



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

The 21st EURL-*Salmonella* workshop

9 June 2016, Saint Malo, France

RIVM Report 2016-0045

K.A. Mooijman



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

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Colophon

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Synopsis

The 21st EURL-*Salmonella* workshop

9 June 2016, Saint Malo, France

This report contains a summary of the presentations given at the 21st annual workshop for the European National Reference Laboratories (NRLs) for *Salmonella* (9 June 2016). The aim of the workshop is to facilitate the exchange of information on the activities of the NRLs and the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*).

Annual ring trials

A recurring item at the workshops is the presentation of the results of the annual ring trials organised by the EURL. These ring trials give information on the quality of the NRL laboratories tested. The 2015 trial showed that all NRLs were able to detect *Salmonella* in whole liquid egg. Detailed information on the results per ring trial is given in separate RIVM-reports.

Molecular techniques

Several presentations provided information on the use of molecular techniques for *Salmonella* typing. These techniques analyse the DNA of the bacterium, and are often used to trace pathogens in food, animals or humans. Each strain has its own unique molecular typing pattern.

Storage of molecular typing results

The European Food Safety Authority (EFSA) presented a database for storage of the molecular *Salmonella* typing results. This database has been available since early 2016 and will make it possible to check whether a specific strain is found in different countries and products.

The annual workshop is 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 2016

Publiekssamenvatting

De 21^e EURL-*Salmonella* workshop

9 juni 2016, Saint Malo, Frankrijk

Dit rapport bevat een bundeling van verslagen van de presentaties van de 21^e jaarlijkse workshop voor de Europese Nationale Referentie Laboratoria (NRL's) voor *Salmonella* (9 juni 2016). Het doel van de workshop is dat het overkoepelende orgaan, het Europese Referentie Laboratorium (EURL) voor *Salmonella* en de NRL's informatie uitwisselen.

Jaarlijkse ringonderzoeken

Een terugkerend onderwerp is de ringonderzoeken die het EURL jaarlijks organiseert en waarmee de kwaliteit van de NRL laboratoria wordt gecontroleerd. De NRL's hadden er in 2015 geen problemen mee om *Salmonella* in ei te vinden. In dit rapport staan de ringonderzoeken kort beschreven. Een uitgebreidere weergave van de resultaten wordt per ringonderzoek gepubliceerd.

Moleculaire technieken

Een aantal verslagen geeft informatie over het gebruik van moleculaire technieken om *Salmonella* te typeren. Met deze technieken wordt het DNA van de bacterie aangetoond. Deze technieken worden steeds vaker gebruikt bij het opsporen van ziekmakende bacteriën in voedsel, dieren en bij de mens. Iedere bacteriestam heeft namelijk een eigen unieke moleculaire typering.

Opslag moleculaire typering resultaten

De European Food Safety Authority (EFSA) geeft verslag van een databank die sinds begin 2016 beschikbaar is. In deze databank kunnen alle Europese landen moleculaire typering resultaten van *Salmonella* opslaan. Dit geeft informatie of een bepaalde ziekmakende bacteriestam in meerdere landen en producten voorkomt.

De organisatie van de 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 2016

Contents

Summary — 9

1 Introduction — 11

2 Thursday 9 June 2016: the day of the workshop — 13

- 2.1 Opening and introduction — 13
- 2.2 Results 20th interlaboratory comparison study on typing of *Salmonella* (2015) – serotyping and PFGE — 13
- 2.3 Update on the joint EFSA/ECDC molecular typing database — 15
- 2.4 How will joint cluster management work in practice? An introduction to EPIS-FWD — 16
- 2.5 Use of WGS for typing of *Salmonella* at PHE — 18
- 2.6 *Salmonella* monitoring data and food-borne outbreaks for 2014 in the European Union — 19
- 2.7 Update on activities in ISO and CEN — 21
- 2.8 Results interlaboratory comparison study Food on detection of *Salmonella* in whole liquid egg (2015) — 26
- 2.9 Preliminary results interlaboratory comparison study on detection of *Salmonella* in boot socks (2016) — 28
- 2.10 Nordic cooperation for Proficiency Testing of regional laboratories — 29
- 2.11 Recent investigations on *Salmonella* Enteritidis contamination in the poultry production in France — 30
- 2.12 Work programme EURL-*Salmonella* second half 2016, first half 2017, discussion on general items and closure—31

3 Evaluation of the workshop — 35

- 3.1 Introduction — 35
- 3.2 Evaluation form — 35
- 3.3 Discussion and conclusions of the evaluation — 41

References — 43

Acknowledgements — 47

List of abbreviations — 49

Annex 1 Participants — 51

Annex 2 Programme of the workshop — 53

Annex 3 Workshop evaluation form — 56

Summary

On 9 June 2016, the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*) organised its annual workshop in Saint Malo, France. Participants of the workshop were representatives of: the NRLs for *Salmonella* from 27 EU Member States, two European Free Trade Association (EFTA) countries, and two (potential) EU candidate countries. Also present were representatives of the European Commission Directorate General for Health and Food Safety (DG-Sante), of the European Food Safety Authority (EFSA) and of the European Centre for Disease Prevention and Control (ECDC). In total 5 participants of NRLs from two EU Member States (Belgium and Malta), one EFTA country (Switzerland) and two (potential) candidate countries (Bosnia and Herzegovina and Turkey), were unable to come to the workshop due to lack of staff or due to problems with public transport. A total of 45 participants attended the workshop.

During the workshop, presentations were given on several items. The results of the interlaboratory comparison studies organised by the EURL-*Salmonella* in the past year were presented. This concerned the studies on detection of *Salmonella* in whole liquid egg (September 2015) and in samples from the primary production stage (February 2016) and the study on typing of *Salmonella* (October/November 2015).

An EFSA representative presented the most recent European summary report on Zoonoses. This report gives an overview of the number and types of zoonotic microorganisms that caused health problems in Europe in 2014. For several years, the number of health problems caused by *Salmonella* has been declining, but it remains the second most important cause of zoonotic diseases in Europe, after *Campylobacter*. Additionally, the EFSA representative gave an update on the joint EFSA/ECDC molecular typing database.

A representative of ECDC presented how joint cluster management works in practice, related to the joint molecular typing database. For this, an introduction to the web-based communication platform EPIS-FWD was given (Epidemic Intelligence Information System for Food and Waterborne Diseases).

A presentation was given on the use of Whole Genome Sequencing (WGS) for typing of *Salmonella*.

A summary was given in relation to standardisation of methods in ISO and CEN.

A Swedish representative of the NRL gave a presentation on the cooperation between Nordic countries regarding the organisation of Proficiency Tests for regional laboratories.

A representative of the NRL in France gave a presentation on the investigations on *Salmonella* Enteritidis in poultry production in France. The workshop concluded with a presentation on the EURL-*Salmonella* work programme for the current and coming year.

All workshop presentations can be found at:

http://www.eurlsalmonella.eu/Workshops/Workshop_2016

1 Introduction

In this report, the abstracts of the presentations given at the 2016 EURL-*Salmonella* workshop are presented, as well as a summary of the discussion that followed the presentations. The full presentations are not provided in this report, but are available on the EURL-*Salmonella* website: http://www.eurlsalmonella.eu/Workshops/Workshop_2016

The layout of the report is consistent with the workshop programme. All abstracts of the presentations are given in chapter 2. The evaluation of the workshop is summarised in chapter 3 and the (empty) evaluation form is given in Annex 3. The list of participants is given in Annex 1. The programme of the workshop is given in Annex 2.

2 Thursday 9 June 2016: the day of the workshop

2.1 Opening and introduction

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

Kirsten Mooijman, head of the EURL-*Salmonella*, opened the 21st workshop of the EURL-*Salmonella*, welcoming all participants to Saint Malo, France.

The workshop was attended by 45 participants, including representatives of the National Reference Laboratories (NRLs) for *Salmonella* from the EU Member States, (potential) candidate EU countries, and member countries of the European Free Trade Association (EFTA). Furthermore, representatives from the EC, Directorate General for Health and Food Safety (DG-Sante), the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) were present. Apologies were received from representatives of two NRLs (Malta and Bosnia and Herzegovina). Additionally, 3 participants (from Belgium, Switzerland and Turkey) were unable to attend due to problems with public transport (at the time of the workshop, public transport in France was disrupted by strike actions).

After a roll call of the delegates, the results of the evaluation of the last five workshops (2011 - 2015) were compared, showing variable results for the five workshops. The opinion on the scientific programme was the same for all workshops: good to excellent.

The workshop started after presentation of the programme and general information concerning the workshop.

The workshop programme is presented in Annex 2.

2.2 Results 20th interlaboratory comparison study on typing of *Salmonella* (2015) – serotyping and PFGE

Wilma Jacobs, EURL-Salmonella, Bilthoven, the Netherlands

In November 2015, the 20th interlaboratory comparison study on serotyping and PFGE typing of *Salmonella* was organised by the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*, Bilthoven, the Netherlands). A total of 34 laboratories participated in this study. These included 29 National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) in the 28 Member States of the European Union (EU), two NRLs of the (potential) EU candidate countries Former Yugoslav Republic of Macedonia and Turkey, and three NRLs of the EFTA countries Iceland, Norway and Switzerland. The main objective of the study was to evaluate whether typing of *Salmonella* strains by the NRLs-*Salmonella* within the EU was carried out uniformly, and whether comparable results were obtained.

All 34 laboratories performed serotyping. A total of 20 obligatory *Salmonella* strains plus one additional optional *Salmonella* strain from an uncommon type were selected for serotyping by the EURL-*Salmonella*. The strains had to be typed with the method routinely used in each

laboratory, following the White-Kauffmann-Le Minor scheme (Grimont and Weill, 2007).

The individual laboratory results on serotyping, as well as an interim summary report on the general outcome, were e-mailed to the participants in February 2016. The O-antigens were typed correctly by 31 of the 34 participants (91%). This corresponds to 99% of the total number of strains. The H-antigens were typed correctly by 21 of the 34 participants (62%), corresponding to 97% of the total number of strains. A total of 19 participants (56%) gave correct serovar names to the full set of strains, corresponding to 97% of all strains evaluated. A completely correct identification by all participants was obtained for eleven *Salmonella* serovars: Agama (S1), Eastborne (S5), Virchow (S7), Emek (S8), Teddington (S13), 1,4,[5],12:i:- (S14), Meleagridis (S15), Typhimurium (S16), Infantis (S17), Enteritidis (S19), and Montevideo (S20). Most problems occurred with the serovar Kintambo (S9). Four laboratories had difficulties assigning the correct serovar name to this strain.

All but two participants serotyped the additional strain S21, being a *Salmonella* Miami. All 32 laboratories correctly serotyped the O-antigens and the H-antigens for this strain, but in order to be able to correctly name this strain, some additional biochemistry was required.

