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

The 20th EURL-*Salmonella* workshop

28 and 29 May 2015, Berlin, Germany

RIVM Report 2015-0083
K.A. Mooijman

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Colophon

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De twintigste EURL-*Salmonella* workshop

28 en 29 mei 2015, Berlijn, Duitsland

Het RIVM heeft de verslagen gebundeld van de presentaties van de twintigste jaarlijkse workshop voor de Europese Nationale Referentie Laboratoria (NRL's) voor de bacterie *Salmonella* (28 en 29 mei 2015). Het doel van de workshop is dat het overkoepelende orgaan, het Europese Referentie Laboratorium (EURL) *Salmonella*, en de NRL's informatie kunnen uitwisselen. Daarnaast worden de resultaten gepresenteerd van de ringonderzoeken van het EURL, waarmee de kwaliteit van de NRL-laboratoria wordt aangegeven. Een uitgebreidere weergave van de resultaten wordt per ringonderzoek in aparte RIVM-rapporten opgenomen.

Nieuwe technieken steeds belangrijker

Een aantal verslagen geeft informatie over het gebruik van nieuwe technieken om overeenkomsten tussen verschillende *Salmonella*-stammen aan te tonen. Veelal zijn dit moleculaire technieken die het DNA van de bacterie aantonen. Deze technieken worden steeds vaker gebruikt bij het opsporen van de ziekmakende bacterie in voedsel, dieren en bij de mens. Iedere bacteriestam heeft namelijk een eigen unieke moleculaire typering.

Een databank voor unieke moleculaire typering resultaten

De European Food Safety Authority (EFSA) geeft verslag van een databank in oprichting. In deze databank kunnen alle Europese landen moleculaire typeringsresultaten van *Salmonella* opslaan. Zo is het mogelijk om na te gaan of een bepaalde ziekmakende bacteriestam in meerdere landen en producten voorkomt.

NRL's presenteren hun activiteiten

In vier verslagen wordt informatie gegeven over de activiteiten van de NRL's voor *Salmonella* uit Noord-Ierland, Portugal, Spanje en Slovakije.

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 2015

Synopsis

The 20th EURL-*Salmonella* workshop
28 and 29 May 2015, Berlin, Germany

In this report, the RIVM presents a summary of the presentations given at the 20th annual workshop for the European National Reference Laboratories (NRLs) for *Salmonella* (28 and 29 May 2015). The aim of this workshop is to facilitate the exchange of information on the activities of the NRLs and the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*). An important item on the agenda is the presentation of the results of the annual ring trials organised by the EURL, which provide valuable information on the quality of the work carried out by the participating NRL laboratories. Detailed information on the results per ring trial is described in separate RIVM-reports.

New techniques more important
Several presentations provide information on the use of new techniques to show similarities between different *Salmonella* strains. These are often molecular techniques, analysing the DNA of the bacterium. These techniques are often used to trace the pathogen in food, animals or humans, as each strain has its own unique molecular typing pattern.

A database for unique molecular typing results
The European Food Safety Authority (EFSA) presented their pilot database in which molecular typing results of *Salmonella* can be stored. This will make it possible to check whether a specific strain is found in different countries and products.

NRLs present their activities
In four presentations information is given of the activities performed by the NRLs for *Salmonella* of Northern-Ireland, Portugal, Spain and the Slovak Republic.

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 2015

Contents

Summary

On 28 and 29 May 2015, the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*) organised its annual workshop in Berlin, Germany. Participants of the workshop were representatives of: the NRLs for *Salmonella* from 26 EU Member States, three European Free Trade Association (EFTA) countries, and four (potential) EU candidate countries. Also present were representatives of the European Commission Directorate General for Health and Food Safety (DG-Sante) and of the European Food Safety Authority (EFSA). Excuses were received from representatives of two EU Member State NRLs, due to lack of staff (Malta) or for medical reasons (Luxembourg). A total of 48 participants attended the workshop.

During the workshop, presentations were given on several items.
The results of the interlaboratory comparison studies as organised by the EURL-*Salmonella* in the past year were presented. This concerned the studies on detection of *Salmonella* in animal feed (September 2014) and in samples from the primary production stage (March 2015) and the study for typing of *Salmonella* (November 2014).
An EFSA representative presented the most recent European summary report on Zoonoses. This report gives an overview on the number and types of zoonotic microorganisms that caused health problems in Europe in 2013. For several years, the number of health problems caused by *Salmonella* has been declining, but it retains the second most important cause of zoonotic diseases in Europe, after *Campylobacter*.
A representative of EC DG-Sante gave a short update of policy issues.
A presentation was given on the use of a molecular serotyping method.
Three presentations were held on outbreaks caused by different *Salmonella* serovars in different products.
EFSA gave an update on the pilot database for molecular typing data collection from food, animal feed and animals.
In two presentations, summaries were given of the activities with the standardisation of methods in ISO and CEN, related to the activities of the NRLs for *Salmonella.*
Representatives of four NRLs for *Salmonella* gave presentations on their activities related to the tasks and duties of being an NRL.
The workshop was 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_2015

1 Introduction

In this report, the abstracts of the presentations given at the EURL-*Salmonella* workshop of 2015 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_2015

The layout of the report is consistent with the workshop programme.
All abstracts of the presentations of the first day are given in chapter 2.
All abstracts of the presentations of the second day are given in chapter 3.
The evaluation of the workshop is summarised in chapter 4 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 28 May 2015: day 1 of the workshop

2.1 Opening and introduction

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

Kirsten Mooijman, head of the EURL-*Salmonella*, opened the 20th workshop of the EURL-*Salmonella*, welcoming all participants to Berlin, Germany. Special thanks were given to Istvan Szabo of the NRL-*Salmonella* from Germany for his help in organising this year's workshop in Berlin.
At this workshop, 48 participants were present, 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), and the European Food Safety Authority (EFSA) were present. Excuses were received from representatives of two NRLs, due to lack of staff (Malta) and due to medical problems (Luxembourg).
After a roll call of the delegates, the results of the evaluation of the last four workshops (2011, 2012, 2013 and 2014) were compared, showing variable results for the four workshops. The opinion on the scientific programme was the same in all workshops: very 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 EU *Salmonella* monitoring data, food-borne outbreaks and antimicrobial resistance

Frank Boelaert, EFSA, Parma, Italy

The role of the European Food Safety Authority (EFSA) is to assess and communicate on all risks associated with the food chain. Within its remit, EFSA collects and analyses data to ensure European food safety risk assessment is supported by the most complete scientific information available. The European Union (EU) Directive 2003/99/EC (EC, 2003a) obligates the EU Member States (MS) to collect data on zoonoses and zoonotic agents every year, and requests EFSA to analyse these data and to publish an annual European Union Summary Report (EUSR) on zoonoses. EFSA's Biological Hazards and Contaminants Unit (BIOCONTAM) is charged with the production of these annual EUSRs, in collaboration with the European Centre for Disease Prevention and Control (ECDC). The monitoring and reporting systems used are based on those in place at the Member States (MSs) level, and in a few cases this is harmonised by EU legislation to the extent that the results from the monitoring are directly comparable between MSs. The most recent EUSRs on zoonoses, food-borne outbreaks and antimicrobial resistance, related to data collected in 2013, were published at the beginning of 2015 (EFSA and ECDC, 2015a and 2015b). The information reported on *Salmonella* in humans, food and animals in the EU were presented and discussed.

