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

The 26th EURL-*Salmonella* workshop

28 May 2021, Online

RIVM report 2021-0130
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Synopsis

The 26th EURL-*Salmonella* workshop
28 May 2021 – Online

The 26th workshop for the European National Reference Laboratories (NRLs) for *Salmonella* took place in May 2021. RIVM has created a collection of the reports of the various presentations. The workshop takes place each year, the aim being to enable information sharing between the European body, the European Union Reference Laboratory (EURL) for *Salmonella*, and the NRLs. Due to the SARS-CoV-2-virus pandemic, the workshop was held online for the second time.

Each workshop devotes a lot of attention to the Proficiency Tests organised by the EURL to monitor the quality of the NRLs. The three final Proficiency Tests were presented on this most recent occasion. The 2020 Proficiency Test examined hygiene swab samples, with that of 2021 examining liquid egg. The third Proficiency Test focused on the typing of various *Salmonella* strains using standard techniques and DNA techniques, with Whole Genome Sequencing (WGS) primarily used for the latter, which allows very precise typing of micro-organisms.
The NRLs achieved high scores for the 2020 and 2021 Proficiency Tests. Below is a brief outline of the Proficiency Tests. More information is available in the individual reports published for each Proficiency Test.

One particular presentation discussed the system that is currently being set up for the storage of the WSG data of all countries in the European Union. This data will be able to be used effectively in the investigation of sources of infection with a *Salmonella* bacteria.

The NRLs for *Salmonella* of the Czech Republic, Ireland and Slovenia provided a presentation of how they carry out their statutory remit.

The EURL-*Salmonella*, which is part of the National Institute for Public Health and the Environment (RIVM), is responsible for organising the workshop. A core task of the EURL-*Salmonella* is to monitor the quality of the National Reference Laboratories concerning this bacterium in Europe.

Keywords: EURL-*Salmonella*, NRL-*Salmonella*, *Salmonella*, workshop 2021

Publiekssamenvatting

De 26e EURL-*Salmonella* workshop

28 mei 2021, Online

In mei 2021 is de 26e workshop voor de Europese Nationale Referentie Laboratoria (NRL's) voor *Salmonella* georganiseerd. Het RIVM heeft de verslagen van de presentaties gebundeld. Deze workshop wordt elk jaar georganiseerd. Het doel is dat het Europese orgaan, het Europese Referentie Laboratorium (EURL) voor *Salmonella*, en de NRL's informatie delen. Door de uitbraak van het coronavirus is de workshop voor de tweede keer online gehouden.

In elke workshop is er veel aandacht voor de ringonderzoeken die het EURL organiseert om de kwaliteit van de NRL's te controleren. Dit keer zijn de drie laatste ringonderzoeken gepresenteerd. In het ringonderzoek van 2020 zijn hygiënedoekjes onderzocht, en in die van 2021 vloeibaar ei. In het derde ringonderzoek zijn verschillende *Salmonella*-stammen getypeerd met gewone technieken en met DNA-technieken. Voor dit laatste is vooral Whole Genome Sequencing (WGS) gebruikt, waarmee micro-organismen heel precies kunnen worden getypeerd.
De NRL's scoorden goed bij de ringonderzoeken van 2020 en 2021. De ringonderzoeken zijn hier kort beschreven. Meer informatie is te vinden in de rapporten die over elk ringonderzoek apart worden uitgegeven.

Een presentatie ging in op het systeem dat wordt gebouwd om de WGS-data van alle landen van de Europese Unie op te slaan. Deze data kunnen goed gebruikt worden om de bron van besmettingen met een *Salmonella*-bacterie te onderzoeken.

De NRL's-*Salmonella* van Tsjechië, Ierland en Slovenië lieten zien hoe zij hun wettelijke taken invullen.

Het EURL voor *Salmonella*, dat onderdeel is van het RIVM, organiseert deze workshop. Een belangrijke taak van het EURL-*Salmonella* is de kwaliteit van de nationale referentielaboratoria voor deze bacterie in Europa controleren.

Kernwoorden: EURL-*Salmonella*, NRL-*Salmonella*, *Salmonella*, workshop 2021

Contents

Summary

On 28 May 2021, the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*) organised its annual workshop. Due to the SARS-CoV-2-virus pandemic it was still not possible to organise a physical meeting. Hence, for the second time, the workshop was organised as a virtual meeting. Participants in the workshop were representatives of the National Reference Laboratories (NRLs) for *Salmonella* from the 27 European Union (EU) Member States, three European Free Trade Association (EFTA) countries, and five (potential) EU candidate countries. Also present were representatives of the European Commission Directorate-General for Health and Food Safety (EC DG SANTE) and of the European Food Safety Authority (EFSA). Thanks to the fact that this workshop was organised as a virtual meeting, it was possible to host more participants compared to a physical workshop. In total 74 participants attended.

During the workshop, presentations were given on several topics:

- An EFSA representative gave an update on *Salmonella* in the EU based on the EU One Health 2019 Zoonoses report.
- Representatives of the EURL-*Salmonella* presented the results of the Proficiency Tests (PTs) organised in the past year, namely the PT on detection of *Salmonella* in hygiene swab samples (October 2020), the PT on detection of *Salmonella* in liquid whole egg (March 2021), and the PT on *Salmonella* typing (November 2020). For the cluster analysis of the latter study, also results of additional WGS analysis performed by the EURL-*Salmonella* were presented.
- Another EFSA representative presented the state of implementation of the 'One Health' system for the collection and analysis of WGS data from food/animal isolates and the new cgMLST scheme for *Salmonella*.
- A representative of the typing department of RIVM showed the results of an in-house validation of *Salmonella* serotyping with Whole Genome Sequencing (WGS).
- Representatives of the NRLs-*Salmonella* from the Czech Republic, Ireland and Slovenia presented the activities undertaken to fulfil their NRL tasks and duties.

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

The workshop presentations are available on the EURL-*Salmonella* website: https://www.eurlsalmonella.eu/workshop-2021

1 Introduction

This report includes the abstracts of the presentations given at the 2021 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 (when the author has given permission for publication):
https://www.eurlsalmonella.eu/workshop-2021

The layout of the report is consistent with that of the workshop programme.
Chapter 2 includes the abstracts of the presentations given on the day of the workshop.
The workshop is evaluated in Chapter 3; 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 Friday 28 May 2021

2.1 Opening and introduction

Kirsten Mooijman, Head of EURL-Salmonella, Bilthoven, the Netherlands

Kirsten Mooijman, head of the European Union Reference Laboratory (EURL) for *Salmonella*, opened the 26th workshop of the EURL-*Salmonella*, welcoming all participants to this second virtual EURL-*Salmonella* workshop.

In total, 74 participants attended, including representatives of the National Reference Laboratories (NRLs) for *Salmonella* from the 27 EU Member States, five (potential) candidate EU countries, and three member countries of the European Free Trade Association (EFTA). Additionally, representatives of the European Commission Directorate-General for Health and Food Safety (EC DG SANTE) and of the European Food Safety Authority (EFSA) attended.

The evaluation of the first online workshop organised in 2020 was presented, showing high scores for all questions raised. Opinions on the scientific programme of the first online workshop were good to very good, meaning that despite the fact that this workshop was a virtual meeting, this did not influence its quality.

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

2.2 Update on *Salmonella* in the EU, based on EU One Health 2019 Zoonoses report

Frank Boelaert, EFSA, Parma, Italy

The One Health Zoonoses report 2019 of the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) presents the results of zoonoses-monitoring activities carried out in 2019 in 36 European countries (28 EU Member States (MS) and eight non-EU MS).
Salmonellosis was the second most commonly reported gastrointestinal infection in humans after campylobacteriosis, and was an important cause of food-borne outbreaks in the EU/EEA. In 2019, 87 923 confirmed cases of salmonellosis in humans were reported with an EU notification rate of 20 cases per 100 000 population, which was at the same level as in 2018. The trend for salmonellosis in humans has been stable (flat) over the last five years, after a long period of decline. The trend of *S*. Enteritidis cases in humans acquired in the EU stabilised in 2015–2019.
In total, 926 salmonellosis food-borne outbreaks were reported by 23 EU MS in 2019, causing 9 169 illnesses, 1 915 hospitalisations (50,5% of all outbreak-related hospitalisations), and seven deaths. *Salmonella* caused 17,9% of all food-borne outbreaks in 2019. The vast majority (72,4%) of the salmonellosis food-borne outbreaks were caused by *S*. Enteritidis. The four most implicated food vehicles in

strong-evidence salmonellosis food-borne outbreaks were 'eggs and egg products', followed by 'bakery products', 'pig meat and products thereof' and 'mixed food', as in previous years.

Official control samples verifying compliance with food safety criteria according to Regulation (EC) No. 2073/2005 (EC, 2005) found the highest percentages of *Salmonella*-positive samples in poultry meat, including fresh meat (3,5%), minced meat and meat preparations intended to be eaten cooked (8,3%), and in meat products intended to be eaten cooked (6,4%). For 2019, 66 113 'ready-to-eat' and 191 181 'non ready-to-eat' food sampling units were reported by 21 and 25 MS with 0,3% and 1,5% positive samples, respectively. Within the category of 'ready-to-eat' food samples, positive samples were from diverse food products; 'meat and meat products', 'milk and milk products', 'fruits, vegetables and juices', 'fish and fishery products', 'spices and herbs', 'salads', 'other processed food products and prepared dishes', 'cereals and nuts', 'infant formulae and follow-on formulae', 'other food' and 'cocoa and cocoa preparations, coffee and tea'. Within the category of 'non ready-to-eat' food samples, positive samples also originated from diverse food products, mostly from 'meat and meat products', notably from fresh meat from broilers and from turkeys. Significantly lower percentages of *Salmonella*-positive pig carcases were reported based on food business operators' self-monitoring data, compared with official control data from the competent authorities. The same observations were made for 2018 and 2017 data.