Six laboratories noted that they had not done any biochemical tests on this strain, and three participants therefore correctly named this strain 9,12:1:1,5. The other three laboratories named this strain Miami (2x) or II (1x, incorrect), but without any supporting 'evidence'. The majority of the participants named S21 Miami, ruling out the possibility of an *S. enterica* subspecies *salamae* (II) result by testing on e.g. malonate or tartrate. However, the 'proof' on how they differentiated between Miami and Sendai was not always given.

At the EURL-*Salmonella* workshop in 2007, the EURL-*Salmonella* proposed a definition for good performance of the NRLs regarding the serotyping. Using this definition, 33 participants achieved good performance. The one laboratory that did not achieve this level participated in the (obligatory) follow-up study consisting of ten additional strains for serotyping. The EU-NRL concerned obtained good scores in this follow-up study (May 2016).

The individual laboratory results on the PFGE typing part were reported to the 16 participants in April 2016. The participants were asked to test 10 *Salmonella* strains using their own routine PFGE method for digestion with XbaI. This year, the evaluation of the (optional) analysis of the gel in Bionumerics was introduced as well. A total of 12 participants also sent in their analysed gel data for evaluation.

The PulseNet Guidelines were used for the quality grading of the PFGE gel images, based on scoring seven parameters with 1 (poor) point to 4 (excellent) points. Some variation in the quality of the gel images was still observed, but also clear improvements were seen compared with the first study in 2013.

The analysis of the gel in Bionumerics was evaluated following the guidelines used in the External Quality Assessment schemes for the FWD laboratories. These guidelines use five parameters which are scored with 1 (poor), 2 (fair/good) or 3 (excellent) points. The majority of the participants scored 'Excellent' for the parameters 'Position of gel',

'Strips', and 'Normalisation'. Improvements could mainly be advised for the parameters 'Curves' and 'Band assignment'.

PFGE typing, concerning the quality of PFGE gel image as well as optional gel analysis in Bionumerics, will be part of the 2016 interlaboratory comparison study on typing of *Salmonella*. MLVA on *S. Typhimurium* will not yet be part of the 2016 study, as only a limited number of 6-7 workshop participants considered this to be of potential relevance to their laboratory practices.

More details can be found in the interim summary reports (Jacobs et al., 2016a and 2016b).

Discussion

Q: Is it possible to provide suggestions for *Salmonella* serovars for inclusion in the serotyping study? Especially 'complicated' serovars found by NRLs may be of interest.

A: The EURL is happy to receive suggestions.

2.3 Update on the joint EFSA/ECDC molecular typing database

Valentina Rizzi, EFSA, Parma, Italy

Molecular typing through microbial DNA fingerprinting has developed rapidly in recent years. Data on the molecular testing of food-borne pathogens such as *Salmonella*, *Listeria monocytogenes* and verotoxigenic *Escherichia coli* (VTEC) could substantially contribute to the epidemiological investigations of food-borne outbreaks and to the identification of emerging health threats, as well as to source attribution studies. For the purpose of the data collection and subsequent linkage with corresponding data from human isolates, ensuring comparability of typing data from food-borne pathogens isolated from food, feed, animals and the related environment, as well as from human sources, is essential.

A Commission vision paper following the Enterohaemorrhagic *Escherichia coli* (EHEC) crisis was endorsed by the Member States (MS) in December 2012 (EC, 2012). Thereafter, the Commission asked EFSA to provide technical support regarding the collection of molecular typing data of food, feed and animal isolates of *Salmonella*, *Listeria monocytogenes* and VTEC, and a similar request was made to ECDC on molecular typing data of human isolates. In addition, the Commission asked EFSA and ECDC to establish a joint database for the molecular typing data of these foodborne pathogens of human and non-human origin. The aim of the joint EFSA-ECDC database is to enhance routine surveillance and outbreak identification by enabling detection of microbiological links between isolates of human and of non-human origin.

The data collection covers molecular typing results obtained through Pulsed Field Gel Electrophoresis (PFGE) for *Listeria monocytogenes*, *Salmonella* and VTEC, and Multiple-Locus Variable number tandem repeat Analysis (MLVA) for *Salmonella* Typhimurium only. Molecular typing data production, interpretation and submission shall be performed according to defined Standard Operating Procedures and technical specifications (Caprioli et al., 2014, EFSA et al., 2014, Jacobs et al., 2014, Roussel et al., 2014). A specific Collaboration Agreement

has been signed by the parties involved to address issues with regards to data ownership, availability, access, use, and publication. Data confidentiality is guaranteed by the limited sharing of data in the joint database and by the restricted access to sensitive information. Curation of human isolates is performed by ECDC; curation of non-human isolates is carried out by the European Union Reference Laboratories (EURLs) for the specific pathogen. The joint cluster analysis of both human and non-human isolates is carried out by EFSA, ECDC and their respective curators in the joint database, according to a specific procedure agreed between the parties. The official nomination of MS representatives for this data collection is ongoing and the technical coordination and support from EFSA to laboratories has started.

Discussion

Q: How many representatives of the NRLs are aware of the molecular typing database?

A: Only a limited number of representatives are aware of the database. This may be due to a lack of communication between the Competent Authorities and the NRLs. The NRLs are advised to contact the relevant Competent Authority to draw attention to this database.

Q: How to deal with the problem if the people submitting the data do not have a laboratory background and do not have any knowledge of BioNumerics?

A: This is indeed complicated. However, it is up to the Competent Authority to do the nomination for data submission from the Member State. A solution may be to separate the tasks: one person (e.g. from a laboratory) submits the data, and a second person (e.g. Competent Authority) approves/validates the data.

Q: What data can a user see in the database?

A: Users can only see non-sensitive data, like serovar names, PFGE profiles, and whether it is of food or veterinary origin (in general terms). No details on the source are visible, nor of the country of origin. It may be possible to see more details from your own country.

Q: Is the database for antimicrobial resistance the same as this molecular typing database?

A: No this is a different database.

Remark: More detailed information on the molecular typing database will be distributed through the EURL-*Salmonella* (by e-mail, website or newsletter). Additionally, a questionnaire will be distributed (also through the EURL) on the use of Whole Genome Sequencing.

2.4 How will joint cluster management work in practice? An introduction to EPIS-FWD

Karin Johansson, ECDC, Stockholm, Sweden

The abbreviation EPIS-FWD stands for Epidemic Intelligence Information System for Food and Waterborne Diseases. It concerns a web-based, restricted access communication platform hosted by ECDC. The purpose of this system is to ensure the early detection and coordination of the response to multi-state outbreaks through the timely sharing of cross-sectorial information. EPIS-FWD is currently available to public health epidemiologists, microbiologists, policymakers and risk managers from

all 28 EU Member States, some EFTA countries and some third countries inside and outside Europe.

Data of communicable disease surveillance collected by all EU Member States and EEA countries are reported to the European Surveillance System (TESSy), a password-protected, fully anonymised database hosted by ECDC.

In EPIS-FWD, a distinction is made between Urgent Inquiry (UI) and Molecular Typing Cluster Investigation (MTCI).

Urgent Inquiry:

- A structure that is used to launch a request for information about an unusual event with the potential for international spread.
- Available to representatives of all Member States and some international partners.
- Urgent Inquiries are generally launched for one of two main reasons:
 - Based on an unusual public health event detected in a Member State;
 - Based on a human cluster discussion (MTCI) which is deemed to be of interest to the whole network.

Molecular Typing Cluster Investigation (MTCI):

- Structure that allows exchange of information about a specific cluster.
- Only available to representatives of the Member States that have isolates in the cluster.
- ECDC and ECDC curators also have access by default.

EPIS-FWD could also be of use for joint and non-human cluster management, as:

- it already supports human cluster management and can be upgraded to also support non-human and joint cluster management;
- it already implements restricted access to cluster evaluation for involved Member States only, and can be upgraded to also support restricted access based on sector.
- it is an opportunity to allow representatives from national food and veterinary authorities and laboratories to exchange information with counterparts from national public health authorities and laboratories.

The development of EPIS-FWD with support for non-human and joint cluster management is ongoing. The joint MTCI functionality is expected in September 2016, and the joint UI functionality is expected in November 2016. EFSA will manage a list of all users from the food and veterinary sector. The access will be country-specific, but all users from the same country have access to the same information. ECDC will issue all users from the EFSA list with login credentials. Once the joint cluster management upgrade is operational, training will be provided by EFSA with support of ECDC.

Discussion

Q: Is it possible to contact ECDC if a match in molecular data is seen, but not all information is visible because it is from another country?

A: ECDC/EFSA/curators will also perform a regular (weekly) search. If it concerns a cluster with human data, the involved countries decide whether to scale it up or not. If agreed, more countries will get access to the data. If it concerns a joint cluster of food and human data, the involved countries from both sectors will decide if it will go for wider access or not.

Q: Who can use EPIS?

A: EPIS has only nominated users. Currently only the public health sector has access. For the non-human side, this still needs to be arranged. EFSA will ask for nominations of users from the non-human side (food, feed, veterinary). It is possible to have multiple users per country. Different user groups can be put into the system.

2.5 Use of WGS for typing of *Salmonella* at PHE

Elizabeth de Pinna, PHE, London, United Kingdom

Following the investment by Public Health England (PHE) in equipment and infrastructure for the introduction of whole genome sequencing (WGS), *Salmonella* was selected as one of the first organisms for a WGS project.

A variety of different methods were being used for the identification, characterisation and typing of *Salmonella*, such as: PCR assays, serotyping, biochemical tests, phage typing, pulsed-field gel electrophoresis (PFGE) and multi-locus variable-tandem repeat analysis (MLVA). The aim of the project was to use WGS to replace these methods for the identification and typing of *Salmonella*.

The project was divided into three stages:

- First phase validation
- Second phase validation
- WGS adopted for routine *Salmonella* identification and typing.

Identification of *Salmonella* by WGS is based on the multi-locus sequence type (MLST). The MLST is based on the sequences of seven housekeeping genes, and it can be used to identify natural genetic clusters. In general, clusters defined by MLST correspond on a one-to-one basis with the serovar.

The validation of WGS was carried out in two phases. The first phase used 1500 strains selected from the *Salmonella* isolates received in 2012. The second phase was started in April 2014, and all the *Salmonella* isolates received in the year up to the end of March 2015 had routine identification using traditional methods and WGS.

The results from the validation projects showed a high correlation between the results from the traditional methods and the WGS. In April 2015, WGS was adopted for routine *Salmonella* identification and typing. During the validation phases, automated pipelines for the analysis of the WGS data and uploading the results to the laboratory information management system were developed.

The *Salmonella* typing data is used for surveillance and outbreak detection and investigation. A method of analysing the WGS data based on single nucleotide polymorphisms (SNPs) was developed to replace the

sub-typing methods of phage typing, PFGE and MLVA. The SNP analysis results in a seven-digit hierarchical SNP address. SNP addresses are compared on a phylogenetic tree which groups genetically similar isolates together; these are called clusters. A cluster of genetically similar isolates can indicate a possible outbreak, which can then be investigated. SNP analysis has been used in the investigation of several outbreaks. The introduction of WGS for routine *Salmonella* identification and typing has enabled most of the traditional methods to be discontinued. In an outbreak situation, further analysis of the WGS data can be carried out to establish the similarity of the *Salmonella* isolates without the need for further typing. SNP typing has also detected outbreaks that would not have been highlighted with the traditional typing methods.