The decreasing EU trend in confirmed human salmonellosis cases observed in recent years has continued. Most Member States met their *Salmonella* reduction targets for poultry. In foodstuffs, the reported EU-level of *Salmonella* non-compliance in fresh poultry meat decreased. A total of 5196 food-borne outbreaks, including water-borne outbreaks, were reported in the EU. Most food-borne outbreaks were caused by *Salmonella*, followed by viruses, bacterial toxins and *Campylobacter*, however the causative agent was unknown in 28.9% of all outbreaks. Important food vehicles in strong-evidence food-borne outbreaks were eggs and egg products, followed by mixed food, and fish and fish products. In *Salmonella* from humans, high proportions of isolates were resistant to ampicillin, sulfonamides and tetracyclines, while proportions of isolates resistant to third-generation cephalosporins and clinically non-susceptible to fluoroquinolones generally remained low. In *Salmonella* isolates from fowl, pigs, cattle, and the meat thereof, resistance to ampicillin, tetracyclines and sulfonamides was commonly detected, while resistance to third-generation cephalosporins was generally uncommon. High to very high resistance to (fluoro)quinolones was observed in *Salmonella* from turkeys, fowl and broiler meat. Multi-resistance and co-resistance to critically important antimicrobials in both human and animal isolates were uncommon. A minority of isolates from animals belonging to a few *Salmonella* serovars (notably Kentucky and Infantis) were high-level resistant to ciprofloxacin.

Discussion

Q: This is more a 'data-warehouse' than a report?
A: This report concerns a harmonisation exercise. The data are collected, validated and stored in the data warehouse. The data will become available to the MSs after a log-in. The data warehouse is currently only operational in EFSA, but the intention is to make it available to all EU MSs. For this, agreements are needed on who can access the data.
Q: When is this going to be available to the MSs?
A: It is the intention to have it available for the user group in 2016.
Q: Who will be the user group?
A: Quite likely, this will be the competent authority of each MS. For other groups this is still under discussion.
Q: What will be the format of the reports?
A: These will be pre-defined reports, graphs, tables, etc. It should all be more user friendly, and the information will become available in a printable format.
Q: The submission of data is mostly done by the Competent Authority of a MS, but they often have difficulties with interpretation of the data. Would it be better if laboratories submit the data?
A: It is up to the Competent Authority of the MS to decide on who will analyse the data and who will submit the data.
Q: Will the data fields remain the same? So far these have changed every year.
A: Indeed this is not yet optimal. In November we will review all changes that may be needed for the next year, and in January the data files will be distributed. It is true that things change now and then, but all changes are evaluated based on their need.

2.3 Update of the European Commission

Klaus Kostenzer, DG-Sante, Brussels, Belgium

DG SANTE has been renamed from DG SANCO and stands for Directorate General for Health and Food Safety. The new Commissioner is aiming at improving crisis preparedness on the public health and food side. Therefore a focus will be put on preparedness and early detection of possible hazards, including *Salmonella* as one of the main agents identified for foodborne outbreaks.

The development of a sound base of expertise in outbreak detection and management and other areas is supported by DG SANTE's 'Better training for safer food (BTSF)' programme. In 2015, various courses for official staff are organised on zoonoses including *Salmonella*, e.g. on outbreak investigations, testing of foodstuffs for Third Countries, and zoonoses control at primary production.

The Commission has asked EFSA and ECDC to establish a molecular database for foodborne pathogens which will enable a comparison of molecular patterns of *Salmonella* in isolates from humans, food, animals, and feed.

At the European Union level, procedures have been established to enable a close collaboration between the relevant sectors. Consequently, rapid outbreak assessments of foodborne outbreaks with a multinational dimension are investigated by ECDC and EFSA in close collaboration with Member States and the Commission.

A harmonised monitoring of antimicrobial resistance in food and primary production has been established. This will enable a comparison between human and animal resistance patterns. A progress report on the European action plan on antimicrobial resistance (AMR) has recently been published. The Commission will also publish guidelines for the prudent use of antimicrobials in veterinary medicine.

The process hygiene criteria for *Salmonella* for carcasses of pigs and poultry at slaughter have been reinforced in Reg. (EC) No 2073/2005 (EC, 2005).

Following the approval of their *Salmonella* control programme, the Ukraine has been added to the list of Third Countries from which class A eggs can be imported into the Union.

The antigenic formula for monophasic *Salmonella* Typhimurium in the target regulations for *Salmonella* for poultry has been corrected (EC, 2015).

Discussion

Q: Molecular data collection is a big issue, especially for use in outbreaks. Would it be possible to already collect sequence data (NGS) in the molecular database?

A: Currently EFSA has started the pilot phase of the molecular database for PFGE and MLVA (*Salmonella* Typhimurium) data of *Salmonella*,

STEC, and *Listeria monocytogenes*. It is the intention to look at other molecular data, in 3-4 years' time.
Q: Is there any discussion at EU level on criteria for *Campylobacter*?
A: There is already a lot of data and risk analysis, and indeed it is being discussed at EU level. However, introducing legislation at EU level has become more difficult due to new rules. Currently the item is being discussed at the highest level, but as yet, no criteria have been established.

2.4 Results interlaboratory comparison study on detection of *Salmonella* in animal feed III (2014)

*Angelina Kuijpers, EURL-*Salmonella*, Bilthoven, the Netherlands*

In September 2014, the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*) organised the third interlaboratory comparison study on the detection of *Salmonella* in samples from animal feed. The matrix of concern was mixed meal for laying hens.
The participants included 34 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 and members of the European Free Trade Association (EFTA)), and one NRL from a non-European country.

