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

The 24th EURL-*Salmonella* workshop

28 and 29 May 2019, Amersfoort, the Netherlands

RIVM Report 2019-0135

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Colophon

DOI 10.21945/RIVM-2019-0135

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This investigation was performed by order, and for the account, of European Commission, Directorate-General for Health and Food Safety (DG SANTE), within the framework of RIVM project E/114506/19 European Union Reference Laboratory for *Salmonella* (2019)

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

Synopsis

The 24th EURL-*Salmonella* workshop
28 and 29 May 2019, Amersfoort, the Netherlands

This report gives a summary of the presentations held at the 24th annual workshop for the European National Reference Laboratories (NRLs) for *Salmonella* (28–29 May 2019). The aim of the workshop was to facilitate the exchange of information on the activities of the NRLs and the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*).

Annual Proficiency Tests
A recurring item at the workshops is the presentation of the results of the annual Proficiency Tests organised by the EURL. These provide information on the quality of the participating NRLs tested. The NRLs had high scores in the 2018–2019 studies; detailed information on the results per Proficiency Tests is available in separate RIVM reports.

***Salmonella* in food and animals**
Salmonella should not be present in food and animals. However, the bacterium is occasionally found in various products. Examples were given of *Salmonella* found in poultry, animal feed, fresh edible leaves, and shellfish. Other information presented at the workshop included the application of Whole Genome Sequencing, a relatively new technique, for characterisation of *Salmonella*.

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

Keywords: EURL-*Salmonella*, NRL-*Salmonella*, *Salmonella*, workshop 2019

Publiekssamenvatting

De 24e EURL-*Salmonella* workshop
28 en 29 mei 2019, Amersfoort, Nederland

Het RIVM heeft de verslagen gebundeld van de presentaties van de 24e jaarlijkse workshop voor de Europese Nationale Referentie Laboratoria (NRL's) voor *Salmonella* (28-29 mei 2019). Het doel van de workshop is dat het overkoepelende orgaan, het Europese Referentie Laboratorium (EURL) voor *Salmonella*, en de NRL's informatie uitwisselen.

Een terugkerend onderwerp zijn de ringonderzoeken die het EURL jaarlijks organiseert om de kwaliteit van de NRL-laboratoria te controleren. De NRL's scoorden goed in de studies van 2018-2019. In dit rapport staan de ringonderzoeken kort beschreven. Een uitgebreidere weergave van de resultaten wordt per ringonderzoek gepubliceerd.

Salmonella mag niet in voedsel en dieren zitten. Toch wordt de bacterie soms gevonden in verschillende producten. Zo is *Salmonella* aangetroffen in pluimvee, diervoeder, eetbare bladeren en schelpdieren. Andere informatie gaat over het gebruik van Whole Genome Sequencing, een relatief nieuwe techniek, om *Salmonella* gedetailleerd te karakteriseren.

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

Kernwoorden: EURL-*Salmonella*, NRL-*Salmonella*, *Salmonella*, workshop 2019

Contents

Summary

On 28 and 29 May 2019, the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*) held its annual workshop in Amersfoort, the Netherlands. Participants in the workshop were representatives of the National Reference Laboratories (NRLs) for *Salmonella* from 27 European Union (EU) Member States, three European Free Trade Association (EFTA) countries, and four (potential) EU candidate countries. Also present was a representatives of the European Food Safety Authority (EFSA). A representative of the NRL-*Salmonella* of one EU Member State was unable to join the workshop. A total of 51 participants attended the workshop.

During the workshop, presentations were given on several topics. The results of the Proficiency Tests (PTs) organised by the EURL-*Salmonella* in the past year were presented, namely the PT on detection of *Salmonella* in boot socks containing chicken faeces (October 2018), the PT on detection of *Salmonella* in flaxseed samples (March 2019), and the PT on *Salmonella* typing (November 2018).

The EFSA representative gave a presentation on the recent stalling in the reduction of human *Salmonella* infections and assessed the current EU reduction targets. Additionally, the EFSA representative gave an update on the joint European Centre for Disease Prevention and Control (ECDC)–EFSA molecular typing database and on the findings of the EFSA–ECDC working group on Whole Genome Sequencing (WGS).

Three presentations dealt with the detection of *Salmonella* in different products. A representative of the NRL-*Salmonella* of the United Kingdom (UK) gave a presentation on the detection of *Salmonella* in (imported) fresh edible leaves; a representative of the NRL-*Salmonella* of Germany gave a presentation on the detection of *Salmonella* in animal feed; and a staff member of the EURL-*Salmonella* gave a presentation on *Salmonella* in bivalve molluscs. The investigation of bivalve molluscs has become part of the work package of the EURL/NRL-*Salmonella* network since the EURL for monitoring bacteriological and viral contamination of bivalve molluscs ceased to exist (on 01/01/2019).

A guest speaker from Wageningen Food Safety Research in the Netherlands gave a presentation on the rapid detection of *Salmonella* species, *Salmonella* Typhimurium, and *Salmonella* Enteritidis by multiplex real-time PCR.

In two presentations information was given on multi-country events. A representative of the NRL-*Salmonella* of the Czech Republic gave a presentation on a multi-country outbreak of *Salmonella* Bareilly, and a staff member of the EURL-*Salmonella* gave a presentation on a multi-country cluster of *Salmonella* Coeln.

Additionally, a representative of the NRL-*Salmonella* of the UK gave a presentation on WGS-based typing of *Salmonella* spp. and molecular analyses, and a staff member of the EURL-*Salmonella* gave a

presentation on recent activity related to the standardisation of microbiological methods in ISO and CEN.

Five NRL-*Salmonella* representatives (Denmark, Italy, France, Latvia, and Switzerland) gave a summary of their activities.

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

The workshop presentations can be found at:
https://www.eurlsalmonella.eu/en/workshop-2019

1 Introduction

This report includes the abstracts of the presentations given at the 2019 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 (subject to publication permission): https://www.eurlsalmonella.eu/en/workshop-2019

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

2 Tuesday 28 May 2019: 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 24th workshop of the EURL-*Salmonella*, welcoming all participants to Amersfoort, the Netherlands. At this workshop, 51 participants were present, including representatives of the National Reference Laboratories (NRLs) for *Salmonella* from 27 EU Member States, four (potential) candidate EU countries, and three member countries of the European Free Trade Association (EFTA). A representative of the European Food Safety Authority (EFSA) also attended the workshop. Apologies were received from the representative of the NRL-*Salmonella* in Malta.

The evaluations of the last eight workshops (2011–2018) were compared. The opinion on the scientific programme was the same in all workshops: very good to excellent.

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

2.2 The stalled *Salmonella* situation in the EU and assessment of current EU reduction targets

Frank Boelaert, EFSA, Parma, Italy

The stalled *Salmonella* situation (EFSA and ECDC, 2018)
In 2017, 91 662 confirmed human salmonellosis cases were reported in the EU by all the Member States (MS). The EU notification rate was 19,7 cases per 100 000 population and was slightly below (2,9% decrease) the value of 2016 (20,4 cases per 100 000 population). A statistically significant decreasing trend of confirmed salmonellosis cases has been observed in the EU/EEA between 2008 and 2017 (taking into account the 25 countries that reported consistently during this period). However, during the last 5 years (2013–2017) the overall EU/EEA trend has not shown any statistically significant increase or decrease. Seven MS reported an increasing trend and four MS a decreasing trend over the period 2013–2017.

The top five most commonly reported serovars in human cases acquired in the EU during 2017 were, in decreasing order: *S*. Enteritidis, *S*. Typhimurium, monophasic *S*. Typhimurium, *S*. Infantis and *S*. Newport.

The proportion of human salmonellosis illnesses due to *S.* Enteritidis continued to increase in 2017, whether considering all cases or only cases infected in the EU. This was mainly due to one large MS starting to report case-based serovar data. When excluding this MS, the proportion was at the same level as in 2016. The data reported on food and animals showed that *S*. Enteritidis was mainly associated with laying

hens, and secondarily with broiler meat. Between 2012 and 2017 a similar evolution was observed in the proportion of *S*. Enteritidis illnesses in humans acquired in the EU and the EU flock prevalence of *S*. Enteritidis in laying hens.

The proportions of human salmonellosis illnesses acquired within the EU due to *S*. Typhimurium, monophasic *S*. Typhimurium, and *S*. Infantis decreased compared with 2016, whereas the proportion of illnesses due to *S*. Newport remained unchanged. According to the reports of distinct serovars from food-producing animals, *S*. Typhimurium was isolated to different extents from almost all food-animal sources analysed; for the monophasic variants of *S.* Typhimurium the strong association with the pig chain was confirmed and this group was also related to the broiler chain. *S*. Infantis was markedly associated with broiler flocks and meat. Finally, *S*. Newport was associated with both turkey and broiler sources. *Salmonella* was the most frequently reported causative agent in the EU (1 241 foodborne outbreaks and no waterborne outbreaks; 24,4% of total outbreaks, 25 MS). Outbreaks of salmonellosis had the highest impact in terms of human cases (9 600, 22,1% of all outbreak cases), hospitalisations (2 227, 49,0% of all hospitalisations) and deaths (11, 33,3% of all deaths). *S*. Enteritidis was by far the most frequently reported *Salmonella* serovar, accounting for 61,1% (N=758, 23 MS) of *Salmonella* foodborne outbreaks (FBOs), corresponding to 14,9% (about one in seven) of all reported FBOs at the EU level. Two MS (Poland and Slovakia) together accounted for the 63,3% of all outbreaks caused by this serovar in the EU.

For 2017, and different from previous years, only the single sample results collected by Competent Authorities and labelled as objective sampling were summarised from the food monitoring data reported by the EU MS according to Regulation (EC) No 2073/2005 (EC, 2005) on microbiological criteria. These data guarantee a satisfactory level of harmonisation for future trend watching. However, data were too scarce and unrepresentative to describe the EU-level situation. In general, the highest number of *Salmonella*-positive units was reported for meat categories intended to be eaten cooked. Process hygiene criterion monitoring data related to *Salmonella* on pigs' carcases were reported by eight MS, with samples taken both by the Competent Authorities (official control samples) and by the food business operators (self-monitoring). For seven of these MS the estimated number of *Salmonella*-positive samples from self-monitoring was significantly lower than of the official control samples.

At primary production level, in the context of the National Control Programmes, the EU-level flock prevalence of target *Salmonella* serovars in breeding hens, laying hens, broilers, and fattening turkeys decreased or remained unchanged compared with 2016, whereas in breeding turkeys it slightly increased due to the presence of *S*. Typhimurium. This last finding seems to be related to the situation in few MS. The analyses of the time trends, since the implementation of the National Control Programmes from 2007 to 2010, showed an overall decreasing prevalence of flocks remaining positive to target *Salmonella* serovars in all poultry species, with the exception of breeding turkeys, where a stationary trend with minor fluctuations was observed.

Moreover, there was an increasing prevalence of *Salmonella*-positive flocks in all poultry categories. This increase seemed to be related to the increased reporting of non-target serovars. Still in the context of National Control Programmes (broilers, and fattening and breeding turkeys) the prevalence of *Salmonella*-positive flocks based on official control samples taken by the CAs was generally higher than that resulting from sampling by FBOs. These differences were more evident for some MS.

Assessment of current EU reduction targets (EFSA, 2019)
The annual number of reported confirmed human salmonellosis cases in the EU increased after 2014. This triggered an investigation of two potential *Salmonella* control options in poultry flocks and their potential public health impact by (a) changing the target serovars in breeding hens (Enteritidis, Typhimurium, Infantis, Virchow, and Hadar) while maintaining the current EU target (1%) and (b) reducing the target for laying hens for *S.* Enteritidis and *S.* Typhimurium from 2% to 1%.

Investigation (a) was carried out by analysing the annual serovar data in breeding, laying, and broiler flocks reported by MS in 2014–2016. For (b) a '*Salmonella* source attribution model' was developed using 2016 data of 28 serovars from 23 MS considering different food and animal sources (EFSA data, laying hens, broilers, turkeys, and pigs) attributing to human *Salmonella* infections (ECDC data).

A modification in the list of target serovars in breeding hens is expected to be effective in reducing the salmonellosis burden at EU level, but it was not possible to quantitatively assess this impact.

There were an estimated 4,08 million CrI_{95}[2,22; 7,39] true cases of human salmonellosis of which 11,7% CrI_{95}[5,7; 22,2] or 465 200 cases CrI_{95}[212 100; 979 800 cases] was attributed to laying hens; 41,5% CrI_{95}[23,1; 59,7] to pigs, 24,9% CrI_{95}[11,1; 45,7] to broilers and 7,5% CrI_{95}[4,7; 10,6] to turkeys. If the target in layers was set at 1%, it was estimated that the number of human cases attributable to this origin would be reduced by 53,38%, meaning an overall estimated true cases reduction by 6,2%.

A stricter target for serovars in laying hens could benefit public health by reducing human cases of this origin by a half. More complete data from MS would facilitate more precise impact analyses.

Discussion
Q: Would it be better to control for all *Salmonella* serovars instead of a 'top 5'?
A: It is up to the risk managers to determine whether this is economically feasible. The control should at least include the *Salmonella* serovars most relevant to public health.
Q: Is the decrease of *Salmonella* in poultry due to the introduction of vaccination against *Salmonella* Enteritidis and *Salmonella* Typhimurium?
A: How FBOs use interventions is not part of the EU zoonoses summary report. It is up to the Competent Authorities of the relevant countries to determine control measures. EFSA does not have the necessary data to analyse the efficacy of vaccination.