Of the 26 Member States reporting on *Salmonella* control programmes in poultry populations, 18 met all the reduction targets, compared to 14 in 2018. The number of MS not meeting the *Salmonella* reduction targets was five in breeding flocks of *Gallus gallus*, four in laying hen flocks, one in broilers flocks, zero in breeding turkey flocks, and one in fattening turkey flocks. Among the target *Salmonella* serovars in the context of national control programmes in poultry, the reported flock prevalence was highest for *S*. Enteritidis in breeding flocks of *Gallus gallus* and laying hens. For broilers, the flock prevalence of *S*. Enteritidis and of *S*. Typhimurium were comparable, whereas for turkeys (both breeding and fattening flocks), the flock prevalence of *S*. Typhimurium was highest. In the context of national poultry control programmes, proportions of *Salmonella* target serovars-positive broiler and fattening turkey flocks reported by food business operators were significantly lower than those reported by competent authorities. A significant increase was noted in estimated *Salmonella* prevalence in breeding flocks of *Gallus gallus*, laying hens, and breeding turkeys in the past 4–6 years. In contrast, the trends in prevalence of *Salmonella* target serovar-positive flocks have been quite stable (flat) from 2015 for all animal categories, with some fluctuations for breeding turkey flocks.

Of all serotyped *Salmonella* isolates reported by MS from food and animal sources, 70% originated from the broiler source, 12% from the pig source, while the laying hen and turkeys sources each accounted for approximately 7%, and isolates from the cattle source about 1%. The top five serovars responsible for human infections were distributed as follows among the serotyped isolates (17 176) from these food–animal sources: *S*. Infantis accounted for 29,7%, *S*. Enteritidis 6,9%,

monophasic variant of *S*. Typhimurium 4,5%, *S*. Typhimurium 3,9%, and *S*. Derby 3,7%.

More information can be found in EFSA and ECDC, 2021.

Discussion
Q: Is this dashboard on foodborne outbreaks a new feature of the EFSA website?
A: This dashboard was published early 2021 and provides information of foodborne outbreaks for all agents. In this dashboard, validated data of foodborne outbreaks are published in a more user-friendly format and the information is updated annually.
Q: Why is swine meat and products thereof (cured ham and cured sausages like salami) a main source of salmonellosis?
A: This is not fully clear. It could be caused by the emergence of monophasic *Salmonella* Typhimurium, but this needs to be investigated in more detail. The fact that pig meat is highly ranked in the report may also be due to some *Salmonella* outbreaks in a few EU MS related to dry sausages in 2019.

Relevant links:
Information on Rapid Outbreak Assessments (ROA) of EFSA can be found on the following link:
https://www.efsa.europa.eu/en/topics/topic/food-incident-preparedness-and-response
The online (public) dashboard on foodborne outbreaks 2019 can be found on the following link:
https://app.powerbi.com/view?r=eyJrIjoiZTQzYWQ0ZmItNWRmOC00NmFmLTk1NjctODYxN2MxOGEyNzA1IiwidCI6ImM0ODdkZDVhLTM3NjktNDQyYy1hYjc3LTI5MTkwODFkODVmYyIsImMiOjl9

2.3 Results EURL-*Salmonella* Proficiency Test Primary Production Stage and Food 2020 – Detection of *Salmonella* in hygiene swab samples

Irene Pol-Hofstad, EURL-Salmonella, Bilthoven, the Netherlands

In October 2020, the combined EURL-*Salmonella* Proficiency Test on the detection of *Salmonella* in food and primary production stage samples (PPS) was organised. A total of 65 National Reference Laboratories (NRLs) for *Salmonella* participated in this study: 37 NRLs PPS and 28 NRLs Food. The NRLs originated from 28 EU Member States (MS) including United Kingdom and Northern Ireland, five from other European countries (EU candidate or potential EU candidate MS and members of the European Free Trade Association (EFTA)), and one from a non-European country. Two participants did not report results. Participation was obligatory for all EU Member State NRLs responsible for the detection of *Salmonella* in primary production stage samples.

In this study, NRLs had to analyse hygiene swab samples artificially contaminated with background flora and a diluted culture of *Salmonella* Typhimurium at the EURL-*Salmonella* laboratory.

Each NRL received sixteen blindly coded samples consisting of ten hygiene swab samples artificially contaminated with background flora and two different concentrations of *Salmonella* Typhimurium: six low contaminated samples (MPN concentration: 3,3 cfu/sample) and four high contaminated samples (MPN concentration: 35 cfu/sample). Additionally, four negative hygiene swab samples (no *Salmonella* added, but with background flora) and two control samples had to be analysed. The control samples consisted of a procedure control blank (only Buffered Peptone Water) 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 28 September 2020, the 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 on Monday 5 October 2020.

The majority of laboratories (45) used the prescribed EN ISO 6579-1:2017 method. Twelve laboratories indicated that they had followed the recently published amendment of EN ISO 6579-1 (EN ISO 6579-1:2017/A1:2020). One laboratory reported using a PCR method only.

Of the 65 participating laboratories, 63 scored well with the analysis of both the procedure control and their own positive control sample. Two laboratories reported their positive control to be accidently negative for *Salmonella*. These laboratories scored a moderate performance.

All laboratories were able to detect *Salmonella* in the hygiene swab samples contaminated with a low level of *Salmonella*. Three laboratories tested one of the six samples negative for *Salmonella*, another laboratory tested three of the six samples negative for *Salmonella*. These results are still within the criteria set for good performance, which permit three negative samples. The sensitivity rate was 98,4% for these samples.

Almost all laboratories detected *Salmonella* in all four high level samples. One laboratory scored one of the four high-level samples negative. This is still within the criteria set for good performance which permit one negative sample. The sensitivity rate was 99,6% for these samples.

All negative samples were scored correctly negative, resulting in a specificity rate of 100%.

Overall, the laboratories scored well in this Proficiency Test with an accuracy of 99,2%. Of the 65 laboratories, 63 fulfilled the criteria for good performance. Two laboratories scored moderate, due to incorrect reporting of their results of the positive control sample.

More details can be found in the full report of this PT (Pol-Hofstad and Mooijman, 2021).

Discussion

Q: Did you see any effect of the long and relatively warm transport of some samples on the results?
A: No, the results did not seem to be affected by this.
Q: According to our national rules, we have to use MKTTn broth in addition to MSRV agar for selective enrichment of *Salmonella* from

samples from the primary production stage. For PPS samples we incubate the MKTTn broth at 41,5 °C (following the information in the Note of subclause 9.3.3. of EN ISO 6579-1:2017). Was this indicated as a technical deviation in the report?
A: We will check if this was indicated as a technical deviation. *Note: This was checked after the workshop and this was not indicated as a technical deviation. MSRV agar is prescribed for analysing PPS samples, and conditions used for MSRV agar were performed correctly*.
Q: According to EN ISO 17604:2015, (carcass) swab samples shall be analysed as soon as possible after receipt, or within 24 h after sampling when stored at 3 °C ± 2 °C. Would it be possible to use the results of this PT to show that storage for e.g., 96 h does not affect the results?
A: It may depend on the strain whether storage of the samples has an effect on the results or not. We chose a relatively stable *Salmonella* strain for our PTs. However, the results could be different for other strains.

2.4 Preliminary results EURL-*Salmonella* Proficiency Test Food 2021 – Detection of *Salmonella* in liquid whole egg

Robin Diddens, EURL-Salmonella, Bilthoven, the Netherlands

In March 2021, an EURL-*Salmonella* Proficiency Test (PT) for the detection of *Salmonella* in food was organised for the NRLs-*Salmonella.* The matrix under analysis was liquid whole egg.
In total, 33 NRLs-*Salmonella* participated: 28 NRLs from 27 EU Member States (MS) and 5 NRLs from third countries (EU candidate MS, members of the European Free Trade Association (EFTA), and United Kingdom).
The most important objective was to test the performance of the participating laboratories in their detection of *Salmonella* Enteritidis (SE) in the artificially contaminated liquid whole egg samples. The prescribed method for the detection of *Salmonella* spp. was EN ISO 6579-1:2017 (including Amd.1:2020). The participants were asked to report *Salmonella* 'detected' or 'not detected' for each sample (after confirmation).

Prior to the start of the PT, pre-tests were conducted to ensure the samples were fit for use, especially the stability of the artificially contaminated samples at different storage temperatures (5 °C and 10 °C) was tested. Additionally, the concentration of the natural background flora (aerobic count and *Enterobacteriaceae*) in the liquid whole egg was measured. The aim was to prepare stable liquid whole egg samples with a low level of SE of approximately 5-10 cfu/test portion and with a high level of SE of approximately 50-100 cfu/test portion.
The results of the pre-tests showed that the artificially contaminated liquid whole egg samples containing 7 cfu SE/25 g were stable at 5 °C for up to three weeks. The same liquid whole egg samples were (slightly) less stable at 10 °C; after three weeks of storage, four out of six samples were still positive for *Salmonella*.
The number of aerobic bacteria in the liquid whole egg samples remained relatively stable when stored at 5 °C for up to three weeks. Storage at 10 °C showed an increase in the number of aerobic bacteria after three weeks of storage. The number of *Enterobacteriaceae* in the liquid whole egg samples remained <10 cfu/g during storage at 5 °C

and 10 °C for up to three weeks. Based on these results, the aim was to inoculate the low level liquid whole egg samples with approximately 10 cfu SE/25 g.

Each laboratory received 14 samples, each containing 25 g of liquid whole egg. These samples consisted of four samples with a high level of SE (inoculum 69 cfu/sample), six samples with a low level of SE (inoculum 10 cfu/sample) and four negative samples (no *Salmonella* added). The PT samples were artificially contaminated with a diluted culture of SE at the EURL-*Salmonella* laboratory. In addition, each participating laboratory had to test two control samples: a procedure control (only Buffered Peptone Water) and a positive control with *Salmonella*.

All 33 laboratories fulfilled the criteria for good performance in the EURL-*Salmonella* Proficiency Test for detection of *Salmonella* in liquid whole egg samples.
The accuracy rate of all control samples was 100%. The sensitivity rate of the liquid whole egg samples artificially contaminated with SE was 99,7%. The accuracy rate of all liquid whole egg samples for all participating laboratories was 99,8%. The specificity rate of the negative liquid whole egg samples was 100%.

