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

The 27th EURL-*Salmonella* workshop

23 and 24 May 2022, Online

RIVM report 2022-0107
K.A. Mooijman

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Synopsis

The 27th EURL-*Salmonella* workshop

23 and 24 May 2022, Online

In May 2022, the EU Reference Laboratory (EURL) for *Salmonella* held the workshop for the European National Reference Laboratories (NRLs) for the 27th year. The aim of the workshop is to exchange information between the EURL and the NRLs. The workshop was held online for the third time due to the coronavirus pandemic.

Each workshop devotes ample attention to the ring trials that the EURL organises to monitor the quality of the NRLs. This edition of the workshop included two ring trials conducted in autumn 2021 being presented. The first ring trial from 2021 studied chicken faeces adhering to boot socks. The second ring trial entailed various strains of *Salmonella* being typed using ordinary techniques and DNA techniques. For the most part, the DNA techniques pertained to Whole Genome Sequencing (WGS). This enables extremely precise typing of micro-organisms. The NRLs scored well in both ring trials. In addition, a summary of 25 years of ring trials was provided.

Another presentation gave information on *Salmonella* bacteria infection rates among humans and animals and contamination of foods in the European Union. There was also a presentation on the European system that is being constructed for the purposes of storing the WGS data on this bacterium. This data will provide a solid basis for research into the source of *Salmonella* infection.

One presentation also provided information on vaccinating chickens to combat *Salmonella* infection in these animals. Lastly, the *Salmonella* NRLs from Belgium, Norway and Sweden illustrated how they are fulfilling their statutory duties.

The EURL for *Salmonella* forms part of the National Institute for Public Health and the Environment (RIVM) and organises this workshop every year. One important aspect of the *Salmonella* EURL's remit is monitoring the quality of the European national reference laboratories for this bacterium.

Keywords: EURL-*Salmonella*, NRL-*Salmonella*, *Salmonella*, workshop 2022

Publiekssamenvatting

De 27e EURL-*Salmonella* workshop

23 en 24 mei 2022, Online

In mei 2022 organiseerde het Europese Referentie Laboratorium (EURL) voor *Salmonella* voor het 27e jaar de workshop voor de Europese Nationale Referentie Laboratoria (NRL's). Het doel van de workshop is om informatie uit te wisselen tussen het EURL en de NRL's. Door de uitbraak van het coronavirus is de workshop voor de derde 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 onder andere de resultaten van twee ringonderzoeken gepresenteerd die in het najaar van 2021 zijn gehouden. In het eerste ringonderzoek van 2021 zijn overschoentjes met kippenmest onderzocht. In het tweede ringonderzoek zijn verschillende *Salmonella*-stammen getypeerd met gewone technieken en met DNA-technieken. Voor de DNA-technieken is vooral Whole Genome Sequencing (WGS) gebruikt. Hiermee kunnen micro-organismen heel precies worden getypeerd. De NRL's scoorden goed in beide ringonderzoeken. Ook is een samenvatting gegeven van de organisatie van 25 jaar ringonderzoeken.

Een andere presentatie gaf informatie over het aantal besmettingen van mensen, dieren en levensmiddelen met een *Salmonella*-bacterie in de Europese Unie. Ook was er een presentatie over het Europese systeem dat wordt gebouwd om de WGS-data van deze bacterie op te slaan. Deze data kunnen goed gebruikt worden voor onderzoek naar de bron van een *Salmonella* besmetting.

Ook gaf een presentatie informatie over het vaccineren van kippen om besmetting met *Salmonella* bij deze dieren tegen te gaan. Ten slotte toonden de NRL's-*Salmonella* van België, Noorwegen en Zweden hoe zij hun wettelijke taken invullen.

Het EURL voor *Salmonella* is onderdeel van het RIVM en organiseert deze workshop elk jaar. 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 2022

Content

Summary

On 23 and 24 May 2022, 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 third time, the workshop took place 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, 103 participants attended.

During the workshop, presentations were given on several topics:

- Three EFSA representatives gave information on: a) EU monitoring of *Salmonella* and of salmonellosis foodborne outbreaks in 2020; b) The status of progress and timelines of the new One Health WGS system for the collection and analysis of WGS data from food/animal isolates; c) The assessment of multi-country foodborne outbreak events at EU level.
- Representatives of the EURL-*Salmonella* presented the results of the Proficiency Tests (PTs) organised in 2021, namely the PT on detection of *Salmonella* in chicken faeces adhering to boot socks (September 2021), and the PT on *Salmonella* typing (November 2021). Additionally, also 25 years of *Salmonella* serotyping PTs was presented.
- Another representative of the EURL-*Salmonella* presented the ongoing activities in ISO and CEN for standardisation of methods.
- Two guest speakers from the Dutch Royal GD (Animal Health Service) gave information on *Salmonella* vaccination in poultry and tests to differentiate between vaccine and field strains.
- Representatives of the NRLs-*Salmonella* from Belgium, Norway and Sweden 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-2022

1 Introduction

This report includes the abstracts of the presentations given at the 2022 EURL-*Salmonella* workshop, as well as a summary of the discussions that followed the presentations. The full presentations are available on the EURL-*Salmonella* website (if the author has given permission for publication): https://www.eurlsalmonella.eu/workshop-2022

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

2 Monday 23 May 2022: Day 1 of the workshop

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 27th workshop of the EURL-*Salmonella*, welcoming all participants to this third virtual EURL-*Salmonella* workshop.

In total, 103 participants attended, including representatives of the National Reference Laboratories (NRLs) for *Salmonella* from the 27 EU Member States (MS), 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 online workshops organised in 2020 and 2021 was presented, showing high scores for all questions raised.

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

2.2 EU monitoring of *Salmonella* and of salmonellosis foodborne outbreaks in 2020

Frank Boelaert, EFSA, Parma, Italy

The One Health Zoonoses 2020 report of the EFSA and the European Centre for Disease Prevention and Control (ECDC) presents the results of zoonoses monitoring activities carried out in 2020 in 27 EU MS and nine non-MS (EFSA and ECDC, 2021). Salmonellosis was the second most commonly reported foodborne gastrointestinal infection in humans after campylobacteriosis and was an important cause of foodborne outbreaks in EU MS and non-MS countries. In 2020, *Salmonella* reporting recorded the lowest number of human cases since 2007, when salmonellosis surveillance started, owing to the impacts of the withdrawal of the United Kingdom from the EU on the one hand and the SARS-CoV-2-virus pandemic on the other hand. In 2020, the number of confirmed cases of human salmonellosis was 52 702, corresponding to an EU notification rate of 13,7 per 100 000 population. This was a decrease of 29,7% and 32,8% compared to the rate in 2019 (19,5 and 20,4 per 100 000 population) with and without the 2019 data from the United Kingdom, respectively. Notwithstanding, the overall trend for salmonellosis in 2016–2020 did not show any statistically significant increase or decrease. The proportion of hospitalised cases was 29,9%, which was lower than in 2019, with an EU case fatality rate of 0,19%. The top five *Salmonella* serovars involved in human infections overall were distributed as follows: *S*. Enteritidis (48,7%), *S*. Typhimurium (12,4%), monophasic *S*. Typhimurium (1,4,[5],12:i:-) (11,1%), *S*. Infantis (2,5%) and *S*. Derby (1,2%).

In total, 694 foodborne outbreaks of *Salmonella* were reported by 22 MS in 2020, causing 3 686 illnesses, 812 hospitalisations and seven deaths. *Salmonella* caused 22,5% of all foodborne outbreaks in 2020. The majority (57,9%) of the reported foodborne outbreaks of *Salmonella* were caused by *S*. Enteritidis. The three food vehicles most commonly involved in strong-evidence foodborne salmonellosis outbreaks were 'eggs and egg products', followed by 'pig meat and products thereof' and 'bakery products'.

Sampling to verify compliance with food safety criteria, according to Regulation (EC) No 2073/2005 (EC, 2005), at distribution level, found the following three categories with the highest proportions of *Salmonella*-positive samples: 'meat products made from poultry meat intended to be eaten cooked': 7,6%, 'fresh poultry meat': 7,3% and 'minced meat and meat preparations made from poultry meat intended to be eaten cooked': 5,7%. Next, for 'mechanically separated meat' and 'minced meat and meat preparations made from other species than poultry intended to be eaten cooked' and 'meat products intended to be eaten raw, excluding products where the manufacturing process or the composition of the product will eliminate the *Salmonella* risk', about 1% of the collected official samples was positive for *Salmonella*. Sampling to verify compliance with process hygiene criteria found significantly lower proportions of *Salmonella*-positive carcases of pigs, broilers, turkey and cattle in industry sampling, compared with the official control samples at EU level. For 2020, 69 898 'ready-to-eat' food sampling units collected according to an 'objective sampling' strategy were reported by 22 MS with 0,15% positive samples overall. Within each food category, 1,6% of 'meat and meat products from broilers', 0,8% of 'spices and herbs', 0,6% of 'meat and meat products from pigs', 0,5% of 'meat and meat products from turkeys' and 0,5% of 'other meat and meats products' were positive for *Salmonella*.