The main objective of the study was to test the performance of the participating laboratories for the detection of *Salmonella* at different contamination levels in animal feed. For this purpose, chicken feed samples of 25 grams artificially contaminated with *Salmonella* Senftenberg (SSE) at various contamination levels, were analysed. The performance of the laboratories was compared with the criteria for good performance. In addition, a comparison was made between the prescribed method (ISO 6579, 2002) using selective enrichment in Rappaport Vassiliadis Soya (RVS) broth and Mueller Kauffmann Tetrathionate novobiocin broth (MKTTn), and the requested method (Annex D of ISO 6579, 2007), using selective enrichment on Modified Semi-solid Rappaport-Vassiliadis (MSRV) agar.

The samples consisted of chicken feed artificially contaminated with a diluted culture of *Salmonella* Senftenberg (SSE) at a low level (approximately 15-20 cfu/25 g of feed), at a high level (approximately 50-100 cfu/25 g of feed) 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 chicken feed had to be tested by each participant for the presence or absence of *Salmonella*. These samples consisted of six blank samples, six samples with a low level of SSE (inoculum 20 cfu/sample) and six samples with a high level of SSE (inoculum 61 cfu/sample). Additionally, three control samples had to be tested: two blank control samples (procedure control (BPW)

and matrix control sample (chicken feed) and one own (NRL) positive control sample (with *Salmonella*).

The laboratories found *Salmonella* in almost all (contaminated) samples, resulting in a sensitivity rate of 99%. A comparison between the different media was made. Isolation on Xylose Lysine Deoxycholate agar (XLD) gave a significantly higher chance of finding *Salmonella* Senftenberg in chicken feed in comparison to other isolation media (most often Brilliant Green Agar – BGA). The difference was 3-7%, and was independent of the selective enrichment medium used (RVS, MKTTn or MSRV). There was a higher chance of finding *Salmonella* after selective enrichment on MSRV compared with RVS and MKTTn, but this was not significant (difference only 1%). Longer incubation (two times 24 h) of MSRV gave 2-3% more positive results.

For the positive control, the majority of the participants (20 laboratories) used a diluted culture of *Salmonella*. The *Salmonella* serovars used for the positive control sample were *S*. Enteritidis (15) and *S.* Typhimurium (9). The concentration of the positive control varied between 8 – 10^9 cfu/sample. For the positive control it is advisable to use a concentration close to the detection limit and a *Salmonella* serovar not often isolated from routine samples.

PCR was used as an own method by six participants, of which five found the same results as with the bacteriological culture method. Most of them used a real-time PCR.

Thirty-two of the 34 laboratories achieved the level of good performance. One NRL reported a positive result for a blank procedure control sample; another NRL reported a negative result for their own positive control sample. Both laboratories showed correct results for the samples with animal feed contaminated with *Salmonella*. However, those results are not reliable, due to the deviations in the positive or negative control samples. The results of those two laboratories were therefore indicated as 'moderate performance'. One of them showed repeated moderate performance in food and animal feed studies. The EURL staff visited this NRL during a follow-up study organised in February 2015. The laboratory scored all samples correctly and achieved a good performance. The EC, DG Sante, was informed accordingly.

More details of the study can be found in the interim summary report (Kuijpers and Mooijman, 2014).

Discussion

Q: Did you check whether the animal feed used in the interlaboratory comparison study contained antimicrobial additives? We have seen problems with detection of *Salmonella* in animal feed, probably due to the presence of antimicrobial additives.

A: We did not check this, but we can look at the list of ingredients later. However, as all bacteria grew well, it does not seem to be likely that the animal feed contained any antimicrobial additive.

Q: How flexible is the level of good performance? Why does this change sometimes?

A: We use a general level of good performance, but we may adjust this slightly for individual studies. For the low contaminated samples it is expected that approximately 50% of the samples will be tested positive. However, for example, if 5 low level samples are used in the study, the level of good performance should then lie at 2.5 positive samples. This is an impossible number and will be adjusted to 2 or 3 positive samples. In addition, in case of problems in a study (e.g. high amounts of background flora disturbing the growth of *Salmonella,* or the level of contamination of the samples turned out to be lower than expected), the level of good performance will be slightly adjusted.

2.5 Results interlaboratory comparison study on detection of *Salmonella* in pig faeces – PPS XVIII (2015)

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

In March 2015, the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*) organized the 18th interlaboratory comparison study on the detection of *Salmonella* in samples from the primary production stage (pps). The matrix of concern was pig faeces.
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 MSs or potential EU candidate MSs and members of the EFTA), and on request of DG-Sante, one NRL from a non-European country.
The main objective of the study was to test the performance of the participating laboratories for the detection of *Salmonella* at different contamination levels in a matrix from primary production. For this purpose, pig faeces samples were artificially contaminated with monophasic *Salmonella* Typhimurium (STM-mono) at three different contamination levels. The prescribed method was Annex D of ISO 6579 (Anonymous, 2007), using selective enrichment on Modified Semi-solid Rappaport-Vassiliadis (MSRV) agar.

The pig faeces samples were artificially contaminated with a diluted culture of monophasic *Salmonella* Typhimurium to obtain 3 different contamination levels: no *Salmonella* (blank), a low level (approximately 84 cfu/25 g of faeces) and a high level (approximately 530 cfu/25 g of faeces). The samples were stored at -20 °C until transport to the participants. The influence of storage and transport conditions were tested beforehand, and showed survival of detectable concentrations of *Salmonella* and background flora.
It was decided to store the samples at -20 °C, as storage at 5 °C resulted in visible presence of moulds on the faeces after a few weeks.

Eighteen individually numbered, blind samples containing pig faeces had to be tested by the participants for the presence or absence of *Salmonella:* six blank samples, six low level samples and six high level samples. Additionally, three control samples had to be tested: two blank control samples (procedure control (BPW) and matrix control sample (pig faeces), and one positive control sample (inoculated by the laboratories themselves using their own positive control strain).

All laboratories scored well on analysing the procedure control and their own positive control (100% correct). The matrix control was correctly analysed as negative by almost all laboratories.

Unfortunately, the artificially contaminated samples were not as stable as in the pre-studies during the storage and transportation period. Reported results for both low and high level contaminated samples varied considerably. 34 participants scored all 6 blank samples correctly negative for *Salmonella*. Two laboratories tested two respectively three blank samples false positive for *Salmonella*.
For the low-level contaminated samples, 24 laboratories detected monophasic *Salmonella* Typhimurium in one to five samples of the six low level contaminated samples. Twelve laboratories did not detect *Salmonella* in any of the 6 low level samples.
A similar pattern was seen in the high level contaminated pig faeces samples. Only five laboratories detected *Salmonella* in all six high level contaminated samples, and five laboratories only reported one of six high level contaminated samples as being positive.