2.3 *Salmonella* Agona in animal feed in Germany 2018

Istvan Szabo, NRL-Salmonella, Berlin, Germany

At the end of 2017 and the beginning of 2018 several 'events' involving *Salmonella* Agona occurred, including a multi-country outbreak of *Salmonella* Agona, possibly linked to ready-to-eat food.
In December 2017, another outbreak with *Salmonella* Agona occurred. This concerned infections among infants reported in France, where the outbreak was linked to internationally distributed infant milk products.

During the same period, several RASFF notifications reported *Salmonella* Agona detection in different matrices in Germany, including:

- *S*. Agona in marigold from Germany ('with raw material from Egypt' according to the RASFF notification).
- *S*. Agona in rapeseed cake produced in Schleswig-Holstein, northern Germany.
- *S*. Agona in soybean meal produced in Bavaria, southern Germany.

Feed produced from rapeseed cake and soybean meal was fed to different herds and flocks all over Germany before the withdrawal of all implicated feed lots. The detection of S*almonella* in feeding stuffs attracted considerable attention in the German animal and food production chain. If *Salmonella* contamination is found in animal feed which has been placed on the market (including storage for sale), measures has to be taken in accordance with Article 20 of Regulation (EC) No 178/2002 (EC, 2002).

As a result of the unusual high reporting rates of *Salmonella* Agona in different matrices originating from Germany and in outbreaks, the German NRL-*Salmonella* carried out an epidemiological investigation using the NGS platform, searching for an epidemiological link between the different *S*. Agona events. The NRL asked the Federal States of Germany to provide all *S*. Agona strains that had been isolated since July 2017. Subsequent WGS SNP-based cluster analysis of *S*. Agona isolates from 2018 and from the strain collection of the NRL revealed no genetic relationship between those isolates and any of the *S*. Agona occurrences/outbreaks described. Moreover, no genetic link between the two *S*. Agona cases from rapeseed cake and soybean meal could be observed. Based on these analyses, however, we observed a turkey-associated *S*. Agona cluster in these isolates that seems to persist in German poultry livestock.

Discussion
Q: Did the soya beans originate from Germany or were they imported?
A: The soya beans were imported, but the rapeseed originated from Germany.
Remark Norway: The soya beans we import are generally positive for *Salmonella*. Rapeseed cake is made from oil pressing and is heat treated. However, there should be strict separation between the un-heated and the heat-treated products to avoid (cross-) contamination of the heat-treated products. In Scandinavian countries it is compulsory to heat treat feed for food-producing animals.

Q: Is *Salmonella* Agona able to persistently colonise systems and food-producing animals? Has *S.* Agona been present for a long time in Germany?
A: We found *S.* Agona only in this specific case in animal feed. We did not find it in the primary production. So in our case it does not seem to be so stable in the food-producing animal chain.
Q: Do you sequence all isolates in Germany?
A: We currently sequence all *Salmonella* Enteritidis isolates and we will extend this to other serovars in the future. Additionally we sequence when there is a problem to investigate, e.g. in the event of outbreaks and in the case of the persistence of *Salmonella* Infantis in broilers.

2.4 *Salmonella* contamination of (imported) fresh edible leaves

Marie Anne Chattaway, NRL-Salmonella, London, United Kingdom

Public Health England receives approximately 9 000 isolates each year, including isolates from food samples. In 2011, high levels of *Salmonella* contamination were found in imported leaves (Bangladesh 24%, India 7%), which led to a ban on imports of betel leaves from Bangladesh and a request for India and Thailand to give sampling results before import was permitted. Analysis of leaves was performed by the food, water and environment (FW&E) laboratory in Birmingham.

Since 2011 over 400 isolates have been serotyped and since 2014 WGS has been performed (on 55 isolates). Studies show that rates of contamination are reduced from the border inspection posts when sampled at the retail level. The *Salmonella* serovars are very heterogeneous and only two human cases were within 5 SNPs of the 44 *S*. *enterica* isolates. An MSc project looked at the transmission of all imported leaves and found potential similarities in EnteroBase using cgMLST of up to 15 alleles from Africa and south Asia. Only three isolates had antimicrobial resistance (two with multi-drug resistance). The strains that caused the *S*. Agona outbreak in a street spice festival in 2013 are still being found in sporadic cases and food projects globally.

In 2017, an outbreak of gastroenteritis in England attributed to *Salmonella* Adjame was detected and investigated. With the introduction of WGS for microbial typing, methods for comparing international outbreak data require evaluation. An outbreak case was defined as a person resident in England with a clinical sample between 1 June 2017 and 27 July 2017 from whom *S*. Adjame was isolated. Cases were interviewed and exposures analysed. Backward tracing of food provenance was undertaken. WGS was performed on isolates from cases and historical isolates and compared using Public Health England's SnapperDB high-quality SNP pipeline and EnteroBase's *Salmonella* cgMLST scheme. In total, 14 cases were identified. The majority were vegetarian, probably of South Asian descent, with a median age of 66.5 years with no recent international travel reported. Cases consumed a range of fresh food products including herbs and spices bought from South Asian grocers. Backward tracing did not identify a common source. WGS typing showed sub-clustering and considerable genetic variation across human samples. cgMLST allele-based analysis was comparable to SNP-derived phylogenetic analysis and clusters were

defined using each method. Imported herbs or spices were suspected vehicles. The cases were linked in time and place but WGS showed marked heterogeneity, atypical of a point source *Salmonella* outbreak. The application of incorporating SNP or allelic differences into the case definition may not always be appropriate. With further validation, cgMLST could be used for international outbreak alerts when WGS analysis is being undertaken to facilitate comparison. Further information can be found at:

- Adjame: https://www.ncbi.nlm.nih.gov/pubmed/30648934
- Street spice outbreak: https://www.ncbi.nlm.nih.gov/pubmed/30109832
- Public health risks with betel leaves: https://www.ncbi.nlm.nih.gov/pubmed/30889473
- Microbiological quality in edible leaves: https://www.ncbi.nlm.nih.gov/pubmed/29802669.

Discussion
Q: Is it feasible to set a threshold for cgMLST for all *Salmonella* serovars?
A: Perhaps this is possible, but we also may need to be flexible and set different thresholds for each outbreak.

2.5 *Salmonella* in bivalve molluscs

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

As a result of the UK voting to leave the European Union, the EURL for Monitoring Bacteriological and Viral Contamination in Bivalve Molluscs could no longer be located at CEFAS (marine research institute) in Weymouth, UK. Moreover, this EURL was one of the last EURLs to be organised around a matrix. Since the EU prefers EURLs to be organised around pathogens, these EURL responsibilities have not been relocated to another laboratory, but have instead been distributed amongst the relevant EURLs for pathogens. EURL-*E. coli* (located in Rome, Italy) is taking over tasks concerning *E. coli* in bivalve molluscs; EURL Foodborne Viruses (located in Uppsala, Sweden) is taking over tasks related to viruses in bivalve molluscs; EURL Marine Biotoxine (located in Vigo, Spain) is taking over tasks related to the monitoring and classification of production areas; and EURL-*Salmonella* is taking over tasks related to *Salmonella* in bivalve molluscs. The *Vibrio* work in shellfish has not yet been reallocated, since there is no corresponding EURL for *Vibrio*.

Bivalve molluscs are filter feeders and grow on phytoplankton present in the production waters. When grown in contaminated waters, they concentrate pathogens in their flesh over time. If they are consumed raw (e.g. oysters) or lightly cooked (e.g. mussels), consumers can become infected.

Most important pathogens in shellfish are viruses, mainly norovirus and hepatitis A. Much research has been carried out towards the development of suitable methods for the detection and quantification of norovirus and hepatitis A virus (HAV), resulting in the publication of CEN ISO/TS 15216-2 for detection in 2013 and EN ISO 15216-1 for quantification in 2017. However, these methods are not suitable for

high-throughput routine analyses. Therefore, *E.coli* is used as an indicator organism for faecal contamination. Food business operators (FBOs) have to take into account that the uptake and removal of *E. coli* by bivalve molluscs differs from that of many pathogens, especially viruses.
Vibrio is an organism that is more widespread in warmer waters with low salinity. Climate changes might make environmental conditions more favourable for the growth of *Vibrio*. *Salmonella* is mostly found in combination with high levels of *E.coli* and is therefore not included in the monitoring plan as a separate parameter. However, FBOs have to check their batches for the presence of *Salmonella* as part of end-product testing requirements.
The EC has laid down a set of rules to ensure the safe production of bivalve molluscs. The most important regulations are EC No 853/2004 (EC, 2004a), laying down specific hygiene rules for food of animal origin, and EC No 854/2004 (EC, 2004b), laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption. The regulations are described in more detail in the Community guide to the principles of good practice for the microbiological classification and monitoring of bivalve molluscs production and relaying areas with regard to regulation No 854/2004.

Shellfish sold on the market must originate from classified production areas. Classified areas have to be monitored regularly for bacteriological contamination, chemical contamination, and marine biotoxins and their producing phytoplankton. The classification of an area is based on microbiological data. The EC has set criteria for *E.coli* for the different classes. For class A areas, 80% of the samples from the monitoring programme must be below 230 cfu *E. coli*/100 g shellfish flesh and fluid (tolerance: 20% of the samples can have a value up to 700 cfu *E. coli*/100 g). Shellfish from class A areas can be eaten raw. In class B areas, 90% of the samples must be below 4 600 cfu *E. coli*/100 g (tolerance: 10% of samples can have a value up to 46 000 cfu *E. coli*/100 g). Shellfish from class B areas must undergo a post-harvest treatment such as depuration or heat treatment. For class C areas, all shellfish samples must be below 46 000 cfu *E. coli*/100 g; these shellfish cannot be depurated but must undergo an inactivation treatment such as high pressure or high temperature.

Up to 2018, the EURL for bivalve molluscs organised 8 Proficiency Tests (PTs) per year (viruses, *E. coli*, *Salmonella*, *Vibrio*). Testing for *E. coli* and *Salmonella* was combined in four PTs: three PTs using lenticules organised in cooperation with Public Health England (UK), and one whole animal matrix PT with *E. coli* and *Salmonella* in bivalve molluscs. From 2019 onwards, EURL-*E. coli* and EURL-*Salmonella* have to take over these PTs in shellfish. The EC is not allocating extra budget for these extra tasks, therefore the EURL-*Salmonella* is considering which regular PT will be replaced by a PT with bivalve molluscs and what the frequency will be. Furthermore, the EURL needs information on which laboratories are responsible for these tasks. The prescribed method for detection of *Salmonella* in bivalve molluscs is EN ISO 6579-1:2017. The preparation of bivalve molluscs is described in EN ISO 6887-3:2017 and additional information on preparing shellfish samples will be published on the EURL-*Salmonella* website.

Discussion
Q: Should the EURL make a recommendation that the responsibility for testing *Salmonella* in bivalve molluscs is part of the tasks of the NRLs-*Salmonella*?
A: It is up to each MS to decide how to arrange this in their country. The CA in an MS can appoint another laboratory to carry out this task. It is also possible to keep the NRL for bivalve molluscs even though the EURL no longer exists. EURL-*Salmonella* has not yet been provided with a list of NRLs performing the analysis of *Salmonella* in bivalve molluscs. We will (again) consult DG SANTE for this. If such a list is not available, we will consult the NRLs for further information.
Q: Do you have information on the sources (humans? animals?) of *E. coli* contamination of bivalve molluscs?
A: This is not clear; *E. coli* can be introduced through sewage from different sources.
Q: Is the quality of the production area based only on the number of *E. coli*?
A: Currently this is indeed the case. Perhaps in the future analysis for norovirus will be added to the quality control, but this may depend on the outcome of the EFSA baseline study. Testing for *Salmonella* must be performed in end products only.

2.6 Results EURL-*Salmonella* Proficiency Test on typing of *Salmonella* (2018) - serotyping and PFGE; Introduction to PT on typing 2019

Wilma Jacobs, EURL-Salmonella, Bilthoven, the Netherlands

In November 2018, the 23rd Proficiency Test (PT) on serotyping and PFGE typing of *Salmonella* was organised by the EURL-*Salmonella*. A total of 36 laboratories participated in this study. These included 29 NRLs-*Salmonella* of the 28 EU Member States, three NRLs of EU-candidate countries, three NRLs of EFTA countries, and one non-European NRL. The main objective of the study was to evaluate whether the typing of *Salmonella* strains by the NRLs-*Salmonella* within the EU was carried out uniformly, and whether comparable results were obtained.
All 36 laboratories performed serotyping, but the results of one new participant were not taken into account in the overall results of the PT, due to its limited set of antisera. A total of 20 obligatory *Salmonella* strains plus one optional *Salmonella* strain from an uncommon type were selected for the study by the EURL-*Salmonella*. The strains had to be typed in accordance with the method routinely used in each laboratory, following the White-Kauffmann-Le Minor (WKLM) scheme (Grimont and Weill, 2007).

The individual laboratory results on serotyping, as well as an interim summary report on the general outcome, were emailed to the participants in February 2019. The O-antigens were typed correctly by 28 of the 35 participants (80%). This corresponded to 98% of the total number of strains. The H-antigens were typed correctly by 23 of the 35 participants (66%), corresponding to 97% of the total number of strains. As a result, 20 participants (57%) gave the correct serovar names to the full set of strains, corresponding to 96% of all strains evaluated.