Discussion

Q: What about the staff originally performing the classical methods?

A: For the new method, a reduced number of staff is needed. The laboratory is restructured and training sessions were organised on the new methods.

Q: In EU legislation, it is indicated that in specific cases, positive strains should be stored to be able to perform phage typing at a later stage. What do we do when PHE stop producing phages at the end of this year?

A: The EC, together with the EURL, may need to have a closer look at this and if necessary, consider amending the legislation. If specific problems are foreseen, please forward them to DG-Sante.

Q: Do you still perform serotyping?

A: Yes, sometimes we still do this for building up the database. Especially for rare serovars, we need to add serotyping results to be able to link WGS results to a serovar name.

Q: What is needed to start with WGS. What is the right moment to switch to WGS?

A: Some (financial) investments are needed, as well as training of staff, and knowledge of bioinformatics. It is a growing process; some laboratories are ahead, and some are further behind. This is normal when new techniques are introduced. Additionally, standardisation may be needed for analysis of data.

Q: Is the use of WGS allowed instead of serotyping, from a legislation point of view?

A: According to legislation, alternative methods are permitted if they are validated. However, the ISO procedure for validation of confirmation and typing methods is still under development. The developments in the field of WGS are going very fast. DG-Sante, together with EFSA and the EURL, will draft a questionnaire to get information on the use of WGS at the NRLs. The EC needs to take into account that not all countries are at the same stage with the new methods. The results of the questionnaire will give a better idea on the use of WGS in different countries. If necessary, legislation needs to be amended, but this will be discussed between DG-Sante, the EURL and the Member States (NRLs).

2.6 *Salmonella* monitoring data and food-borne outbreaks for 2014 in the European Union

Valentina Rizzi, EFSA, Parma, Italy

The European Union (EU) Directive 2003/99/EC (EC, 2003a) requires EU Member States (MS) to collect data on zoonoses and zoonotic agents every year, and requests the European Food Safety Authority (EFSA) to analyse these data and to publish annual European Union Summary Reports (EUSRs) on zoonoses, foodborne outbreaks (FBOs) and antimicrobial resistance (AMR). EFSA is charged with the production of these annual EUSRs, in collaboration with the European Centre for Disease Prevention and Control (ECDC) that collects and analyses human data. The most recent EUSRs on zoonoses, FBOs and AMR, related to 2014 data, were published at the end of 2015 and the beginning of 2016 (EFSA and ECDC, 2015 and 2016). An update about the reporting tool for data collection was given, as well as on *Salmonella* data in humans, food and animals in the EU.

For the first year, the data has to be submitted exclusively using the EFSA's Data Collection Framework (DCF). Salmonellosis is confirmed as the second most frequently reported zoonose in humans in the EU in 2014, after campylobacteriosis. The declining EU trend in confirmed human salmonellosis cases observed in recent years has continued. Most MS met their *Salmonella* reduction targets for poultry populations. In foodstuffs, the categories with the highest level of non-compliance to the microbiological criteria were minced meat, meat preparation and meat product, whereas the reported EU level of *Salmonella* non-compliance in fresh poultry meat decreased. No major changes were observed with regards to the contamination of foodstuffs with *Salmonella* spp. compared with previous years, and *Salmonella* was most frequently detected in poultry meat, and less often in pig or bovine meat, and rarely in table eggs. The analysis of the serovar distribution and trends in different animal populations and food categories shows the emergence of some serovars (e.g. *S. Infantis*, *S. Kentucky*) in specific geographical areas and food production chains.

A total of 5251 FBOs, including water-borne outbreaks, were reported in the EU, and *Salmonella* was the second most recognised causative agent after viruses. In total, 1048 *Salmonella* FBOs were reported, of which 225 were supported by strong evidence. Important food vehicles in strong-evidence FBOs were eggs and egg products, followed by bakery products and pig meat and products thereof. In addition, one waterborne strong-evidence outbreak caused by *Salmonella* was reported.

With regard to the AMR monitoring, in 2014 a specific focus was on poultry populations. A frequent resistance to fluoroquinolones was observed, but low resistance to other critically important antimicrobials, and low occurrence of Extended-spectrum beta-lactamase (ESBL)/AmpC producers. No carbapenemase producers were detected. The transferable resistance to colistin has recently been reported. This confirms the continuously evolving threat from emerging antimicrobial resistance and the need to review the data collected, interpret the findings, and to assess trends. Data show marked variations between *Salmonella* serovars, with *S. Infantis* and *S. Kentucky* contributing significantly to the overall numbers of multi-resistant *Salmonella* and displaying high-level resistant to ciprofloxacin. Variations were also observed between reporting countries.

Discussion

Q: Are there any plans to increase the control in pigs?

A: Currently there are no new plans.

2.7 Update on activities in ISO and CEN

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

Kirsten Mooijman of the EURL-*Salmonella* presented an overview of activities in ISO and CEN in relation to *Salmonella*.

The relevant groups in ISO and CEN are:

- ISO/TC34/SC9: International Standardisation Organisation, Technical Committee 34 on Food Products, Subcommittee 9 – Microbiology;
- CEN/TC275/WG6: European Committee for Standardisation, Technical Committee 275 for Food Analysis – Horizontal methods, Working Group 6 Microbiology of the Food Chain.

Both groups held their plenary meetings in Paris, France from 9 to 13 May 2016. The progress on the *Salmonella* documents was presented at these meetings by Kirsten Mooijman.

A summary was given on standardisation items relevant for the NRLs for *Salmonella*.

EN ISO 6579, part 1 (CEN lead)

Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 1: Horizontal method for the detection of *Salmonella*.

FDIS voting took place from 12 November 2015 to 12 January 2016. The outcome was: 100% positive in CEN (20 approvals, 13 abstentions) and 96% positive in ISO (24 approvals, 1 disapproval). The total outcome was positive, with 13 pages of comments, mainly editorial. A few technical comments were given which had to be taken into account. For that reason, a written consultation of ISO Resolution No. 686 took place from 9 March to 20 April 2016. In this resolution, agreement was asked for:

1. Addition of the following text in the Introduction at the end of the first paragraph: 'The main changes listed in the Foreword, introduced in this International Standard compared to ISO 6579:2002, are considered as minor changes.'
2. Change annex F from normative into informative, and change the third paragraph of 9.1 accordingly into: 'For specific products, follow the procedures given in ISO 6887 (all parts). For the preparation of test portions and initial suspensions of milk and milk products, Annex F should be followed. In case of discrepancies between the procedures described in Annex F and ISO 6887-5, follow ISO 6887-5.'

The outcome of the consultation for Resolution No. 686 was the following:

- Q1 (Main changes considered as minor changes): 24 approvals, 14 abstentions.
- Q2 (change Annex F from normative to informative & change text in 9.1): 18 approvals, 1 disapproval (UK against changing annex F into informative, but agrees with proposed changes to text), 15 abstentions.

For the final publication of the standard, it was necessary to draft a justification to ask CEN central for approval of the changes. After approval of CEN/TC275, the document can be finalised as ISO central has already approved the changes.

Note: By mid-June, CEN decided that a second formal vote (second FDIS vote) is needed prior to the publication of the document, which will result in a further delay of the publication of EN ISO 6579-1.

PCR monophasic *Salmonella* Typhimurium (cooperation ISO and CEN)

This concerns a cooperation between ISO WG10 (convenor Kirsten Mooijman) and CEN TAG3 (project leader Burkhard Malorny).

- In June 2015, Recommendation N383 was taken at the meeting of CEN/TC275/WG6: 'TAG3 will continue technical work to develop PCR for identification on monophasic *Salmonella* Typhimurium. Once the method is available, the work will be transferred to ISO/TC34/SC9 WG10'.
- The following was agreed in meetings of TAG3 (April 2015), EURL-*Salmonella* workshop (May 2015), and WG6 (June 2015):
 - Priority should be given to a protocol for identification of monophasic *S. Typhimurium* lacking the second phase (1,4,[5],12:i:-). For the time being, the protocol does not yet have to be able to also identify the monophasic variant lacking the first phase (1,4,[5],12:-:1,2).
 - A gel-based and real-time PCR method covering monophasic variant *S. 1,4,[5],12:i:-* should be standardised including data of their performance characteristics.
 - The protocol(s) will be published as amendment to ISO/TR 6579-3 and will become a guidance document.
- 2 February – 1 March 2016: requests were sent to CEN-TAG8 ('Detection of *Salmonella*'), ISO-WG10 ('Serotyping of *Salmonella*') and NRLs-*Salmonella* to indicate interest in reviewing draft PCR protocols; to participate in a future verification study to determine performance characteristics; and to indicate possible interesting strains for use in verification study. In total, 23 replies (including one from the USA) were received. 14 laboratories indicated being interested in reviewing the draft protocols, 21 laboratories indicated being interested in participating in an interlaboratory study for determining performance characteristics, and 21 laboratories indicated that they have interesting strains for the interlaboratory study.
- March 2016: first draft protocol prepared by Burkhard.

The following was discussed at the meetings of ISO-SC9 and CEN-WG6. It was agreed that it would be preferable to prepare a new part 4 of ISO 6579 instead of making the method an annex to ISO/TR 6579-3, as it concerns a different technique. Furthermore, it was preferred to publish the document as a Technical Specification (TS). As soon as the technical work is finished and CEN-TAG3 agrees on the draft document, it should be moved to ISO-WG10, so that a New Work Item Proposal (NWIP) can be launched. Currently, WG10 could include the work as a Preliminary Work Item (PWI). The secretariat of SC9 will launch a call for additional participants for WG10 with expertise for PCR of monophasic *Salmonella* Typhimurium; TAG3 members especially will be invited to join WG10. It

was also suggested to invite Burkhard Malorny, the current project leader at TAG3, to become co-project leader in WG10. For the PWI the following title was suggested: 'Part 4: Identification of monophasic *Salmonella* Typhimurium (1,4,[5],12:i:-) by Polymerase chain reaction'. The organisation of the interlaboratory study for determining performance characteristics will be organised once the final draft standard document is available.

New proposal: standardisation of a method for molecular (PCR) serotyping of *Salmonella*

At the last ISO meeting (May 2016), the US delegation proposed to standardise a method for molecular (PCR) serotyping of *Salmonella*. Information on the method was distributed before the SC9 meeting and was explained during the meeting. According to the information, it is an open method (not proprietary) and it can be useful for serotyping either pure cultures of *Salmonella* or 24h pre or enriched mixed cultures. It concerns a multiplex PCR assay for the 'top 30' clinical *Salmonella* serovars associated with foodborne outbreaks. It should be possible to perform the method in 'any laboratory', it is not necessary to be a specialised laboratory. The Food and Drug Administration (FDA) in US has performed a single laboratory validation, and is planning to organise an interlaboratory study in the near future. At the ISO meeting, it was agreed that a written enquiry would be launched to ask for the need for a standard for molecular serotyping of *Salmonella*. With this enquiry, the (summarised) method should be included, as well as the available validation data from the FDA.