Considering the large variation in results and the instability of this *Salmonella* strain in these frozen samples, the performance of the participating laboratories could not be evaluated.

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

Discussion
Q: Will a follow-up study be organised?
A: Unfortunately it will not be possible to organise another study on *Salmonella* in samples from the primary production stage (pps) again this year. For a new pps study, it will be necessary to wait for spring 2016.
Q: Did you look at the influence of the matrix (pig faeces) on the growth of *Salmonella*? We have seen unexplainable negative results in pig faeces, although pre-tests were fine.
A: For this years' study we were not able to use chicken faeces due to Aviaire influenza in flocks in the Netherlands, therefore we had to change to another matrix. When the pig faeces was stored at 5 °C, we saw no problems with the growth of *Salmonella*, but moulds became visible. Therefore it was decided to store the pig faeces at -20 °C, but this affected the growth of *Salmonella*. Although this effect was small in the pre-tests, it became obvious in the interlaboratory comparison study. We therefore think that the high number of negative samples was mainly caused by freezing of the samples.
Q: What have you learned from this study?
A: Difficult to say, as the results in the pre-tests were fine. Some of the problems may have been caused by the fact that pig faeces is more wet than chicken faeces.
Q: What was the temperature of the samples during transport? Could this have influenced the results?
A: The results are not yet available, but there is not a specific geographical clustering of results visible, so it does not seem likely that the problems were caused by the temperature during transport of the samples.
Q: Would it be possible to share the protocol for the preparation of this type of samples?

A: The preparation of the samples and the pre-tests are summarised in the relevant reports. The results may vary per *Salmonella* serovar and per strain.
Q: What criteria are used for the selection of the strains for artificially contaminating the matrix samples?
A: We use strains of own culture collection (preferably a strain isolated from the same matrix) or strains from a culture collection.
Q: When NRLs organise PT schemes and would face similar problems, would it then be allowed to skip the follow-up study?
A: This will depend on your resources and possibilities, and should be discussed at national level. In our case, a follow-up study was not organised due to lack of time on the side of the EURL as well as on the side of the NRLs.
Q: A few laboratories seem to have found good results. Did they use alternative methods?
A: There were no trends visible, so this could not be investigated further.
Q: Does the EURL participate in the study as well?
A: Yes, and we also found a very low number of positive samples.

2.6 Update on EFSA's molecular typing project

Frank Boelaert, EFSA, Parma, Italy

Molecular typing or microbial DNA fingerprinting has developed rapidly in recent years. Data on the molecular testing of food-borne pathogens such as *Salmonella*, *Listeria monocytogenes* and Shiga toxin-producing *Escherichia coli* could substantially contribute to the epidemiological investigations of food-borne outbreaks and to the identification of emerging health threats. The molecular testing data may also be very useful for source attribution studies when estimating the contributions of different food categories or animal species as sources of human infections. The European Food Safety Authority received the mandate from the European Commission to provide technical support to the development of a database on molecular typing data on isolates of *Salmonella*, *Listeria monocytogenes* and Shiga toxin-producing *Escherichia coli* from food, feed, animals, and the related environment. For the purposes 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. The project on database development comprises two phases: a pilot data collection phase and database set-up, followed by a fully functional data collection and database management. The present report addresses the pilot phase that covers molecular typing data based on Pulsed Field Gel Electrophoresis for *Salmonella*, *L. monocytogenes* and Shiga toxin-producing *Escherichia coli*, together with Multiple Loci variable-number tandem repeat Analysis for *Salmonella Typhimurium*. The purpose of the pilot phase is to test the functionality of the database including its technical components, as well as the operational process underlying the collection, exchange, curation and analysis of the data. A technical report published by EFSA (EFSA, 2014) addresses all technical aspects for the design of the database and its functionalities. In addition, specific information about the procedures for data submission and accessibility are also covered.

Discussion
Q: How will you guarantee the quality of the molecular data?
A: The relevant EURLs will perform the curation of the PFGE data uploaded in the database. Furthermore, PFGE has recently been included in the EURL-*Salmonella* interlaboratory comparison studies on typing of *Salmonella*. This can help to improve the quality of the data of the NRLs. The relevant EURLs, as well as the curator of the ECDC database will also cooperate to harmonise curation of the PFGE data as much as possible.
Q: The database is currently only for storage of molecular typing data (PFGE, MLVA) of *Salmonella*, STEC and *Listeria monocytogenes*. Will this be extended to other microorganisms in the future?
A: The pilot phase will be restricted to these three microorganisms, but in the future this may be extended to other microorganisms, e.g. *Campylobacter* and/or to data obtained with other molecular typing techniques (like Whole Genome Sequencing). The ECDC molecular typing database already includes Mycobacterium.
Q: Who can use the data in the database? What are the restrictions on use?
A: We share your concern on the confidentiality of the data and this will be covered in the collaboration agreement which still needs to be signed by all parties involved. The data in the joint EFSA-ECDC database are intended for outbreak analysis, but not all (sensitive) data will be placed in the joint database. For use of the data in publications it has to be sure that this is agreed by the data providers as well.

2.7 Comparison of a rapid molecular serotyping method (Check and Trace Test) to conventional serotyping

Doris Mueller-Doblies, APHA Weybridge, United Kingdom

During 2009 and 2012, a Defra-funded project evaluated alternative molecular methods of *Salmonella* detection with the aim of providing evidence for a rapid *Salmonella* alerting system that could be used in the UK at some point.
Three arrays were tested: a luminex based array (LUM array), a linear probe based array (SGSA) and an SNP-based array (Check & Trace)

The Check&Trace (C&T) *Salmonella* is a rapid genetic test based on a microarray platform. Each position on the microarray represents a specific DNA marker associated with a unique *Salmonella* target sequence. Spots only become visible if the DNA markers exactly match the corresponding DNA sequences of the *Salmonella* isolate. The combination of present and absent spots yields a pattern. The Check-Points Tube Reader and the Check&Trace Software confirm the presence of *Salmonella* and match the pattern to a specific serovar.

Currently, the C&T array includes the determination of several hundred of the most commonly reported *Salmonella* serovars, and new sequences are added as and when needed. It has accreditation for 102 serotypes with the AOAC-RI, and 22 of those serovars with current and future regulatory significance also have International OIE certification.

A total of 2135 isolates representing 171 serovars were tested in the project. These included a panel of 104 well-characterised isolates,

twenty-four non-*Salmonella* spp., and 2007 field isolates, mainly received at APHA Weybridge in 2011.