The NRLs-*Salmonella* were given the opportunity to analyse the samples using a second detection method if this method was (routinely) used in their laboratories. These results could also be reported, but only the results obtained with EN ISO 6579-1:2017(/A1:2020) were used to assess the performance of each NRL.
Twelve laboratories used a second method for detecting *Salmonella* in the liquid whole egg samples. As second detection method, most laboratories used a PCR or a real-time PCR. The results of the second detection methods were all equal to those obtained with EN ISO 6579-1:2017(/A1:2020).

More details can be found in the full report of this PT (Diddens and Mooijman, 2021).

2.5 State of implementation of the 'One Health' system for the collection and analysis of WGS data from food/animal isolates and new cgMLST scheme for *Salmonella*

Mirko Rossi, EFSA, Parma, Italy

In 2019, the European Commission (EC) sent a mandate to ECDC and EFSA to implement and manage two interoperable systems for the collection and analysis of Whole Genome Sequencing (WGS) data of *Salmonella enterica*, *Listeria monocytogenes,* and *Escherichia coli* isolates from human and non-human origins, respectively. The aim is to provide a robust and efficient tool for rapid detection of multi-country foodborne outbreaks with the ultimate purpose of serving public health interests and protecting European consumers.

The EFSA One Health WGS System is implemented in the EFSA Azure Cloud infrastructure and it will allow appointed users from EU/EEA countries to submit WGS-based typing data and epidemiological data to

the EFSA database, and to manage their submission. It has a user-friendly interface for data provision, access, analysis, and visualisation. The cgMLST analysis is performed automatically by applying the EFSA One Health WGS pipeline, which consists of several open source modules, each performing bioinformatic analyses, organized by means of a container technology. The system is designed to interact with the ECDC Molecular Typing system and exchange cgMLST profiles and epidemiological data with it according to a Collaboration Agreement and a procedure for the use of Molecular Typing data agreed by EFSA and ECDC. In addition, the system gives users the opportunity to explore the content of the EFSA database and to compare their submissions with what is available in the system, in addition to comparing their submissions with those available in the ECDC system. These services are designed respecting confidentiality and data protection.

The food safety users from EU/EEA countries will be able to submit WGS data of isolates of non-human origin on a voluntary basis for the purpose of molecular surveillance and continuous monitoring of foodborne pathogens. In the case of ongoing multi-country foodborne outbreaks, the EU/EEA countries shall provide such data to EFSA and the EC for the purpose of supporting the investigation of the event. The EFSA One Health WGS System represents the single collection point in EFSA of such WGS data of isolates of non-human origin. EFSA will use these data for risk assessment, in accordance with Article 33 of Regulation (EC) No 178/2002 (EC, 2002).

Note: At a later stage (this year or next year) demos and/or webinars will be organised to train users on how to submit data to the database.

2.6 Results additional (WGS) analysis on isolates of EURL-*Salmonella* Proficiency Test Typing 2019 & Results EURL-*Salmonella* Proficiency Test Typing 2020 – serotyping and cluster analysis

Wilma Jacobs-Reitsma, EURL-Salmonella, Bilthoven, the Netherlands

In November 2020, the 25th *Salmonella* typing Proficiency Test (PT) was organised by the EURL-*Salmonella*. The study's main objective was to evaluate whether the typing of *Salmonella* strains by NRLs-*Salmonella* in the European Union was carried out uniformly, and whether comparable results were obtained.
A total of 37 laboratories participated in this study. These included 29 NRLs-*Salmonella* from the 27 EU Member States plus the United Kingdom, two NRLs of (potential) EU candidate countries, three NRLs of EFTA countries, and three additional participants to compare their WGS-based results.

All 37 laboratories performed serotyping. A total of 20 obligatory *Salmonella* strains plus one optional *Salmonella* strain were selected by the EURL-*Salmonella* for serotyping. The strains had to be typed according to 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 March 2021.

The O-antigens were completely typed correctly by 29 of the 37 participants (78%). This corresponds to 99% of the total number of strains. The H-antigens were completely typed correctly by 31 of the 37 participants (84%), corresponding to 98% of the total number of strains. As a result, 28 participants (76%) gave the correct serovar names, corresponding to 97% of all strains evaluated.
A completely correct identification was obtained for nine *Salmonella* serovars: Bousso, Hadar, Zega, Typhimurium, Larochelle, Virchow, Enteritidis, Benfica, and Infantis.
Interestingly, some inconsistencies were seen in the submitted results for strains S3 (Hadar) and S5 (Muenchen), especially by the four participants using WGS. Both strains belong to the pairs of serovars in *Salmonella* serogroup C_2 which differ only by the minor antigen $O:6_1$ that can show variable expression (also described as 'colonial form variation', Hendriksen et al., 2009; Mikoleit et al., 2012). Laboratory 73 reported to confirm separately for presence of O:6. Retrospectively, laboratory 29 also reported to have checked for the presence of both O:6 and O:8 by agglutination, and both antigens were found. Therefore, the O-antigens should have been reported as 6,8 and not just 8, as mistakenly done initially. The other two laboratories may not have this particular option of additional testing in their routine WGS pipelines/protocols.

All but three participants tried to serotype optional strain S21, a *Salmonella enterica* subsp. *diarizonae* (IIIb). Some laboratories did not have access to the required antisera to finalise this (50:r:1,5).
Overall, the performance of the participants in the PT Serotyping 2020 was very good, including the performance of the four participants submitting WGS-based results. All participants met the level of good performance at the first stage of this PT and there was no need to organise a follow-up study.

Nineteen NRLs and two external partners also performed additional typing at DNA level (PFGE and/or MLVA and/or WGS) to investigate an additional set of ten *Salmonella* strains using cluster analysis.
In the previous and first pilot PT on cluster analysis, an unexpected variability of some of the strains was observed, especially in the WGS results. Extended investigations revealed that this was more likely to be due to the biological origin (sub-culturing, long-term storage) than the technical origin (participants' wet-lab/dry-lab protocols, QC data assessments).
Based on the information gained from the first pilot in 2019, the second pilot PT Cluster Analysis 2020 mimicked an outbreak situation with a monophasic *Salmonella* Typhimurium ST34, MLVA type 3-14-13-NA-211 as reference strain. The selection of suitable (stable) PT strains, primarily based on WGS analysis, was improved by including more pre-testing of the strains.
Raw WGS data of this reference strain (fastq-files) were made available through a secure ftp server. Participants were asked to analyse the ten strains and to report per strain if a clustering match with the reference strain was found or not.
Evaluation of the participants' cluster analysis results was done by comparing the participants' results to the expected results in the outbreak investigation setting, as pre-defined by the EURL-*Salmonella.*

The individual laboratory results on the cluster analysis part, as well as an interim summary report on the general outcome, were sent to the participants on 27 May 2021.

The analysis results of the two PFGE participants were in complete agreement. Five of the six participants reported the MLVA-based cluster analysis results as expected. All but one of the 23 submissions (two participants with both an SNP-based and a cgMLST-based submission) reported the WGS-based cluster analysis results as expected. The technical duplicate strains 20SCA06/20SCA08 were expected to be reported as (part of) one cluster. This was the case in 2/2 PFGE submissions, in 6/6 MLVA submissions, and in 22/23 WGS submissions.

More details can be found in the full reports of the PTs Typing 2019 and 2020 (Jacobs-Reitsma et al., 2021a, 2021b).

Discussion

Q: Would it be possible to share the genomes of the multiple colonies with the network and with EFSA, to check if the same variability is also visible with other workflows? This will help reduce the noise and better understand the biological significance of these results (in addition this could be very useful for aligning the workflows).
A: This may indeed be a good idea. Please contact Wilma Jacobs if you want to do some testing with the genomes in your own workflows.

2.7 In-house validation of *Salmonella* serotyping with WGS

Maaike van den Beld, RIVM, Bilthoven, the Netherlands

For more than 50 years, *Salmonella* isolates have been serotyped at the National Institute for Public Health and the Environment (RIVM) for diagnostic and surveillance purposes using slide agglutination with an extensive panel of antisera, supplemented in the last six years with a screening method based on Luminex technology. *Salmonella* serovars are based on the detection of O- and H-antigens. Over 2500 different serovars have been described, most of which belong to *S. enterica* subspecies *enterica*. The genetic code for the antigens of these serovars enables *in silico* detection. From this perspective, a pipeline for serotyping *Salmonella* using Whole Genome Sequencing (WGS) data was developed, and its use for diagnostics and surveillance in the Netherlands was validated in accordance with EN ISO 15189:2015 and EN ISO 16140-6:2019.

A selection of 503 *Salmonella* isolates, comprising 181 different serovars from various origins and 100 non-*Salmonella* isolates were tested to assess the suitability of this approach against the criteria of EN ISO 15189:2015 and EN ISO 16140-6:2019. For this accuracy (inclusivity), analytical specificity (exclusivity), measurement trueness, and measurement precision were determined. Short read Illumina sequencing data were generated using the NextSeq platform, then processed with an in-house pipeline ('Juno') for *de-novo* assembly, including trimming and quality control algorithms. An *in silico Salmonella* serotyper pipeline based on SeqSero2 microassembly-mode was developed using Snakemake workflows and reproducible conda

environments. The pipeline generates a multi-report containing the seroformula (e.g., 9:g,m:-) and a predicted serovar name (e.g., Enteritidis).

After identification, none of the 100 non-*Salmonella* isolates were designated as *Salmonella,* while 473/503 (94%) *Salmonella* isolates were correctly identified by the *in silico* serotyper. Retesting the 30 discrepant isolates with slide agglutination resolved 15 cases where the stored serovar differed from that of the sequenced isolate. In 9 of the remaining 15 discrepancies, an antigen was detected genetically, but was not expressed phenotypically. Because this outcome is not incorrect, these results were excluded from the accuracy-analysis. In four other cases, the seroformula found was correct, but a serovar name was incorrectly assigned. This was corrected in the pipeline. In the remaining two cases, an O-antigen (O:25) was phenotypically found but not detected in the sequence reads. These results accumulate to an accuracy (inclusivity) of 99% and a 100% score for analytical specificity (exclusivity) and measurement trueness. To determine the measurement precision, md5sum hashes of the multireports, generated multiple times under different conditions, were compared and 100% agreement was found. After retesting, all results were within the acceptability limits of both EN ISO standards.