It was agreed that the EURL-*Salmonella* will keep the NRLs updated on the subject.

Harmonisation of incubation temperature

Experiments at the laboratory of Adria, France (Daniele Sohier) were performed to test the influence of incubation temperature (35°C or 37°C) on the growth of *Salmonella* and several *Enterobacteriaceae* species. These experiments showed no difference in growth of *Salmonella* spp. at both temperatures, but some impact on the growth of some (other) *Enterobacteriaceae* species.

In 2015, it was proposed to set up a protocol to test the influence of the incubation temperature with a larger group of laboratories (the members of ISO and CEN), especially to test the influence on the growth of *Enterobacteriaceae*.

The first final draft protocol and reporting form were drafted by Daniele Sohier and Kirsten Mooijman and were tested at both laboratories. Next the protocol was further updated. The SC9 members were requested to make an inventory of which laboratories in their countries intend to participate in this study, and to report this to the secretariat of SC9 before 1 July 2016. Barbara Gerten (Germany) will ask IDF/SC5 for participation in the study, and Kirsten Mooijman will ask the NRLs for *Salmonella* for participation. This latter was done at the EURL-*Salmonella* workshop and it was agreed that the EURL will send the protocol to the NRLs so that they can decide whether to participate or not.

The data should be sent to the ad hoc group by February 2017, so that the data can be analysed and a presentation of the results prepared before the next SC9 meeting.

CEN mandate M381

This project started in 2007, with the aim of standardising and validating methods that are referred to in legislation, in order to support the EU food policy. The project exists of 15 sub-projects, including international standardisation and validation of 15 microbiological methods. One of these sub-projects concerns the validation of the method for detection of *Salmonella* in samples from the primary production stage (pps). The performance characteristics for detection of *Salmonella* in pps samples have been determined from EURL-*Salmonella* interlaboratory studies of 2008 (chicken faeces), 2012 (pig faeces) and 2013 (boot socks – combined EURL/CEN mandate study).

The CEN mandate project ends in June 2017. By then, all 15 EN/ISO standards, including the performance characteristics have to be published. At the last CEN meeting, the following was agreed:

- To store the raw data at DG-Sante and at CEN, so that it is available for possible future recalculations;
- To publish all validation studies in a special issue of the International Journal of Food Microbiology. The participants of each study will be mentioned in the acknowledgements of the relevant study, and their agreement for publication will be requested.

Pre-enrichment step

The CEN Task group, TAG9, was set up in 2012 to try to come to an optimal pre-enrichment medium for detection of several (mainly Gram negative) pathogenic bacteria, in order to resuscitate stressed or damaged cells. The group has performed experiments to test chemically defined products as a replacement of 'peptone'. This resulted in poor growth of several strains. Furthermore, different strains were tested for their growth in BPW prepared from different batches of peptones, which gave variable results. At a meeting of TAG9 in spring 2016, it was agreed to start the development of a protocol for determination of performance characteristics for the pre-enrichment step (e.g. in BPW). Additionally, the impact of modifications on the sample preparation will be considered for the pre-enrichment step, like soaking of dry products, pooling of test portions, different dilution factors, pre-warming of BPW, and alternative BPW formula for acidifying food items.

EN ISO 6887 parts 1 to 4

It is expected that the FDIS voting on parts 1 to 4 of ISO 6887 will be launched in the second half of 2016

Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination

- Part 1: General rules for the preparation of the initial suspension and decimal dilutions (including information on pooling of samples and verification protocol for pooling)
- Part 2: Specific rules for the preparation of meat and meat products
- Part 3: Specific rules for the preparation of fish and fishery products
- Part 4: Specific rules for the preparation of miscellaneous products (e.g. animal feed, eggs, cocoa products, acidic products)

For part 5 the revision will (probably) start soon under IDF leadership (part 5: Specific rules for the preparation of milk and milk products).

ISO 16140 'Microbiology of food and animal feeding stuffs – Protocol for the validation of alternative methods' (Anonymous 2003). This document is under revision and is divided into six parts:

Microbiology of the food chain – Method validation

- Part 1 'Vocabulary'.
- Part 2 'Protocol for the validation of alternative (proprietary) methods against a reference method'.
- Part 3 'Protocol for verification of reference and alternative methods in a single laboratory'. This document describes a procedure for internal verification of methods, which is especially of interest in case a method is performed under accreditation.
- Part 4 'Protocol for in-house (single) laboratory method validation'
- Part 5 'Protocol for factorial interlaboratory method validation'
- Part 6 'Protocol for the validation of microbiological confirmation and typing methods'. The EURL-*Salmonella* is project leader of this part.

Parts 1 and 2 were published in June 2016.

For part 3, a second Working Draft was published in April 2016, and for parts 4 to 6, the Committee Draft (CD) voting was held from early 2016 until mid-April 2016. The CD voting was positive for all 3 parts, and included several comments. Comments on the second Working Draft of part 3 were also received. These will be introduced in a draft CD version for part 3 and in a draft DIS version for parts 4 to 6, and will be discussed in the ISO working group in autumn 2016 before launching the CD and DIS voting.

EN-ISO 7218:2007/Amendment 1:2013 'Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations'. The revision of this document will include (amongst others):

- Inclusion of information/reference to 'new' techniques;
- Reduction of the calculation section by giving reference to Excel calculators;
- Reduction of the equipment section.

ISO 11133:2014 'Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media

ISO 11133 was published in 2014, but does not yet include the performance testing of confirmation media and reagents. The ISO working group is therefore preparing an amendment for this. Another amendment will be prepared for correction of errors and to clarify some aspects in the current ISO document. After finalisation of the two amendments, it is planned to revise the full document again.

ISO working group on WGS

In 2014, a new working group was set up under ISO/TC34/SC9, to take a closer look at the options for standardisation of protocols for Whole Genome Sequencing. The project leader of this group is located in the

USA. In the past year, the working group has prepared an outline of the future standard with, as initial target organisms, foodborne prokaryotes. The future standard will be divided into three parts:

- Part 1: Wet laboratory sequencing and analysis of sequence data.
- Part 2: Validation of data and methods.
- Part 3: Metadata and sequence repository.

Discussion

Q: Is the new range for the incubation temperature at 37°C, 36°C ± 2°C?

A: No, the range is 34°C to 38°C. If 36°C ± 2°C is prescribed, it means that the temperature of the incubator has to be set at 36°C. However, by giving a range, the incubator can be set at any temperature between 34°C and 38°C, as long as the minimum temperature is 34°C and the maximum temperature is 38°C.

Q: Is it necessary to do a revalidation when introducing ISO 6579-1 in the laboratory?

A: The CEN Task Group has indicated the changes in the new ISO 6579-1 as being minor compared to ISO 6579:2002, meaning that a full revalidation is not necessary. However, this may be required if the national accreditation body still requires a (limited) verification when introducing the new ISO 6579-1. This needs to be discussed with the national accreditation body.

Q: When are the ISO standards for WGS expected to be published?

A: The working group is very active, but it will take some time (a few years) before a standard is finalised. First working draft versions of the three parts may be expected next year.

2.8 Results interlaboratory comparison study Food on detection of *Salmonella* in whole liquid egg (2015)

Angelina Kuijpers, EURL-Salmonella, Bilthoven, the Netherlands

In September 2015, the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*) organised the seventh interlaboratory comparison study on the detection of *Salmonella* in samples from food. The matrix of concern was whole liquid chicken egg.

The participants were 36 National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*): 30 NRLs from the 28 EU Member States (EU-MS), 4 NRLs from third countries within Europe (EU candidate MS or potential EU candidate MS, member of the European Free Trade Association (EFTA)) and one NRL from a non-European country.

The most important objective of the study was to test the performance of the participating laboratories for the detection of *Salmonella* at different contamination levels in a food matrix. For this purpose, whole liquid egg samples of 25 grams artificially contaminated with *Salmonella* Enteritidis (SE) at various contamination levels, were analysed. The performance of the laboratories was compared with the criteria for good performance. The participants were not given a Standard Operating Procedure (SOP) but were asked to follow FDIS ISO 6579-1 (Anonymous, 2015) according to normal routine procedure for detection of *Salmonella* in 'official' samples. According to this document, in addition to Mueller Kauffmann Tetrathionate novobiocin broth (MKTTn), either Rappaport Vassiliadis

Soya broth (RVS) or Modified Semi-solid Rappaport-Vassiliadis (MSRV) can be used for selective enrichment.

For the results, the participants were asked to note what would have been reported if these samples had been routine samples, meaning that the indication 'positive' (1) or 'negative' (0) per sample (after confirmation) was sufficient (independent of the combination of selective enrichment medium and isolation medium). Hence, the results per medium are no longer visible for the EURL-*Salmonella*.

The samples consisted of whole liquid egg artificially contaminated with a diluted culture of *Salmonella* Enteritidis (SE) at a low level (approximately 15-20 cfu/25 g of egg), at a high level (approximately 50-100 cfu/2 g of egg) and with no *Salmonella* at all (blank samples). The samples were artificially contaminated at the laboratory of the EURL for *Salmonella*. Before the start of the study, several experiments were carried out to make sure that the samples were fit for use in an interlaboratory comparison study (e.g. choice of *Salmonella* serovar, stability at different storage temperatures, and influence of background flora).

Eighteen individually numbered blind samples with whole liquid egg had to be tested by the participants for the presence or absence of *Salmonella*. These samples consisted of six blank samples, six samples with a low level of SE (inoculum 21 cfu/sample) and six samples with a high level of SE (inoculum 101 cfu/sample). Additionally, two control samples had to be tested: one blank control sample (procedure control (BPW)) and one own (NRL) positive control sample (with *Salmonella*). The laboratories found *Salmonella* in all (contaminated) samples, resulting in a sensitivity rate of 100%.

PCR was used as an own method by nine participants, and eight of these laboratories used a real-time PCR. All nine laboratories found the same results as when using the bacteriological culture method.

The majority of participants (27) used all three selective enrichment media (MKTTn, MSRV and RVS) and nine laboratories chose between RVS and MSRV in addition to MKTTn.

For the positive control, the majority of the participants (21 laboratories) used a diluted culture of *Salmonella*. The *Salmonella* serovars most often used for the positive control sample were *S. Enteritidis* (16) and *S. Typhimurium* (8). The concentration of the positive control varied between 5 and 10^7 cfu/sample. For the positive control, it is advisable to use a concentration level close to the detection limit, and a *Salmonella* serovar not often isolated from routine samples (to distinguish it more easily in case of cross contamination).

The egg samples needed to be stored at 5°C after receipt at the participating laboratory. Unfortunately, this was not always the case and temperatures up to 10°C were observed, which could have resulted in less stable samples and more difficulties when detecting *Salmonella*.

For this study, four different batches of whole liquid chicken egg were used. The background flora in the batch used in the main study was much lower compared to the batch used in the pre-test. This made it slightly easier to detect *Salmonella* in the samples used in this study.