Of the 26 MS reporting on *Salmonella* control programmes 14 met the reduction targets for all poultry populations, compared to 18 in 2019. The number of MS that did not meet the *Salmonella* reduction targets was three for breeding flocks of *Gallus gallus*, seven for laying hen flocks, three for broiler flocks, one for breeding flocks of turkeys and three for fattening turkey flocks. In the context of *Salmonella* control programmes in poultry, the prevalence of target *Salmonella* serovars in broiler and fattening turkey flocks reported by food business operators was significantly lower than that reported by the Competent Authorities at EU level. A significant increase in the estimated prevalence of *Salmonella* was noted for laying hens and breeding turkeys in 2020 compared with 2014 and 2015, respectively, when prevalence reached the lowest level in these poultry populations. Flock prevalence trends for target *Salmonella* serovars were, in contrast, fairly stable over the last few years for all poultry populations.

Considering the top five serovars responsible for human infections and the major putative sources (broilers, cattle, turkeys, laying hens and pigs, isolated from both animals and food products from animals), a panel of 17 877 serotyped isolates from food and food-producing animals was reported. *S*. Enteritidis was primarily related to broiler sources and to layers and eggs. *S*. Typhimurium was mainly linked with

broiler and pig sources. Monophasic *S*. Typhimurium (1,4,[5],12:i:-) was related mainly to pig and secondly to broiler sources. *S*. Infantis was strictly related to broiler sources, whereas *S*. Derby was primarily linked with pigs.

The links to the relevant EFSA webpages are the following.
EFSA foodborne outbreaks (FBO) story map:
https://multimedia.efsa.europa.eu/fbo-storymaps/index.html
EFSA FBO dashboard:
https://www.efsa.europa.eu/en/microstrategy/FBO-dashboard

Discussion
Q: Do I understand correctly that there is an increase in *Salmonella* spp. prevalence, but a decrease in the top 5 target serovars?
A: This is indeed what we see for laying hens and breeding turkeys, but it could also be a reporting artefact. It is difficult to say if the increase is related to a specific *Salmonella* serovar as reporting of non-target serovars is not mandatory. Flock prevalence trends for target *Salmonella* serovars were fairly stable over the last few years for all poultry populations.
Q: Do you have more information about the decline in salmonellosis? Were there less human *Salmonella* cases in 2020, or was this due to less reporting?
A: The SARS-CoV-2-virus pandemic quite likely affected the number of reported cases in several ways. Due to constraints on human resources, as staff was needed for management of the pandemic, a few countries filed incomplete reports. Also, during the SARS-CoV-2-virus pandemic restriction periods, patients with symptoms related to food- and waterborne diseases were unlikely to visit doctors or confirm the diagnosis in a laboratory, resulting in under reporting or under diagnosis. However, it is also possible that the number of cases were indeed lower than before. During the lockdown(s) there were no social eating habits (e.g. no event catering, no buffets over the summer, restaurants were closed). Some restrictive measures implemented against COVID-19, such as frequent hand washing and disinfection and the lockdowns, may have had a direct effect on limiting the spread of *Salmonella*. Moreover, the number and proportion of travel-related cases (both outside and within the EU) dramatically dropped as a direct consequence of reduced travelling abroad during the lockdowns. The decrease in salmonellosis cases was probably also due to exclusion of the United Kingdom from the ECDC reporting data due to its withdrawal from the EU. It will be interesting to see what will happen with the figures in 2021 and 2022.

2.3 Results EURL-*Salmonella* Proficiency Test Primary Production Stage 2021 – Detection of *Salmonella* in chicken faeces adhering to boot socks

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

In September 2021, the EURL-*Salmonella* Proficiency Test (PT) on detection of *Salmonella* in samples from the primary production stage (PPS) was conducted. A total of 35 National Reference Laboratories (NRLs) for *Salmonella* participated in this study: 27 NRLs-*Salmonella*

from the 27 EU-Member States (MS), seven NRLs from third European countries (EU candidate MS or potential EU candidate MS and members of the European Free Trade Association (EFTA)), and one NRL from a non-European country.

In this study, chicken faeces adhering to boot socks contaminated with a diluted culture of *Salmonella* Infantis at the EURL-*Salmonella* laboratory, was used as a matrix (referred to as boot sock samples in this report). Each NRL received 16 blindly coded samples consisting of 10 boot sock samples artificially contaminated with two different concentrations of *Salmonella* Infantis: six low-level contaminated samples (MPN concentration: 2,3 cfu/sample) and four high-level contaminated samples (MPN concentration: 35 cfu/sample). Additionally, each NRL had to analyse four negative boot sock samples (no *Salmonella* added) and two control samples. The control samples consisted of a blank procedure control sample and a positive control sample. For the latter, the participants had to use their own positive control strain. The samples were stored at 5 °C until the day of transport. On Monday 20 September 2021, EURL-*Salmonella* packed and sent the boot sock samples to the NRLs. The NRLs were asked to store the samples at 5 °C on arrival until the start of the analysis on Monday 27 September 2021.

All laboratories used the prescribed method EN ISO 6579-1:2017. The majority of the laboratories also indicated that they followed Amendment 1 of EN ISO 6579-1 (EN ISO 6579-1:2017/A1:2020). One laboratory reported to be NRL-*Salmonella* for samples from the primary production stage but used Rappaport-Vassiliadis soya (RVS) broth instead of modified semi-solid Rappaport-Vassiliadis (MSRV) agar for the selective enrichment. This is not in line with the prescribed method in EN ISO 6579-1:2017 for analysing PPS samples.
Six laboratories also used a PCR method as second detection method for analysing the samples. Not all laboratories found identical results using the PCR method compared to the results found with EN ISO 6579-1:2017(/A1:2020).

Of the 35 participating laboratories, 34 scored well, analysing both the procedure control sample as well as their own positive control sample correctly. One laboratory made an administrative error by accidentally reporting their positive control as tested negative for *Salmonella*. This laboratory scored a moderate performance.
All laboratories detected *Salmonella* in the boot sock samples contaminated with a low level of *Salmonella*. Eight laboratories tested one of the six samples negative for *Salmonella.* Five laboratories tested two of the six samples negative for *Salmonella*. These results are still within the criteria for good performance, which permit three negative samples. The sensitivity rate for these samples was 91,4%.

Almost all laboratories detected *Salmonella* in all four high-level samples. Only four laboratories scored one of the four high-level samples as negative. This is still within the criteria for good performance, which permit one negative sample. The sensitivity rate for these samples was 97,1%.

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

Overall, the laboratories scored well in this Proficiency Test, with an accuracy of 95,5%. Thirty-four laboratories fulfilled the criteria of good performance. One laboratory scored a moderate performance due to an administrative error. It switched the results of the positive and the negative control samples.

More details can be found in the report of this PT (Pol-Hofstad and Mooijman, 2022).

Discussion
Q: Have you experienced before that no *Enterobacteriaceae* were present in the chicken faeces?
A: No, never. In the current study we did not detected *Enterobacteriaceae* in the chicken faeces used for the pre-tests only, while in the chicken faeces used for the PT, the number of *Enterobacteriaceae* was 10^7 cfu/g! Remarkably, the chicken faeces for both the pre-tests and the PT were collected from the same chicken flock.
Q: Do you still accept the results from laboratories for which the parcels arrived late due to long transport time?
A: When we analyse the results of a laboratory we also consider the possible negative effect of long transport time, in combination with the temperature during transport (information obtained from the temperature recorder in the parcel). In case of long and/or warm transport of the PT samples in combination with an unexpected high number of samples tested negative for *Salmonella* by a laboratory, the results are no longer used for determining the performance of this laboratory. Luckily, in the current study we did not see any effect on the results due to long/warm transport times.

2.4 Results EURL-*Salmonella* Proficiency Test Typing 2021 – serotyping and cluster analysis

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

In November 2021, EURL-*Salmonella* organised the 26th PT on typing of *Salmonella*. A total of 35 laboratories participated in this PT, consisting of an obligatory serotyping part and an optional part on cluster analysis. Participants included 27 NRLs-*Salmonella* in the 27 EU-MS and 8 NRLs from third countries (EU candidate or potential EU candidate Member States, EFTA countries, and the United Kingdom). The main objective of this PT was to evaluate the performance of the NRLs for serotyping of *Salmonella*.

For the serotyping study, EURL-*Salmonella* selected a total of 20 obligatory *Salmonella* strains plus one additional *Salmonella* strain from an uncommon type. The strains had to be typed by means of the method routinely used in each laboratory, following the White-Kauffmann-Le Minor scheme (Grimont and Weill, 2007). EURL-*Salmonella* e-mailed the individual laboratory results on serotyping, as well as an interim summary report on the general

outcome, to the participants in February 2022. Out of the 35 participants, 32 (91%) typed the O-antigens completely correctly. This corresponds to nearly 100% of the total number of strains. The H-antigens were typed completely correctly by 26 of the 35 participants (74%), corresponding to 98% of the total number of strains. As a result, 26 participants (74%) reported all serovar names correctly, which corresponds to 98% of all strains evaluated. A completely correct identification was obtained for ten *Salmonella* serovars: Enteritidis (S1), Chingola (S2), Braenderup (S6), Montevideo (S7), Wien (S9), Virchow (S10), Infantis (S13), Hadar (S15), Anatum (S17), and Typhimurium (S19). All but three participants tried to serotype optional strain S21, a *Salmonella enterica* subsp. *diarizonae* (IIIb). A few laboratories did not have access to the required antisera to finalise this (50:r:1,5).
Overall, the performance of the NRLs in the PT Serotyping 2021 was very good. One EU Member State NRL did not meet the level of good performance at the first stage of the PT and EURL-*Salmonella* organised a follow-up study for this laboratory. The ten additional strains were correctly serotyped in this follow-up study.

