	5 (Excellent)	No opinion

Remarks:_____

3. What is your opinion on how easy (high score) or difficult (low score) it was to reach the meeting venue?

1 (Very poor)	2	3	4	5 (Excellent)	No opinion

Remarks:_____

4. What is your opinion of the hotel room?

1 (Very poor)	2	3	4	5 (Excellent)	No opinion

Remarks:_____

5. What is your general opinion of the meeting room?

1 (Very poor)	2	3	4	5 (Excellent)	No opinion

Remarks:

6. What is your opinion on the readability of the presentations on the screen?

1 (Very poor)	2	3	4	5 (Excellent)	No opinion

Remarks:

7. What is your opinion on the technical equipment in the meeting room (computer, screen, microphones, etc)?

1 (Very poor)	2	3	4	5 (Excellent)	No opinion

Remarks:

8. What is your opinion on the catering provided during the workshop (coffee, tea, lunch, dinner)?

1 (Very poor)	2	3	4	5 (Excellent)	No opinion

Remarks:

9. What is your opinion on the scientific programme of the workshop?

1 (Very poor)	2	3	4	5 (Excellent)	No opinion

Remarks:

10. Are there specific presentations you want to comment on, or did you miss information on certain subjects?

11. What is your general opinion of the workshop?

1 (Very poor)	2	3	4	5 (Excellent)	No opinion

Remarks:

12. Do you have any remarks or suggestions that we can use for future workshops?

Thank you very much!

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