Apart from some spelling errors in the reporting, a completely correct identification was obtained for 11 *Salmonella* serovars: Enteritidis (S1), Southampton (S3), Hadar (S5), Typhimurium (S6), Derby (S12), Lawndale (S13), Brandenburg (S14), Lagos (S15), 1,4,[5],12:i:- (S16), Chester (S18), and Goldcoast (S19). Strain S11, Cannstatt (1,3,19:m,t:-), clearly gave the most problems. Nine laboratories did not name this strain correctly. In six cases the error was caused by a mistake in the phase 1 H-antigen determination: reporting g,m,t (Kouka) instead of m,t (Cannstatt).

All but five participants tried to serotype strain S21, a *Salmonella enterica* subsp. *salamae* (II). Only a few laboratories did not have access to the required antisera to finalise this (55:k:z_{39}). Historically, serovar 55:k:z_{39} was named Tranoroa, but this serovar name has now been withdrawn from the WKLM scheme (Grimont and Weill, 2007). Serovar names have been maintained only for subspecies of *enterica* serovars. Serovars of the other subspecies of *S. enterica*, as well as those of *S. bongori*, are designated only by their antigenic formula.

At the EURL-*Salmonella* workshop in 2007 (Mooijman, 2007), criteria for 'good' performance by NRLs regarding serotyping were defined. Two participants did not meet the level of good performance at the initial stage of the typing study. A follow-up study was organised in April/May 2019, consisting of 10 additional strains for serotyping. Both participants scored a good performance for this follow-up study.

The individual laboratory results on the PFGE typing part were reported to the 12 participants a few weeks before the workshop. The participants were asked to test 11 *Salmonella* strains, using their own routine PFGE method for digestion with XbaI. Quality grading was done according to the guidelines used in the External Quality Assessment (EQA) schemes for the ECDC–FWD network (following the PulseNet Guidelines). These are based on seven parameters, scored from 1 (poor) point to 4 (excellent) points. In general, an acceptable quality (>1 points) should be obtained for each parameter, since a low-quality score in just one category can have a high impact on the ability to further analyse the image and compare to other profiles. Two participants scored only 1 point for the parameters 'Image acquisition/Running conditions' and 'Bands', respectively 'Restriction' and 'DNA Degradation', and therefore need to make specific improvements on this. The quality of the other 10 gel images was sufficient to allow inter-laboratory profile comparisons.

The evaluation of the analysis of a gel in BioNumerics was optionally included. As in the previous study, a common gel was provided to all participants. A total of 11 participants sent in their analysed gel data for evaluation, which was done according to the guidelines used in the EQAs for the FWD laboratories. These guidelines use five parameters, which are scored with 1 (poor), 2 (fair/good) or 3 (excellent) points. The analysis of one participant was completely in agreement with the reference analysis for all 10 strains. One participant mistakenly did not assign bands at around 40 kb, but this would be easy to correct. Apart from this mistake, 3 strains were correctly analysed by all participants. All deviations in the other 7 strains were seen in the assignment of double bands as single bands (5 strains) or in the assignment of a

doublet instead of a triplet (2 strains), both being well known difficulties in the analysis of PFGE images.
This way of evaluating PFGE typing in the PT, concerning the quality of PFGE gel images and gel analysis in BioNumerics, will no longer be offered in the 2019 PT on typing of *Salmonella*. Instead, a cluster analysis on 11 *Salmonella* isolates, using PFGE and/or MLVA and/or WGS, will be offered as a pilot in 2019.

More details on the 2018 typing study can be found in the (interim) summary reports (Jacobs-Reitsma et al., 2019a, b)

Discussion
Q: Some Member States find *Salmonella* Enteritidis in pig and cattle. Can this be caused by problems with serotyping?
A: It is not very likely. The NRLs-*Salmonella* generally score well in the PTs for serotyping of *Salmonella*.
Q: What type of information should we report in the pilot PT for cluster analysis?
A: Some organisations already have experiences with this type of studies, like EURL-*E. coli*, EURL-*Listeria monocytogenes*, and Statens Serum Institute (Denmark). We will consult these organisations to learn from their experiences in the set-up of the study and reporting of the results.
Q: How many laboratories are using WGS for serotyping of *Salmonella*?
A: A consultation at the workshop revealed that approx. 3-4 NRLs-*Salmonella* use WGS for serotyping.

2.7 Results EURL-*Salmonella* Proficiency Test Primary Production 2018 - Detection of *Salmonella* in boot socks with chicken faeces

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

In October 2018, the EURL-*Salmonella* organised a Proficiency Test (PT) on detection of *Salmonella* in primary production stage (PPS) samples. A total of 36 NRLS-*Salmonella* participated in this study: 29 NRLs from the 28 EU Member States (MS), six from EU candidate or potential EU candidate MS and members of EFTA, and one from a non-European country. Participation was obligatory for all EU MS NRLs responsible for the detection of *Salmonella* in PPS samples.

In this study, boot socks with chicken faeces from a pathogen-free farm were used. The boot sock samples were artificially contaminated with a diluted culture of *Salmonella* Infantis at the EURL laboratory.

Each NRL received 18 blindly coded boot sock samples with faeces, consisting of 12 boot sock samples artificially contaminated with *Salmonella* Infantis at two different levels – 6 x low (10 cfu) and 6 x high (53 cfu) and 6 negative boot socks with chicken faeces (no *Salmonella* added). In addition, 2 control samples were included consisting of a blank procedure control and a positive control sample. For the positive control, each participant used its own positive control strain. The samples were stored at 5 °C until the day of transport. On Monday 24 September 2018, the samples were packed and sent to the NRLs-*Salmonella*. On arrival,

the NRLs were asked to store the samples at 5 °C until the start of the analysis.

Most laboratories used EN ISO 6579-1:2017 for the detection of *Salmonella* in the boot sock samples. Two laboratories used EN ISO 6579:2002/Amd.1:2007 (Annex D), and three laboratories used another method.

All laboratories achieved good results when analysing both the blank procedure control and their own positive control sample.
All but two laboratories detected *Salmonella* in the six boot sock samples contaminated with a low level of *Salmonella*. Two laboratories (lab codes 1 and 3) found one of these samples negative for *Salmonella*. This is still well within the criteria for good performance, which permit three negative results.
All but one of the laboratories detected *Salmonella* in all six high-level samples. One laboratory (lab code 26) scored one of the six high-level samples negative. This is still within the criteria for good performance, which permit one negative result. The sensitivity rate was 99.3% for these samples. One laboratory (lab code 35) experienced problems with its samples. It tested five of the six low-level samples negative for *Salmonella* and one of the six high-level samples negative. This was most likely due to temperature abuse during transport, as the parcel arrived at the laboratory after eight days of transport, and the samples had experienced temperatures of 26–28 °C for several days. For that reason, the quality of the samples could not be guaranteed, and the results of this laboratory were not included in the evaluation.
All negative boot sock samples were scored correctly negative, resulting in a specificity of 100%.

Overall, the laboratories scored well in this PT. The accuracy rate was 99,5%. Thirty-five laboratories fulfilled the criteria of good performance. The results of one laboratory were not included in the evaluation because of temperature abuse during sample transport.

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

2.8 Preliminary results EURL-*Salmonella* Proficiency Test Food-Feed 2019 - Detection of *Salmonella* in flaxseed

Robin Diddens, EURL-Salmonella, Bilthoven, the Netherlands

In March 2019, a EURL-*Salmonella* Proficiency Test (PT) on detection of *Salmonella* in a food/feed matrix was organised for the NRLs-*Salmonella.* The matrix under analysis was flaxseed. Flaxseed is used as a food product as well as an ingredient of animal feed. Participation was obligatory for the NRLs from EU Member States responsible for the analysis of *Salmonella* in food samples. For the NRLs-*Salmonella* that analyse animal feed products, participation was optional.
In total, 42 NRLs-*Salmonella* participated in this study: 37 NRLs from the 28 EU Member States (MS) and five NRLs from (potential) EU candidate MS and members of EFTA.

The objective of this PT was to test the performance of the participating laboratories in detecting different concentrations of *Salmonella* in flaxseed samples. The prescribed method for the detection of *Salmonella* was EN ISO 6579-1:2017. 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 make sure that the samples were fit for use concerning the choice of the *Salmonella* serovar, stability of the samples at different storage temperatures (5 °C and 10 °C) for approximately 3 weeks, and the concentration of natural background flora (aerobic count and *Enterobacteriaceae)*. In the pre-tests only the most critical samples were tested, being the low-level flaxseed samples. It was aimed to inoculate these low-level samples with a diluted culture of *Salmonella* Typhimurium (STm) at a level of 5–10 cfu/g. The results of the pre-test showed that the aerobic count in the flaxseed varied between 10^6 and 10^7 cfu/g and the concentration of *Enterobacteriaceae varied* between 10^5 and 10^7 cfu/g during the 2–3 weeks of storage.

Parcels were prepared for each laboratory containing the flaxseed samples, control samples, cooling blocks, and a device to record the temperature during transport and cooling.
Forty-one parcels arrived within two days at the NRLs-*Salmonella* and one parcel arrived after seven days, because it was held by customs. The temperature recorder of this parcel showed that the parcel was stored at -12 °C for one day, followed by storage at 1,5–2 °C. The temperature of the other parcels was between 0-5 °C during transport and generally between 0-7,5 °C during storage.

Each laboratory received 18 samples of 25 g of flaxseed. These comprised six negative samples (no *Salmonella* added), six samples with a low level of STm (inoculum 10 cfu/25 g) and six samples with a high level of STm (inoculum 105 cfu/25 g). The laboratories also had to test two control samples: a blank procedure control and an own positive *Salmonella* control.

Forty-one laboratories detected *Salmonella* in all low-level flaxseed samples. One laboratory detected *Salmonella* in five out of the six low-level samples, which is above the criteria for good performance of at least three positive samples. All the laboratories did not detect *Salmonella* in the negative samples and detected *Salmonella* in all high-level samples. The specificity rate for the negative samples was 100% and the accuracy rate for all artificially contaminated flaxseed samples was 99,9%.

Laboratory 13 swapped the results of the control samples when reporting their results. This laboratory scored a 'moderate' performance. All the other laboratories scored a good performance.
The laboratories also reported technical details such as the selective media used, incubation temperatures and times, concentrations of novobiocin, and pH of the different media used. Three laboratories did not use MKTTn broth for selective enrichment, despite the fact that it is prescribed in EN ISO 6579-1:2017. Laboratory 1 and 24 used only MSRV agar as selective enrichment medium. Laboratory 9 used RVS broth and MSRV agar as selective enrichment media.

In addition to the prescribed method, the NRLs-*Salmonella* could analyse the flaxseed samples with a second detection method, if this was (routinely) used in their laboratories. Thirteen laboratories also used a second method for detection of *Salmonella* in the flaxseed samples. The methods used were PCR, qPCR, and mini VIDAS®. The results of the second detection methods were all equal to the results obtained with EN ISO 6579-1:2017.

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

Discussion
Q: What is the effect of incubation of the pre-enrichment broth (BPW) for a different time than indicated in EN ISO 6579-1?
A: The idea is to keep the incubation time short to prevent overgrowth of *Salmonella* by background flora. This is especially important for 'dirty' samples.

2.9 Rapid detection and differentiation of *Salmonella* species, *Salmonella* Typhimurium, and *Salmonella* Enteritidis by multiplex real-time PCR

Bart Wullings, Wageningen Food Safety Research (WFSR), Wageningen, the Netherlands

An early and fast detection of *Salmonella* species and identification of the serovars Typhimurium and Enteritidis in the food chain facilitates effective intervention and prevents further distribution of contaminated food products. Therefore, a multiplex real-time PCR was developed for the rapid and simultaneous detection of *Salmonella* spp., *S.* Typhimurium, and *S.* Enteritidis in samples from the food chain and compared with culture-based methods. Three primer and probe sets were designed to target the *InvA* gene, the STM4200 gene, and the SEN1392 gene to detect *Salmonella* spp., *S.* Typhimurium, and *S.* Enteritidis, respectively. The multiplex real-time PCR was validated and the selectivity was analysed by using 225 *Salmonella* isolates and 35 non-*Salmonella* isolates from various sources. Furthermore, the level of detection (LOD) was examined for 10 different matrices by artificial contamination of samples of 25 g at four different inoculation levels. The inclusivity of the multiplex real-time PCR was 100% for all 225 *Salmonella* isolates, including 72 *S.* Typhimurium and 53 *S.* Enteritidis isolates. The exclusivity was 100%, 100%, and 94,6% for *Salmonella* spp., *S.* Enteritidis, and *S*. Typhimurium, respectively. The PCR to detect *S*. Typhimurium showed cross-reaction with *S*. Derby, *S*. Rissen, and *S*. Goldcoast.

The validation showed that the method is horizontally applicable for *Salmonella* detection in the food chain, since for more than five different matrices the performance of the multiplex real-time PCR was comparable to the performance of EN ISO 6579:2002 and the MSRV method (EN ISO 6579:2002/Amd.1:2007). By using the multiplex qPCR method, instead of conventional culture methods, for the screening of enrichments broths, the analysis time of samples is reduced from 48 h to 24 h. In 2018, over 11 000 samples were tested negative after qPCR

screening. The ability to differentiate between *S.* Enteritidis and *S.* Typhimurium makes it a robust tool to easily detect both serovars as requested by regulation No 2073/2005 (EC, 2005). However, positive results for *S.* Typhimurium must be further confirmed. This method facilitates fast and effective intervention when contaminated food products are on the market.

More details can be found in Heymans et al., 2018.

Discussion
Q: Do you use an internal PCR control?
A: Yes, we use four targets per PCR.
Q: Do you use this PCR method for all samples you analyse for *Salmonella*?
A: Not all, but for almost all we use this PCR method. It is Regulatory driven and project driven whether we can use the PCR method or whether EN ISO 6579-1:2017 has to be used.
Q: Is the PCR method validated by MicroVal or Afnor?
A: It is not a proprietary method and therefore we performed an in-house validation, based on EN ISO 16140-2:2016.