The individual laboratory results on the cluster analysis part, as well as an interim summary report on the general outcome, were e-mailed to the participants in the week following the workshop.
A total of 19 NRLs participated in the optional cluster analysis; all 19 performed WGS analysis and 5 participants also performed MLVA analysis.
EURL-*Salmonella* selected strains to be suitable for analysis by using either MLVA or WGS. A set of 15 human surveillance strains, collected and sequenced in 2019, were re-cultured from storage and submitted for MLVA and WGS analysis both directly and after sub-culturing for 10 times. Subsequently, 9 strains were selected for inclusion in the PT. The 10^{th} strain was to be a technical duplicate.
The PT Cluster Analysis 2021 was mimicking an outbreak situation, with a *Salmonella* Enteritidis ST11, MLVA type 3-10-4-4-1 as the reference strain. Raw WGS data of this strain (21SCA-REF_R1.fq.gz and 21SCA-REF_R2.fq.gz) were made available through a secure ftp server. For this particular PT 2021 situation, the cluster definition was set at maximum 7 allelic differences from the reference sequence. For MLVA, the cluster definition was set at no loci with a different number of repeats.

Participants were asked to analyse the 10 *Salmonella* strains and to report per strain whether a clustering match with the reference strain was found or not.
Evaluation of the participants' cluster analysis results took place by comparing the participants' results to the expected results in the outbreak investigation setting, as pre-defined by the EURL-*Salmonella.*
No specific performance criteria were set for this PT on cluster analysis. As a minimum, it was expected that participants would report the technical duplicate strains 21SCA06 and 21SCA09 to be (part of) one cluster.
Based on the given cluster definition, MLVA-based results were expected to indicate strains 21SCA04, 21SCA06 (reference strain), 21SCA08 and 21SCA09 (technical duplicate of the reference strain) to be a clustering match with the reference outbreak strain as detailed in the PT Typing

2021. All 5 participants reported the MLVA-based cluster analysis results completely as expected.
Based on the given (cgMLST-)cluster definition, WGS-based results were expected to indicate strains 21SCA06, 21SCA08, and 21SCA09 to be a clustering match with the provided reference outbreak strain 21SCA-REF data as detailed in the PT Typing 2021. Fourteen of the 23 submissions (3 participants made multiple submissions) reported the WGS-based cluster analysis results completely as expected. All deviations concerned results on strain 21SCA08. Based on their distance matrix data, all but one of the 13 cgMLST submissions were reported in accordance with the PT 2021 cluster definition of a maximum of 7 allelic differences, even though this subsequently ended up in a deviation from the expected result. Because no cluster definition was specified for SNP-based analysis, the 10 SNP submissions were based on the participants' internal criteria. The apparent variety in these internal criteria may, partly, explain the differences in cluster analysis results reported for strain 21SCA08.

More details can be found in the interim summary reports of the PT Typing 2021 (Jacobs-Reitsma et al., 2022a, 2022b).

2.5 Activities of the NRL-*Salmonella* to fulfil tasks and duties in Sweden

Erik Eriksson, NRL-Salmonella*, Uppsala, Sweden*

The NRL-*Salmonella* of Sweden is located at the National Veterinary Institute (SVA) in Uppsala, approximately 60 km North from Stockholm. The institute has 380 employees and the NRL is accommodated at the Section for Bacteriology with 24 employees. Laboratory veterinarian Erik Eriksson and laboratory technician Jenny Eriksson are responsible for the activities at the NRL.

The *Salmonella* analyses performed at SVA in 2021 involved 35 000 culture analyses with EN ISO 6579-1:2017. Most of the samples were faecal and environmental samples from life stock production (22 319 samples) and feedstuff related samples (9 132 samples). In 2021, serotyping was performed on 1 162 *Salmonella* strains whereof most strains originated from SVA's analyses but also from other regional labs. Typing is performed on isolates from veterinary, food and feed related samples.
At the institute serological analysis for antibodies were also performed in 2021, including samples from poultry (3 832 analysis), cattle (4 542 analysis) and swine (3 046 analysis).

SVA has performed Whole genome sequencing of *Salmonella* since 2021. Accreditation is under way in 2022 and an evaluation of the process is performed by typing *Salmonella* strains in parallel with conventional serotyping and WGS typing. Data is analysed with SISTR for serotyping and cgMLST and wgSNP for clustering. Data from WGS typing is exchanged ad hoc with other authorities in Sweden.

Other activities that SVA carries out as NRL-*Salmonella* are:

- Organisation of meetings with regional laboratories and other authorities;
- Giving advice to regional laboratories concerning analyses for *Salmonella*;
- Providing education for regional laboratories in *Salmonella* diagnostics;
- Cooperation with the National food administration in their contact with official laboratories that analyse *Salmonella* in food;
- Organisation of laboratory comparisons/ proficiency tests for regional laboratories, in cooperation with other NRLs in the Nordic countries (Sweden, Norway, Denmark, Finland) and Estonia.

At the workshop two topical subjects regarding *Salmonella* in Sweden were presented.
Salmonella Cholerasuis has not been isolated from swine in Sweden since the seventies. However, currently a specific *S*. Cholerasuis strain seems to have established itself in in the wild boar population. This has resulted in the fact that this specific strain of *S*. Cholerasuis has been isolated from three Swedish swine herds between 2020-2022. For this reason, extensive screening for *Salmonella* is currently performed in the Swedish swine and wild boar population.
Every year, during late winter/ early spring outbreaks of *Salmonella* occur among passerine birds in Sweden. The birds get infected by specific types or *S*. Typhimurium strains which are associated with this wild bird population. Harsh winters and lack of food lead to the fact that the birds migrate into the communities and seek for food at feeding tables where *Salmonella* is spread among the birds. Outdoor cats get infected after they have catched a sick bird and eaten it. In 2019, 1 179 cats were infected with *Salmonella* and in 2020, 1 209 cats were infected. These specific *S*. Typhimurium strains are also found in Swedish human cases, mostly children.

Discussion
Q: Are there specific bird species showing this contamination with *Salmonella* Typhimurium?
A: Yes we see this in certain passerine species such as Eurasian siskins (*Carduelis spinus*), Eurasian bullfinches (*Pyrrhula pyrrhula*), common redpolls (*Carduelis flammea*) or greenfinches (*Carduelis chloris*) (Söderlund et al., 2019).
Q: Did you use WGS to detect multiple related isolates in private laboratories?
A: Yes indeed.
Q: How long does it take to serotype by WGS?
A: Answered by Maaike van den Beld (RIVM): in our case the time for WGS serotyping does not differ much from 'classical' serotyping by serology as we analyse the isolates by batch twice a week.

2.6 Activities of the NRL-*Salmonella* to fulfil tasks and duties in Norway

Bjarne Bergsjø and Julia Storesund, NRL-Salmonella*, Oslo, Norway*

The Norwegian NRL-*Salmonella* is located at the Norwegian Veterinary Institute at Ås.
Seven different private official laboratories are testing the samples of the *Salmonella* surveillance programme taken at slaughterhouses and cutting plants. The Norwegian Veterinary Institute is also an official laboratory, testing the samples from live animals.

Activities of the Norwegian NRL-*Salmonella*:

- Participation in the PTs organised by the EURL-*Salmonella* and participation in the EURL-*Salmonella* annual workshops.
- Sharing relevant strains and whole genome sequences during outbreaks.
- Accredited (mandatory) for the relevant work field.
- Use of EN ISO 6579-1:2017 as standard method. Given a validation, alternative methods are approved.
- The official laboratories report their ring-trial results to the NRL. The laboratory that tests the samples from live animals, need to participate in a ring-trial with real faeces samples. The laboratory is referred to 'The Scandinavian interlaboratory comparison study of *Salmonella* in material from animal production' from the Swedish NRL-*Salmonella*.
- The NRL organises annual meetings with the official laboratories and the Norwegian Food Safety Authority. During these meetings, the NRL informs about its activities, and gives an overview about the epidemiological situation at EU-level and at national level. The NRL sums up the latest outbreaks. The NRL shares information from the EURL on these meetings and on a day-to-day basis, e.g. during outbreaks.
- The NRL coordinates the *Salmonella* surveillance control programmes and compiles the annual *Salmonella* report. The surveillance programme comprises approximately 10 000 samples from live animals and 11 500 samples from slaughterhouses and cutting plants.
- In Norway, any detection of *Salmonella* in food, feed, water and animals is notifiable. All non-human *Salmonella* isolates are referred to the NRL-*Salmonella* for confirmation and serotyping. Isolates of the top five serovars, isolated from animals and from food, are whole genome sequenced. All isolates are stored in a biobank at the NRL.
- The NRL reports about the epidemiological situation for *Salmonella* daily during outbreaks and in the annual *Salmonella* report.

Discussion
Q: Were there less samples tested in 2020-2021 than in former years?
A: Yes, there were less samples than in former years, due to the SARS-CoV-2-virus pandemic.

2.7 Activities of the NRL-*Salmonella* to fulfil tasks and duties in Belgium and Northern Ireland

*Cristina Garcia Graells and Inge van Damme, NRL-*Salmonella*, Brussels, Belgium*

The laboratory of Foodborne Pathogens of Sciensano (Institute of Public Health, Belgium), was designated as NRL for *Salmonella* in food, feed and primary production in 2009.
As stated in Regulation (EU) 2017/625 (EC, 2017), the National Reference Laboratory performs the following tasks.