This validation in accordance with EN ISO 15189:2015 and EN ISO 16140-6:2019 shows that the *in silico* serotyper based on WGS data is a reliable method for determining the serovar of a *Salmonella* isolate. As a result, this approach has been implemented for isolates submitted to the RIVM starting January 2021. To prevent any incorrect results, isolates with results known to cause difficulties in this *in silico* method will still be confirmed using slide agglutination.

Discussion

Q: I found O:6 bias in both SeqSero2 and SISTR (meaning that these tools are not really able to find serotypes with O:6); did you find the same problem?
A: Yes we found similar problems and therefore we still perform a serological confirmation for these isolates.
Q: Will you open an issue in github for Rostock and Blegdam for this? It would be useful if they could fix this issue.
A: We have not yet done so, but have programmed it in our pipeline.
Q: Did automatically trimming improve the quality of the SeqSero2 prediction?
A: Yes, in our tests an improvement was seen.
Q: Will you publish this interesting validation study?
A: Yes we are working on the publication of the work. We will share the information when the study is published.
Q: Do you have an estimate of the costs for serotyping of one *Salmonella* isolate with WGS?
A: At our institute, one serotyping with WGS costs approximately €130 including staff costs. This is more than the costs for traditional serotyping. On the other hand, the WGS data are also used for our monitoring tasks, for outbreak detection, AMR gene detection, virulence profiling, and for many more (future) analyses.

2.8 Activities of the NRL-*Salmonella* to fulfil tasks and duties in Czech Republic

Tomás Cerný, NRL-Salmonella*, Prague, Czech Republic*

The Czech NRL for *Salmonella* is located in the State Veterinary Institute in Prague. The main workload of the institute is laboratory diagnostics of infectious and non-infectious animal diseases, monitoring of residues of veterinary drugs and contaminants in the food chain of humans, animals and the environment and analysis of food, feed and water quality and food adulteration. The institute also has a training center for organising training, educational and professional activities.

The NRL-*Salmonella,* as part of the Czech *Salmonella* network, is led by the State Veterinary Administration. Its main tasks include the coordination of official laboratories, collection and analysis of data from diagnostic activities, and organisation of Proficiency Tests. Proficiency Tests are organised annually with the usual rotation of detection and serotyping of *Salmonella* spp.

Annually, approximately 800 isolates of *Salmonella* spp. are serotyped in the NRL laboratory. About one half of these isolates is further typed molecular-genetically (MLVA, WGS) and subsequently epidemiologically analysed. The tested isolates come from two different sources: from animals and their environment, and from meat and food of animal origin. A different prevalence of *Salmonella* was observed in these groups. *Salmonella* Enteritidis is the most common serotype from animal and environmental samples. *Salmonella* Infantis is most often isolated from meat and food of animal origin.

Our plans for the near future are to organise two Proficiency Tests (detection of *Salmonella* in carcass swabs and serotyping) and to focus mainly on building the capacity of WGS testing and building an online network for data sharing between stakeholders in the veterinary and human sector.

Discussion
Q: Do you produce the sampling kits yourself which you mentioned in your presentation?
A: No, we only designed them, after which they are prepared and produced by a manufacturer.

2.9 Activities of the NRL-*Salmonella* to fulfil tasks and duties in Ireland

William Byrne, NRL-Salmonella*, Celbridge, Ireland*

The Irish National Reference Laboratory for *Salmonella* is located in the Department of Agriculture, Food and the Marine (DAFM) Laboratories in Backweston Campus, Celbridge, Co. Kildare, Ireland.
With regard to the NRL for *Salmonella*, DAFM Laboratories are accredited by the Irish National Accreditation Board (INAB) to EN ISO/IEC 17025:2017 for *Salmonella* spp. detection, Maldi-ToF, serotyping of *Salmonella* spp. isolates by slide agglutination, PCR for confirmation of *S.* Typhimurium and monophasic *S*. Typhimurium and

Whole Genome Sequencing (WGS) for cluster analysis and serotype prediction of *Salmonella* isolates.

The NRL participates in EURL Proficiency Tests for *Salmonella* spp. detection in primary production and in food and feed, *Salmonella* serotyping and cluster analysis using WGS, and in commercially available schemes such as the VETQAS PT (organised by Animal and Plant Health Agency (APHA), UK) for *Salmonella* spp. detection in poultry samples. The NRL plays a role in supervising the network of official laboratories, which operate under DAFM or under the Department of Health, and commercial laboratories who are approved for detection of *Salmonella* from foodborne outbreak check samples, from poultry primary production, and from animal by-products' operators. All official and approved laboratories are required to complete an annual questionnaire in which the details of their participation and results of an appropriate Proficiency Trial Scheme must be reported to the NRL. In addition, commercial laboratories must apply to be approved for testing of samples for the National *Salmonella* Control scheme and are subject to having that approval withdrawn if their performance is unsatisfactory.

In 2021, the Department of Agriculture, Environment and Rural Affairs (DAERA) of Northern Ireland requested that the NRL *Salmonella* in DAFM laboratories be the National Reference Laboratory for Northern Ireland; this was agreed between DAFM and DAERA.

The NRL tests all of DAFM's official samples collected for *Salmonella* detection as part of the National *Salmonella* Control Plan in poultry flocks and other *Salmonella* testing required for official testing programs, such as process Hygiene Criteria testing of meat and carcass swab samples, and schemes for tallow, greaves, animal by-products, petfood, feed and compost.
Of 1 437 official poultry flock samples tested in 2020, *Salmonella* spp. was detected in just one sample from a layer flock; a *Salmonella* Kentucky isolate.
Furthermore, the NRL serotypes all *Salmonella* isolates from primary production, food or feed, animal by-products, tallow, greaves, petfood, and compost submitted from approved laboratories and from other DAFM laboratories. Of 27 isolates serotyped from poultry primary production environmental samples, none were found to be of any of the target serovars of *Salmonella* spp. indicating that commercial Irish poultry flocks were not infected throughout 2020 with those *Salmonella* serovars.

The NRL-*Salmonella* also assists with investigation of outbreaks and suspected case(s) of *Salmonella* and applies Next Generation Sequencing for source attribution and for detection of laboratory cross-contamination, for which an example of each such application was provided.

Discussion
Q: I notice that several NRLs make use of VETQAS PT0088 (UK) for testing the performance of their network of private laboratories for detection of *Salmonella*. Are there any other commercial, accredited PTs known for detection of *Salmonella* in samples from the primary production stage?

A: In Europe this is the only commercial PT labelled for primary production, despite the fact that the samples are freeze dried (mixed) cultures and not matrix samples. Another drawback of this PT is the relatively high contamination level of *Salmonella* in the samples. If NRLs have information on alternative PTs they are asked to share this with the network.

2.10 Activities of the NRL-*Salmonella* to fulfil tasks and duties in Slovenia

Jasna Micunovic, NRL-Salmonella*, Ljubljana, Slovenia*

The Slovenian NRL for *Salmonella* is situated at the National Veterinary Institute in Ljubljana. The activities of the National Veterinary Institute are the following:

- diagnosis of animal diseases, determination of the compliance of food of animal origin and feed;
- scientific support, confirmation of results, training and organisation of Proficiency Tests (by NRLs);
- pathomorphological diagnostics and veterinary-hygiene service;
- review of animal reproduction at National level;
- review of the health status and treatment of fish and bees.

The organisation is accredited to EN ISO/IEC 17025:2017 for 155 procedures, including detection and serotyping of *Salmonella*.

The different NRL activities include:

- cooperation with EURL-*Salmonella*;
- participation in Proficiency Tests (organised by EURL, APHA (VETQAS), WHO, FAPAS, FEPAS);
- forward information from the EURL at National level;
- coordination and organisation of National Interlaboratory Comparison assays (ILC);
- expert support on request of the Administration of the Republic of Slovenia for Food safety, Veterinary sector and Plant Protection (AFSVPP);
- testing official samples;
- maintaining the collection of *Salmonella* isolates and the database;
- performing confirmatory testing and serotyping of *Salmonella* strains (approx. 350 isolates/year);
- performing differentiation of vaccine strains from wild strains;
- monitoring *Salmonella* antimicrobial resistance (together with NRL for antimicrobial resistance);
- participation in preparation of the annual zoonosis report (published by EFSA);
- daily support of laboratories participating in the National control program for *Salmonella*;
- coordination of official laboratory activities;
- performing multiplex PCR for determination of monophasic *S.* Typhimurium;
- performing real-time PCR for *S.* Infantis (e.g., to confirm for variant 6,7:-:1,5 or 6,7:r:-);
- performing WGS (mainly for research projects).

Every year, the NRL-*Salmonella* organises interlaboratory comparison studies for all nominated laboratories in Slovenia and for other laboratories on request. Depending on the matrix, 8-12 laboratories participate in each study. The studies include isolation of *Salmonella* from three or four different matrices and identification to serogroup level. The different matrices include: food (meat or milk products), animal feed, primary production stage samples (poultry faeces or boot swabs), environmental samples (sponges).

2.11 Work programme EURL-*Salmonella* second half 2021, first half 2022, concluding remarks workshop 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 second half of 2021 and for early 2022.

Due to late adoption of the Multi-annual Financial Framework (MFF) 2021-2027 and of the new Single Market Program Regulation (SMP) by the Council and the European Parliament, the grant applications for EURL's work programs could be submitted only in the course of 2021. To ensure the continuity of activities, all EURLs had to informally submit the annual work program 2021 to the relevant DG SANTE technical desk officer. In January 2021, the desk officer at DG SANTE informally agreed with the EURL-*Salmonella* work program of 2021.
The template for the work programme follows Regulation EU No 625/2017 (EC, 2017), Article 92 (2).

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:

- to standardise methods (ISO and CEN);
- to keep track of developments in (alternative) methods;
- to provide NRLs with information on developments of relevant (standardised/new) analytical methods.