2.10 Multi-country outbreak of *Salmonella* Bareilly

Tomas Cerny, NRL-Salmonella*, Prague, Czech Republic*

The National Institute of Public Health (NIPH) in the Czech Republic informed all stakeholders about the increased incidence of *Salmonella enterica* subsp. *enterica* serovar Bareilly since August 2017 at the regular meeting of the Working Group for Zoonoses (WGZ) in spring 2018. After obtaining this information, the State Veterinary Administration (SVA) activated subordinate organisations in order to detect the potential source of *S.* Bareilly. This *Salmonella* serovar may originate from animals or from food of animal origin.

In 2017–2018, the Veterinary Research Institute (VRI) and NIPH carried out an investigation to confirm the outbreak and to identify the source. The NIPH and VRI performed molecular typing of selected strains of the outbreak period. In the course of 2018, an adapted trawling questionnaire generating a common hypothesis was distributed in the Czech Republic and the Slovak Republic.

Up to October 2018, 325 human cases had been identified in both countries. *Salmonella* Bareilly strains from 84 cases were analysed by PFGE. Of these, 78 had pulsotype congruent with XbaI.2667 (according to the TESSy reference strain). Additionally, WGS analysis of isolates from 16 *S.* Bareilly cases was performed. These strains showed very close relationships and confirmed the existence of an outbreak cluster. Four *S.* Bareilly isolates were detected in dried egg products produced in one processing plant from September to October 2018. All these isolates showed the outbreak pulsotype. One *S.* Bareilly strain was analysed with WGS. This strain was genetically linked to the outbreak cluster based on cgMLST analysis.
Subsequent epidemiological investigations conducted by the SVA showed possible ways of spreading of *S.* Bareilly through contaminated

materials (dried egg matter) and the role of long-term contaminated technology (egg dryer) when moving it between two companies.The analysis of this foodborne outbreak helped to improve communication and cooperation in the network of public health and veterinary authorities in the Czech Republic. The need for close and timely cooperation between stakeholders has also been confirmed.

2.11 Multi-country cluster of *Salmonella* Coeln in 2018: involvement of EURL/NRL-*Salmonella* network

Angela van Hoek, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands

In December 2018, the EURL-*Salmonella* was contacted by EFSA following the identification of a multi-country cluster of *Salmonella* Coeln by the ECDC. The EURL-*Salmonella* was asked to send a request to its NRL network for information on the isolation of *S*. Coeln from food, feed, and animals in 2017 and 2018. A total of 31 NRLs responded to the request and submitted information concerning the isolation of *S*. Coeln. WGS sequences were shared by several NRLs, and the EURL-*Salmonella* also collected over 25 strains, from which a sub-set was sequenced. An explorative analysis was performed with the obtained sequences, including over a hundred *S*. Coeln strains from the public databases ENA and EnteroBase. MLST analysis (7 housekeeping genes) revealed the presence two sub-types (STs) among the dataset, i.e. ST1995 and ST2015. The majority of the human isolates from the multi-country cluster belonged to ST1995. cgMLST analysis using the EnteroBase scheme, which includes over 3 000 genes, was conducted with the ST1995 isolates only. This resulted in the clustering of apparently unrelated human cases from 2012 to the 2018/2019 cluster with an equal distance as some isolates from the NRLs. In addition, it was difficult to define a clear cut-off (cgMLST distance threshold) for the cluster. The available epidemiological data did not point to any connection between the cases and the different sources.
Additional analysis identified the overrepresentation of the virulence gene *sopE* and the absence of virulence features such as the sodC1 gene and the STM1043 prophage among the isolates of the multi-country cluster.

Discussion
Q: Did you use a dendrogram in addition to the minimum spanning tree for cluster analysis?
A: No, as the program we are using does not provide this option. However, it might be an idea to do so for further analysis.
Q: SNP analysis may have a higher discriminatory power than cgMLST; did you use SNP analysis as well?
A: The program does not provide this option either, but it is certainly a good suggestion to have a further look at.

2.12 Update on activities in ISO and CEN

Kirsten Mooijman, EURL-Salmonella, Bilthoven, the Netherlands

Kirsten Mooijman of the EURL-*Salmonella* presented an overview of activities in ISO and CEN 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;
- CEN/TC275/WG6: European Committee for Standardization, Technical Committee 275 for Food Analysis – Horizontal methods, Working Group 6 – Microbiology of the Food Chain.

The last annual meeting of both groups was organised from 18 to 22 June 2018, and the next meeting is organised in Milan, Italy from 8 to 12 July 2019.

Amd.1 to EN ISO 6579-1 'Detection of *Salmonella*'

After the publication of EN ISO 6579-1 in 2017, a mistake was detected in the composition of Selenite cystine broth in Annex D.3: L-cystine solution should be 10 ml instead of 100 ml. From 22/12/2017 until 26/02/2018 the members of ISO/TC34/SC9 and CEN/TC275/WG6 were asked to check for any other errors in EN ISO 6579-1:2017. In June 2018 their findings were discussed at the annual meeting of ISO-SC9 and CEN-WG6 and it was agreed to draft an amendment to EN ISO 6579-1.

The first draft of Amd.1 was sent to the members of ISO-SC9 and CEN-WG6 (draft Resolution N842) by the end of 2018. Resolution N842 included:

- A request for agreement to skip the CD (Committee Draft) vote and go straight to Draft International Standard (DIS) voting;
- An invitation to nominate active experts;
- An invitation to comment on the draft.

In December 2018, Resolution 842 was adopted with 22 approvals, no disapprovals, 11 abstentions, and some comments. Seven experts were nominated from seven different countries. In January–February 2019, two additional comments were received, which also had to be taken into account. All comments were discussed with the expert group and incorporated in draft DIS Amd.1, which was sent to the secretariat of ISO-SC9 in April 2019. The DIS voting was to be held between 08/07/2019 and 30/09/2019.

Content of DIS Amd.1 EN ISO 6579-1:2017:

- Suggested title: 'Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSRV and SC' (where SC stands for selenite cystine medium);
- For incubation of selective media the temperature range has been extended from 37 °C ± 1 °C to 34–38 °C throughout the document (like for incubation of non-selective culture media). The following note has been added to sub-clause 6.3 (incubator 34–38 °C): 'The range 34 °C to 38 °C for incubation of media includes the use of incubators set at 35 °C ± 1 °C, 36 °C ± 2 °C or 37 °C ± 1 °C.'
- A comment was made about a mistake in the final concentration of $MgCl_2$ in MSRV agar (Annex B.4). This has been corrected in Amd.1: The final concentration of $MgCl_2$ in MSRV agar should be

10.9 g/l. However, the composition given in EN ISO 6579-1:2017 results in a final concentration of 14.9 g/l $MgCl_2$. In Annex B.4 (MSRV agar from individual ingredients) the concentrations of the ingredients of solution A, the base medium, and complete medium have been corrected.
- The status of Annex D ('Detection of *Salmonella* Typhi and *S.* Paratyphi') has been changed from normative to informative to prevent further confusion as to whether Annex D should always be followed or not: Annex D should be followed only if *S*. Typhi and *S*. Paratyphi are specifically sought.
- The correction of the concentration of L-Cystine solution in selenite cystine medium from 100 ml to 10 ml in 1 L base medium.

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

The activities for this subject are carried out jointly by CEN/TC275/WG6 TAG3 (project leader in TAG3: Burkhard Malorny, Germany) and ISO/TC34/SC9 WG10 (convenor Kirsten Mooijman).

- From 2016 to 2017 several Working Drafts (WDs) of ISO/TS 6579-4 were prepared by Burkhard Malorny (NRL-*Salmonella* Germany), including 3 PCR protocols.
- The 3 PCR protocols needed to be tested and for this a call for test strains was made by the EURL-*Salmonella* in early 2017.
- In March 2017, approx. 400 strains (target and non-target) were received from several NRLs-*Salmonella*.
- Until the autumn of 2017 the EURL-*Salmonella* performed typing of all strains and repeated the typing where there were discrepancies.
- In 2018, 172 strains (target and non-target) were selected and tested with the 3 PCR protocols by the NRL-*Salmonella* in Germany (BfR) and by the EURL-*Salmonella*. For the selection of the strains, information of prEN ISO/DIS 16140-6:2017 was used.
- Early in 2019, the results of BfR and EURL were compared. Strains with different results between BfR and EURL with the same PCR protocol are retested with all three PCR protocols.
- Differences between the 3 PCR protocols were mainly found in isolates tested as *Salmonella* Typhimurium with the 'Tennant' PCR protocol and as monophasic *Salmonella* Typhimurium with the two other PCR protocols. These strains are retested with slide agglutination.
- In the autumn of 2019 the results of the testing of the 172 strains, including the additional tests, will be presented in CEN-TAG3. If necessary, the PCR protocols will be updated.
- When the draft is ready, the New Work Item Proposal (NWIP) will be launched in ISO-WG10 and in parallel in CEN/TC275/, together with a call for experts, in early 2020.

Other subjects

Parts 3–6 of EN ISO 16140 on Method validation are under development. In the past year the following activities relating to these parts took place:

- Part 3 on method verification: In 2018 the DIS version of Part 3 was approved at ISO level, but not at CEN level. ISO/TC34/SC9 WG3 has made a great effort to solve the main issues of the countries voting negative, which seems to have been successful, as on the pre-FDIS (Final Draft International Standard) vote only one negative vote was received. After discussion of the comments at a meeting of WG3, the project leaders will prepare the FDIS document.
- WG3 has drafted a guidance document for implementing ISO 16140-3 once it is published. As it has been decided that verification according to ISO 16140-3 can only be done for validated methods, a transition period is suggested for the application of Part 3 to non-validated methods until 01/01/2027. This period can then be used to perform validation studies of ISO methods not yet validated. Methods already validated/verified in user laboratories do not need to be re-verified after publication of ISO 16140-3 (as long as no major changes are introduced in the verified method). When the voting for FDIS 16140-3 is launched, the guidance document will also be sent around for further comments.
- Part 4 on in-house (single lab) validation, Part 5 on factorial design validation, and Part 6 on validation of confirmation and typing methods: the pre-FDIS of the three documents was approved in 2018 with no negative votes. The comments on the pre-FDIS were discussed at the meeting of WG3 in December 2018 and the FDIS vote was launched in June 2019 for an 8-week period.

The revised version of EN ISO 22117 'Microbiology of the food chain – Specific requirements and guidance for proficiency testing by interlaboratory comparison' was published in April 2019. In comparison with the former version the main amendments that have been made are:

- The document has become a full ISO instead of a Technical Specification (TS);
- Updates have been made to align the document with ISO 13528:2015 'Statistical methods for use in proficiency testing by interlaboratory comparison'.

In ISO/TC34/SC9, WG25 is drafting an ISO document on Whole Genome Sequencing. The New Work Item Proposal was launched in spring 2018. The voting on draft ISO 23418 was positive, but with approximately 300 comments. The working group has addressed the comments and prepared a second draft document, which was launched for CD voting by 15 May 2019, until 7 July 2019.

Discussion

Q: What method do you use at the EURL-*Salmonella* for the identification of monophasic *Salmonella* Typhimurium?
A: We use the 'Tennant protocol' with some modifications. However, real-time PCR is more specific, so perhaps in future we will change to this PCR protocol.
Q: We do not use a PCR method for the identification of monophasic *Salmonella* Typhimurium; would it be sufficient to perform

phase inversion two or three times to identify the isolate as monophasic *S.* Typhimurium?
A: You may need to discuss this with your Competent Authority. The reason for performing an identification by PCR is to exclude the possibility that the isolate is the monophasic variant of a serovar other than Typhimurium, as the Regulations are more stringent for *S.* Typhimurium than for other serovars. In the Netherlands we had this discussion with the Competent Authority and the official (Dutch) laboratories performing serotyping of *Salmonella* isolated from poultry several years ago. In 2012, the following procedure was agreed in the Netherlands:

- The official laboratories follow the White Kauffmann Le Minor scheme for serotyping of *Salmonella*;
- If the second H-phase can still not be determined after performing the phase inversion twice, and the O-antigens and the first H-phase indicate that the isolate is the monophasic variant of *Salmonella* Typhimurium, then it is reported to the client that the monophasic variant of Salmonella Typhimurium was found, so that actions can be taken immediately;
- To validate this procedure, the isolate is sent to the Dutch NRL-*Salmonella* for identification with PCR, to confirm that the isolate is indeed the monophasic variant of *Salmonella* Typhimurium and not the monophasic variant of another *Salmonella* serovar.

So far, all isolates received by the Dutch NRL-*Salmonella* following this procedure have been confirmed as monophasic *Salmonella* Typhimurium with PCR.
Q: Can EN ISO 6579-1:2017 also be used for the detection of *Salmonella* in pig and cattle faeces?
A: Yes, the method is applicable over a broad range of matrices.