Thirty-five of the 36 laboratories achieved the level of good performance. One NRL reported two positive results for a blank whole liquid egg sample. In a follow-up study, this laboratory scored all samples correctly, eventually resulting in good performance by all laboratories.

More details of the study can be found in the interim summary report (Kuijpers and Mooijman, 2015).

2.9 Preliminary results interlaboratory comparison study on detection of *Salmonella* in boot socks (2016)

Irene Pol, EURL-Salmonella, Bilthoven, the Netherlands

In February 2016, the 19th EURL-*Salmonella* interlaboratory comparison study on the detection of *Salmonella* in samples from the primary production stage was organised. In total, 36 NRLs participated in this study: 29 NRLs from 28 EU-Member States (MS), 6 NRLs from third countries within Europe (EU candidate MS or potential EU candidate MS and members of the European Free Trade Association (EFTA)), and on request of DG-Sante, one NRL from a non-European country.

The study design consisted of pairs of boot socks to which 10g of *Salmonella* free chicken faeces was added. The chicken faeces originated from a pathogen free broiler breeder's farm and tested negative for *Salmonella*. The boot socks with chicken faeces samples were artificially contaminated with *Salmonella* Typhimurium (STM) at the laboratory of the EURL-*Salmonella*.

Each NRL analysed a total of 20 blindly coded samples: 12 samples of boot socks with chicken faeces artificially contaminated with two different levels of *Salmonella* Typhimurium (six low (11 cfu/sample) and six high (95 cfu/sample)), six blank samples and two control samples. The control samples consisted of a procedure control blank and a control sample to be inoculated by the participants using their own positive control strain. The samples were stored at 5°C until the day of transport. On Monday 15 February 2016, the contaminated boot sock samples were packed and sent to the NRLs. On arrival at the NRLs, samples had to be stored at 5°C until the start of the analysis.

All laboratories used the prescribed method (Annex D of ISO 6579; Anonymous, 2007) with selective enrichment on MSR.V.

All laboratories scored well with both the procedure control and their own positive control samples. Both samples were scored 100% correctly. One laboratory (lab code 32) made an error in copying raw data to the electronic report, and received a moderate performance.

All laboratories detected *Salmonella* in all boot sock samples artificially contaminated with a high level of *Salmonella*. In addition, almost all laboratories detected *Salmonella* in all six low-level boot sock samples. Two laboratories (lab codes 6 and 20) scored one of the six low-level contaminated samples negative for *Salmonella*. This is still well above the

criteria for good performance. The sensitivity score was 99.5% for these samples.

The specificity of the study is given by the correctly scored blank samples, and reached 99% for this study. Only 1 laboratory did not score all 6 blank samples negative. This laboratory scored three of the six blank samples positive for *Salmonella*, and received a poor performance.

Overall, the laboratories scored well in this year's study with an accuracy of 99%. Thirty-four laboratories fulfilled the criteria of good performance, one laboratory scored a moderate performance, and one laboratory scored poor performance.

More details of the study can be found in the interim summary report (Pol-Hofstad and Mooijman, 2016).

2.10 Nordic cooperation for Proficiency Testing of regional laboratories

Lennart Melin, NRL-Salmonella Sweden

This joint venture regarding Proficiency Tests (PT) among the four Nordic countries started in 2006. At that time, the participants were Denmark, Norway and Sweden. There was no follow up in 2007, but from 2008 onwards an annual cooperative PT has been held for the regional laboratories of these countries. From 2010 onwards, regional laboratories from Finland have also participated. The Finnish require that each laboratory performs the PT at least once every third year. The time cycle of the PT starts every year in late August (see Figure 1), with a mutual decision on matrix and type of *Salmonella* serovars. This is agreed by e-mail between all four NRLs.

From 2008, the NRL in Sweden has performed several practical activities as listed below.

- Inviting the participating laboratories selected by each NRL giving them, in addition to the requirement to participate, the following background for participation:
 - A PT gives each laboratory an opportunity to:
 - evaluate its own ability to detect different *Salmonella* serovars in different matrixes.
 - detect, and attend to, possible inability to detect *Salmonella*.
 - A PT gives each NRL an opportunity to:
 - evaluate the performance of the regional laboratories in their own country.
 - help and guide any regional laboratory if needed.
 - work with quality assurance regarding its field of work in a national perspective.
 - compare different analytical methods ability to detect different *Salmonella* serovars, both in the same laboratory as well as between different laboratories.
 - evaluate the performance of different media.
- Producing the freeze dried ampoules containing the different *Salmonella* serovars.
- Preparing and sending 25 ampoules with freeze-dried *Salmonella* in various concentrations together with 300 g of the chosen

matrix to the participants, that can only be recognised by a Lab-ID.

- Make a compilation of the results and send that to the NRLs and each participant respectively.
- Providing the NRLs with a copy of the individual test result from the participants in its own country.
- Sending out a new set of samples to those laboratories that did not perform well.

Each NRL will take responsibility for the laboratories in its own country and, if necessary, takes the actions needed on behalf of each laboratory's result in the PT. Typically, 20 to 30 laboratories have participated in each of the past years, and one or two have performed below 'Good Performance'. However, the results over the years have shown improvement.

The PT is open to more participants if there is any interest.

For more information, see files at the EURL-*Salmonella* webpage on the 2016 Workshop in Saint Malo. It is also possible to contact any of the Nordic NRLs.

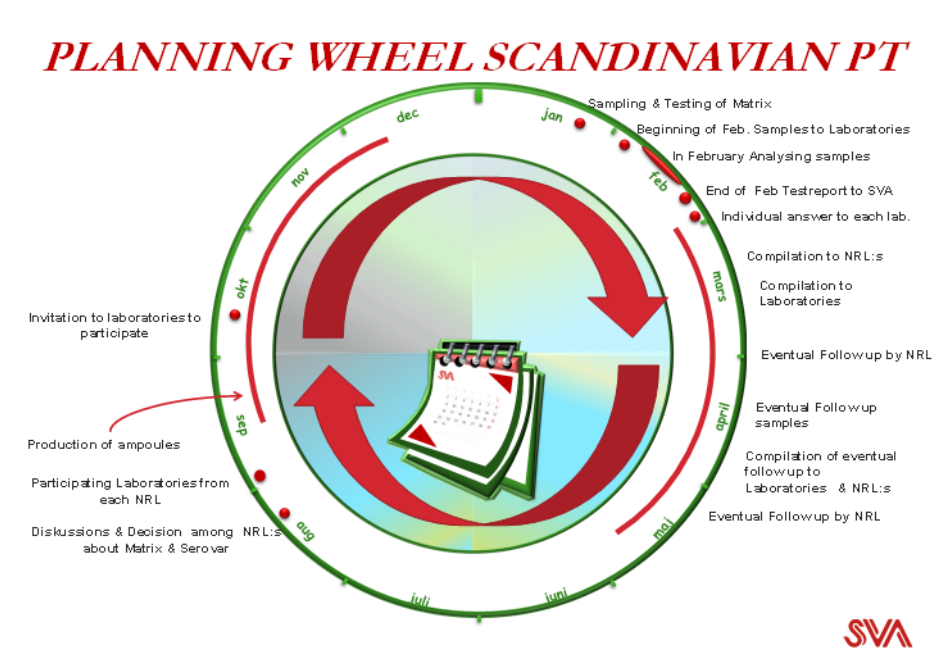


Figure 1 Annual planning of the Scandinavian Proficiency Tests

2.11 Recent investigations on *Salmonella* Enteritidis contamination in the poultry production in France

Laetitia Bonifait, NRL-Salmonella France

Salmonella spp. is the most important cause of food-borne bacterial gastroenteritis in developed countries. Poultry products are often associated with salmonellosis. In the EU, the monitoring of *Salmonella* in poultry flocks is laid down by the regulation (EC) No 2160/2003 (EC, 2003b). In this context, the National Reference Laboratory (NRL) is appointed to keep all *Salmonella* strains for at least 2 years. Despite this control program laid down at the primary production level, *Salmonella*

Enteritidis is still a major problem in some production areas in France. The aim of this study is to characterise *S. Enteritidis* strains collected at different stages of the poultry production in one French department and its neighbouring departments in order to trace the contamination and establish clonal relationships between the isolates.

All *Salmonella* strains are isolated according to Amendment 1 of ISO 6579 (Anonymous, 2007), from poultry farms at different steps (breeding, production flocks of laying hens, broilers and turkeys), from different departments in France, and from different source samples (faeces, bootsocks, socks, etc.). A total of 311 isolates of *S. Enteritidis*, were collected between 2008 and 2014, and analyzed by PFGE (Pulsed Field Gel Electrophoresis) using the restriction enzyme XbaI.

This investigation showed that *S. Typhimurium* and *S. Enteritidis* continue to be among the principal serovars found in poultry flocks and emphasises the increase of some non-regulated serovars such as *S. Senftenberg*. The PFGE dendrogram of *S. Enteritidis* revealed distinct pulsotypes with a diversity index varying between 0.7 and 0.9 according to the year. Several persistent and common isolates of *S. Enteritidis* were identified circulating through poultry rearing steps, over time, and across the departments. Contamination at the breeding level suggested the diffusion of the contamination at the following stages and appeared to originate from one particular department.

It was concluded that the strain collection of the French NRL is an important tool of the monitoring system implemented. *S. Enteritidis* contamination persisted across the years and across the poultry productions, as similar isolates were found among the circulating ones. Through this monitoring, the data highlighted the need to reinforce sanitary barriers and take corrective measures not only between the flocks, but also between the departments.

Discussion

Q: Were you able to identify the source of infection?

A: Unfortunately, we could not identify the source. We have seen the infection throughout the production chain. It could have been a common source, like animal feed.

Q: Did you look for *Salmonella* in mice and rats? We have found this to be a problem, especially in the area where the feed is stored.

A: No, we did not look at this.

Q: Did you perform further identification to distinguish wild strains from vaccine strains?

A: No, we have not yet performed this type of identifications.

2.12 Work programme EURL-*Salmonella* second half 2016, first half 2017, 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 2016 and for early 2017.

Interlaboratory comparison studies

Three interlaboratory comparison studies are planned in the coming year:

- Detection of *Salmonella* spp. in a food matrix: September/October 2016. Experiments have been performed at the laboratory of the EURL-*Salmonella* to prepare stable materials when inoculating minced turkey meat with a diluted culture of *Salmonella*. For this, two different *Salmonella* serovars have been tested and the materials have been stored at different temperatures (-20°C, +5°C and +10°C). The results so far are promising.
- Typing of *Salmonella* spp.: October/November 2016. As in former typing studies, this study will contain an obligatory part for serotyping 20 different *Salmonella enterica* serovars (and additionally, one optional non-*enterica* isolate) and an optional part for PFGE testing 10 different *Salmonella* serovars. A short inventory among the participants of the workshop revealed that the number of NRLs performing MLVA of *Salmonella* Typhimurium is still too low (<10 laboratories) to also include MLVA in the 2016 study. If the number of NRLs performing MLVA will increase in the (near) future, inclusion of MLVA in the typing studies will be reconsidered.
- Detection of *Salmonella* spp. in a sample from the primary production stage: February/March 2017. The choice of the matrix will be decided later.