In the first run, 93.4% of results matched with serotyping results according to the White-Kauffmann-Le Minor scheme (Grimont and Weill, 2007). 4.4% of the inaccuracies were due to human error or reaction failures. These samples were repeated, after which the match was increased to 97.8%.

All samples containing *Salmonella* were identified as *Salmonella* and all non-*Salmonella* were identified as non-*Salmonella*. 100% matching results were achieved for *S*. Enteritidis, *S*. Typhimurium and the remainder of 15 commonly seen serovars (1473 isolates).
Cases where a complete match could not be achieved included isolates where only a '*Salmonella* Genovar result' could be achieved, where more than one *Salmonella* species was proposed, or where related species were proposed (e.g. overlaps).

Discussion
Q: What were the human failures mentioned in your presentation?
A: It concerns the fact that the staff need to get used to the method.
Q: Are there countries that do not perform serotyping of *Salmonella* in accordance with the White-Kauffmann-Le Minor scheme?
A: It may be the case that some (small) laboratories in certain countries have not (yet) introduced conventional serotyping. For them it may be easier/cheaper to introduce an alternative serotyping method.
Q: Is use of an alternative method for serotyping allowed?
A: This is not allowed for the analysis of official samples, but it may be useful for other samples. An important issue for the use of alternative methods is the fact that these have to be validated. However, currently there is no standard procedure describing how to validate confirmation/typing methods. An ISO working group (WG3) is working on this subject and is drafting a procedure which will eventually be published as part 6 of ISO 16140.

2.8 Input of sequencing data for Food-borne Outbreak investigations: the recent French experience

Renaud Lailler, ANSES, Maisons-Alfort, France

The French *Salmonella* surveillance system relies on the participation of a variety of actors which collect data all along the agro-food chain. The quality of these data and their representativeness should allow investigation of epidemiological events in order to achieve public health goals. Modern and efficient ways are needed to collect and share information and to analyse it quickly, especially during health warning situations.
In 2014, two food-borne outbreaks (FBO) were observed in France due to *S.* Kedougou and *S*. Havana. In this framework, Whole Genome Sequence (WGS) analyses were assessed in parallel with routine investigations in order to discriminate isolates and to confirm the source of contamination.
The French national reference centre at the Pasteur Institute detected an abnormally high number of *S*. Kedougou isolates in May 2014 (n=25) compared to the average annual rate. Epidemiological investigations

identified some unpasteurised cheese consumers among cases, but the causal relationship was not strongly established. Finally, one strain isolated from Reblochon (a semi-soft and washed-rind mountain cheese) was identified by the French *Salmonella* network in June. Analyses of SNPs and Whole Genome (WG)-MLST results confirmed the epidemiological link between the food product and human cases. WGS has proven to be very powerful in discriminating isolates, and the involvement of different batches of food could be assumed.
In the same period (May-July 2014), S.Havana caused a second FBO as a result of the consumption of sausages. A panel of strains harbouring a large diversity of origin was characterized. PFGE results were homogenous, assuming either a high clonal relationship between strains or a poor PFGE discrimination power. Globally, WGS analyses have shown similar results, but when looking at the details, SNPs or WG-MLST have revealed few differences between each strain. These results have highlighted the need to define a threshold concerning the maximum of molecular differences required to conclude that two strains are epidemiologically linked.
Through these two situations, sequencing methods appeared to be very informative and of interest for alert investigations, even if routine use of these analyses is limited due to cost and time. WGS applications have a low added value for investigations on rare serovars. The detection of isolates and serovar determination could be sufficient to implement control measures.
However, WGS provides access to a deep characterisation of genome, allowing a great resolution for FBO. Because WGS results can be compared with previous molecular typing results (PFGE, MLVA or MLST) by in-silico determination, information available in databases remains useful. Thereby, diversity in origin of isolates and matrices are valuable for interpreting molecular results.
Reference genomes are needed to facilitate data mapping and epidemiological thresholds have to be defined for each serovar to facilitate the interpretation of SNPs and WG-MLST results. In conclusion, a surveillance based on WGS seems increasingly feasible, and sharing of these data would be very informative, to move forward with the implementation of Standard Operating Procedures.

2.9 A large outbreak of *Salmonella* Thompson related to smoked salmon in the Netherlands, 2012

Anjo Verbruggen, RIVM, Bilthoven, the Netherlands

An outbreak of salmonellosis due to *Salmonella* Thompson affected the Netherlands between 2 August and 19 October 2012 (Friesema et al., 2014). 1149 cases were confirmed with a median age of 44 years; 63% were female and 36 were hospitalized. On 15 August 2012, the National Institute for Public Health and the Environment noticed an increase in the number of *S*. Thompson; in a normal year an average of 7 strains are found. An outbreak investigation was started. A matched case-control study was conducted by sending a similar version of the questionnaire to four controls per case, matched on year, birth, sex and municipality. On 26 September 2012, the Netherlands Food and Consumer Product Safety Authority (NVWA) held an inspection at the fish production site and took samples from different batches of smoked salmon products. Subsequently, all smoked salmon from this producer

was recalled, starting Friday 28 September 2012. Isolates from patients and smoked salmon were subjected to molecular typing analysis by means of pulsed-field gel electrophoresis (PFGE) according to the PulsNet international protocol. The PFGE patterns from patients and salmon were indistinguishable using BioNumerics. Previous outbreaks due to *S.* Thompson were related to contaminated fresh coriander, rucola lettuce, pet treats, cow's milk, roast beef, and egg albumen. In earlier outbreaks related to salmon, *S*. Montevideo or *S.* Enteritidis were found.

Discussion
Q: Did it concern hot or cold smoked salmon?
A: It concerned problems with cross contamination through the plates in the transport line of salmon in general.
Q: Is there any molecular information of the *Salmonella* Thompson strain, e.g. does it concern a more virulent strain?
A: Some first results of recent experiments showed that the specific outbreak strain seems to be more invasive compared to another *S.* Thompson strain isolated from chicken.
Q: When did the company detect the problem?
A: They did not discover it themselves. There were no problems with their procedures, but the porous material of the (new) transport plates was the cause of the problem.