3 Wednesday 29 May 2019: Day 2 of the workshop

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

Søren Aabo, NRL-Salmonella, Lyngby, Denmark

The Danish Veterinary and Food Administration (DVFA) and the National Food Institute (DTU-Food) at the Technical University of Denmark share responsibility for the NRL function. The main areas where DTU-Food supports the DVFA are: (i) Reference laboratory responsibility and food crisis support; (ii) counselling in microbiological food safety; (iii) surveillance and data exchange; (iv) research and general improvement of competences. DTU-Food is designated as the NRL for *Salmonella* according to EU regulation No 882/2004 (EC, 2004c), article 32, and responsibility for the laboratory rests with the public DVFA food control laboratory, Ringsted, which participates in EURL Proficiency Tests. Accreditation according to EN ISO/IEC 17025:2005 is assessed by DANAK as is the audit of proficiency testing in private laboratories doing testing according to food legislation. The DVFA is accredited to the *Salmonella* methods EN ISO 6579, NMKL71, NMKL187 (MSRV), BAX-PCR. Additionally, WGS serotyping of *Salmonella* is accredited. The laboratory takes part in several Proficiency Tests, generally with 'good' performance. DTU-Food supports the DVFA with qualitative and quantitative risk assessments on foodborne hazards, with a particular focus on *Salmonella*, *Campylobacter*, *Listeria*, *Yersinia*, VTEC, and antibiotic resistance (e.g. ESBL and MRSA). DTU-Food supports the DVFA in surveillance activities on microbiological hazards and provides statistical and epidemiological data management. DTU-Food also participates in outbreak investigations of zoonotic diseases and hosts a national collection of strains from food, which is central in outbreak investigations and source tracking. DTU-Food coordinates and publishes the *Annual Report on Zoonoses* and the *Annual Report on Antibiotic Resistance and Consumption* (DANMAP) and contributes to the reporting to EFSA and to national surveillance projects. The agreement between the DVFA and DTU-Food also states that the University must carry out research that supports solving problems relevant for the DFVA. This includes the development of detection methods for foodborne bacteria, antibiotic resistance, and viruses, and this activity supports the infrastructure of the NRL laboratory. Similarly, DTU-Food supports the routine WGS analysis carried out at the NRL laboratory and provides stepping stones for future implementation of metagenome analysis and bioinformatics. Other research areas include emerging antibiotic resistance in animal production, with a focus on possible zoonotic transmission, and the development of platforms for source attribution, which is a key area of interest for the DVFA. Internationally, DTU-Food hosts the EU reference laboratory for antibiotic resistance and is WHO Collaborating Centre for antibiotic resistance and genomics in foodborne pathogens. Participation in EFSA and Codex Alimentarius working groups and in the BioHaz panel are examples of other activities that are part of its collaboration with the DVFA.

Discussion
Q: How many people work at your laboratory?
A: In total approximately 50, including 4 microbiological researchers and more than 20 technicians. Separate groups work on different pathogens.
Q: How many *Salmonella* isolates have you sequenced?
A: Since we started in 2017, approximately 3 000 isolates from food products.
Q: What program(s) do you use for WGS serotyping?
A: Our program is based on several others, including SeqSero. However, I do not know how many (or which) other programs are included.

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

Veronica Cibin, NRL-Salmonella, Legnaro, Italy

The Italian NRL-*Salmonella* is located at the Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe). The IZSVe is a public veterinary institute which conducts prevention, control, and research activities in three main areas: animal health and welfare, food safety, and environmental protection. It was founded in June 1929 and is part of a national network that consists of nine other Italian public veterinary institutes (IZSS), each of which covers a specific geographical area. The headquarters of the IZSVe are located in Legnaro (Padua) and the geographical area that is covered by IZSVe corresponds to the north-east part of Italy.
The NRL-*Salmonella* was designated in 1999 by the Ministry of Health and in 2007 it was recognised by the OIE as a reference laboratory for animal salmonellosis.

The Italian NRL-*Salmonella* collaborates with the EURL-*Salmonella* by participating in training courses, in Proficiency Tests, and in the annual meeting and by sending data and strains when requested.
It coordinates the activities of the Official Laboratories (OLs), which are located in IZSS, through the Enter-Vet network, which was set up in 2002 and whose main aim is to collect data on *Salmonella* from non-human sources (a web-based database is dedicated to this function).
The NRL-*Salmonella* disseminates to OLs and Competent Authority (CA) the relevant information that the EURL supplies via the Moodle platform, the annual meeting, and email.

The Italian NRL organises Proficiency Tests to evaluate both the ability of the participants to isolate *Salmonella* from primary production samples and their ability to identify the serovars by serotyping. As regards isolation PTs, the NRL organises two different studies: one is dedicated to OLs and the other (two editions per year) to the private laboratories (PLs). PLs that analyse samples in the framework of the National *Salmonella* Control Plans must participate in the PTs and they can be authorized only in case of satisfactory results.
Additionally, the Italian NRL-*Salmonella* provides assistance to the CA by: supporting the drafting of national monitoring and control plans, cooperating in the recognition of private laboratories, performing analysis within the framework of the National *Salmonella* Control Plan in case of confirmatory samples, participating in national working groups,

supporting the evaluation of epidemiological situations, and assisting in the event of outbreaks.

As OIE reference laboratory the Italian NRL: carries out and/or coordinates scientific and technical studies upon OIE request, provides training for professionals from OIE member counties, and places expert consultants at the disposal of the OIE.

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

Laetitia Bonifait, NRL-Salmonella, Ploufragan, France

Salmonella is one of the most important causes of foodborne bacterial gastroenteritis in the world. Poultry products are often associated with salmonellosis. In the EU, monitoring of *Salmonella* in poultry flocks is laid down by regulation EC No 2160/2003 (EC, 2003). In France, the National Reference Laboratory (NRL) for *Salmonella* was designated by the French Ministry of Agriculture. As the national representative of the European Reference Laboratory, the French NRL-*Salmonella* has several responsibilities: to provide daily support and advice to the official lab network, to contribute to the salmonellosis surveillance programme, to perform confirmatory testing and serotyping of *Salmonella* strains, and to maintain the collection of *Salmonella* isolates and the database of the related data. The strain collection of the French NRL is an important tool of the monitoring system. The NRL is required to keep all *Salmonella* strains for at least two years and performs analysis of *Salmonella* trends in the poultry production in France. Despite the control programme enforced at the primary production level, *S*. Enteritidis and *S*. Typhimurium are still a major problem in France; they continue to be among the principal serovars found in poultry flocks. Nevertheless, for all poultry production types the presence of some non-regulated serovars such as *S*. Senftenberg and *S*. Montevideo is increasing. Genotyping has shown that several persistent and common isolates of *S.* Enteritidis are circulating through the poultry-rearing steps (breeding, production flocks of laying hens, broilers, and turkeys), both over time and across departments. Despite the preventive measures taken by farmers, according to the isolates received at the NRL, *Salmonella* is still a concern in poultry flocks.

Discussion

Q: Are all *Salmonella* serovars notified to the French ministry?
A: Yes. It is a legal requirement to notify any *Salmonella* isolated from poultry flocks.
Q: Do you have an explanation for the increase in *Salmonella* (predominantly *S.* Enteritidis) during the summer? Do you see this increase in humans or in animals?
A: We see this increase in *Salmonella* in both humans and animals, but the reason behind it is unclear. Perhaps it is (partly) caused by the fact that barbecuing (and the higher risk of undercooked meat and cross-contamination) is more popular during summer than during other seasons.

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

Madara Streikisa, NRL-Salmonella, Riga, Latvia

The Latvian National Reference Laboratory for the control of *Salmonella* and other specified foodborne zoonotic agents is part of the Scientific Institute of Food Safety, Animal Health and Environment (BIOR). In 2009, the restructuring of the Food and Veterinary Service (FVS) and the state agency Latvia Fish Resource Agency was made. The institute took over the functions of the National Diagnostics Centre of the FVS and a part of the functions of Latvia Fish Resource Agency, and began operating as BIOR on 1 January 2010.

The laboratory uses various techniques, including microbiological culture, biochemical confirmation, MALDI-TOF MS, serotyping, antimicrobial resistance testing, and molecular biological methods for *Salmonella* spp. detection from food, feed, and primary production stage samples. All laboratories are accredited according to EN ISO/IEC 17025:2005.
Salmonella detection is performed following EN ISO 6579-1:2017 and additional molecular methods. Serotyping of *Salmonella* strains is performed by following CEN ISO/TR 6579-3:2014, including the White-Kauffmann-Le Minor scheme (Grimont and Weill, 2007).
The major activities and functions of the NRL-*Salmonella* are:

- Cooperation with the EURL (participation in PTs, annual workshops, training courses, etc.);
- Organisation of interlaboratory comparative tests between official state laboratory and self-control laboratories;
- Organisation of training courses;
- Cooperation with the Latvian competent authority (Ministry of Agriculture, Food and Veterinary Service);
- Surveillance programmes for official samples testing;
- *Salmonella* serotyping;
- AMR monitoring;
- MLST typing (*S.* Enteritidis*/S.* Typhimurium);
- Performing WGS (outbreaks/epidemiological investigations).

Salmonellosis is one of the most common intestinal infections in Latvia. The main sources are contaminated products, mainly poultry, pork, eggs, and egg products; less often sea products and vegetables. The most common *Salmonella* serovars in humans are *S.* Enteritidis and *S.* Typhimurium. In Latvia there are active surveillance programmes for *Salmonella* in poultry, food, feed, and environmental samples.
In total, 8 988 samples (6 487 food and 2 501 animal and feedstuffs) were investigated in 2018. Most often, *Salmonella* was detected in meat and meat products and in the faeces of production birds (poultry, broilers). In 2018, approximately 250 isolates were serotyped by the NRL, including those received from official and self-control laboratories. The most common serovars in Latvia are *S.* Enteritidis*, S.* Derby*, S.* Infantis*, S.* Typhimurium*, S.* Coeln*,* and *S.* Newport.
In 2018, BIOR started to perform Next Generation Sequencing for the first time to gain experience in this field. More than 300 *Salmonella* isolates have been analysed by Whole Genome Sequencing, of which

50% were human isolates from outbreaks or sporadic cases, 31% were veterinary isolates, and 16% were food isolates.
Last year, in cooperating with the Latvian Centre for Disease Prevention and Control, the following six *Salmonella* outbreaks were analysed using WGS:

- 1 x *S.* Typhimurium – no similarity between human and veterinary/food isolates was observed.
- 2 x *S.* Enteritidis – no similarity between human and veterinary/food isolates was observed.
- 1 x *S.* Enteritidis – 8 alleles distance was observed between the human and veterinary sample obtained two weeks before the first human case (the cluster alert threshold for *Salmonella* spp. as defined by Ridom SeqSphere was 7).
- Prolonged outbreak of *S.* Infantis – a perfect match (0 allele distance) was observed between the human isolate, one food isolate, and three veterinary isolates.
- Outbreak of *S.* Coeln in several European countries in 2018 – the BIOR performed WGS of 8 *S.* Coeln strains, isolated from various sources originated from Latvia: 1 human isolate and 7 non-human isolates (6 poultry and 1 zoo animal). In early 2019 these sequence data were reported to the EURL-*Salmonella*, EFSA, and the ECDC.

Discussion
Q: Do you also find *Salmonella* Enteritidis in pigs?
A: Yes, sometimes we do, but not very often. *S.* Derby is more common in pigs than *S.* Enteritidis.
Q: In one of your slides you mention finding *Salmonella* 61:k:5,7. From what product/animal did this isolate originate?
A: It was isolated from the faeces of a zoo animal.

3.5 Activities of the NRL-*Salmonella* to fulfil tasks and duties in Switzerland

Gudrun Overesch, NRL-Salmonella, Bern, Switzerland

Current data on *Salmonella* spp. in Switzerland show that in 2018 1 580 notified cases of human salmonellosis were reported. Incidence levels have been stable since 2009. Most cases are caused by *S*. Enteritidis, *S*. Typhimurium, and monophasic *S*. Typhimurium. Results of the European baseline studies, several years ago, already demonstrated the low prevalence of *Salmonella* spp. in Swiss poultry and pigs. Cases of *Salmonella* in animals occur very rarely (approximately 100 cases per year, mostly in cattle). Fresh Swiss poultry meat is contaminated at a very low level (<1%). A study with raw milk cheese showed that none of the approximately 1 000 samples tested positive. In 1990, Switzerland started a programme to combat *Salmonella* in poultry. Today a national control programme equivalent to the EU legislation on *Salmonella* spp. in poultry is in force.

National reference laboratories for *Salmonella* spp. are designated for *Salmonella* in humans (NENT: National Centre for Enteropathogenic Bacteria, Institute of Food Safety and Hygiene, Vetsuisse faculty of Zurich), for *Salmonella* in food (Institute for Food Safety and Hygiene,

Vetsuisse faculty of Zurich, since 2018), for *Salmonella* in poultry (National Reference Centre for Poultry and Rabbit Diseases, Institute of Veterinary Bacteriology, Vetsuisse faculty of Zurich), for *Salmonella* in feed (Agroscope, federal institute, Bern), for *Salmonella* in farm animals (excluding poultry) and for antimicrobial resistance (ZOBA: National Centre for Zoonoses, Bacterial Animal Diseases and Antimicrobial Resistance, Institute of Veterinary Bacteriology, Vetsuisse faculty of Bern).
The last has the mandate to organise Proficiency Tests (PTs) for the approved laboratories in Switzerland to guarantee good performance of *Salmonella* detection in the framework of the national control plan. Proficiency testing has been conducted with chicken faeces from a *Salmonella*-free herd and lenticules containing *Salmonella* at several defined contamination levels (blank, low, and high). When the first PTs were carried out, in 2009, the overall accuracy was 73% at a contamination level of 100 cfu/lenticule. More PTs were conducted in 2010, 2011, 2013, and 2017, and the accuracy rate has increased to 98% at a contamination level of 10 cfu/lenticule. For the future, PTs on *Salmonella* spp. 'pre-serotyping' and on detection of *Salmonella* spp. are planned.