Supporting activities

The 'research' performed by the EURL-*Salmonella* always has a relation to the activities of the EURL. The following is planned or will be continued in the next year:

- Continuation of the activities for the standardisation organisations, ISO (at international level) and CEN (at European level).
- Laboratory activities for development of the standard for PCR identification of monophasic *Salmonella* Typhimurium: collection of test strains to verify the performance of the draft PCR protocols. Organisation of a 'verification study' with a (selected) group of NRLs-*Salmonella* to set performance characteristics of the protocols for identification of monophasic *Salmonella* Typhimurium. Depending on the progress with the pre-work (finalisation draft protocols, collection of strains and testing of draft protocols with different test strains), the verification study will be organised in 2017 or later.
- Performing experiments to test the influence of incubation temperature (35°C versus 37°C) on selective enrichment of *Salmonella* and background flora in MKTTn.
- Testing different matrices for use in interlaboratory comparison studies.

Assistance to the Commission and communication

- If necessary/requested, experts of the EURL-*Salmonella* will participate in working groups of EFSA and of DG-Sante.
- EURL-*Salmonella* will perform ad hoc activities (on its own initiative or on request) and, if needed, will support DG-Sante or EFSA in case of outbreaks.

- The EURL regularly receives questions for information or advice from NRLs, DG-Sante and third parties. Replies are given as quickly as possible, but may sometimes be delayed due to the fact that literature and/or other experts need to be consulted.
- As before, the newsletter will be published four times a year through the EURL-*Salmonella* website. The NRLs are requested to provide any relevant information of interest to the other NRLs for publication in the newsletter.
- The EURL-*Salmonella* website will be kept up to date with information on new activities/results.
- Results of interlaboratory comparison studies and workshops are summarised in (RIVM) reports. These reports are published on the RIVM and EURL-*Salmonella* websites, and NRLs are informed about the publication of the reports by e-mail.

Training

- Trainings can be given by EURL-*Salmonella* at the EURL premises or at the NRL laboratory, and can be on request of an NRL or indicated by the EURL (in case of poor performance).
- In July 2016, a training on the use of BioNumerics was held, and will be repeated in 2017. This training is organised in cooperation with the EURLs for *Listeria monocytogenes* and STEC. Per EURL network, 4 NRLs can participate, resulting in a total of 12 NRLs per training. In 2016, the training was organised at the premises of the EURL for *Listeria monocytogenes* (France), and in 2017 it will be held at the premises of the EURL for STEC (Italy).

Molecular typing

Activities in relation to molecular typing foreseen for the coming year are:

- Including (again) PFGE typing in the EURL-*Salmonella* interlaboratory comparison study on typing of *Salmonella*;
- Continue participation in the EFSA steering committee on molecular typing database;
- Training NRLs on *Salmonella* molecular typing and use of BioNumerics;
- Curators meetings with the other EURLs involved in the EFSA molecular database (EURLs for STEC and for *Listeria monocytogenes*) and with the Statens Serum Institute (SSI) in Denmark, the curator of the ECDC database;
- Curation of molecular data (PFGE) for the EFSA (pilot) database;
- Perform WGS analysis and/or analysis of WGS data in case of outbreaks;
- Cooperate with DG-Sante and EFSA for organising an inventory amongst the NRLs on the use of WGS.

Workshop 2017

The date(s) as well as the location for the workshop of 2017 are still under investigation.

Discussion

Q: We face problems because the producer of a *Salmonella* vaccine recently stopped production of the culture media needed to differentiate

the vaccine strain from wild strains. Do other NRLs have the same problem?

A: It may be possible to make the medium yourself, the composition of the medium should be open. Alternatively, it is possible to use a validated Real time PCR method for identification of the vaccine strain.

Q: In our country, we have problems with *Salmonella* 9,46:-:-. Do others find this serotype as well?

A: No other NRLs have seen this serotype in their countries. It is possible to send the strain to the EURL for confirmation of the type.

3 Evaluation of the workshop

3.1 Introduction

At the end of the workshop, an evaluation form was given to all participants to ask for their opinion of the workshop (see Annex 3). A total of twelve questions were asked. For ten of these questions, participants were asked to give a score ranging from 1 to 5 as an answer to the questions. The scores represent: very poor (1), poor (2), average (3), good (4) and excellent (5). In addition, it was possible to add comments. Two questions were 'open' questions, in which the participants were asked to give their opinion. The evaluation form was handed to 43 participants of the workshop and 37 completed forms were returned, a response rate of 86%.

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

3.2 Evaluation form

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

Figure 2 shows that the majority of the respondents considered the information given in advance of the workshop as good or excellent (scores 4-5).

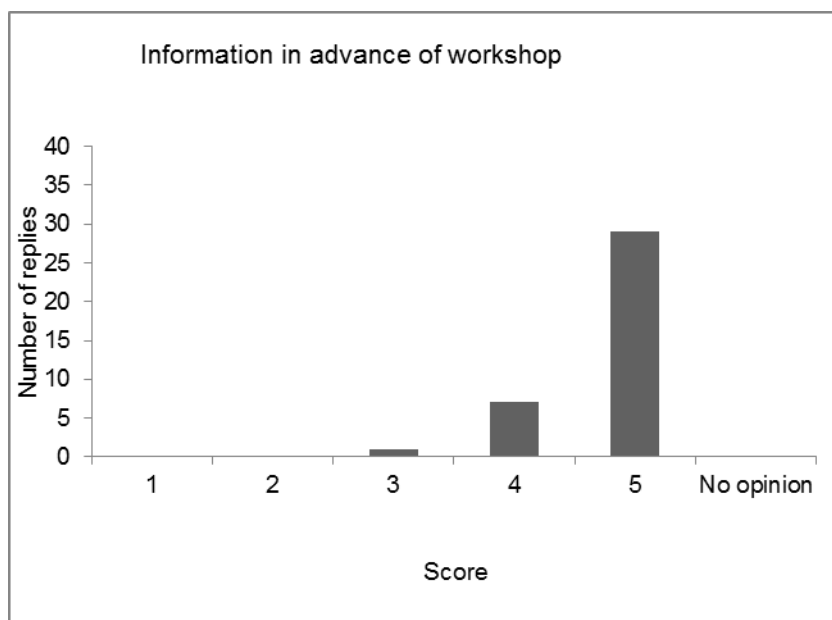


Figure 2 Scores given to question 1 'Opinion on information given in advance of the workshop'

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

For this year's workshop, the majority of participants booked the tickets themselves. The few participants for whom tickets were arranged by the EURL were very satisfied. Participants who booked their own ticket

indicated 'no opinion' (see Figure 3). It was remarked twice that it would have been preferred if the EURL had booked the tickets.

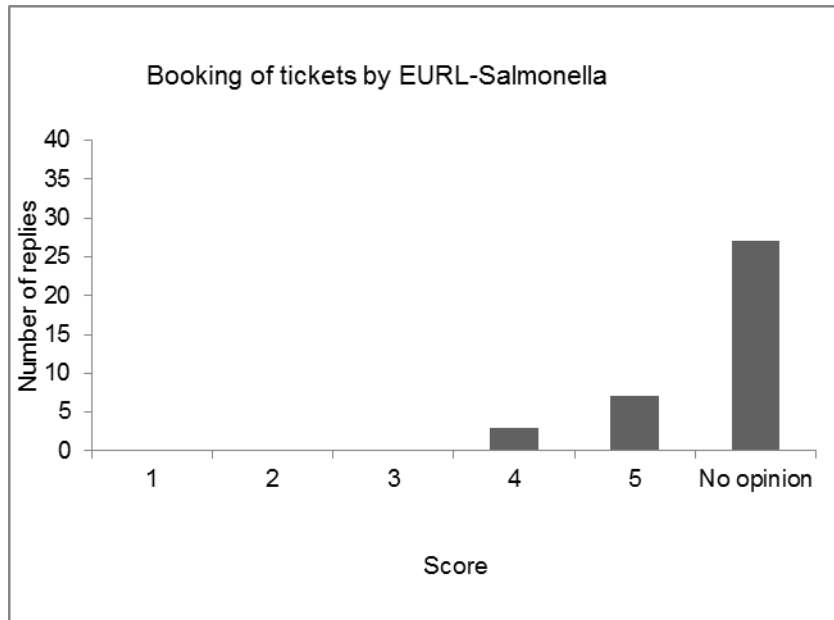


Figure 3 Scores given to question 2 'Opinion on the booking of the tickets by the EURL-Salmonella'

3. What is your opinion on the accessibility of the meeting venue?

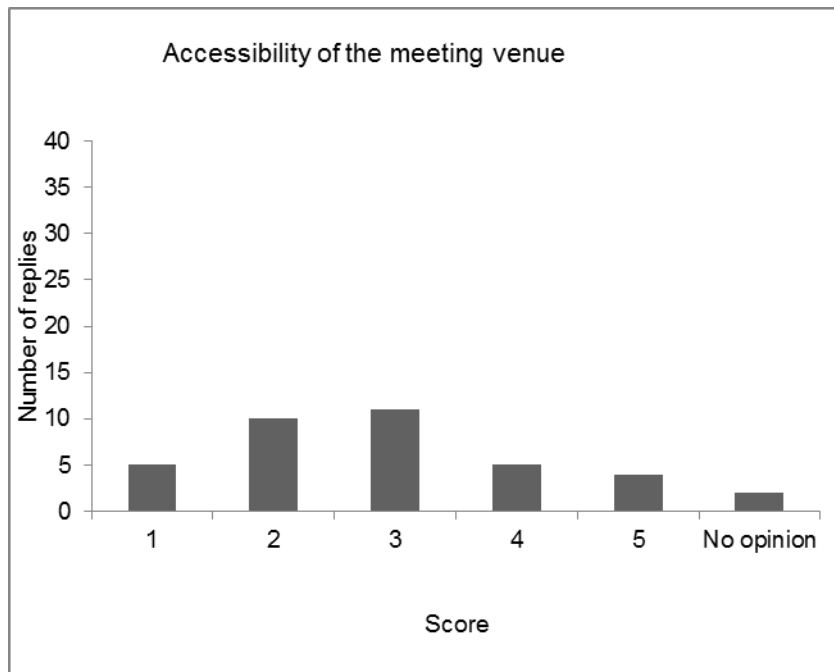


Figure 4 Scores given to question 3 'Opinion on the accessibility of the meeting venue'

To be able to organise the workshop in conjunction with the International Symposium for *Salmonella* and Salmonellosis (IS), the meeting venue was in Saint Malo, France. For the majority of the participants it was a

long journey. Additionally, in the period of the symposium and the workshop, public transport strikes were being held in France. This resulted in extra delay in the travel time for many participants. These transport problems are clearly visible in the evaluation results (Figure 4). The majority of the replies were in the range of very poor (1) to average (3). Still, some replied that the accessibility of the meeting venue was good (4) to excellent (5). Some remarks were given as well:

- 'Accessibility was very poor due to strikes and flight delay' (1x);
- 'Still I would do it again' (1x);
- 'Due to strike, very long traveling time' (1x);
- 'In addition to the fact that I needed 18h to get to Saint Malo, I could not find a plane connection that would take me back on the same day' (1x).