This activity includes activities for ISO and CEN:

- ISO-WG10 (convenor) – drafting CEN ISO/TS 6579-4 'Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 4: Identification of monophasic *Salmonella* Typhimurium (1,4,[5],12:i:-) by polymerase chain reaction (PCR)'. From 18 May until 16 August 2020, the voting for the New Work Item Proposal (NWIP) for draft CEN ISO/TS 6579-4 took place. The outcome was 100% approval in ISO and CEN with few comments. From September 2020 until April 2021, two more Working Draft versions of ISO/TS 6579-4 were prepared and discussed in the meetings of ISO-WG10 (November 2020 and March 2021). From 22 April until 26 May 2021, the voting on draft ISO/CD TS 6579-4 among the members of ISO/TC34/SC9 took place. Additionally, the document was also sent to the NRLs-*Salmonella* for comments. Comments were received from only 2 members of ISO/TC34/SC9 and from 8 NRLs-*Salmonella*. The next steps are to discuss the comments with the

members of ISO-WG10 (second half 2021), to prepare the next draft version of ISO/TS 6579-4 and to start the preparation for the organisation of an interlaboratory study to determine the performance characteristics (probably to be organised in 2022).

- ISO-WG3 Method validation (co-project leader and member).
  In January 2021, EN ISO 16140-3 was published ('Protocol for the verification of reference and validated alternative methods implemented in a single laboratory'). In March 2021, the Dutch Standardisation Organisation (NEN) organised a webinar to explain the use and application of this EN ISO document. Supporting materials to facilitate the implementation of the EN ISO 16140 series, as well as calculation tools in Excel, are available at the website of ISO/TC34/SC9 (https://www.eurlsalmonella.eu/publications/analytical-methods - Validation and verification).
  In 2020, ISO-WG3 started with the development of Amd.1 of EN ISO 16140-2:2016, and with the development of EN ISO 16140-7 ('Protocol for validation of identification methods of microorganisms'). As a member of ISO-WG3, EURL-*Salmonella* will follow the development of these documents and give comments when relevant.
  Also in 2020, the revision of EN ISO 17468:2016 ('Microbiology of the food chain - Technical requirements and guidance on establishment or revision of a standardized reference method') started. EURL-*Salmonella* is co-project leader of this activity.
- ISO-AHG1 (project leader) on harmonisation of ISO/CEN standards for microbiology of the food chain: updating guidance document for publication of edition 3 in 2021/2022.
- ISO-AHG4 (member) made an inventory of the validation status of ISO/CEN standards of the Food chain. For each EN ISO document, it is checked whether performance characteristics are valid for a broad range of foods, or only for a limited number of food categories. If an EN ISO document is not validated for a broad range of foods, it is checked if additional data can be used from validation studies of proprietary methods (published by MicroVal and AFNOR validation). EURL-*Salmonella* checks this for validation data of EN ISO 6579-1.
- ISO-WG25 Whole genome sequencing (member). Development of EN ISO 23418 'Microbiology of the Food Chain - Whole genome sequencing for typing and genomic characterization of foodborne bacteria - General requirements and guidance'. The voting for the Draft International Standard (DIS) took place from 18 September until 11 December 2020. The outcome was 100% approval in ISO and CEN, but with a substantial number of comments. The comments were discussed at a meeting of ISO-WG25 in April 2021 and agreements were made on updating the document for the next voting round (FDIS).

Sub-activity 1.2 joint EURLs working group on NGS
Objectives:

- to promote the use of NGS across the EURL networks;
- to build capacity for producing and using NGS data within the EU;
- to ensure liaison between the work of the EURLs and the work of EFSA and ECDC on NGS.

The working group includes 8 biological EURLs, and 9 activities have been defined in relation to NGS. For each activity, guidance documents are prepared and published on the EURLs' websites. By the time of the workshop, the guidance documents of 7 activities were available on the website of the EURL leading the activity. Other EURLs provide a link to the documents published by colleague EURLs (also see https://www.eurlsalmonella.eu/publications/analytical-methods - Next Generation Sequencing (NGS))
On 25 September 2020, an online conference was organised by the working group, with support of the Med-Vet-Net association. This conference was titled: 'Modern technologies to enable response to crises: Next Generation Sequencing to tackle food-borne diseases in the EU'. In total, more than 500 participants attended this online conference. The organisation of a second conference to follow-up on questions arising in the first conference is being discussed. The timing of this second conference will depend on the SARS-CoV-2 pandemic situation. This is also the case for the organisation of the joint EURL training on NGS.

Sub-activity 1.3 Proficiency Tests
Objective:
Organisation of Proficiency Tests (PTs) to gain information on the performance of the NRLs-*Salmonella* for detection and typing of *Salmonella.*

In the coming year, three PTs are foreseen:

1. Detection of *Salmonella* in samples from the primary production stage (PPS). This study will be held in September/October 2021 and the matrix will be chicken faeces adhering to boot socks.
2. Detection of *Salmonella* in food samples. This study may become a combined study with the PT on detection of *Salmonella* in PPS in September/October 2022 as it may be the case that the interlaboratory study for determination of the performance characteristics of draft ISO/TS 6579-4 (identification of monophasic *S.* Typhimurium) will be organised in the first half of 2022.
3. Typing of *Salmonella* (serotyping, molecular typing). This study is foreseen for November 2021 and will include serotyping of *Salmonella* (obligatory) and a part on cluster analysis for which a free of choice molecular method can be used (MLVA and/or WGS).

Activity 2 To provide scientific and technical assistance to NRLs
Sub-activity 2.1 Workshop
Objective:
To exchange information on the activities of the NRLs-*Salmonella* and the EURL-*Salmonella* and on (new) developments in the relevant work field.

Whether the 2022 workshop will again be organised as an online meeting or as a physical meeting depends on the SARS-CoV-2-virus pandemic situation. The workshop will probably be organised at the end of May 2022.

Sub-activity 2.2 Training courses

Objective:

To train NRLs-*Salmonella* in a specific work field.

The physical training courses also depend on the situation with the SARS-CoV-2-virus pandemic and may concern:

1. training on request of an NRL (requests for 2020 are postponed to 2021/2022);
2. training following advice from the EURL (e.g., in case of repeated poor performance in PTs);
3. joint EURLs training on WGS (basics), organised in cooperation with other EURLs (postponed to 2021/2022).

Sub-activity 2.3 Scientific advice and support of NRLs
Objectives:

- to provide scientific and technical assistance to the NRLs-*Salmonella* for the relevant work field;
- to perform confirmatory testing (samples/isolates) for NRLs when needed;
- to perform WGS analysis of isolates of NRLs-*Salmonella* for outbreak investigations;
- to maintain the EURL-*Salmonella* website and keep the information up to date. By the end of 2020/early 2021, several extensive updates were made to the website (see www.eurlsalmonella.eu);
- to inform NRLs on the activities of the EURL and other parties in the relevant work field, as well as on developments in this field;
- to publish four 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:

- to provide scientific and technical assistance to EC DG SANTE for the relevant work field;
- to provide assistance to DG SANTE, EFSA, and (NRLs of) Member States in the event of (international) *Salmonella* outbreaks;
- to collaborate with EFSA and ECDC for the relevant work field;
- to cooperate with other biological EURLs.

Description:

- ad hoc scientific and technical assistance of DG SANTE;
- member of the joint EFSA–ECDC Steering Committee for management of the (joint) EFSA-ECDC molecular typing database;
- assistance of DG SANTE, EFSA, NRLs, and ECDC in the event of outbreaks, e.g., consultation of NRL network for specific information, (sub)typing of suspect isolates (MLVA, NGS), and analysis of data.

Activity 4 Reagents and reference collections
Sub-activity 4.1 Reference strains and reference materials
Objective:
To supply information on available culture collections and suppliers of microbiological reference materials and to investigate the possibility for setting up a reference collection of WGS data.

Description:

- Reference to culture collections and reference materials on the EURL-*Salmonella* website;
- Maintenance of the in-house culture collection;
- Provide sets of reference strains of *S.* Enteritidis and *S.* Typhimurium for MLVA typing;
- Publication of a reference collection of genomes obtained from Proficiency Tests;
- Provide a link to the White Kauffmann Le Minor (WKLM) scheme, and keep contact with the WHO reference centre.

In recent months, the EURL-*Salmonella* website was updated with more detailed information on culture collections and reference materials (https://www.eurlsalmonella.eu/publications/analytical-methods - Reference materials). The page on reference materials now includes definitions, references to relevant EN ISO documents, a list of culture collections (not exhaustive), and a list of producers of microbiological (certified) reference materials (not exhaustive).

Discussion
Q: It may be interesting to add some *Enterobacter* strains to the PT samples, to challenge the detection of *Salmonella* on MSRV agar.
A: In the PTs with matrix samples (like faeces or food), we do not add artificial background flora as this is already (naturally) present in the matrix. In the PTs with hygiene swabs, we did add artificial background flora. In the latest PT with hygiene swabs (2020) we added *Escherichia coli* and *Citrobacter freundii*, as the latter may also challenge the detection of *Salmonella* on XLD agar. However, in a next study with hygiene swabs, we may also consider including an *Enterobacter* strain.

3 Evaluation of the workshop

3.1 Introduction

At the end of the workshop, a link to an evaluation form was sent to the participants asking them for their opinion by answering 10 questions (see Annex 3). For several questions, participants were asked to give a score from 1 to 5. The scores represent: very poor (1), poor (2), fair (3), good (4) and very good (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 sent to all participants, but the staff members of the EURL-*Salmonella* were excluded from the evaluation, making a total of 66. In total, 48 participants completed the evaluation form, a response rate of 73%. This is a higher response rate compared to the first online workshop in 2020 (response rate 53%). This may be a confirmation of the fact that participants are getting used to online activities after living with the pandemic for more than one year.

In section 3.2, the scores for each question are presented and a summary of the remarks is given.

3.2 Evaluation form

1. What is your opinion on the information given in advance of the workshop?
Figure 3.1 shows that the majority of respondents scored the information given in advance of the workshop as very good (score 5).