Discussion
Q: In Switzerland we want to organise PTs with low-level samples, but these are hard to obtain. Do you have any suggestions?
A: The NRL-*Salmonella* in Sweden prepares freeze-dried ampules with low levels of *Salmonella*. These ampules are used in PTs in Sweden, Norway, and Denmark. The participants add an ampule to a matrix sample before analysis. If other countries are interested in the materials, they should contact the NRL-*Salmonella* in Sweden.

3.6 Whole Genome Sequencing (WGS)-based typing of *Salmonella* spp. and molecular analyses

Katharine Newton, NRL-Salmonella, *Addlestone, United Kingdom*

At the Animal and Plant Health Agency (APHA) methods are developed for reference laboratory typing of *Salmonella* from animals, animal feed, and livestock production environments using whole genome sequencing (WGS)-based serotyping and downstream molecular analyses. The ambition is to facilitate real-time sharing and use of WGS data for outbreak identification and investigation across the veterinary, food, and human sectors, as well as between countries. The focus is on the development and validation of methodologies required for transition to *Salmonella* serotyping based on WGS. Accurate results are produced by linking three analytical tools into a '*Salmonella* Pipeline', which produces consensus results that are more accurate than outputs from single pipelines.

Within this project, the accuracy of the developed '*Salmonella* Pipeline was assessed by comparison with the 'gold standard' CEN ISO/TR 6579-3:2014 serotyping method for a total of 1 980 sequenced genomes, selected to fulfil and supplement the recommended criteria of prEN ISO/DIS 16140-6:2017. As well as providing additional assurance needed for UK surveillance testing, with an aim to achieve UKAS

accreditation to EN ISO/IEC 17025:2005 for *Salmonella* serotyping using WGS. The reproducibility and the time required to obtain results were tested by repeated serotyping of selected isolates by the pipeline, on three separate occasions. In addition to serotyping, a WGS-based method was developed for differentiation of *S*. Typhimurium, *S*. Enteritidis, and *S*. Gallinarum/*S*. Pullorum vaccine isolates from field strains. This is a unique added feature of the APHA *Salmonella* Pipeline.

Discussion
Q: What do you do with new *Salmonella* serovars? Do you have to upload new serovars in the program(s)?
A: In the case of new serovars we perform conventional serotyping. It may depend on the program how the information is added. The MOST program was developed by Public Health England (PHE) and is not self-learning. PHE performs conventional serotyping in cases where MOST does not recognise a (new) *Salmonella* serovar. It may be discussed what to do with novel serovars. Do we have to develop a system which fits 100% with the White Kauffmann Le Minor scheme, or should we define novel serovars genotypically instead of by name?
Q: What do you use in SeqSero: FASTQ files assembly or de novo assembly?
A: We use FASTQ files.
Q: Does WGS serotyping takes less time than conventional serotyping?
A: No; generally more time is needed for WGS serotyping (in total approximately 5 days) than for conventional serotyping.

3.7 Update on the joint ECDC–EFSA molecular typing database and outcome of the EFSA–ECDC working group on WGS

Frank Boelaert, EFSA, Parma, Italy

Following the Shiga toxin-producing *Escherichia coli* (STEC) crisis in 2011, a 'Vision paper on the development of databases for molecular testing of food-borne pathogens in view of outbreak preparedness' was prepared by the EC, in consultation with EFSA, ECDC, and the relevant EURLs (EC, 2012). Next, the European Commission (EC) asked EFSA and ECDC to provide technical support for the collection of molecular typing data on *Salmonella*, *Listeria monocytogenes*, and Verotoxigenic *Escherichia coli* (VTEC) isolates from human and non-human (food, feed, and animals) samples; and to develop a joint EFSA–ECDC database for the joint analysis of these data. The aim of the joint EFSA–ECDC database is to enhance routine surveillance and outbreak identification by enabling detection of microbiological links between isolates of human and of non-human origin. During 2012, EFSA received a mandate from the EC to provide technical support for the development of a database of molecular typing data on isolates of *Salmonella*, *Listeria monocytogenes*, and STEC from food, feed, animals, and the related environment. For the purposes of the data collection and subsequent linkage with corresponding data from human isolates, it is essential to ensure the comparability of typing data from foodborne pathogens isolated from food, feed, animals, and the related environment as well as from human sources. A report published by EFSA addresses all the technical aspects of the design of the database and its functionalities and provides

information about the procedures for data submission and accessibility (EFSA, 2014).

In 2017, EFSA received from the EC a new mandate on the collection and analysis (in collaboration with ECDC) of WGS data for foodborne pathogens from human and non-human samples. Initially, the mandate covered a feasibility study (deadline 30 April 2019) aimed at evaluating the possible solutions for the collection and analysis of WGS data for at least *Salmonella*, *Listeria monocytogenes*, and VTEC. A follow-up mandate will be issued on the implementation of the solution chosen by the EC.

WGS offers great potential in the investigation, assessment, and management of microbiological food safety issues, outbreaks, and illnesses. It allows the identification and characterisation of micro-organisms with a level of precision not previously possible, allowing the detection of diffuse multi-country outbreaks. The use of these new data for public health purposes could potentially increase the workload for EFSA, in particular with regard to the number of joint Rapid Outbreak Assessments (ROAs) to be produced in collaboration with ECDC.
Activities are ongoing in EFSA to build capacity for the application of WGS methods for outbreak investigations and other public health applications (e.g. monitoring of antimicrobial resistance).
Molecular typing data have already successfully supported the investigation of multi-country foodborne outbreaks and the production of joint ECDC–EFSA ROAs.

Discussion
Q: How do you assure comparability between the ECDC database (WGS) and EFSA database (PFGE, MLVA)?
A: The ECDC database for human molecular data has already moved to WGS, and is a bit ahead the EFSA database. The curators of the databases meet regularly to assure harmonisation.
Q: Why did only 11 Member States sign the agreement to submit molecular data on isolates from food, feed, and animals to the EFSA database?
A: The challenge is that the Competent Authorities (CAs) of the Member States have to sign the agreement and have to appoint a laboratory (e.g. the NRL) to upload the data into the database. The CAs have been reminded several times of the existence and importance of the database, but they may not always see the advantage of sharing data in this database.
Q: Is there perhaps reluctance about sharing metadata?
A: Perhaps, but only very few metadata are shared and in the agreement it is clearly stated what is shared and what is not.

3.8 Work programme EURL-*Salmonella* second half 2019, first half 2020, discussion on general items and closure

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

Kirsten Mooijman summarised the information on the work programme of the EURL-*Salmonella* for the rest of 2019 and for early 2020.

In December 2018, the EURL-*Salmonella* had submitted a two-year work programme (2019–2020) to EC DG SANTE. The template for the work programme follows Regulation EU No 625/2017 (EC, 2017), Article 92 (2). Approval by DG SANTE of the work programme and the budget for 2019–2020 was received in April 2019.

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 the activities for ISO and CEN:

- ISO-Ad hoc group on drafting Amd.1 of EN ISO 6579-1 (project leader);
- CEN-TAG3/ISO-WG10 – drafting ISO/TS 6579-4 PCR identification of monophasic *Salmonella* Typhimurium (project leader of ISO-WG10): re-testing some strains; amendment of document; launching NWIP; organisation of validation study (at DIS stage);
- ISO-WG3 Method validation, especially ISO 16140-6 (validation of alternative confirmation and typing procedures; project leader);
- ISO-Ad hoc group on harmonisation of ISO/CEN standards for microbiology of the food chain (project leader): updating guidance document;
- ISO-Ad hoc group 'General aspects' (member): includes drafting of general information for in the scopes and introduction of ISOs;
- CEN-TAG9 Improvement of the pre-enrichment step (member);
- ISO-WG25 Whole genome sequencing (member).

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 eight EURLs, and eight activities have been defined in relation to NGS. Summaries will be drafted on collected information of different activities and on the outcome of the surveys conducted amongst NRLs on the use of 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. This study will be held in September/October 2019 and the matrix of choice is likely to be chicken faeces.
2. Detection of *Salmonella* in food samples. This study is foreseen for February/March 2020 and the matrix has not yet been decided. As the EURL-*Salmonella* also has to organise PTs for the detection of *Salmonella* in bivalve molluscs, it may be the case that this matrix will be used in the PT for food of 2020.
3. Typing of *Salmonella* (serotyping, molecular typing). This study is foreseen for November/December 2019, and will include serotyping of *Salmonella* (obligatory) and a pilot for cluster analysis for which a molecular method free of choice can be used (if there is sufficient interest).

For each PT, the results of each NRL-*Salmonella* will be compared to pre-set definitions of 'good' performance. The performance criteria were set several years ago and have proved to be useful.

Performance criteria for PTs for serotyping of Salmonella

- **4 penalty points:** Incorrect typing of *S.* Enteritidis, *S.* Typhimurium (including the monophasic variant), *S.* Hadar, *S.* Infantis, or *S.* Virchow **or** assigning the name of one of these 5 serovars to another strain.
- **1 penalty point:** Incorrect typing of any other *Salmonella* serovar.
- For each NRL-*Salmonella* the total amount of penalty points is determined and 'Good' performance is less than 4 penalty points.
- A follow-up is obligatory for NRLs of EU-MS with 4 penalty points or more.

Performance criteria for PTs for detection of Salmonella

When 6 matrix samples per contamination level and 2 control samples are used in a PT, the performance criteria as indicated in Table 1 are used. However, if the matrix samples prove to be less stable than expected, the required percentages positive may be amended for the specific PT.

If the results of an NRL-*Salmonella* do not fulfil the criteria for good performance, the EURL will contact the NRL for possible technical clarification(s). A follow-up study will then be organised, which is obligatory for NRLs of EU-MS. In the event of repeated poor performance, the NRL must undergo a training course. This may involve a visit of the EURL-*Salmonella* to the NRL or training of NRL staff at the premises of the EURL-*Salmonella.* Additionally, the EURL will inform EC DG SANTE.

*Table 1. Criteria for good performance used in EURL-*Salmonella *Proficiency Tests for detection of* Salmonella

Matrix samples	**Percentage positive**	**No of positive samples/ total no of samples**
Negative samples[a]	20% max	1/6 max
Low-level contaminated samples	≥ 50%	≥ 3/6
High-level contaminated samples	≥ 80%	≥ 5/6
Control samples		
BPW	0%	0/1
Own positive control	100%	1/1

[a] 100% *Salmonella*-free matrix cannot be guaranteed; therefore, 1 positive out of 6 samples is still considered acceptable (20%).

In addition to 'good' and 'poor' performance, an NRL-*Salmonella* can score 'moderate' performance in the PTs.
Examples of reasons for scoring moderate performance are:

- Mixing up of samples;
- Electricity breakdown (samples stored at elevated temperatures);
- Error when copying raw data into electronic results form.

Initially these results will be judged as poor performance. If the NRL-*Salmonella* can show raw data clarifying the reasons for the error, the result may be changed to moderate performance.

No follow-up will be organised after one PT with moderate performance. However, after repeated moderate performances, e.g. after the third study in a row with moderate performance, or in combination with earlier poor performance in PTs, a follow-up will be organised. The type of follow-up will be decided case by case.

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 2020 workshop will be the 25th workshop and is likely to be held in the Netherlands.

Sub-activity 2.2 Training courses

Objective:

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

The following training courses are foreseen:

1. Training upon request of an NRL;
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 EURL-STEC and EURL-*Listeria monocytogenes*; four NRLs per network can participate (12 participants in total). This training course will be held at the premises of EURL-*Listeria monocytogenes* (Maisons-Alfort, France) in October 2019.

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 maintain the EURL-*Salmonella* website and keep the information on the website up to date;
- 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;
- To draft a harmonised guidance document, in a joint working group of five EURLs, for the organisation of PTs by NRLs for national OLs, including partial outsourcing.

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;
- Participation in working groups/scientific committees of DG SANTE and EFSA, such as the EFSA–ECDC Steering Committee of the molecular database;
- Curation of PFGE data in the EFSA molecular 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.

Description:

- Providing a link to WKLM scheme and keeping contacts with WHO reference centre;
- Reference to culture collections and reference materials at the website;
- Maintenance of in-house culture collection;
- Provision of sets of reference strains (*S.* Enteritidis and *S*. Typhimurium) for MLVA typing;
- Sub-activity 4.1 is merged with 2.3 (supporting NRLs; keeping information on website up to date).

4 Evaluation of the workshop

4.1 Introduction

At the end of the workshop, an evaluation form was given to the participants to ask for their opinions (see Annex 3).
A total of 12 questions were asked. For 10 of these questions, participants were asked to answer using a score ranging from 1 to 5. The scores represent: very poor (1), poor (2), fair (3), good (4) and excellent (5).
In addition, it was possible to add comments. Two questions were 'open' questions, in which the participants were asked to give their opinion.
The evaluation form was handed to 47 workshop participants;
41 completed forms were returned, a response rate of 87%.