- Analytical methods: In 2021, the NRL-*Salmonella* together with the NRL for Food Microbiology has verified the alternative method IQ Check for the rapid screening of *Salmonella* spp. in food products analysed in the framework of foodborne outbreak investigations. The verification of the method has been done according to EN ISO 16140-3:2021. An extension to primary production samples and environmental samples is foreseen.
- Proficiency tests: The NRL organises biannual PTs for the detection of *Salmonella* spp. in primary production. Chicken faeces are currently used as matrix in these PTs, resulting in good performance by all official laboratories designated by the national authority.
- The NRL conducts outbreak investigations concerning suspicion of *Salmonella* spp. In this respect, national human health and food safety authorities collect samples and send them to the corresponding National Reference Centre and NRL-*Salmonella*/ NRL FBO for *Salmonella* spp. detection. The laboratory uses reference method EN ISO 6579-1:2017 and an alternative method, followed by serotyping and MLVA typing. In addition, the NRL performs WGS analysis and trace back studies for cluster analysis and identification of the origin of the contamination.
- Providing training courses for particular topics to official laboratories. This activity is performed upon request. In 2019, a laboratory was trained on the use of EN ISO 6579-1:2017.
- The transfer of knowledge and dissemination of the information provided by the EURL-*Salmonella* to the national authorities is assured by participating in the annual workshop and reporting to the competent authority.

NRL-*Salmonella* Northern Ireland.
In 2022, the laboratory of Foodborne Pathogens of Sciensano (Institute of Public Health, Belgium), was designated as NRL for *Salmonella* in food and feed by the Food Standards Agency (FSA) for Northern Ireland. The official laboratories in Northern Ireland are supported by the UK NRLs, and by Sciensano to provide the required NRL responsibilities and tasks under Regulation (EU) 2017/625 (EC, 2017).

3 Tuesday 24 May 2022: Day 2 of the workshop

3.1 Assessment of multi-country foodborne outbreak events at EU level

Eleonora Sarno, EFSA, Parma, Italy

A multi-country foodborne outbreak is an incident in which two or more people from at least two EU countries experience the same disease or infection that follows the consumption of a common contaminated food. In this case, the European Commission or the European Centre for Disease Prevention and Control (ECDC) can request the European Food Safety Authority (EFSA) to work with ECDC on a joint assessment. The assessment of these events, carried out by EFSA in collaboration with ECDC, generates two types of outputs: a joint rapid outbreak assessment (ROA) and/or a joint notification summary (JNS).
A ROA is produced when there is a need to communicate to the general public about an event. It provides an in-depth analysis of the information available in the Rapid Alert System for Food and Feed (RASFF), identifying the contaminated food, its origin, and the point of contamination along the food production line. It is used by risk managers to identify the control measures needed to mitigate the risk of occurrence of new cases of infections.
A JNS is a working document produced when there is a need to inform risk managers rapidly about an event so as to support national investigations. It provides a summary of the information related to the contaminated food, a description of its traceability (where it was produced and distributed) and of the control measures implemented. A JNS relies on the data and information already shared by Member States in RASFF. It is shared only with the appointed competent authorities through dedicated platforms, the European Reference Laboratories and National Reference Laboratories of the involved countries, and with the European Commission.
Examples of recently published rapid outbreak assessments (ROA) on multi-country foodborne outbreaks were also provided.

3.2 Status of progress and timelines of the new EFSA One Health WGS system

Mirko Rossi, EFSA, Parma, Italy

In 2019, EFSA and ECDC received a mandate from the European Commission (EC) to implement and manage two interoperable systems for the collection and analysis of Whole Genome Sequence (WGS) data of *Salmonella enterica*, *Listeria monocytogenes* and *Escherichia coli*, including Shiga toxin-producing *E. coli* (STEC) from humans and from food, feed, animals and the related environment, to contribute to the epidemiological investigations of food-borne outbreaks and to the identification of emerging health threats. As response to this EC mandate, EFSA has developed the EFSA One Health WGS System that interoperates with the ECDC Molecular Typing system exchanging core genome Multi Locus Sequence Typing (cgMLST) profiles and minimum metadata. Interested EU-MS and European Economic Area (EEA)

countries can submit their typing data from food, feed and animal samples to the EFSA One Health WGS system either through an user-friendly interface or programmatically. The presentation describes the EFSA One Health WGS system, including its architecture, the users and their relative data visibility, as well as data ownership and intellectual property.

3.3 25 Years of *Salmonella* serotyping Proficiency Tests

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

As task resulting from Council Directive 92/117/EEC (EC, 1992), the Community Reference Laboratory for *Salmonella* (CRL-*Salmonella*, situated at the RIVM, Bilthoven, The Netherlands) organised in 1995 the first collaborative study on serotyping of *Salmonella*. All National Reference Laboratories (NRLs) for *Salmonella* (17 at that time) participated in this study. In 2020, the re-named European Union Reference Laboratory (EURL-*Salmonella*) in Bilthoven organised the 25th collaborative study, or Proficiency Test (PT), on serotyping of *Salmonella*, nowadays as task resulting from EC Regulation No. 2017/625 (EC, 2017). A total of 37 laboratories participated in this 25th study. This includes the NRLs-*Salmonella* in the 27 EU Member States, 7 NRLs from third countries, and 3 additional participants to compare with their WGS-based results.
The main objective of all 25 PTs remained the same over the years: to evaluate whether the serotyping of *Salmonella* strains by the NRLs-*Salmonella* is carried out uniformly, and whether they yield comparable results. For this, the participants serotype annually 20 strains of *Salmonella enterica* subspecies *enterica*. Strains have to be typed according to the method routinely used in each laboratory, following the White-Kauffmann-Le Minor scheme (Grimont and Weill, 2007). The results per strain to be reported are the formula for the O-antigens and H-antigens, and the serovar names. These three aspects are evaluated as Correct, Not typable, Partly correct, or Incorrect.
In 2007, EURL-*Salmonella* defined criteria for 'good performance' on identification of the serovar names. Penalty points are given for the incorrect typing of strains. The total number of penalty points is calculated for each participant and the criterion for good performance is set at less than 4 penalty points.

The historical data on the EU NRLs-*Salmonella* show that the percentages of correctly typed strains became stable over time to 95% - 100%, usually showing a better performance for the O-antigens than for the H-antigens.
The total number of penalty points per PT has clearly declined, from 35 points in 2007, to 3 points in 2020. Moreover, the number of EU NRLs with a non-good performance decreased from 6 in 2007, 3 in 2008 and 4 in 2009 to 2 in the period 2010 - 2013, 1 in 2014, 2015 and 2018, and none in the 2016, 2017, 2019 and 2020 PTs.

3.4 Update on activities in ISO and CEN

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

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

- ISO/TC34/SC9: International Organization for Standardization, Technical Committee 34 on Food Products, Sub-committee 9 – Microbiology of the food chain;
- CEN/TC463: European Committee for Standardization, Technical Committee 463 – Microbiology of the food chain.

This years' annual meeting of both groups took place as a virtual meeting from 13 to 17 June 2022.

Development of 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)')
The development of ISO/TS 6579-4 has a long history, starting in 2014 with the agreement to develop this (EN) ISO document. Several draft versions of ISO/TS 6579-4 have been prepared before the voting of the New Work Item Proposal (NWIP) was launched in 2020, followed by a voting on draft ISO/CD 6579-4 in 2021. By the end of 2021, the ISO working group prepared the draft DIS version of the document, as well as a draft protocol for performing an interlaboratory study to determine the performance characteristics.

Draft ISO/TS 6579-4 describes three different PCR methods:

- PCR method 1: a probe-based multiplex real-time PCR. Primers and probes published by Maurischat et al., 2015.
- PCR method 2: an agarose gel-based multiplex PCR. Primers published by EFSA, 2010 and Tennant et al., 2010.
- PCR method 3: an agarose gel-based single target PCR. Primers published by Maurischat et al., 2015; primers internal control published by Gallien, 2003.

The scope of draft ISO/TS 6579-4 indicates that the method is applicable for:

- differentiation of the isolate under analysis between monophasic *Salmonella* Typhimurium and the monophasic variant of another *Salmonella* non-Typhimurium serovar;
- identification of the isolate under analysis being either monophasic *Salmonella* Typhimurium or (biphasic) *Salmonella* Typhimurium.

The design of this interlaboratory study (ILS) is based upon the information of EN ISO 16140-6:2019, including:

- Per PCR method at least 10 valid data sets from at least 10 collaborators will be needed.
- A total of (at least) 24 different strains shall be tested for inclusivity (target strains) and exclusivity (non-target strains).
- The strains will be selected from the set of 172 strains tested during the method evaluation study.

- The results found by the collaborators will be compared to the 'true' identity of the strains. To enable this, EURL-*Salmonella* (re)analysed all selected strains with biochemical galleries, slide agglutination, the 3 PCRs of draft ISO/DTS 6579-4, MALDI-TOF, MLVA and WGS.

In February-March 2022, a call for participants in the ILS was made among the NRLs-*Salmonella*, the members of ISO/TC34/SC9 and of ISO/TC34/SC9-WG10. For PCR method 1, 30 registrations were obtained; for PCR method 2, 21 and for PCR method 3, 17. These numbers for potential participants were sufficient to continue with the organisation of the ILS.
On 16 May 2022, the samples were shipped to the participants and the deadline for reporting the results of the ILS is 1 July 2022.