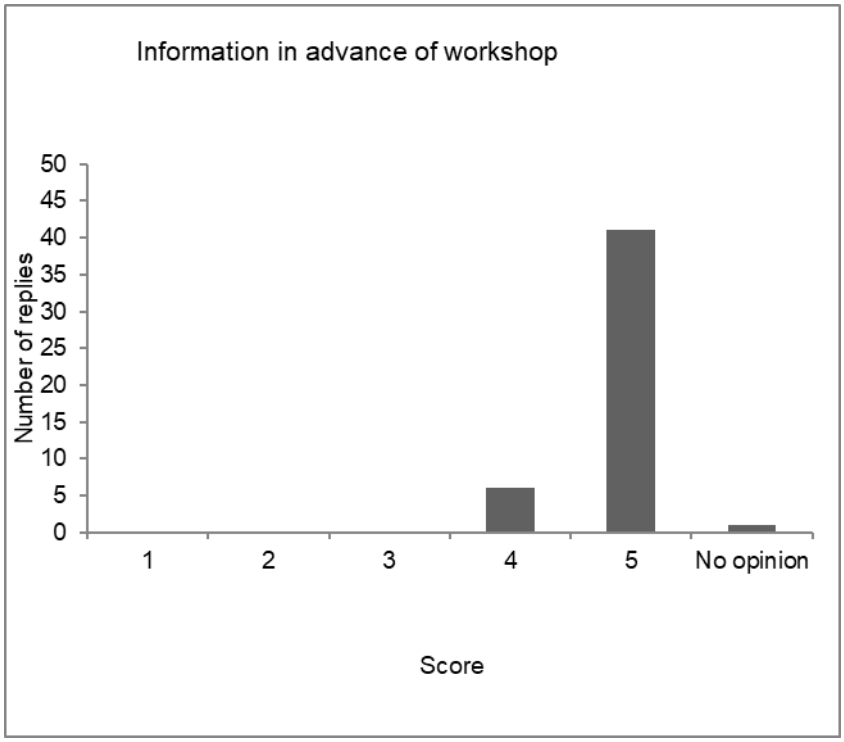

Figure 3.1 Scores given to question 1 'Opinion on information given in advance of the workshop'

2. What is your opinion on the ease of logging into the meeting?
All participants found it easy to login into the online meeting (see Figure 3.2).

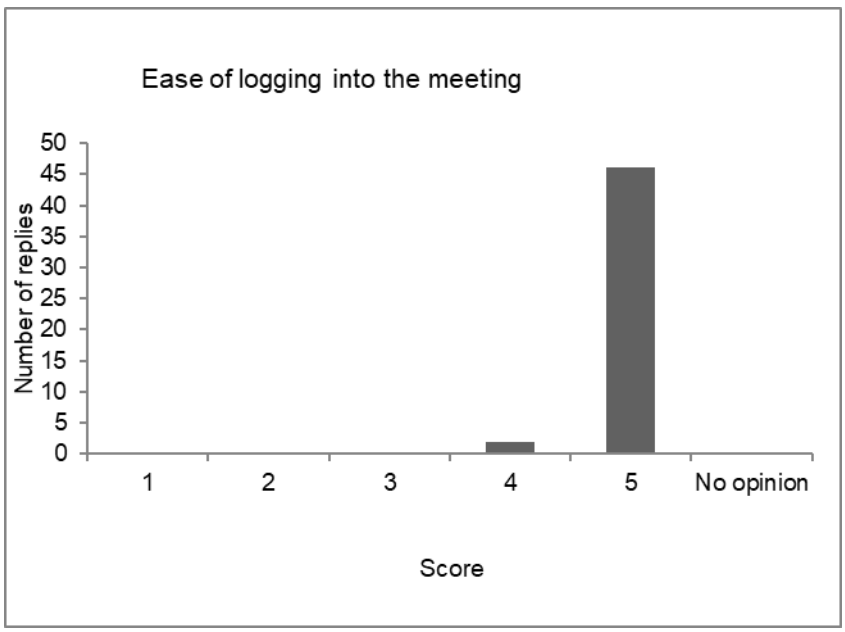

Figure 3.2 Scores given to question 2 'Opinion on the ease of logging into the meeting'

3. Did you face any technical problems during the meeting?
None of the respondents reported any technical problems during the meeting (see Figure 3.3).

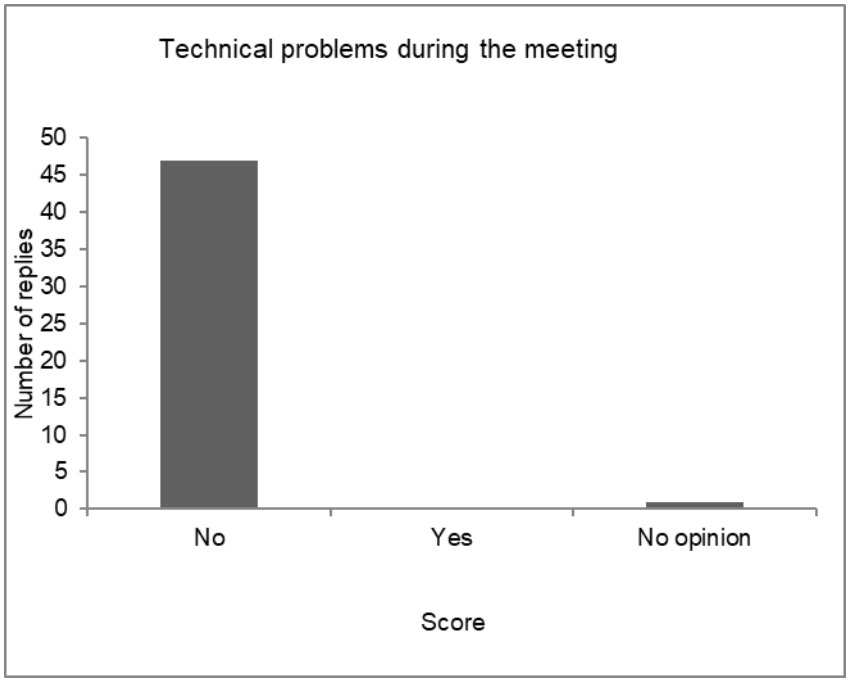

Figure 3.3 Replies given to question 3 'Did you face any technical problems during the meeting?'

4. What is your opinion on the length of the meeting and the number of breaks?
47 of the 48 respondents considered the length of the meeting to be fine (Figure 3.4a) and 43 respondents considered the number of breaks to be fine (Figure 3.4b). Two remarks were made, being: 'Friday is not the best option' and 'a two-day meeting is preferable, with half a day every day'.

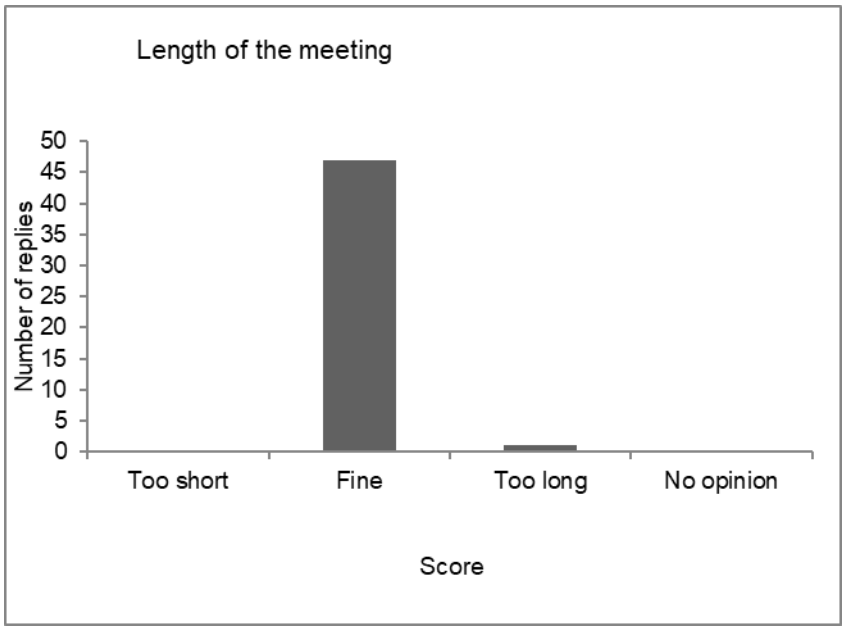

Figure 3.4a Replies given to question 4 'What is your opinion on the length of the meeting?'

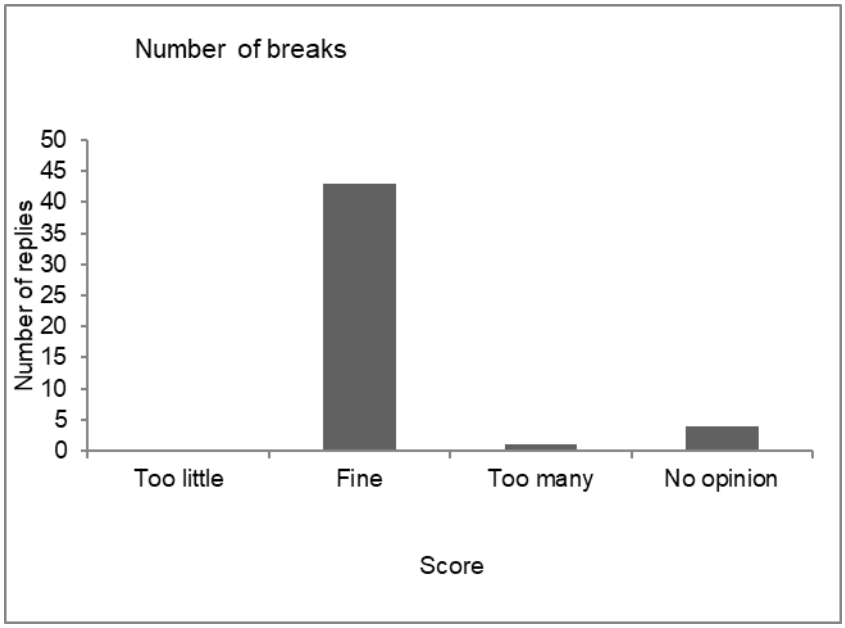

Figure 3.4b Replies given to question 4 'What is your opinion on the number of breaks?'