4. What is your opinion on the hotel room?

This year it was not possible to make a group booking in a hotel for all participants, resulting in participants being situated in approximately 13 different hotels in Saint Malo. The majority of the participants scored their hotel good to excellent (scores 4-5, see Figure 5).

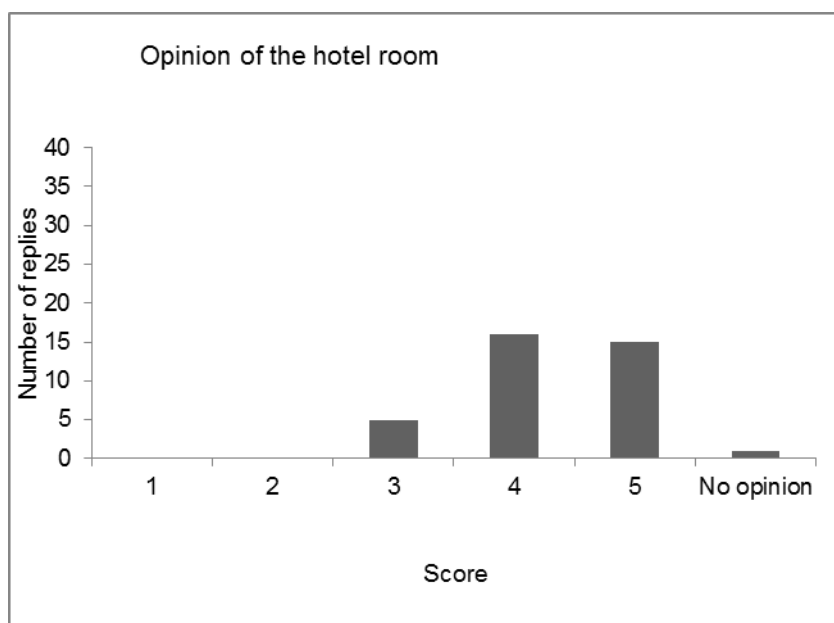


Figure 5 Scores given to question 4 'Opinion on the hotel room'

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

The opinion about the meeting room varied for the majority between average (score 3) and good (score 4; see Figure 6). Unfortunately, the air conditioning was not optimal in the meeting room, resulting in a warm room during the day. Additionally, the microphone caused a disturbing noise in the loudspeakers, so it was decided not to use it. However, this resulted in the fact that some speakers could not be heard at the back of the room. Several remarks were made:

- 'Too hot, no fresh air' (10x);
- 'No microphone' (3x);
- 'Last row too distant from the screen' (1x).

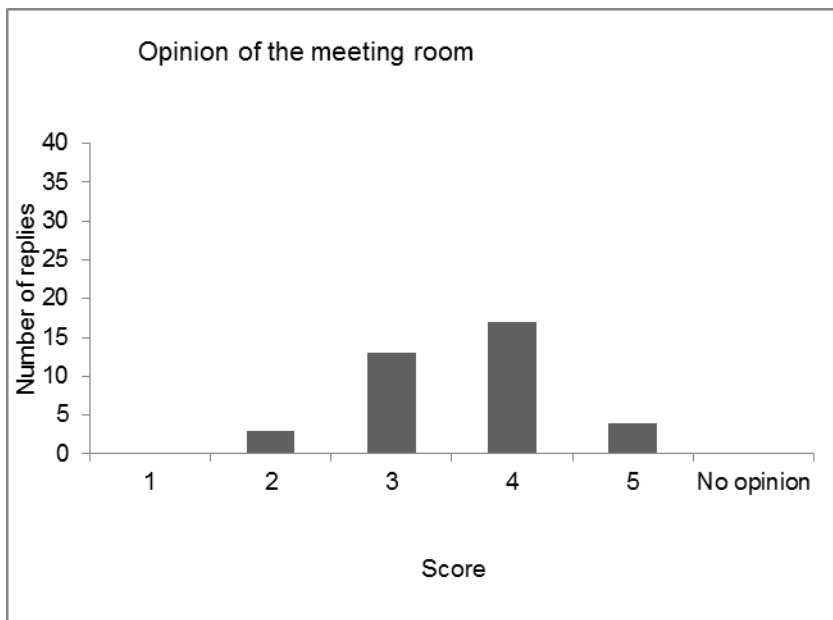


Figure 6 Scores given to question 5 'Opinion on the meeting room'

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

The screen was not very large, resulting in the fact that some participants at the back of the room had difficulties reading the screen. The opinions on this item varied from poor (score 2) to excellent (score 5; see Figure 7). The following remarks were made:

- 'The screen was too small in the back' (3x);
- 'Some presentations were difficult to read' (2x).

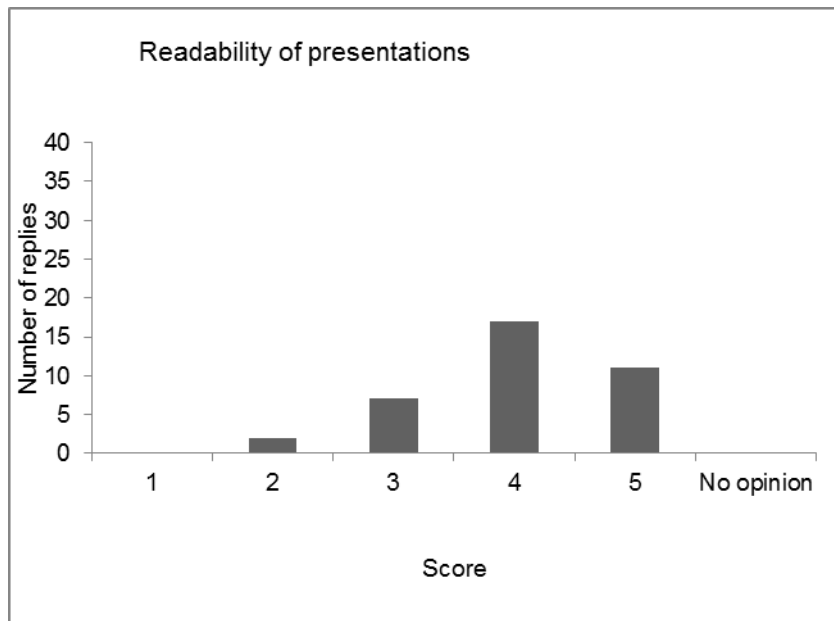


Figure 7 Scores given to question 6 'Opinion on the readability of the presentations on the screen'

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

The majority of the participants considered the technical equipment as average (score 3) to good (score 4), although also some 'poor' (score 2) replies were given (Figure 8). The following remarks were given:

- 'Lack of microphone' (3x);
- 'Air conditioning not cold enough' (2x);
- 'The noise from the air conditioning made it hard to hear some of the speakers' (1x).

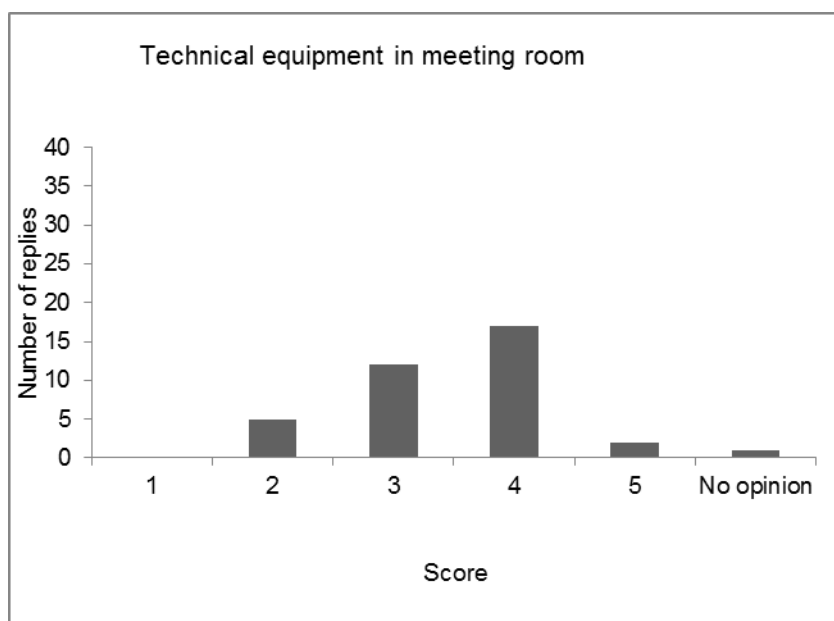


Figure 8 Scores given to question 7 'Opinion on the technical equipment'

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

The majority of the respondents considered the catering as excellent (score 5), see Figure 9.

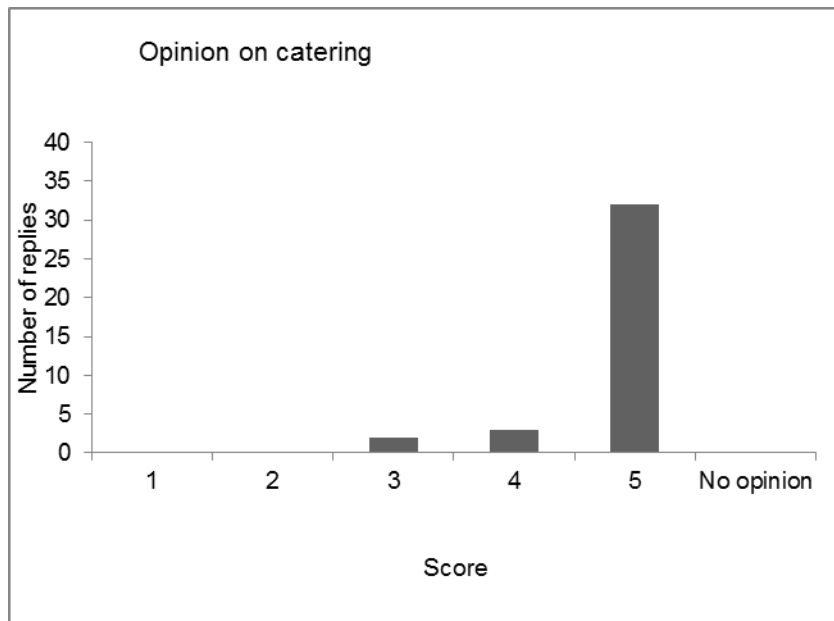


Figure 9 Scores given to question 8 'Opinion on the catering'

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

The majority of the respondents were very satisfied with the scientific programme of the workshop: mainly good (score 4) or excellent (score 5) scores were given (see Figure 10).