Other subjects
EURL-*Salmonella* is also convenor of an ISO Ad hoc group (AHG A) for preparing and/or updating a guidance document for drafting ISO/CEN standards for microbiology of the food chain. This is an internal document to help convenors and project leaders of ISO/TC34/SC9 and of CEN/TC463 with drafting of ISO/CEN documents in an harmonised way. Edition 1 of this guidance document was published in 2018, edition 2 in 2020. AHG A has recently prepared draft edition 3, which was sent for comments to members of ISO and CEN until 27 May 2022.

EURL-*Salmonella* is member and project leader of a subgroup of ISO/TC34/SC9-WG3 ('Method validation'). WG3 is employing several activities in the field of method validation:

- AHG validation status of ISO's. For verification of methods in accordance to EN ISO 16140-3:2021, the (EN ISO) reference methods have to be validated from 2027. The AHG made an inventory on presence of performance characteristics in EN ISO documents of microbiology of the food chain. As a result, the AHG prepared a table of methods needing (additional) validation studies, in order of importance. High in ranking for additional validation studies is EN ISO 6579-1:2017 ('Detection of *Salmonella*').
- Development of Amendment 1 of EN ISO 16140-2:2016, entitled 'Revision of the qualitative method comparison study data evaluation, revision of RLOD calculations in the interlaboratory study, revision of the calculation and interpretation of the relative trueness study, and inclusion of commercial sterility testing protocol of UHT milk'.
- Development of Amendment 1 of EN ISO 16140-4:2020, entitled: 'Validation of a larger test portion size for qualitative methods'. The approach in this document is to compare the level of detection (LOD) of the original test portion size to the LOD of the larger test portion size.
- Development of ISO 16140-7 'Protocol for the validation of identification methods of microorganisms'. This document specifies the general principle and the technical protocol for the validation of identification methods of microorganisms for microbiology in the food chain, in case no reference method is available. When a reference confirmation or typing method is

available, the alternative method shall be validated in accordance to EN ISO 16140-6:2019.
- Revision of EN ISO 17468:2016 'Technical requirements and guidance on establishment or revision of a standardized reference method'. This document is revised to include information of EN ISO 16140-4:2020 (in-house validation), EN ISO 16140-6:2019 (validation of confirmation and typing methods) and EN ISO 11133:2014 (performance testing of culture media); to explain the impact of minor and major changes of a revised EN ISO document; to extend the content for situations where it is not possible to compare a new EN ISO method with a former reference method.
- Together with WG2 ('Statistics'), WG3 forms a joint sub-group which reviews evaluation/validation protocols for ISO standards. This sub-group advises other ISO working groups with drafting of protocols for performing validation studies.

Other ISO working groups working on general subjects are:
- Joint working group JWG5, revising EN ISO 11133:2014 'Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media'. This JWG prepared two amendments for EN ISO 11133: Amd.1 'Corrections and explanations' published in 2018, and Amd.2 'Performance testing of confirmation media and reagents' published in 2020. Additionally, a table with all control strains for performance testing of culture media and reagents from published standards from microbiology of the food chain and from water microbiology is published at the website of ISO/TC34/SC9 (https://committee.iso.org/home/tc34sc9). JWG5 will soon start with the revision of the full document EN ISO 11133.
- WG7 is revising EN ISO 7218:2007 'Microbiology of the food chain - General requirements and guidance for microbiological examinations'. In May 2022, the DIS voting (Draft International Standard) was launched for the revised document and will last until 1 August 2022.
- WG25 has developed (EN) ISO 23418 'Microbiology of the Food Chain - Whole genome sequencing for typing and genomic characterization of foodborne bacteria — General requirements and guidance'. The final document is published in June 2022.

3.5 *Salmonella* vaccination in poultry and tests to differentiate between vaccine and field strains

Christiaan ter Veen and Annet Heuvelink, Royal GD, Deventer, the Netherlands

Salmonellosis is one of the most important foodborne zoonoses. In 2019, almost 88 000 confirmed cases were reported in the EU, most of which attributed to foods of animal origin. Historically, poultry meat and eggs have been an important source of food borne salmonellosis and it remains a major source of salmonellosis today. This prompted *Salmonella* control programmes for poultry on a national and later EU level. The current EU legislation consists of an intensive monitoring

programme, targets for the reduction of salmonellosis in poultry and channelling of products originating from *Salmonella*-positive poultry flocks.
Measures against *Salmonella* in poultry aim towards control in poultry feed, biosecurity, hygiene, prevention of vertical transmission and vaccination. Although information on the use of vaccines within the EU is not available, and vaccination rates may differ between Member States, the use of *Salmonella* vaccines may be considered common. For example, in the Netherlands 100% of layer flocks and 80% of breeder flocks are vaccinated against *Salmonella*.
Both live and killed *Salmonella* vaccines are available and employed within the EU. Live *Salmonella* vaccines may interfere with the current monitoring programme because vaccinated chickens may harbour and shed the vaccine strain for a short period after vaccination. Therefore, information on colonization and shedding of the bacterium after vaccination has to be supplied upon the registration of new vaccines, and a test has to be provided to differ between the vaccine and field strains.

Most *Salmonella* vaccines are applied during the rearing phase, although recently a vaccine became available which can be applied during lay. The chance of isolation of a vaccine strain depends on the growth characteristics of the strain in the different culture media, the time between vaccination and monitoring, and shedding levels. In practice some vaccines are very rarely detected during routine monitoring, while other vaccines are frequently detected shortly after vaccination. In such cases a so called DIVA test (Differentiating Infected from Vaccinated Animals), often based on antimicrobial susceptibility or genetic composition, can be employed to differentiate between field and vaccine strains. Information hereof can be acquired from the pharmaceutical company or from the summary of product characteristics at the national registration office for veterinary medicinal products or European Medicines Agency.

Discussion
Q: Question to the colleagues of the NRLs-*Salmonella*: do you report the presence of vaccine strains?
A: Reply NRL-*Salmonella* PT: yes we do. Reply NRL-*Salmonella* GR: yes we do when the competent authority asks for it.

3.6 Work programme EURL-*Salmonella* second half 2022, first half 2023, 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 2022 and for early 2023.

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 by the end of 2020. In January 2021, the desk officer at DG SANTE informally agreed with the EURL-*Salmonella* work program of 2021. In September 2021, EURL-*Salmonella* officially submitted its work programme for 2021-2022 to the granting authority, the European Health and Digital Executive Agency (HaDEA). By April 2022, it received the Grant Decision for the activities of 2021-2022 (partly already performed).

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, further described in clause 3.4.

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 exists of 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. 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)).

Sub-activity 1.3 Proficiency Tests (PTs) and interlaboratory study (ILS)

Objectives:

Evaluation of the performance of the NRLs-*Salmonella* for detection and typing of *Salmonella* by means of interlaboratory comparisons (Proficiency Tests). Determination of the performance characteristics of (draft) ISO/TS 6579-4 by organisation of an interlaboratory study (ILS).

In the coming year, the following ILS and PTs are foreseen:

1. ILS to determine the performance characteristics of draft ISO/TS 6579-4 (identification of monophasic *Salmonella* Typhimurium) is organised from 16 May until 1 July 2022.
2. Detection of *Salmonella* in primary production stage samples (PPS) and in food samples. This study will be a combined study for NRLs-*Salmonella* analysing PPS samples as well as food samples.

The matrix under analysis will be hygiene swabs and the PT will be organised in September-October 2022.
3. Typing of *Salmonella* (serotyping, molecular typing). This study is foreseen for November 2022 and will include serotyping of *Salmonella* (obligatory) and a part on cluster analysis (with WGS).
4. Another PT on detection of *Salmonella* in food/feed samples is likely to be organised in March 2023.

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.

The next workshop will probably be organised at the end of May 2023 and, if possible, it will be tried to organise this workshop again as a physical meeting.

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. NRLs which individual trainings were postponed due to the pandemic and for which the request still stands and/or NRLs with a new training request, are advised to contact the EURL to try to make a new planning;
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 the other 7 EURLs of the joint EURLs WG NGS. The first joint EURLs training was organised in 2019 and the next training is organised in June 2022 (in Rome, Italy). If possible, and if desired, another joint EURLs training on NGS will be organised in 2023 (location still unknown).

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 (https://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 (WGS), and analysis of data. In 2021, EURL-*Salmonella* took part (to a certain extent) in 7 events/outbreak investigations.

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, for use in PTs; for testing, validation, verification of methods; to be provided to NRLs for specific tests;
- 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.

Discussion

Q: Will the EURL-*Salmonella* organise a PT for detection of *Salmonella* in shellfish in 2023?

A: We do not know yet, but it may be likely that we do not organise such PT before 2024. This year (2022) we do not organise a PT dedicated to the detection of *Salmonella* in a food or feed matrix, but we organise a combined PT for PPS and food samples. For that reason it is likely that in 2023 we will organise a PT with a more general food/feed matrix and perhaps one year later for a specific food matrix like shellfish.

4 Evaluation of the workshop

4.1 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 95. In total, 59 participants completed the evaluation form, a response rate of 62%.

In section 4.2, the scores for 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 4.1 shows that the majority of respondents scored the information given in advance of the workshop as very good (score 5).