5. Were you satisfied with the options for raising questions during the meeting (chat function; discussion time at the end of a presentation and at the end of a session)?
45 of the 48 respondents were satisfied with the options for raising questions. The other 3 respondents had no opinion (Figure 3.5).

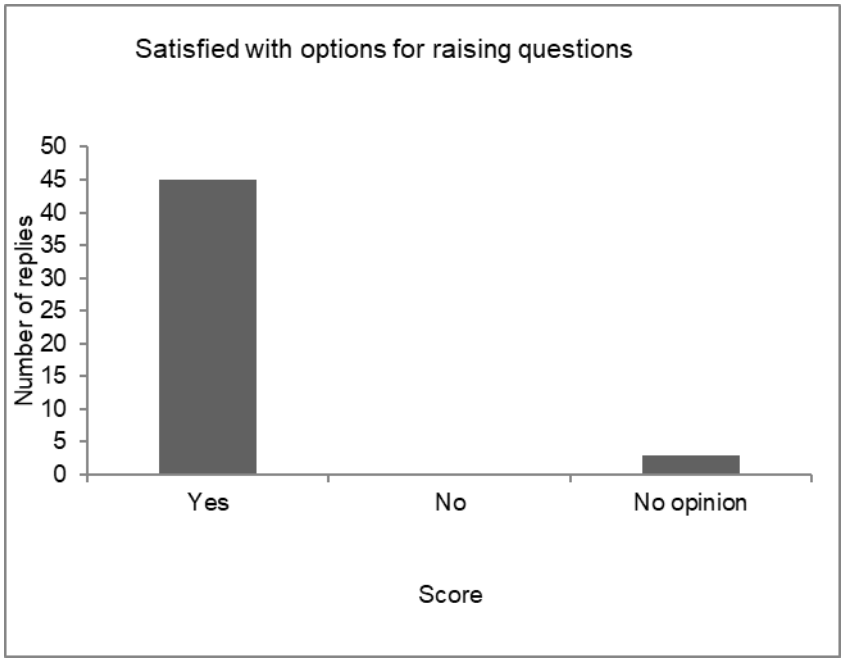

Figure 3.5 Scores given to question 5 'Were you satisfied with the options for raising questions during the meeting?'

6. What is your opinion on the scientific programme of the workshop?
The majority of respondents were satisfied with the workshop's scientific programme; the majority of the scores were good (4) to very good (5), see Figure 3.6.

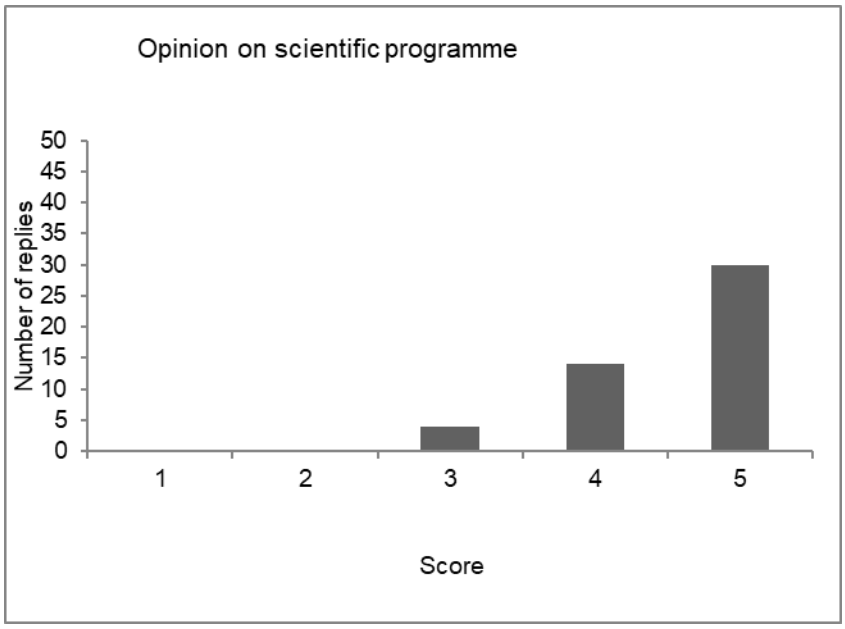

Figure 3.6 Scores given to question 6 'What is your opinion on the scientific programme of the workshop?'

7. Are there specific presentations you want to comment on, or did you miss information on certain subjects?
This was an 'open' question and several responded 'no'. Remarks given were:

- 'Very interesting were the presentations by Mirko Rossi and Maaike van den Beld.'
- 'The EFSA presenter should speak slower. The time was too short for the amount of information. Nevertheless, it was important.'
- 'Especially appreciated the presentation on the validation study NGS versus serotyping.'
- 'All presentations were very good.'

8. What is your general opinion of the workshop?
All but 1 respondents indicated that the workshop as a whole had been good (4) or very good (5), see Figure 3.7.

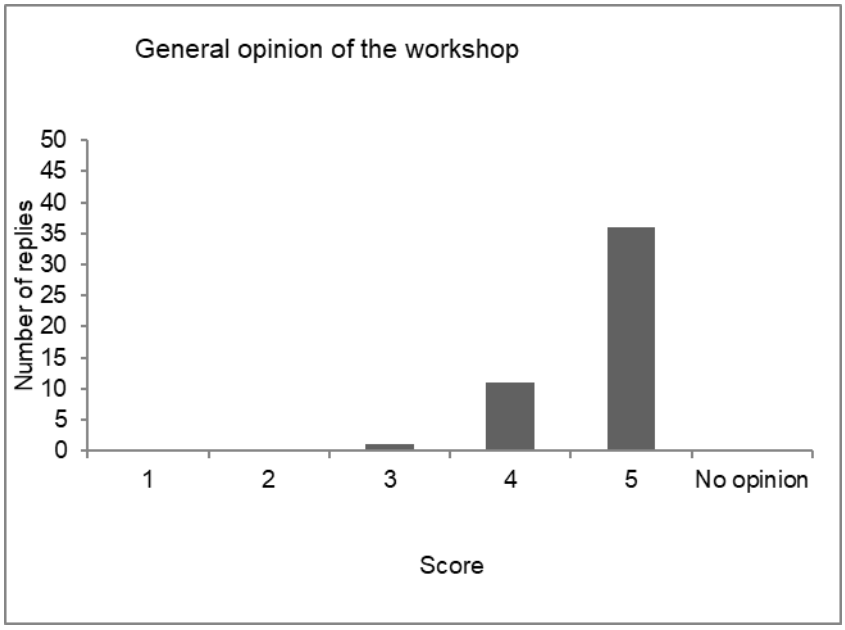

Figure 3.7 Scores given to question8 'What is your general opinion of the workshop?'

9. Due to the circumstances we had to organise this workshop again as an online meeting. When the pandemic is over, what would be your preference for the frequency of physical and online meetings?
The replies to this question varied, but the majority of respondents were not in favour of always organising the workshop online (see Figure 3.8). The number of respondents indicating their preference for organising always a physical workshop (17) was similar to the number of respondents giving preference to organising the workshop every second year online (16).
The following remarks were made:

- 'If technically feasible, it may be a nice add-on to offer parallel 'online broadcasting' to the physical meeting.'
- 'I suggest a hybrid model with an annual physical meeting for one representative from each NRL, plus the option to join online for those that cannot attend (one or two per MS).'
- 'I would suggest online webinars on specific topics (when needed) in addition to annual physical meetings.'
- 'I would prefer to always organise a physical meeting, with the option to participate online in case a trip is not possible.'
- 'Physical meetings promote cooperation and better understanding of the topics presented. However, it would be logical to keep the online option for some special circumstances.'

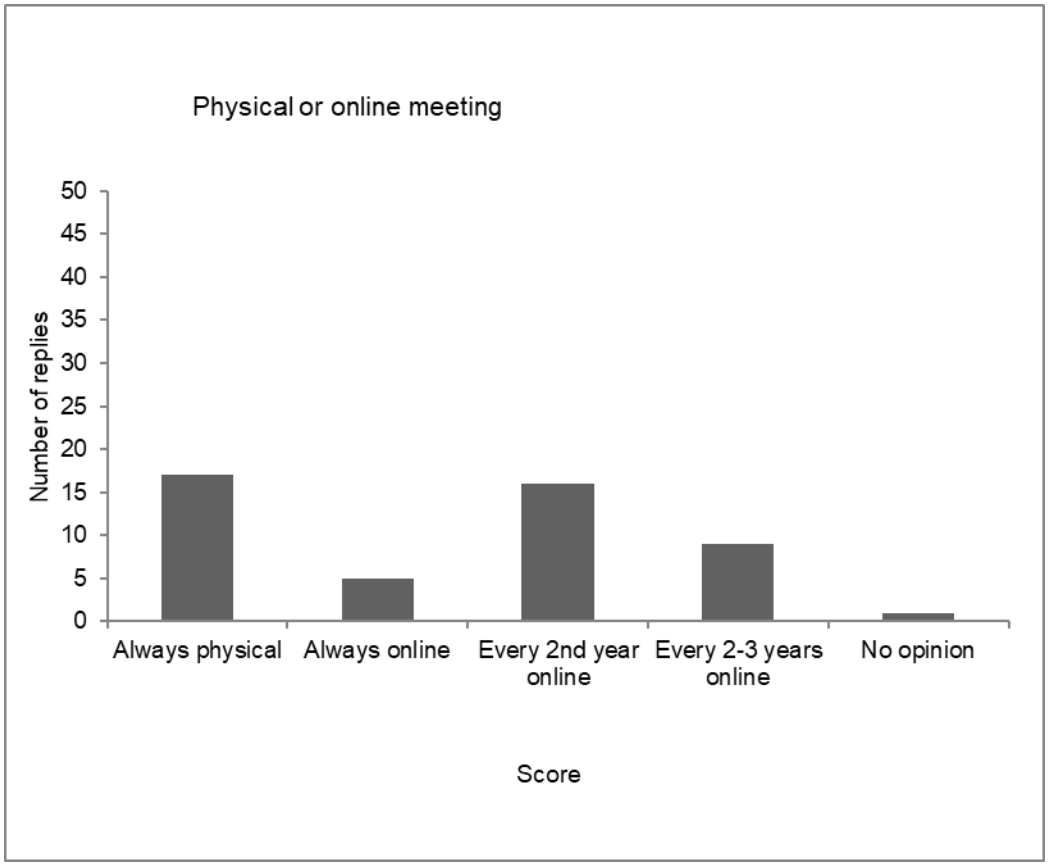

Figure 3.8 Replies given to question 9 'When the pandemic is over, what would be your preference for the frequency of physical and online meetings?'