2.10 International *Salmonella* Newport outbreak in 2011-2012

Petra Hiller, Federal Institute for Risk Assessment, Berlin, Germany

In October/November 2011, a large *Salmonella* Newport outbreak with 106 confirmed cases took place in Germany. Cases also occurred in a hospital in the Netherlands. The multidisciplinary outbreak investigation included an epidemiological study, molecular typing of human and food isolates, and trace-back investigations. A case control study revealed that consumption of sprouts was significantly associated with *S*. Newport infection. Isolates of sprouts and humans were compared with 33 epidemiologically unrelated *S*. Newport strains which had been isolated between 2009 and 2011 from food items (turkey and chicken), reptiles and other animals, and environmental sources. The human outbreak isolates showed an identical PFGE pattern. The PFGE pattern was indistinguishable from the pattern of a mung bean sprout isolate, which originated from a sample taken in October 2011 in Germany during routine food sampling. The sprouts were produced in the Netherlands (producer A). The epidemiologically unrelated isolates showed PFGE and MLVA patterns that were different from the pattern of the outbreak strain. Outbreak isolates were susceptible to a panel of 14 antimicrobial agents. Trace-back investigations revealed that a rehabilitation clinic and six Asian restaurants in Germany as well as the hospital in the Netherlands, where cases had eaten before getting ill, had received sprouts from a sprout producer (producer A) in the Netherlands. The restaurants under investigation reported that sprout preparation varied from brief heating to well cooked. In the rehabilitation clinic, the raw sprouts were served in a salad bar. This outbreak demonstrates once again that sprouts may contain pathogens. Persons with a not fully developed or weak immune system (e.g.

infants, pregnant women, elderly and sick people) should, as a precaution, only eat sprouts after they have been sufficiently heated.

2.11 Standardisation of a method for PCR identification of monophasic *Salmonella* Typhimurium: a status report

Burkhard Malorny, Federal Institute for Risk Assessment, Berlin, Germany

In June 2013, members of CEN/TC275/WG6 asked Task Group (TAG) 3 and TAG 8 to investigate a PCR identification procedure for monophasic *S*. Typhimurium as an amendment to the (CEN) ISO/TR 6579-3 (Anonymous, 2014).

Following this recommendation, activities were initiated to propose and standardise a suitable method in close cooperation with TAG 8 (detection of *Salmonella*) and ISO/TC34/SC9 Working Group (WG) 10 (serotyping of *Salmonella*). TAG 3 nominated Burkhard Malorny from NRL-*Salmonella* in Germany as project leader. Kirsten Mooijman (convenor of TAG 8 and WG 10) contacted members of the groups and NRLs, asking them to submit suitable methods for the PCR identification of monophasic *S*. Typhimurium. A list comparing all methods submitted by laboratories and published in literature based on either agarose gel-based detection or real-time PCR was established and presented on the last TAG 3 meeting (April, 2015). Six laboratories provided their methods. Most laboratories use the multiplex PCR according to Tennant et al. (2010) which has also been recommended in the EFSA Scientific Opinion on monitoring and assessment of the public health risk of '*Salmonella* Typhimurium-like' strains (EFSA, 2010). A PCR to identify the fliC gene encoding the phase 1 flagellin was also submitted. Three real-time PCR methods were collected; two were based on the publication of Prendergast et al. (2013), and one on the publication of Maurischat et al. (2015).
A questionnaire was again circulated between the members and selected NRLs, asking for their opinion on the need of the identification of phase 1 monophasic *S*. Typhimurium, kind of detection (gel-based or/and real-time PCR), validation data and considering an internal amplification control for an assay. Members of TAG 3 agreed that a multiplex real-time PCR based on the publication by Maurischat et al. (2015) is most suitable to identify isolates of monophasic *S*. Typhimurium (4,[5],12:i:-). This assay is not only able to identify the absence of fljB encoding the phase 2 flagellin, but it is also able to identify other possibly deleted regions surrounding fljB. Several recent publications described monophasic *S*. Typhimurium variants where the fljB gene was present, but adjacent DNA sequences were deleted. A gel-based detection PCR assay identifying all such variants is currently not available. Therefore, it needs to be discussed if the Tennant method should be standardised regardless of its weakness to identify only monophasic *S*. Typhimurium lacking the fljB gene. Alternatively, singleplex PCRs could be performed based on the same primer and target sequences used in the real-time PCR by Maurischat et al. (2015).

Once the assay selection has been agreed, a performance study of the protocols will be performed by the EURL-*Salmonella* in collaboration with the NRLs for *Salmonella*.

Discussion
An extensive discussion took place on the method to be standardised. A summary is given below.
It was indicated that it is the intention to draft the PCR method for identification of monophasic *Salmonella* Typhimurium as an annex to ISO/TR 6579-3 (Anonymous, 2014). As this concerns a guidance document, this annex will also become a guidance protocol and will not become normative. Almost 50% of the NRLs for *Salmonella* currently use (or introduce) the gel-based 'EFSA protocol' based on Tennant et al. (2010), and only a few have experiences with real-time PCR. At the time the EFSA opinion on monophasic *Salmonella* Typhimurium was published (EFSA, 2010), the 'Tennant-protocol' was a good method for identification of this monophasic variant. However, methods were further developed and new investigations have shown that a multiplex real-time PCR may currently be more suitable for the identification of this type of strains. Although it is preferable to include 'the best' method as protocol in ISO/TR 6579-3, it should also be a method which can be used by an international group of laboratories. Therefore it is worth considering to include more than one procedure in this new annex: one gel-based PCR and one real-time PCR.
In addition, it was indicated that it would help if the protocol(s) are validated/verified with a 'standard' set of strains. It was agreed that the EURL-*Salmonella* and Burkhard will have a closer look at the (draft) protocols, as well as to the possibility of testing these protocols with a 'standard' set of test strains.
Finally, the NRLs were asked for their opinion if the protocol should only identify monophasic *S.* Typhimurium lacking the second phase (1,4,[5],12:i:-), or whether it should also identify the monophasic variant lacking the first phase (1,4,[5],12:-:1,2). The general opinion was that priority should be given to a protocol for identification of monophasic *S.* Typhimurium lacking the second phase.

2.12 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 Delft, the Netherlands from 22 to 26 June 2015. The progress on the *Salmonella* documents was presented at these meetings by Kirsten Mooijman.
A summary was given on standardisation items with relevance for the NRLs for *Salmonella.*

EN ISO 6579, parts 1 to 3:
Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella*

- Part 1: Horizontal method for the detection of Salmonella. For this part, the prEN/DIS (Draft International Standard) voting took place from 5 June to 5 November 2014.
- Part 2: Enumeration by a miniaturized Most Probable Number technique. This part was published in November 2012.
- Part 3: Guidelines for serotyping of Salmonella spp. This part was published in July 2014. As indicated in the presentation by Burkhard Malorny (see 2.11), it is considered to draft an amendment for ISO/TR 6579-3 to include PCR identification of monophasic Salmonella Typhimurium.