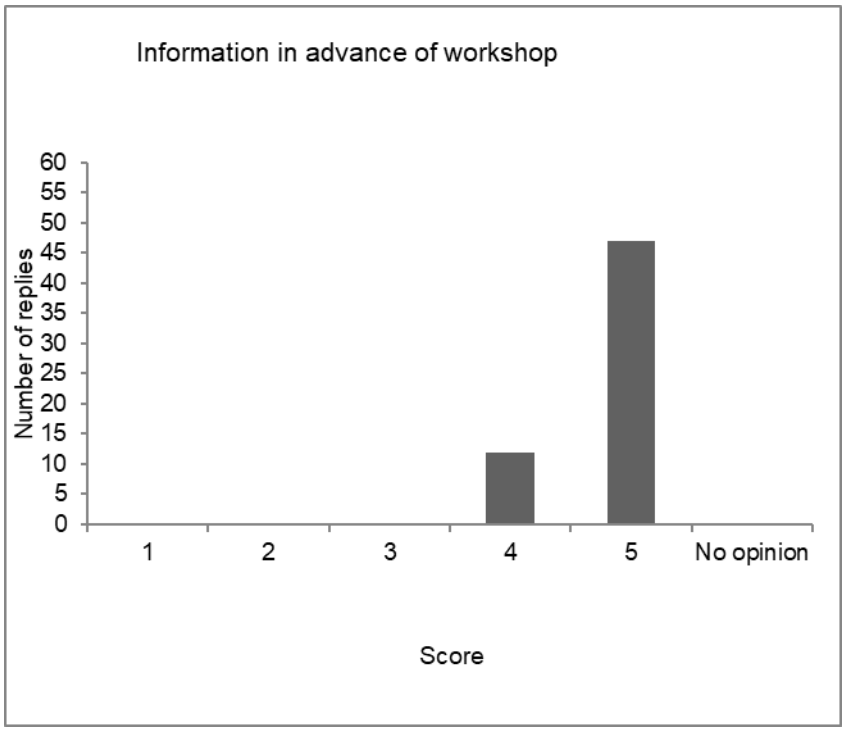

Figure 4.1 Scores given to question 1 'Opinion on information given in advance of the workshop'

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 4.2).

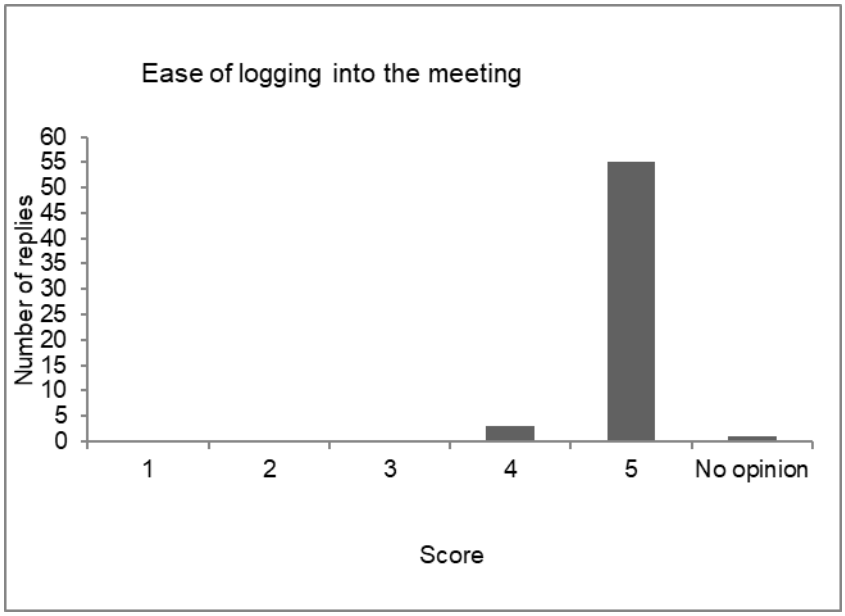

Figure 4.2 Scores given to question 2 'Opinion on the ease of logging into the meeting'

3. Did you face any technical problems during the meeting?
One respondent reported a technical problem during the meeting (see Figure 4.3), as the chat was not available.

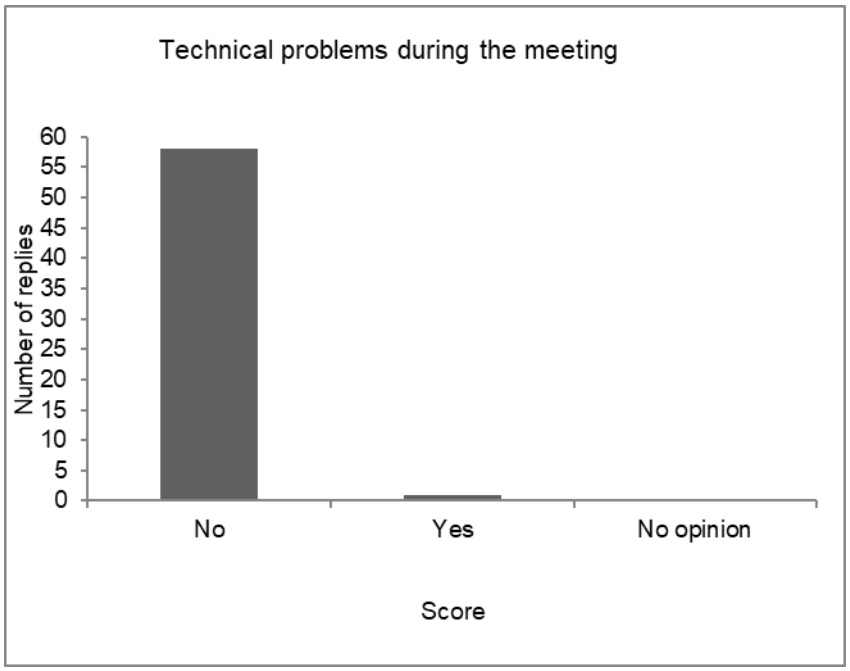

Figure 4.3 Replies given to question 3 'Did you face any technical problems during the meeting?'

4. What is your opinion on the length of the meeting and the number of breaks?
55 of the 59 respondents considered the length of the meeting to be fine (Figure 4.4a) and 54 respondents considered the number of breaks to be fine (Figure 4.4b). The following remarks were made: 'Would prefer not to continue during lunch time'; 'The time for breaks need to be longer'; 'Having the meeting over two half days was very good. The number of breaks was fine, but one of them could perhaps have been 20 minutes?'; 'Breaks of 15-20 minutes maximum acceptable'; 'Two breaks would have been nice'.

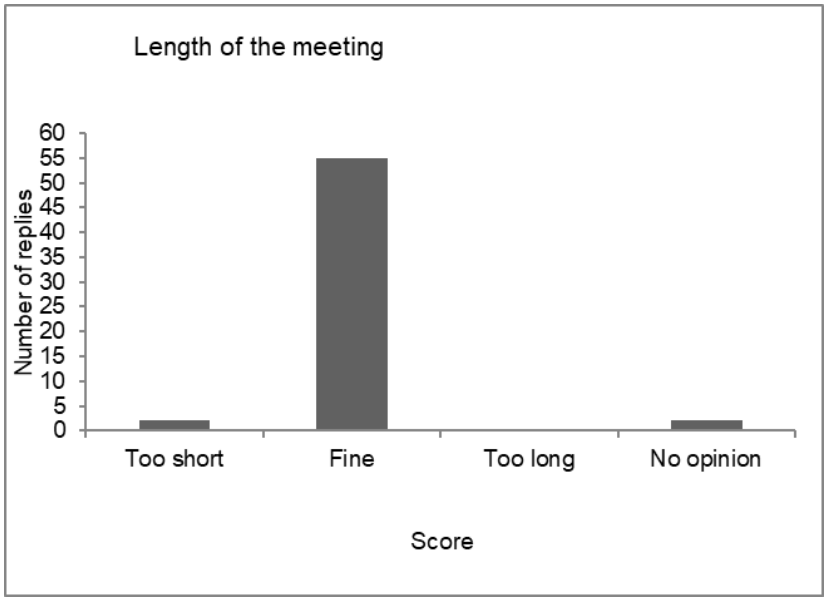

Figure 4.4a Replies given to question 4a 'What is your opinion on the length of the meeting?'

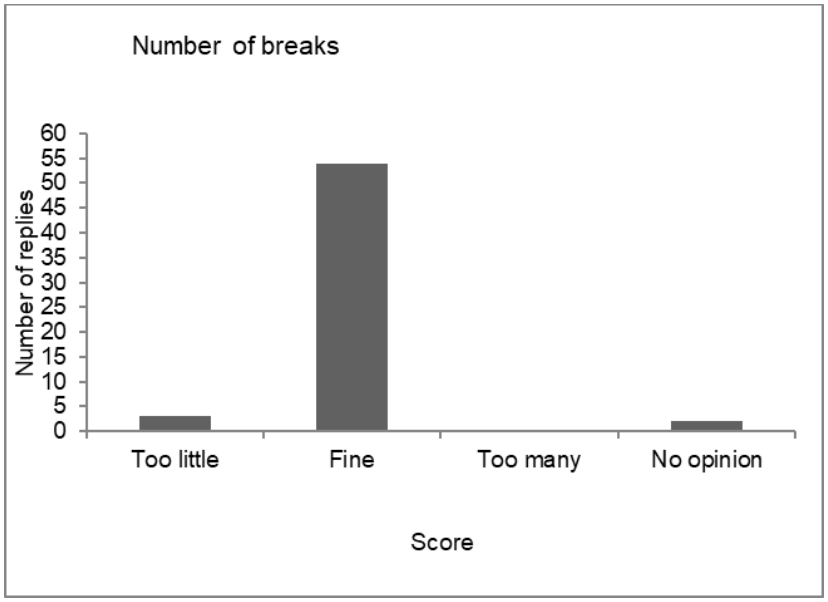

Figure 4.4b Replies given to question 4b 'What is your opinion on the number of breaks?'