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

4.2 Evaluation form

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

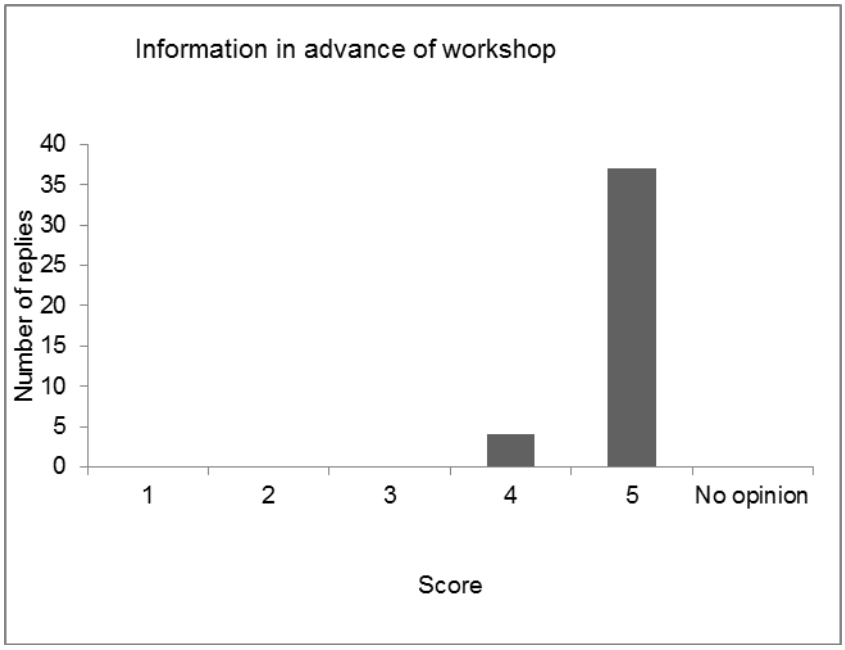

Figure 1. Scores given to question 1 'Opinion on information given in advance of the workshop'

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

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

3. What is your opinion on how easy (high score) or difficult (low score) it was to reach the meeting venue?
The majority of respondents considered the meeting venue easy to reach, as the scores given to this question were either good (score 4) or excellent (score 5), see Figure 3. A few participants gave a fair score (score 3) and referred to the public transport strikes on the first day of the workshop (28 May 2019). However, almost all participants arrived on 27 May and left on 29 May, while the strike was limited to 28 May 2019. Hence, the strike affected only a few participants. Another remark given to this question was 'great instructions'.

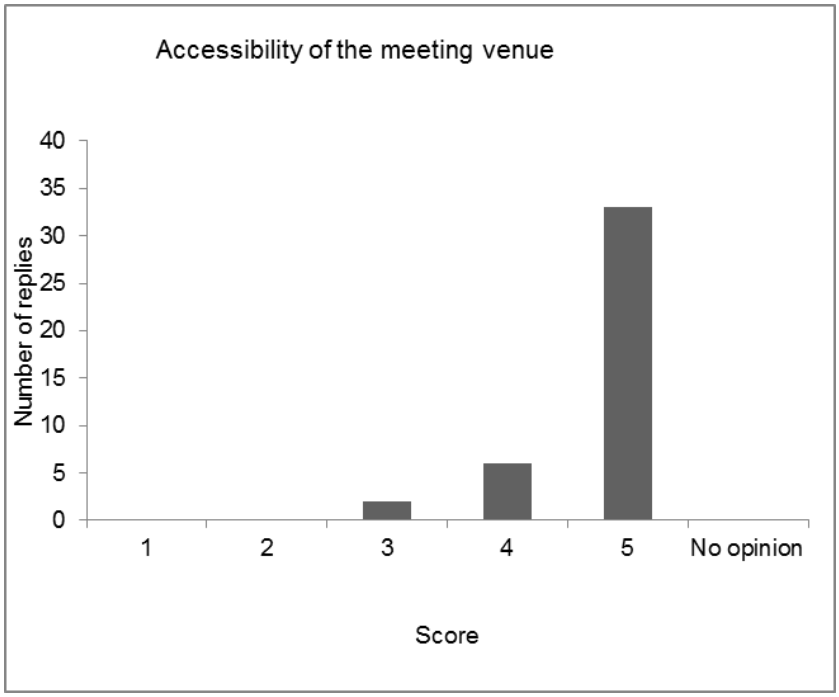

Figure 3. Scores given to question 3 'Opinion on the accessibility of the meeting venue'

4. What is your opinion on the hotel room?
The majority of the participants were satisfied with the hotel rooms (Figure 4). One participant remarked 'clean and spacious with a desk to work'.

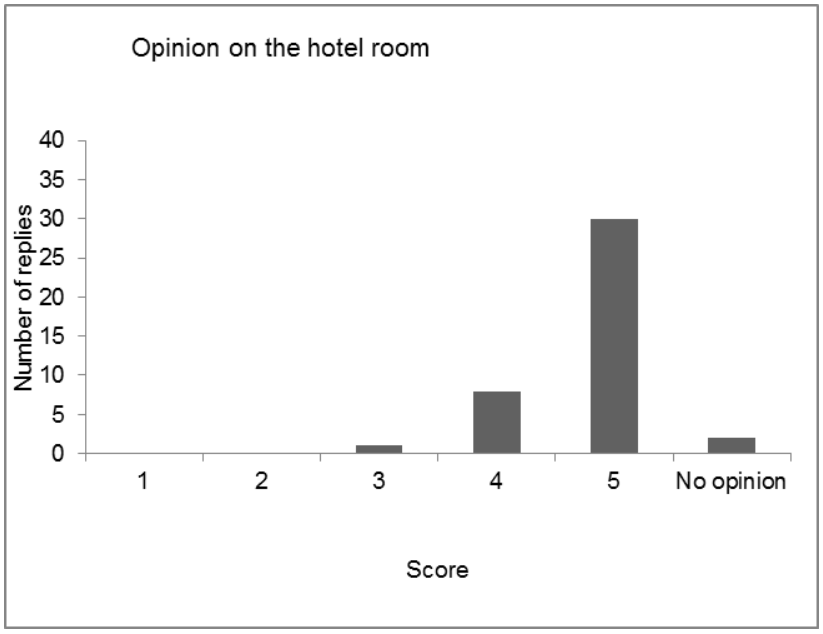

Figure 4. Scores given to question 4 'Opinion on the hotel room'

5. What is your general opinion on the meeting room?
The opinion on the meeting room was, in general, good to excellent (scores 4 and 5; see Figure 5). Additional remarks:
'Excellent view of the double screen, good acoustics and tea at the back of the room.'
'A little cold.'

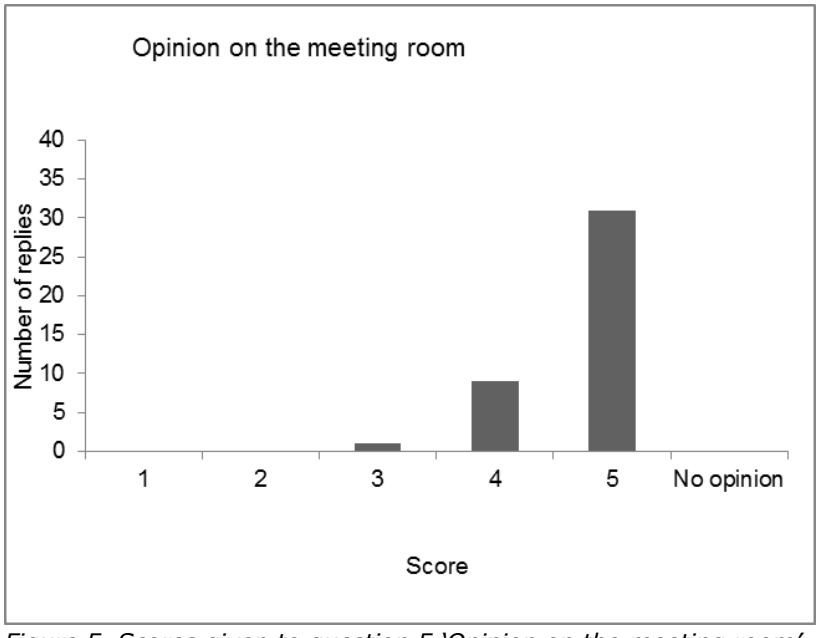

Figure 5. Scores given to question 5 'Opinion on the meeting room'

6. What is your opinion on the readability of the presentations on the screen?
The majority of respondents were satisfied with the readability of the presentations on the screen (see Figure 6). A few remarks were made:
'Readability dependent on the speakers' choice of 'size of fit'.
'Large and clear.'

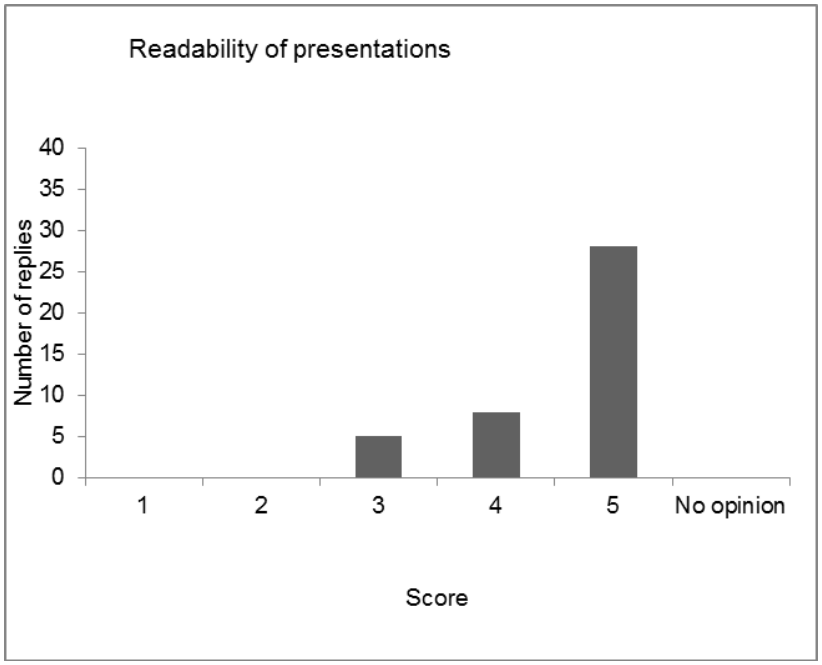

Figure 6. Scores given to question 6 'Opinion on the readability of the presentations on the screen'

7. What is your opinion on the technical equipment in the meeting room (computer, screen, microphones, etc.)?
The majority of respondents were also satisfied with the technical equipment in the meeting room (Figure 7).

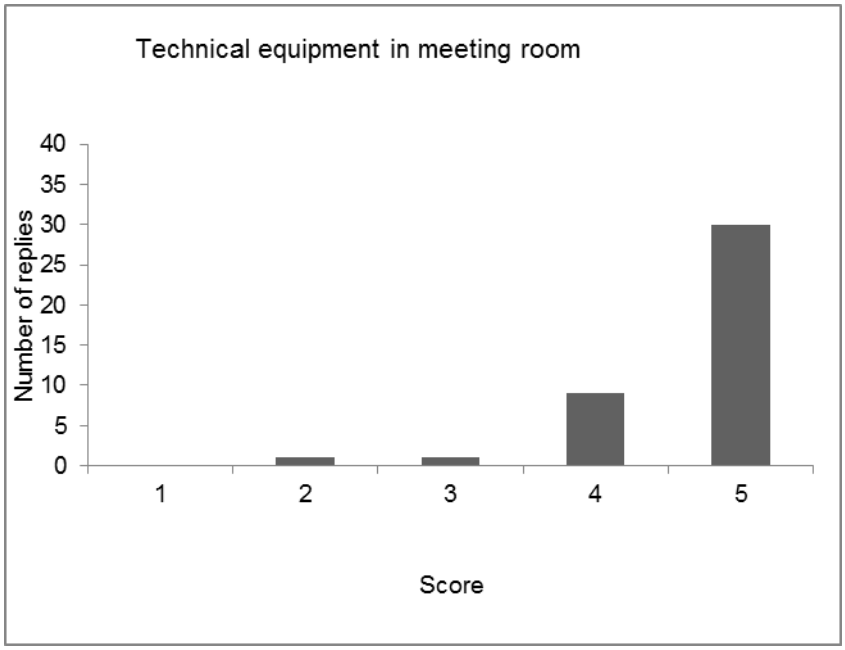

Figure 7. Scores given to question 7 'Opinion on the technical equipment'

8. What is your opinion on the catering provided during the workshop (breakfast, coffee, tea, lunch, dinner)?
The majority of respondents were satisfied with the catering, see Figure 8. The following additional remark was made:
'Great choice, I loved the fresh mint tea.'