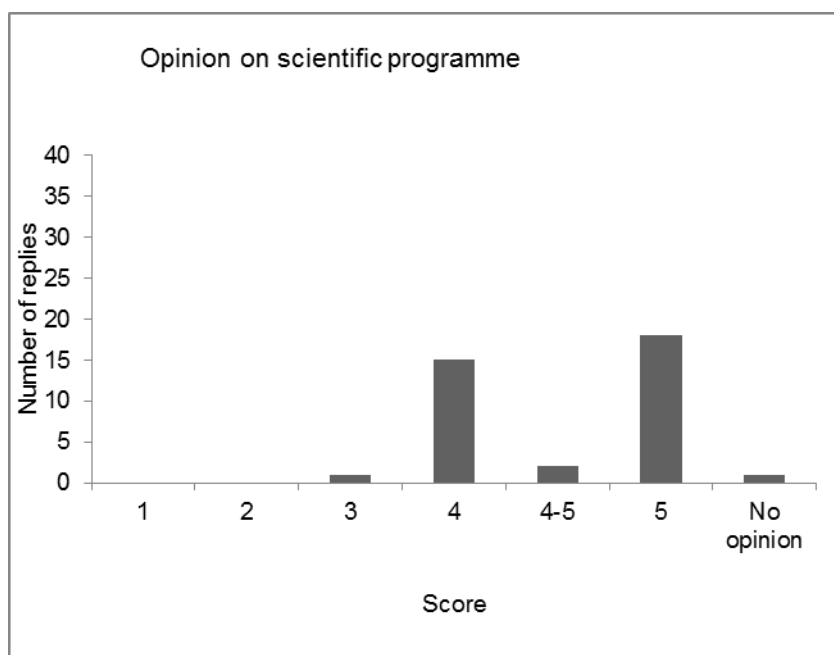


Figure 10 Scores given to question 9 'Opinion on the scientific programme'

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

This concerned an 'open' question and one response was obtained: 'The lecture on WGS was very interesting'

11. What is your general opinion of the workshop?



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

The respondents indicated that the workshop as a whole had been good (score 4) or excellent (score 5), see Figure 11.

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

This concerned an 'open' question and the following responses were obtained:

- 'Thank you for the very interesting workshop.'
- 'Consider Sweden for next year.'
- 'The workshop is good, but 2-days traveling for a one-day workshop is not very efficient. Please let the workshop last 2 days.'
- 'Please do not print 6 slides/page for the hand-outs. This is too small. Print two slides/page or arrange electronic submission.'
- 'It is very important to increase the capacity of the BioNumerics training courses, especially for Member States signed up to the database to ensure standardisation of methods.'

3.3 Discussion and conclusions of the evaluation

The scores for the general opinion of the workshop, as well as for the scientific programme remain high, as for previous workshops, which is a very satisfying result for the workshop organisers. However, some other aspects did not get very high scores. For the current workshop, the accessibility to the meeting venue scored low, and the scores for the meeting room were lower than those of 2015.

For the majority of the participants, the travel time to Saint Malo was long. However, this year, we had the additional bad luck that public transport strikes took place in France. This will certainly have had an effect on the scores for the accessibility of the meeting venue. The aim for the next years' workshop will be to try to retain the high quality of the scientific programme high, and to find an optimal meeting venue.

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Acknowledgements

The author would like to thank Elizabeth de Pinna of Public Health England (PHE), London, England for volunteering again to make notes during the workshop. An effort that is highly appreciated.

List of abbreviations

A	Answer
AMR	Antimicrobial resistance
BPW	Buffered Peptone Water
CD	Committee Draft
CEN	European Committee for Standardization
cfu	colony forming units
DG-Sante	Directorate-General for Health and Food Safety
DIS	Draft International Standard
EC	European Commission
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
EFTA	European Free Trade Association
EPIS	Epidemic Intelligence Information System
EU	European Union
EURL	European Union Reference Laboratory
FBO	Food-borne Outbreak
FDA	Food and Drug Administration
FDIS	Final Draft International Standard
FWD	Food and Waterborne Diseases
FYROM	Former Yugoslav Republic of Macedonia
IDF	International Dairy Federation
ISO	International Organization for Standardization
MKTTn	Mueller Kauffmann Tetrathionate broth with novobiocin
MLST	Multi Locus Sequence Typing
MLVA	Multi-Locus Variable number of tandem repeats Analysis
MPN	Most Probable Number
MS	Member State
MSRV	Modified Semi-solid Rappaport Vassiliadis
NGS	Next Generation Sequencing
NRL	National Reference Laboratory
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
PHE	Public Health England
PPS	Primary Production Stage
PT	Proficiency Test
Q	Question
RIVM	National Institute for Public Health and the Environment
RVS	Rappaport Vassiliadis broth with Soya
SE	<i>Salmonella</i> Enteritidis
SC	Sub Committee
SNP	single nucleotide polymorphism
SOP	Standard Operating Procedure
STEC/VTEC	Shiga toxin-producing/ Verotoxigenic <i>Escherichia coli</i>
TAG	Technical Advisory Group
TC	Technical Committee
USA	United States of America
WG	Working Group
WGS	Whole Genome Sequencing

Annex 1 Participants

European Food Safety Authority (EFSA)	Valentina Rizzi
European Centre for Disease Prevention and Control (ECDC)	Karin Johansson
EC DG-Sante	Kris de Smet
EURL – <i>Salmonella</i>	Kirsten Mooijman Angelina Kuijpers Wilma Jacobs
Guest speaker (United Kingdom)	Elizabeth de Pinna (PHE, London)

National Reference Laboratories for *Salmonella*

AUSTRIA	Heimo Lassnig
BELGIUM	Katelijne Dierick Vicky Jasson absent due to problems with public transport
BOSNIA HERZEGOVINA	-
BULGARIA	Hristo Daskalov
CROATIA	Borka Simpraga
CYPRUS	Eleni Papageorgiou
CZECH REPUBLIC	Tomas Cerny
DENMARK	Gitte Sørensen
ESTONIA	Maiu Kuningas
FINLAND	Henry Kuronen
FRANCE	Frédérique Moury Laetitia Bonifait
FYROM	Mirko Prodanov
GERMANY	Istvan Szabo Burkhard Malorny
GREECE	Aphrodite Smpiraki
HUNGARY	Erzsebet Adrian
ICELAND	Ana Pála Vignisdóttir
IRELAND	Rosemarie Slowey
ITALY	Lisa Barco
LATVIA	Madara Streikisa
LITHUANIA	Asta Pereckiene
LUXEMBOURG	Catherine Ragimbeau
MALTA	-
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Patricia Themudo
Carmen Anca Manea
Jasna Kureljusic
Lubos Mikula
Urska Henigman
Maria Christina de Frutos Escobar
Lennart Melin
Gudrun Overesch absent due to
problems with public transport
Derya Karates Yeni absent due to
problems with public transport
Francesca Martelli

Annex 2 Workshop Programme

Programme of the 21st EURL-*Salmonella* workshop 9 June 2016, Saint Malo, France

General information

Meeting venue:

Grand Hotel de Courtoisville
9 rue Michelet - 69 boulevard Hébert
35400 Saint Malo
Tel: +33 (0)2 99 40 83 83

<http://www.hotel-saint-malo-courtoisville.com/en/>

Information for those giving a presentation:

Presentations: To be able to make hand-outs for all participants, please send your (PowerPoint) **presentation** to Kirsten Mooijman (kirsten.mooijman@rivm.nl) **before 1 June 2016**. Alternatively, bring your own hand-outs.

Abstract: For the preparation of the report of the workshop it is necessary to receive an abstract of your presentation (approximately one page). Please hand this over to Kirsten during the workshop or send it to Kirsten.mooijman@rivm.nl **preferably before 10 June 2016**

Wednesday 8 June 2016

Arrival of participants at St. Malo (if not already present for the I3S symposium)

18.00 – 19.00 Registration and get-together in Grand Hotel de Courtoisville

- Final information concerning the programme
- Administrative aspects

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 Wednesday 8 June, with a maximum of € 30 per person. A receipt will be needed in order to be able to reimburse you for this meal.

Thursday 9 June 2016**Morning Chair: Kirsten Mooijman**

09:00 - 09:30	Opening and introduction	Kirsten Mooijman, EURL- <i>Salmonella</i>
09:30 - 10:00	Results 20 th interlaboratory comparison study on typing of <i>Salmonella</i> (2015) – serotyping and PFGE	Wilma Jacobs, EURL- <i>Salmonella</i>
10:00 - 10:30	Update on the joint EFSA/ECDC molecular typing database	Valentina Rizzi, EFSA
10:30 - 11:00	<i>Coffee/tea</i>	
11:00 - 11:30	How will joint cluster management work in practice? An introduction to EPIS-FWD	Karin Johansson ECDC
11:30 - 12:00	Use of WGS for typing of <i>Salmonella</i> at PHE	Elizabeth de Pinna, PHE, UK
12:00 - 12:30	<i>Salmonella</i> monitoring data and food-borne outbreaks for 2014 in the European Union	Valentina Rizzi, EFSA
12:30 - 13:45	<i>Lunch</i>	

Afternoon Chair: Wilma Jacobs

13:45 - 14:15	Update on activities in ISO and CEN	Kirsten Mooijman, EURL- <i>Salmonella</i>
14:15 - 14:45	Results interlaboratory comparison study Food on detection of <i>Salmonella</i> in whole liquid egg (2015)	Angelina Kuijpers, EURL- <i>Salmonella</i>
14:45 - 15:15	Preliminary results interlaboratory comparison study on detection of <i>Salmonella</i> in boot socks (2016)	Irene Pol, EURL- <i>Salmonella</i>
15:15 - 15:45	<i>Coffee/tea</i>	
15:45 - 16:05	Nordic cooperation for Proficiency Testing of regional laboratories	Lennart Melin NRL- <i>Salmonella</i> Sweden
16:05 -	Salmonellosis or <i>Salmonella</i> infection –	Gudrun

16:25	high nasal colonization rates of <i>Salmonella enterica</i> subspecies <i>diarizonae</i> 61:k:1,5,(7) in Swiss sheep herds	Overesch NRL- <i>Salmonella</i> Switzerland
	This presentation was canceled	
16:25 - 16:45	Recent investigations on <i>Salmonella</i> Enteritidis contamination in the poultry production in France	Laetitia Bonifait NRL- <i>Salmonella</i> France
16:45 - 17:15	Work programme EURL- <i>Salmonella</i> second half 2016, first half 2017 Discussion on general items and closure	Kirsten Mooijman, EURL- <i>Salmonella</i>
18:30 -	<i>Dinner at Grand Hotel de Courtoisville</i>	
----- End workshop -----		

Annex 3 Workshop evaluation form

**Evaluation of the 21st EURL-*Salmonella* workshop
9 June 2016, Saint Malo, France**

We would highly appreciate if you could give us your opinion on the 21st EURL-*Salmonella* workshop, organised in Saint Malo, France, on 9 June 2016. 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 = average 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? Please indicate your hotel name

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

Hotel name:

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 which we can use for future workshops?

--

Thank you very much!

RIVM

Committed to *health and sustainability*