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)?
52 of the 59 respondents were satisfied with the options for raising questions, two respondents were not satisfied and five had no opinion (Figure 4.5). The following remarks were made: 'Virtual attendance does not give an opportunity for further discussion'; 'We were able to both raise hands and ask in the chat which was very good, but it is more difficult to get a proper discussion during a virtual meeting (this applies to any virtual meeting)'; 'To write in the chat is always boring, I would prefer the raise hands option'.

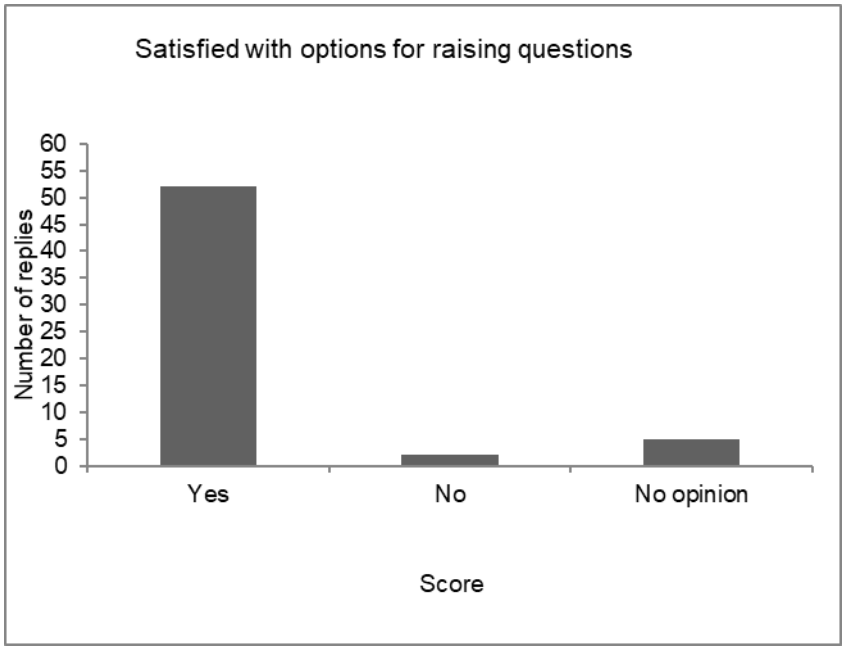

Figure 4.5 Scores given to question 5 'Were you satisfied with the options for raising questions during the meeting?'

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 4.6. The following remarks were made: 'When we are back to physical meetings, I would guess there would be more time for short scientific presentations from countries, and not only how they are carrying out the NRL tasks'; 'Very interesting, also in parts that do not directly concern our laboratory'; 'A lot of information in a short time limit'.

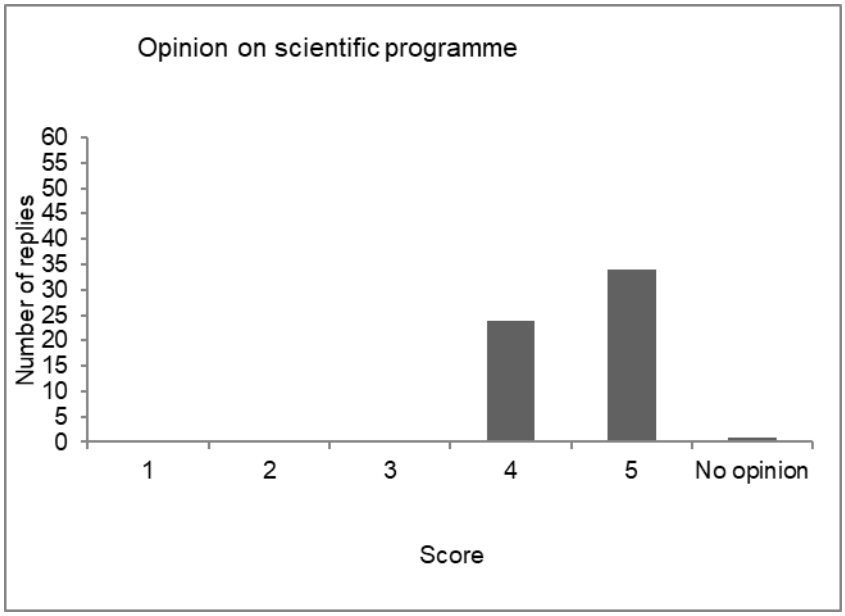

Figure 4.6 Scores given to question 6 'What is your opinion on the scientific programme of the workshop?'

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 presentation was about *Salmonella* vaccination in poultry and tests to differentiate between vaccine and field strains.'
- 'Would welcome recommendations on how best to perform cluster analysis.'
- 'I think the presentations from EFSA on day 2 were interesting. I missed a little bit more focus on *Salmonella* in foods. The tahini/halva outbreak could perhaps have been explored a little more in detail, with presentations from countries working with it.'
- 'A summary of the presentations or the sending of the ppt slides themselves would be very helpful for later reading.'
- 'Noticed some slides had information not in English.'
- 'All presentations were educational, practical and very useful.'
- 'I missed some information, because I had to answer incoming calls, to receive samples and to proceed to the daily laboratory routine duties.'
- 'For the future it would be interesting to hear about the bioinformatical pipelines for WGS installed in the labs, and their experiences.'

8. What is your general opinion of the workshop?
All respondents indicated that the workshop as a whole had been good (4) or very good (5), see Figure 4.7. Remarks given were: 'It is good to have workshops focussing on one issue, as in this case *Salmonella'*

Figure 4.7 Scores given to question 8 'What is your general opinion of the workshop?'

9. This years' workshop was intended to be organised as a hybrid meeting. Unfortunately the number of in person registrations was too limited to justify such meeting. If applicable, can you please inform us what would have prevented your from in person participation?
It was possible to give more than one reply to this question and in total 62 replies were obtained. All different options which would have prevented one from in person participation were indicated, with a majority (>10 replies) for 'I did not want to travel due to measures taken for the SARS-CoV-2 pandemic' (12 replies), 'I prefer virtual meetings' (13 replies) and 'I was not able to travel due to other reasons' (15 replies). The other options indicated were: 'I did not want to travel due to the war in Ukraine' (9 replies), 'My organisation does not allow me to travel when it is also possible to participate virtually in a meeting' (4 replies), and 'other' (9 replies). The results are also shown in Figure 4.8.

The following remarks were made:

- 'I'm not the official representant for my country. This virtual workshop gives other people the opportunity to participate (2).'
- 'My intention was to participate physically (4).'
- 'In a virtual (or hybrid) meeting, more than one person from each institute can participate, while for a physical meeting perhaps only one person is able to travel.'
- 'My organisation does allow only one person to travel because of the limited refund. Participation of other colleagues would be possible via a virtual meeting.'
- 'Through hybrid participation, the number of participants from our laboratory could be more and so the information shared was more fruitful through direct participation.'
- 'Perhaps next time you can start with registration for in person participation only and a few weeks later open the virtual opportunity.'

- ‘I prefer live meetings, because you are free to discuss more than in an online workshop.’
- ‘I was not able to travel due to the tight financial situation (only one participant covered by EU).’

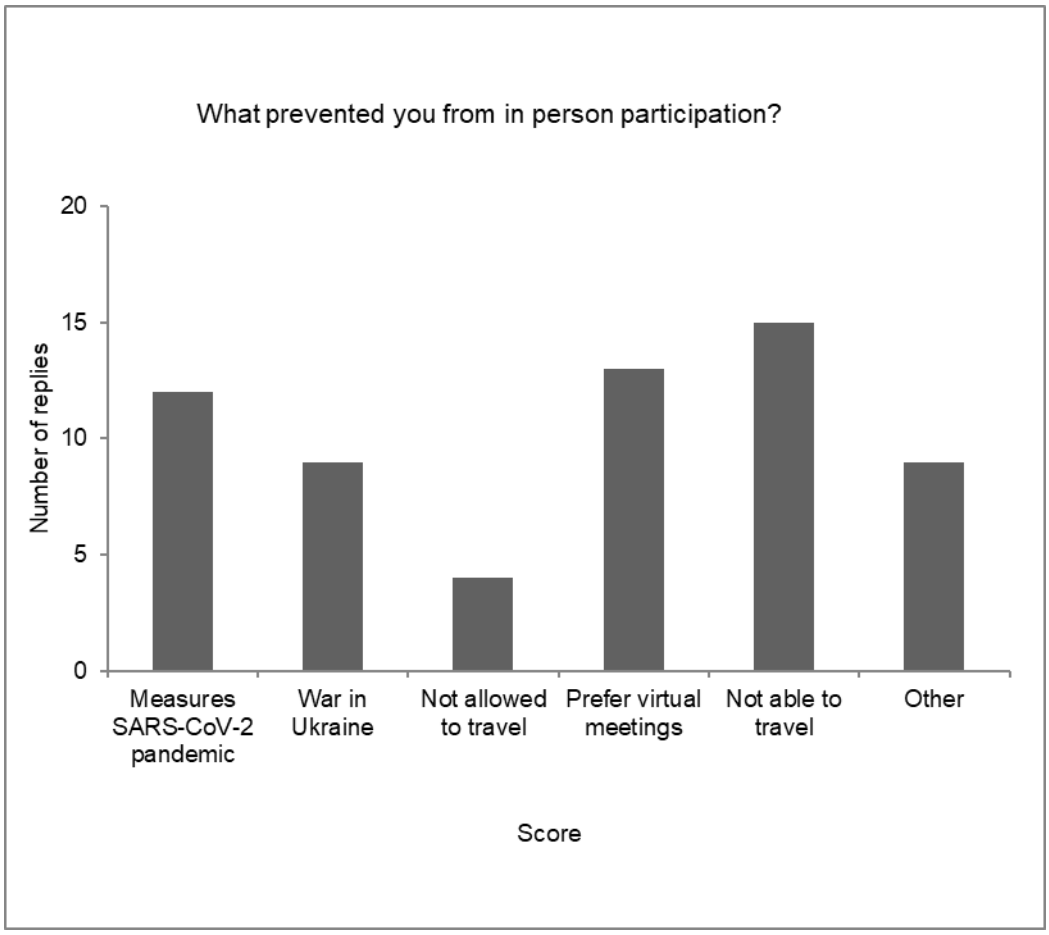

Figure 4.8 Replies given to question 9 ‘If applicable, can you please inform us what would have prevented your from in person participation?’