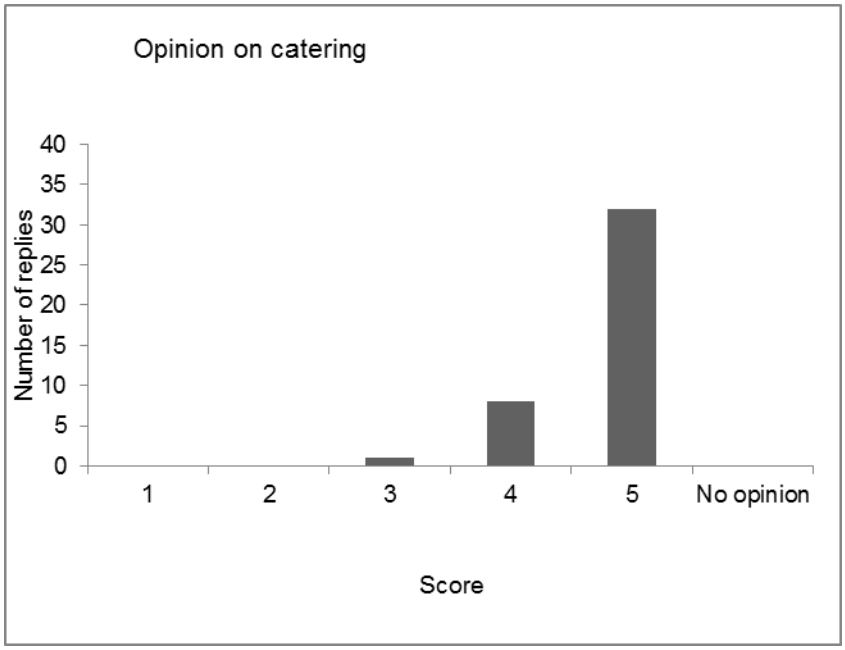

Figure 8. Scores given to question 8 'Opinion on the catering'

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

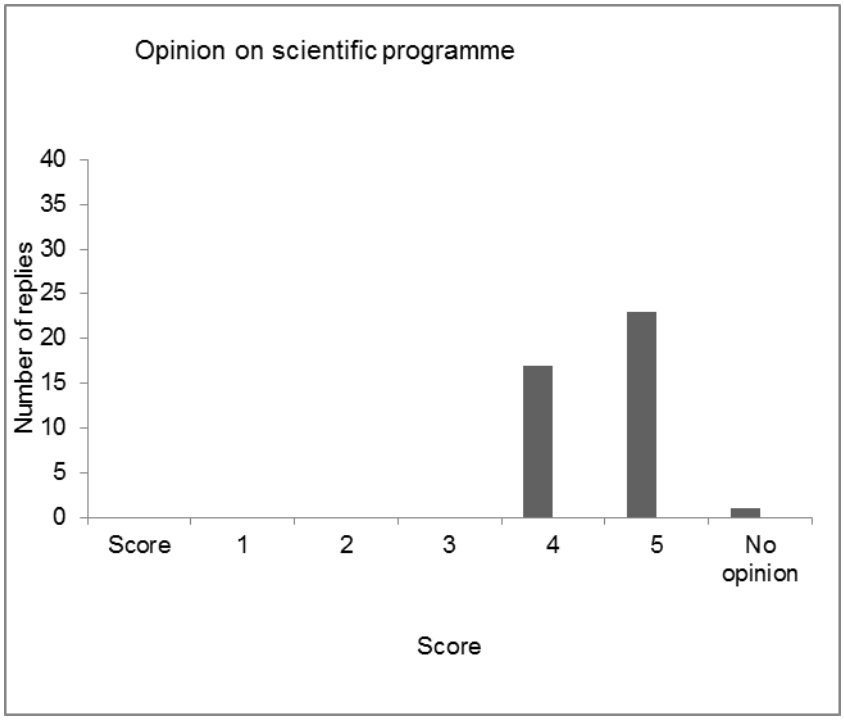

Figure 9. Scores given to question 9 'Opinion on the scientific programme'

The respondents were satisfied with the scientific programme of the workshop; the scores were good (4) to excellent (5), see Figure 9.
Additional remark:
'Great integration of scientific talks, EURL updates and quality summaries.'

10. Are there specific presentations you want to comment on, or did you miss information on certain subjects?
This was an 'open' question and the following responses were obtained:
'If possible, more technical presentations.'
'The *Salmonella* Coeln event was very well presented.'
'The EFSA information could be more focused. Too many details at once.'
'Several excellent presentations.'

11. What is your general opinion of the workshop?
The respondents indicated that the workshop as a whole had been good (4) or excellent (5), see Figure 10. The following additional remark was made:
'Great workshop, discussions and interactions.'

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

12. 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:
'If we have the meeting at an institute it would be interesting to visit a laboratory at that institute that is, of course, a relevant *Salmonella* lab.'
'I suggest inviting speakers from Eastern EU Member States to support those NRLs to give a presentation.'
'Proficiency Testing results to be presented on the second day of the workshop.'
'Thank you for this meeting.'
'Workshop in a good atmosphere.'
'Not clear if we have access to the presentations or the report – it would be good to specify this at the beginning of the meeting.'
'More case studies, practical things in routine work, in order to improve existing work.'

4.3 Discussion and conclusions of the evaluation

In general, high scores were received for this workshop: for almost all items good (4) or excellent (5) scores were given. This time the workshop took place without any technical problems, but unfortunately, there was a country-wide public transport strike on the first day of the workshop. However, this affected only a few participants, as the majority travelled the day before the first day and during the afternoon of the second day of the workshop.

The participants made several interesting remarks on the evaluation forms, which will be used when organising next year's workshop.

Acknowledgements

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

In addition, the author would like to thank William Byrne from the NRL-*Salmonella* of Ireland for taking notes during the workshop. This was of great value when drafting the discussions in this report.

Colleagues from the EURL-*Salmonella* and RIVM – Robin Diddens, Wilma Jacobs-Reitsma, Angela van Hoek, and Irene Pol-Hofstad – are also thanked for their help with the organisation of the workshop, taking notes during the workshop and carefully reading this report.

List of abbreviations

A	Answer
AMR	Antimicrobial resistance
BPW	Buffered Peptone Water
CA	Competent Authority
CD	Committee Draft
CEN	European Committee for Standardization
cfu	colony forming units
cgMLST	core genome Multi-Locus Sequence Typing
CrI_{95}	95% Credibility Interval
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
EQA	External Quality Assessment
EU	European Union
EURL	European Union Reference Laboratory
FBO	Food Business Operator
FDIS	Final Draft International Standard
FWD	Food and Waterborne Diseases and zoonoses
HAV	Hepatitis A virus
ISO	International Organization for Standardization
LOD	Level of Detection
MKTTn	Mueller Kauffmann Tetrathionate broth with novobiocin
MLST	Multi-Locus Sequence Typing
MLVA	Multi-Locus Variable number of tandem repeats Analysis
MS	Member State
MSRV	Modified Semi-solid Rappaport Vassiliadis
NGS	Next Generation Sequencing
NRL	National Reference Laboratory
NWIP	New Work Item Proposal
OIE	World Organisation for Animal Health
OL	Official Laboratory
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
PL	Private Laboratory
PPS	Primary Production Stage
PT	Proficiency Test
Q	Question
qPCR	quantitative Polymerase Chain Reaction
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
STEC	Shiga toxin-producing *Escherichia coli*
STm	*Salmonella* Typhimurium

TAG	Technical Advisory Group
TC	Technical Committee
TESSy	The European Surveillance System
TS	Technical Specification
UK	United Kingdom
WG	Working Group
WGS	Whole Genome Sequencing
WKLM	White Kauffmann Le Minor

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Annex 1 Participants

European Food Safety Authority (EFSA)	Frank Boelaert
EURL-*Salmonella*	Kirsten Mooijman Wilma Jacobs Irene Pol Robin Diddens

Guest speakers

The Netherlands	Bart Wullings (WFSR, Wageningen) Angela van Hoek (RIVM, Bilthoven)
United Kingdom	Katharine Newton (APHA, Addlestone)

National Reference Laboratories for *Salmonella*

ALBANIA	Renis Maçi
AUSTRIA	Christian Kornschober
BELGIUM	Oonagh Paerewijck
BOSNIA HERZEGOVINA	Amira Koro
BULGARIA	Gergana Mateva
CROATIA	Gordan Kompes
CYPRUS	Maria Emmanuel
CZECH REPUBLIC	Tomas Cerny
DENMARK	Søren Aabo Birgitte Nauerby
ESTONIA	Age Kärssin
FINLAND	Henry Kuronen
FRANCE	Laetitia Bonifait Frédérique Moury
GERMANY	Istvan Szabo Jennie Fischer
GREECE	Aphrodite Smpiraki
HUNGARY	Sára Kostyák
ICELAND	Franklin Georgsson
IRELAND	William Byrne
ITALY	Veronica Cibin Marta Leati
LATVIA	Madara Streikisa
LITHUANIA	Aista Darata Brazdilyte
LUXEMBOURG	Joël Mossong
MALTA	-
NETHERLANDS	Anjo Verbruggen
NORTHERN IRELAND	Angela Lahuerta Marin
NORWAY	Bjarne Bergsjø
POLAND	Aleksandra Smialowska Kinga Wieczorek Elzbieta Mackiw
PORTUGAL	Patricia Themudo
REPUBLIC OF NORTH MACEDONIA	Dean Jankuloski

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SERBIA	Jasna Kureljusic
SLOVAK REPUBLIC	Lubos Mikula
SLOVENIA	Jasna Micunovic
SPAIN	Maria Cristina de Frutos
Alexandra Herrera Estévez	
SWEDEN	Lennart Melin
	Jenny Skantz
SWITZERLAND	Gudrun Overesch
UNITED KINGDOM	Marie Chattaway

Annex 2 Workshop Programme

Programme of the 24th EURL-*Salmonella* workshop
28 and 29 May 2019, Amersfoort, the Netherlands

General information

Place of accommodation and Meeting venue:
NH Hotel Amersfoort
Stationsstraat 75
3811 MH Amersfoort
The Netherlands
https://www.nh-hotels.com/hotel/nh-amersfoort

Contact in case of emergencies:
Kirsten Mooijman Email: kirsten.mooijman@rivm.nl

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

Abstract: For the preparation of the report of the workshop it is necessary to also receive an abstract of your presentation (approximately 0,5-1 page). Please hand this over to Kirsten during the workshop or send it to Kirsten.mooijman@rivm.nl **preferably before 1 June 2019**

Monday 27 May 2019

Dinner information
For participants for whom the costs of travel and stay are paid from the budget of EURL-*Salmonella,* the EURL will also cover the expenses of a dinner on Monday 27 May, with a maximum of € 40,- per person. You can use the dinner at the NH Hotel in Amersfoort and ask to have the costs added to the invoice of your room. Alternatively, you can have dinner at another location, for which we will need a receipt in order to reimburse you for this meal.

Tuesday 28 May 2019

08:30 - 09:00 Registration

Morning Chair: Kirsten Mooijman

09:00 - 09:30	Opening and introduction	Kirsten Mooijman, EURL-*Salmonella*
09:30 - 10:00	The stalled *Salmonella* situation in EU and assessment of current EU reduction targets	Frank Boelaert, EFSA
10:00 - 10:30	*Salmonella* Agona in animal feed in Germany 2018	Istvan Szabo Germany
10:30-11:00	*Coffee/tea*	
11:00 - 11:30	*Salmonella* contamination of (imported) fresh edible leaves	Marie Anne Chattaway United Kingdom
11:30 - 12:00	*Salmonella* in bivalve molluscs	Irene Pol,EURL-*Salmonella*
12:00 - 12:30	Results EURL-*Salmonella* Proficiency Test on typing of *Salmonella* 2018 – serotyping and PFGE Introduction to PT on typing 2019	Wilma Jacobs, EURL-*Salmonella*
12:30-13:30	*Lunch*	

Afternoon Chair: Kirsten Mooijman

13:30 - 14:00	Results EURL-*Salmonella* Proficiency Test Primary Production 2018 – Detection of *Salmonella* in boot socks with chicken faeces	Irene Pol EURL-*Salmonella*
14:00 - 14:30	Preliminary results EURL-*Salmonella* Proficiency Test Food-Feed 2019 – Detection of *Salmonella* in flaxseed	Robin Diddens EURL-*Salmonella*
14:30 - 15:00	Rapid detection and differentiation of *Salmonella* species, *Salmonella* Typhimurium, and *Salmonella* Enteritidis by multiplex real-time PCR	Bart Wullings NVWA The Netherlands
15:00-15:30	*Coffee/tea*	
15:30 - 16:00	Multi-country outbreak of *Salmonella* Bareilly confirmed with WGS in the Czech Republic	Tomas Cerny Czech Republic
16:00 - 16:30	Multi-country cluster of *Salmonella* Coeln in 2018 – involvement of EURL/NRL-*Salmonella* network	Angela van Hoek EURL-*Salmonella*
16:30 - 17:00	Update on activities in ISO and CEN	Kirsten Mooijman, EURL-*Salmonella*
17:30-18:30	*Guided walk in Amersfoort*	
19:00 - ….	*Dinner at NH Hotel*	

Wednesday 29 May 2019

Morning Chair: Kirsten Mooijman

09:00 - 10:40	Activities NRLs to fulfil tasks and duties, and information on national *Salmonella* control programs	
09:00 - 09:20	NRL-*Salmonella* Denmark	Søren Aabo
09:20 - 09:40	NRL-*Salmonella* Italy	Veronica Cibin
09:40 - 10:00	NRL-*Salmonella* France	Laetitia Bonifait
10:00 - 10:20	NRL-*Salmonella* Latvia	Madara Streikisa
10:20 - 10:40	NRL-*Salmonella* Switzerland	Gudrun Overesch
10:40 - 11:15	*Coffee/tea*	
11:15 - 11:45	Whole Genome Sequencing (WGS) based typing of *Salmonella* spp. and molecular analyses	Kate Newton United Kingdom
11:45 - 12:15	Update on the joint ECDC–EFSA molecular typing database and outcome of the EFSA–ECDC working group on WGS	Frank Boelaert, EFSA
12:15 - 12:45	Work programme EURL-*Salmonella* second half 2019, first half 2020 Discussion on general items Closure	Kirsten Mooijman, EURL-*Salmonella*
12:45 - 13:45	*Lunch*	

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

Annex 3 Workshop evaluation form

Evaluation of the 24th EURL-*Salmonella* workshop, 28 and 29 May 2019, Amersfoort, the Netherlands

We would highly appreciate if you could give us your opinion on the 24th EURL-*Salmonella* workshop, organised in Amersfoort, the Netherlands, on 28 and 29 May 2019. Thank you very much in advance for completing this questionnaire and returning it to the EURL-*Salmonella* team by the end of the workshop.

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

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

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

Remarks:

---

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

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

Remarks:

---

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

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

Remarks:

---

4. What is your opinion of the hotel room?

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

Remarks:

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

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

Remarks:

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

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

Remarks:

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

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

Remarks:

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

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

Remarks:

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

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

Remarks:

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

11. What is your general opinion of the workshop?

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

Remarks:

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

Thank you very much!

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