The outcome of the voting of prEN/DIS 6579 part 1 was as follows:
ISO: 25 approvals and two disapprovals (Canada and France).
CEN: 22 approvals and one disapproval (France).
Overall the voting result was positive, with in total 25 pages of comments.
On 27 and 28 January 2015, the CEN Task Group (TAG8) met in Brussels to discuss the comments and to update the document.

Below, the main comments for disapproval of prEN DIS 6579-1 are summarised, as well as the TAG8 replies.

Comment from Canada: Change incubation temperature of MKTTn from 37 °C to 41.5 °C (or 42.5 °C).
Reply of CEN-TAG8: Not accepted. Incubation of MKTTn at 37 °C has been decided for the current version of ISO 6579: 2002. Many (European) laboratories have over 13 years' experience (for many types of samples) with the use of MKTTn, incubated at 37 °C. Furthermore, validation data have been obtained with MKTTn incubated at 37 °C in 2000 (see Annex C.1 of ISO 6579, Anonymous, 2002).

Comment 1 from France: According to a French study, many more samples from the primary production stage will be found positive (approx. 23%) if, in addition to selective enrichment on MSRV (incubated at 41.5 °C), selective enrichment in MKTTn broth (incubated at 41.5 °C) is also performed.
Reply of CEN-TAG8: TAG 8 has discussed this information in detail and decided (together with France) not to change the procedure, because there seem to be too many factors involved influencing the data. For example: in the French study, a relatively large number of samples contained lactose positive *Salmonella* (approx. 20%) for which detection was dependent on the chosen isolation medium. It was therefore decided to add the following informative note to clause 9.3.3 (selective enrichment pps):
'NOTE - Sensitivity may be improved by using a second selective enrichment procedure, e.g. MKTTn broth incubated at 41.5 °C for 24 h.'

TAG8 asked the organisers of the French study to publish the results as soon as possible so that reference can be made to this publication in the final publication of EN ISO 6579-1.

Comment 2 from France: Serological testing should become optional instead of mandatory after biochemical testing.

Reply of CEN-TAG8: Partly accepted. Limited serological testing is required (up to group O and group H), especially as the number of mandatory biochemical tests is limited (only tests for Triple sugar/iron (TSI), ureum and L-Lysine decarboxylation (LDC)).

After the TAG8 meeting in January 2015, the document was updated to include the comments from the CEN enquiry/DIS voting. Next, the amended document was sent to the members of TAG8 for a final check in April 2015. After this, a few additional comments were received from some members of TAG8, which were introduced in the final draft version of EN ISO 6579-1. In May 2015, the final draft document was sent to the WG6 secretariat to launch the FDIS (Final Draft International Standard) voting. It is not yet clear when the FDIS voting will be launched, as the ISO CS is very busy.

Harmonisation of incubation temperature
At the meetings of ISO/TC34/SC9 and CEN/TC275/WG6 in 2014, it was agreed to use a broader temperature range for incubation of non-selective media:
34 – 38 °C instead of 37 °C ± 1 °C, to have a better harmonisation with e.g. methods used in the USA. At that time, it was also discussed whether this broader temperature range could also be used for incubation of selective media. It was decided that first, predictive data from strain databases should be consulted, and the next steps be discussed at the following SC9/WG6 meeting (June 2015).

EN ISO 6887 parts 1 to 4:
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)

Little progress has been made with these documents, after the DIS voting ended in April 2014.

EN-ISO 7218:2007/Amendment 1:2013 'Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations'. This document was published in August 2013 and includes improvements to EN ISO 7218:2007. This document is again under revision since 2014.

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 6 parts:
Microbiology of the food chain – Method validation -

- Part 1 'Vocabulary'. This part includes all definitions. The FDIS vote closed on 19 May 2015.

- Part 2 'Protocol for the validation of alternative (proprietary) methods against a reference method'. The FDIS vote closed on 19 May 2015.

The following documents still concern working drafts:

- 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'

EN ISO/TS 17728 'Microbiology of food and animal feed - Sampling techniques for microbiological analysis of food and feed samples': the second voting round finished in February 2015. The outcome was positive and the document may be finalised soon.

ISO/TS 22117 'Microbiology of food and animal feeding stuffs – specific requirements and guidance for proficiency testing by interlaboratory comparison': this document was published in 2010, and it was recently decided to revise the document for two main reasons. 1) To make the document a full standard (instead of a Technical Specification) as a TS is not recognised in some countries, and 2) to take into account new information on statistical aspects for Proficiency Testing (PT) schemes.

ISO working group on WGS
In 2014, a new working group was raised under ISO/TC34/SC9 to have a closer look at the options for standardisation of protocols for Whole Genome Sequencing. The project leader of this group is situated in the USA.

AOAC activities on Salmonella
AOAC International has formed the ISPAM (International Stakeholder Panel on alternative methods) working group on '*Salmonella* methods harmonization'. The main aim of this working group is to determine how and if the US and ISO reference methods for *Salmonella* can be harmonised. The following steps are indicated:

- Provide recommendations for the process of harmonising the US (BAM/MLG) and ISO *Salmonella* reference culture methods;
- Determine matrices for which the US and ISO *Salmonella* methods are statistically equivalent by analysing existing data using ISO 16140;
- Determine which steps should be taken to harmonise the US and ISO *Salmonella* methods.

The working group includes approximately 20 members from e.g. Food and Drug Administration (FDA), Health Canada, Bio-Rad, Biocontrol, bioMerieux, Nestle, EURL-*Salmonella*.

Discussion
Q: Is it mandatory to perform biochemical confirmation in ISO 6579-1?

A: Biochemistry is indeed required, especially for official samples. For other type of samples, alternative methods can be used if validated. However, here again we are confronted with the issue that a standard procedure for validation of confirmation methods is not yet available (see also the discussion at 2.7).

3 Friday 29 May 2015: day 2 of the workshop

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

Gintare Bagdonaite, NRL-Salmonella*, Belfast, Northern Ireland*

The *Salmonella* Veterinary National Reference Laboratory for the UK in Northern Ireland (NI) is based in the Agri-Food and Biosciences Institute (AFBI).
AFBI is a non-departmental public body (NDPB) created in 2006 from the amalgamation of the Department of Agriculture and Rural Development (DARD) Science Service and the Agricultural Research Institute of Northern Ireland (ARINI). AFBI is a leading provider of scientific research and services to government, non-governmental and commercial organisations.