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:

- ‘To my opinion the workshop was good and interesting (4).’
- ‘One of workshops’ breaks need to be longer than others.’
- ‘Although I wish to have more physical meetings, it is good to have the possibility for hybrid meetings as more persons from different institutions can attend.’
- ‘Group photo compiled and shared with all participants for this 'Total virtual' workshop. For future live/hybrid workshops, it may be necessary to compile two separate group photos.’
- ‘I understand that during the year, various labs seek your assistance and possibly a visit is held on site too. How about having the experience from such laboratories through such a visit be documented through a short recorded video interview with

Head of Section and maybe one or two of the participants (Trainer and Trainee)?'
- 'Thanks for the as always good meeting. The meeting will have definitely a different character if it goes live again.'
- 'I think that online workshops prevent direct communication between participants in lecture breaks and exchange of knowledge and experiences.'

4.3 Discussion and conclusions of the evaluation

For the third time EURL-*Salmonella* organised this workshop as a virtual meeting, although it was originally planned to organise this years' workshop as a hybrid meeting (in person participation in combination with online participants). However, the number of in person registrations was too limited to justify such meeting. It was interesting to learn what prevented the NRLs from in person participation, although it was not possible to indicate one major reason why participants did not want to travel. It was frequently remarked that the advantage of a virtual meeting is the fact that more/other people can participate. However, a disadvantage of virtual meetings frequently indicated, was the lack of discussions/interactions compared to physical meetings.
Still, the participants were satisfied with the organisation, the technical aspects, and with the scientific programme of this third online EURL-*Salmonella* workshop.

Acknowledgements

The success of the workshop depends for a large part on the input of several colleagues. Especially the help of the staff members of the EURL-*Salmonella* is very valuable and highly appreciated. The author would, therefore, like to thank the following persons.
Simone Severs-Mommers for all her efforts to find a suitable meeting room for a hybrid meeting, as well as an affordable hotel for the in person participants. And after having it all (almost) organised, to optimistically cancel it again and switch to an (full) online meeting. She is also thanked for her help with the administration and for the technical hints for the online meeting.
Robin Diddens for his valuable help for a smoothly sharing of the presentations.
Wilma Jacobs-Reitsma for giving interesting presentations, for taking care of the slide shows during the breaks, for making the nice group picture and for publishing all presentations and photo's at the EURL-*Salmonella* website after the workshop.
Irene Pol-Hofstad for her input to the organisation of the workshop and for giving an interesting presentation.

Of course also all other colleagues which gave an interesting presentation are thanked. Without their input the workshop could not have been a success.

Thank you all very much!

List of abbreviations

A	Answer
AHG	Ad hoc group
BPW	Buffered peptone water
CD	Committee draft
CEN	European Committee for Standardization
CEN/TC463	European Committee for Standardization, Technical committee 463 - Microbiology of the food chain
cfu	colony forming units
cgMLST	core genome multi-locus sequence typing
DG SANTE	Directorate-General for Health and Food Safety
DIS	Draft International Standard
DTS	Draft International Technical Specification
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
FBO	Foodborne outbreak
HaDEA	European Health and Digital Executive Agency
ILS	Interlaboratory study
ISO	International Organization for Standardization
ISO/TC34/SC9	International Organization for Standardization, Technical Committee 34 on Food Products, Sub-committee 9 – Microbiology of the food chain
JNS	Joint notification summary
LOD	Level of detection
MALDI-TOF	Matrix assisted laser desorption/ionisation time-of-flight analyser
MKTTn	Muller-Kauffmann tetrathionate-novobiocin broth
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
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
SNP	Single-nucleotide polymorphism
TAG	Task group

TC	Technical Committee
TS	Technical Specification
UK	United Kingdom
WG	Working group
WGS	Whole Genome Sequencing
WHO	World Health Organization
WKLM scheme	White-Kauffmann-le Minor scheme

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Annex 1 Participants

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European Food Safety Authority (EFSA)	Frank Boelaert Valentina Rizzi Mirko Rossi Eleonora Sarno
EURL-*Salmonella* (and NL NRL-*Salmonella*)	Robin Diddens Angela van Hoek Wilma Jacobs-Reitsma Kirsten Mooijman Irene Pol-Hofstad Maaike van den Beld Anjo Verbruggen Kim van der Zwaluw

Guest speakers

The Netherlands	Annet Heuvelink (Royal GD, Deventer) Christiaan ter Veen (Royal GD, Deventer)

National Reference Laboratories for *Salmonella*

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BELGIUM	Maria Cristina Garcia Graells Inge van Damme (Northern Ireland representative) Marie Polet
BOSNIA HERZEGOVINA	Amira Koro Emina Residbegovic Edina Turan
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LUXEMBOURG	Dominique Claude
	Catarina Martins
MALTA	Stephen Borg
	Gertrude Gatt Lanzon
	David Sammut
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	Magdalena Zajac
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	Monica Vanghele
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	Iria Uhia
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	Erik Eriksson
SWITZERLAND	Sonja Kittl
	Gudrun Overesch

Annex 2 Programme 27th EURL-*Salmonella* workshop; 23 and 24 May 2022 - Online

Monday 23 May 2022

09:30 - 10:00	Opening and introduction	Kirsten Mooijman EURL-*Salmonella*
10:00 - 10:30	EU monitoring of *Salmonella* and of salmonellosis foodborne outbreaks in 2020	Frank Boelaert EFSA
10:30 - 10:45	*Break*	
10:45 - 11:15	Results EURL-*Salmonella* Proficiency Test Primary Production Stage 2021 – Detection of *Salmonella* in chicken faeces adhering to boot socks	Irene Pol-Hofstad EURL-*Salmonella*
11:15 - 11:45	Results EURL-*Salmonella* Proficiency Test Typing 2021 – serotyping and cluster analysis	Wilma Jacobs-Reitsma EURL-*Salmonella*
11:45 – 12:00	*Break*	
12:00 - 13:00	Activities NRLs to fulfil tasks and duties	
12:00 - 12:20	NRL-*Salmonella* Sweden	Erik Eriksson
12:20 - 12:40	NRL-*Salmonella* Norway	Bjarne Bergsjø and Julia Storesund
12:40 -13:00	NRL-*Salmonella* Belgium	Cristina Garcia Graells and Inge van Damme

---- End day 1 ----

Tuesday 24 May 2022

09:30 - 09:55	Assessment of multi-country food borne outbreak events at EU level	Eleonora Sarno, EFSA
09:55 - 10:15	Status of progress and timelines of the new EFSA One Health WGS system	Mirko Rossi EFSA
10:15 – 10:45	25 years of *Salmonella* serotyping Proficiency Tests	Wilma Jacobs-Reitsma EURL-*Salmonella*
10:45 - 11:00		*Break*
11:00 - 11:30	Update on activities in ISO and CEN	Kirsten Mooijman EURL-*Salmonella*
11:30 – 12:15	*Salmonella* vaccination in poultry and tests to differentiate between vaccine and field strains	Christiaan ter Veen and Annet Heuvelink, Royal GD the Netherlands
12:15 - 12:45	Work programme EURL-*Salmonella* second half 2022, first half 2023 Concluding remarks workshop and closure	Kirsten Mooijman EURL-*Salmonella*

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

Annex 3 Workshop evaluation form

Evaluation of the 27th EURL-*Salmonella* workshop,
23 and 24 May 2022 - online

We would highly appreciate if you could give us your opinion on the 27th EURL-*Salmonella* workshop, organised as online meeting on 23 and 24 May 2022. Thank you very much in advance for completing the questionnaire by 10 June 2022 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?
   a. Length meeting:
      - ☐ Too short
      - ☐ Fine
      - ☐ Too long
   b. 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. It was our intention to organise this years' workshop as hybrid meeting. Unfortunately the number of in person registrations was too limited to justify such meeting. *If applicable,* can you please inform us what would have prevented you from in person participation? (*more than one answer possible*)
   ☐ I did not want to travel due to measures taken for the SARS-CoV-2 pandemic
   ☐ I did not want to travel due to the war in Ukraine
   ☐ My organisation does not allow me to travel when it is also possible to participate virtually in a meeting
   ☐ I prefer virtual meetings
   ☐ I was not able to travel due to other reasons
   ☐ Other _____

Remarks:_____

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

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