10. Do you have any remarks or suggestions which we can use for future workshops?
This was another 'open' question and the following responses were received:

- 'Very nice program, I liked that it was broad and that different NRLs presented their work.'
- 'GoToMeeting worked perfectly. Well done to all participants, and keep it up for next year's workshop.'
- 'Keep up the good work.'
- 'Now that we have so many online meetings, it is difficult to concentrate a whole day at an online meeting. Two half days would be a better option.'

3.3 Discussion and conclusions of the evaluation

Due to the worldwide SARS-CoV-2-virus pandemic, it was again not possible to organise a physical workshop in 2021. Still, the online workshop was considered a good alternative, although several participants indicated their preference for a physical meeting
In general, the participants were satisfied with the organisation, technical aspects, and with the scientific programme of this second online EURL-*Salmonella* workshop.

Acknowledgements

The organisation of the workshop cannot be done without the valuable help of the EURL-*Salmonella* staff. Not only by giving presentations on the different activities of the EURL-*Salmonella* and sharing their knowledge with the NRLs-*Salmonella*, but also by helping with the many administrative, technical and social aspects of the workshop. For all this, the author wants to say a big thank you to Noël Peters-Dirker, Robin Diddens, Wilma Jacobs-Reitsma, and Irene Pol-Hofstad.

List of abbreviations

A	Answer
AHG	Ad hoc group
APHA	Animal and Plant Health Agency
BPW	Buffered Peptone Water
CD	Committee Draft
CEN	European Committee for Standardization
cfu	colony forming units
cgMLST	core genome Multi-Locus Sequence Typing
DG SANTE	Directorate-General for Health and Food Safety
DIS	Draft International Standard
EC	European Commission
ECDC	European Centre for Disease Prevention and Control
EEA	European Economic Area
EFSA	European Food Safety Authority
EFTA	European Free Trade Association
EU	European Union
EURL	European Union Reference Laboratory
FAPAS	Food Analysis Performance Assessment Scheme
FEPAS	Food Examination Performance Assessment Scheme
FDIS	Final Draft International Standard
ISO	International Organization for Standardization
ISO/TC34/SC9	International Organization for Standardization, Technical Committee 34 on Food Products, Sub-committee 9 – Microbiology
MKTTn	Mueller Kauffmann Tetrathionate broth with novobiocin
MLST	Multi-Locus 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
NWIP	New Work Item Proposal
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
PPS	Primary Production Stage
PT	Proficiency Test
Q	Question
RIVM	National Institute for Public Health and the Environment
RVS	Rappaport Vassiliadis broth with Soya
SC	Sub Committee
SE	*Salmonella* Enteritidis
SNP	Single-Nucleotide polymorphism
TC	Technical Committee
TS	Technical Specification
UK	United Kingdom
WG	Working Group
WGS	Whole Genome Sequencing
WHO	World Health Organization
WKLM	White Kauffmann Le Minor

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Annex 1 Participants

EC DG SANTE	Jean-Baptiste Perrin
European Food Safety Authority (EFSA)	Frank Boelaert Valentina Rizzi Mirko Rossi
EURL-*Salmonella*	Robin Diddens Angela van Hoek Wilma Jacobs-Reitsma Kirsten Mooijman Noël Peters Irene Pol-Hofstad

Guest speaker

The Netherlands	Maaike van den Beld (RIVM, Bilthoven)

National Reference Laboratories for *Salmonella*

ALBANIA	Renis Maçi
AUSTRIA	Thomas Pölzler Tanja Urbanke
BELGIUM	Maria Cristina Garcia Graells Laurence Delbrassinne
BOSNIA HERZEGOVINA	Amira Koro Emina Residbegovic
BULGARIA	Hristo Daskalov Gergana Mateva
CROATIA	Borka Simpraga
CYPRUS	Maria Emmanuel
CZECH REPUBLIC	Tomas Cerny
DENMARK	Tina Beck Hansen Lise Bonnichsen
ESTONIA	Age Kärssin Annika Vilem
FINLAND	Henry Kuronen
FRANCE	Laetitia Bonifait Frédérique Moury
GERMANY	Jennie Fischer Marina Lamparter
GREECE	Nikki Mouttotou Aphrodite Smpiraki
HUNGARY	Erzsébet Adrián Sára Kostyák
ICELAND	Pall Steinthorsson
IRELAND	William Byrne Montserrat Gutierrez
ITALY	Lisa Barco Veronica Cibin

KOSOVO	Vlora Gashi
	Besart Jashari
LATVIA	Jelena Avsejenko
	Madara Streikisa
LITHUANIA	Ceslova Butrimaite-Ambrozeviciene
	Tatjana Kutyriova
LUXEMBOURG	Manon Bourg
	Catarina Martins
MALTA	Donald Paul Parsons
	David Sammut
NETHERLANDS	Anjo Verbruggen
	Kim van der Zwaluw
NORWAY	Bjarne Bergsjø
	Bjørn Tore Lunestad
POLAND	Elzbieta Mackiw
	Emilia Mikos
	Kinga Wieczorek
PORTUGAL	Patricia Themudo
REPUBLIC OF NORTH MACEDONIA	Dean Jankuloski
ROMANIA	Maria Ionescu
	Carmen Manea
SERBIA	Jasna Kureljusic
SLOVAK REPUBLIC	Katarina Hollá
	Lubos Mikula
SLOVENIA	Majda Biasizzo
	Jasna Micunovic
	Tina Pirs
SPAIN	Montserrat Agüero
	Cristina de Frutos
	Teresa Rodriguez
SWEDEN	Jenny Eriksson
SWITZERLAND	Jule Horlbog
	Gudrun Overesch

Annex 2 Workshop Programme 26th EURL-*Salmonella* workshop; Friday 28 May 2021 - Online

09:30 - 10:00	Opening and introduction	Kirsten Mooijman EURL-*Salmonella*
10:00 - 10:30	Update on *Salmonella* in the EU, based on EU One Health 2019 Zoonoses report	Frank Boelaert EFSA
10:30 - 10:45	***Break***	
10:45 - 11:15	Results EURL-*Salmonella* combined Proficiency Test Primary Production Stage and Food 2020 - Detection of *Salmonella* in hygiene swab samples	Irene Pol EURL-*Salmonella*
11:15 - 11:45	Preliminary results EURL-*Salmonella* Proficiency Test Food 2021 - Detection of *Salmonella* spp. in liquid whole egg	Robin Diddens EURL-*Salmonella*
11:45 - 12:15	State of implementation of the 'One Health' system for the collection and analysis of WGS data from food/animal isolates and new cgMLST scheme for *Salmonella*	Mirko Rossi EFSA
12:15 – 13:30	***Break***	
13:30 - 14:15	Results additional (WGS) analysis on isolates of EURL-*Salmonella* Proficiency Test Typing 2019 & Results EURL-*Salmonella* Proficiency Test Typing 2020 – serotyping and cluster analysis	Wilma Jacobs EURL-*Salmonella*
14:15 - 14:45	In-house validation of *Salmonella* serotyping with WGS	Maaike van den Beld, The Netherlands
14:45 - 15:00	***Break***	
15:00 - 16:00	Activities NRLs to fulfil tasks and duties	
15:00 - 15:20	NRL-*Salmonella* Czech Republic	Tomás Cerný
15:20 - 15:40	NRL-*Salmonella* Ireland	William Byrne
15:40 -16:00	NRL-*Salmonella* Slovenia	Jasna Micunovic
16:00 - 16:30	Work programme EURL-*Salmonella* second half 2021, first half 2022 Concluding remarks workshop and closure	Kirsten Mooijman EURL-*Salmonella*

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

Annex 3 Workshop evaluation form

Evaluation of the 26th EURL-*Salmonella* workshop, Friday 28 May 2021 - online

We would highly appreciate if you could give us your opinion on the 26th EURL-*Salmonella* workshop, organised as online meeting on 28 May 2021. Thank you very much in advance for completing the questionnaire by 15 June 2021 at the latest.

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

1 (Very poor)	2 (poor)	3 (fair)	4 (good)	5 (very good)	No opinion

Remarks:_____

2. What is your opinion on the ease of logging into the meeting?

1 (Very poor)	2 (poor)	3 (fair)	4 (good)	5 (very good)	No opinion

Remarks:_____

3. Did you face any technical problems during the meeting?
   - ☐ No
   - ☐ Yes, I encountered the following problems: _____

Remarks:_____

4. What is your opinion on the length of the meeting and the number of breaks?

Length meeting:
- ☐ Too short
- ☐ Fine
- ☐ Too long

Number of breaks:
- ☐ Too little
- ☐ Fine
- ☐ Too many

Remarks:_____

5. Were you satisfied with the options for raising questions during the meeting (chat function; discussion time at the end of a presentation and at the end of a session)?
   - ☐ Yes
   - ☐ No opinion
   - ☐ No, but I have a suggestion for improvement _____

Remarks:_____

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

1 (Very poor)	2 (poor)	3 (fair)	4 (good)	5 (very good)	No opinion

Remarks:_____

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

8. What is your general opinion of the workshop?

1 (Very poor)	2 (poor)	3 (fair)	4 (good)	5 (very good)	No opinion

Remarks:_____

9. Due to the circumstances we had to organise this workshop again as online meeting. When the pandemic is over, what would be your preference for the frequency of physical and online meetings?
   - ☐ Always physical
   - ☐ Always online
   - ☐ Every other year an online workshop
   - ☐ Every 2 or 3 years an online workshop
   - ☐ Other frequency, _____

Remarks:_____

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

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