Established in 1992, the NRL UK-NI carry out tasks in accordance to the EC Directive 2003/99/EC (EC, 2003a) on monitoring of zoonoses and zoonotic agents and the Regulation (EC) 2160/2003 (EC, 2003b) on the control of *Salmonella* and other specified food-borne zoonotic agents. The laboratory performs a wide variety of techniques, including serotyping, microbiological culture, biochemical and antimicrobial resistance methods. Laboratory methods for isolation and identification of *Salmonella* spp are ISO 17025 (Anonymous, 2005) accredited. Additionally, a large *Salmonella* strain collection and archive is maintained.
Approximately 8000 samples are tested under Statutory *Salmonella* Control plan (poultry NCP programme) every year. Approximately 1500 isolates are serotyped and characterised further. Isolates for serotyping are received from different sources: as part of the statutory testing programme; from clinical cases through the AFBI-Disease Surveillance & Investigation Branch (DSIB), and also from Regional/Private Veterinary Laboratories within Northern Ireland for private testing.
The laboratory provides data collected as part of the surveillance system of *Salmonella enterica* isolates for several official reports and other epidemiological analyses. In addition, the laboratory provides expert information and advice to DARD and to other governmental agencies in Northern Ireland.

Discussion
Q: How do you decide to serotype 1500 isolates out of 8000?
A: On average we test 8000 samples, of which 1500 are positive for *Salmonella* and these are all serotyped and tested for antimicrobial resistance.
Q: Do all *Salmonella* Dublin strains origin from cattle?
A: No, we also isolated *S.* Dublin from poultry and pigs.

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

Patricia Themudo, NRL-Salmonella*, Portugal*

The Portuguese NRL for *Salmonella* belongs to INIAV, I.P. – the National Institute for Agriculture and Veterinary Research. INIAV is the official laboratory of the Ministry of Agriculture and Sea (MAM), and carries out research activities in agricultural and veterinary areas. INIAV was created in 2012, retaining the tasks relating to agricultural (the L-INIA) and veterinary (L-LNIV) research of the former National Institute of Biological Resources IP (INRB).
The INIAV laboratory activity is organised in eight major sectors: animal health; food safety; plant health; soil / plant nutrition; forestry; genetics and animal breeding; animal nutrition; and viticulture and enology. Currently, laboratories are located in the following centres: Lisboa-Benfica; Lisboa-Lumiar; Lisboa-Tapada da Ajuda; Oeiras; Vairão; Santarém – Fonte Boa; Dois Portos.
It is expected that the three centres located in Lisbon - Benfica, Lumiar and Tapada da Ajuda, will move to Oeiras in January 2016.
INIAV owns the National Reference Laboratories for animal diseases, for food safety and for plants diseases. INIAV is also the Reference Laboratory for the OIE and FAO for Contagious bovine pleuropneumonia (CBPP).

The NRL-*Salmonella* activities are:
1 National Control Programmes:

- Salmonella detection in samples from the official control;
- Salmonella serotyping on strains isolated from the National Control Programmes;
- Salmonella serotyping of isolates from food, feed, environment and veterinary samples isolated in private/regional laboratories;
- Determination of Antimicrobial Resistance (MIC) for Salmonella isolates.

2 Activities with EURL, cooperation with EURL-*Salmonella:*

- Participation in the EURL-Salmonella annual workshops (since 1995);
- Participation in the EURL-Salmonella Proficiency Tests (since 1995);
- Disclosure of relevant information received from EURL-Salmonella.

3 Scientific and Technical assistance:

- Scientific and Technical assistance to the Competent Authority;
- Supervision of regional and private recognized laboratories, that collaborate in monitoring programmes;
- Providing the Competent Authority and EFSA with data of Salmonella serovars and antimicrobial resistance data.

Serotyping of *Salmonella* strains is performed by following the White-Kauffmann-Le Minor scheme (Grimont and Weill, 2007) and antimicrobial resistance is performed by MIC. Both methods have been accredited by IPAC (Portuguese Institute for Accreditation) since July 2013. *Salmonella* detection is performed following ISO 6579 (Anonymous, 2002) and accreditation by IPAC was granted in March 2015. In April 2015 accreditation for ISO 6579/Amd1 (Anonymous, 2007) was requested and the concession audit is expected in July 2015. The approved/recognised laboratories authorised to perform *Salmonella* auto control analyses for the National Control Programs, belong to a

'laboratories list' published by the Competent Authority on their website. This list of laboratories is confirmed every year. A cooperation protocol is signed between the three interested parties (Competent Authority, NRL and the recognised laboratory), establishing the obligations and responsibilities of each party.
The compulsory requirements for laboratories that belong to the list are: 1) participation in collaborative studies of VETQAS PT0088 '*Salmonella* in poultry', 2) being accredited for 'Detection of *Salmonella* following ISO 6579:2002/Amd1:2007' (Anonymous, 2007), and 3) availability for audits by the NRL together with the Competent Authority, when necessary.
The monitoring of the laboratories performance for PT0088 is conducted by the INIAV QA team. Deviating results are followed either by an extra audit, either by a meeting with the laboratory involved, or by evaluation of the action plans and requested evidence.

Discussion
Q: In Portugal we face problems with *Salmonella* Cerro in three broiler flocks in the Lisbon area. Do other countries also see an increase of this serovar?
A: A few countries have also found *S.* Cerro, but none has seen a real increase. It was suggested to Portugal that it may be worthwhile to test for the presence of *S.* Cerro in animal feed, as well as in the hatcheries. Furthermore, other NRLs promised to have a further look at their data 'at home' and to inform Patricia in case more information on the prevalence of this serovar is found.

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

*Cristina de Frutos Escobar, NRL-*Salmonella*, Madrid, Spain*

In Spain there are three *Salmonella* NRLs (food, humans, animals and feed). The Laboratorio Central de Veterinaria (LCV, Madrid), dependent on the Ministry for Agriculture, Food and Environment, has been the Spanish *Salmonella* NRL for animals and feed since 1995.
LCV is divided in two main areas, 'Genetics and Control' and 'Animal Health'. The latter, dealing with the official control of animal diseases and zoonosis in feed and live animals, is in charge of the NRL *Salmonella*. The LCV is accredited according to ISO 17025 (Anonymous, 2005) for the detection, serotyping and antimicrobial resistance of *Salmonella*.

Among the different activities of the LCV as NRL-*Salmonella* are:
Coordination of the laboratories taking part in the Salmonella *National Control Programmes (SNCP).*
In Spain there are 20 official laboratories and 30 business operator laboratories working in the SNCP, although the designation and approval of these laboratories is the competence of the Autonomous Communities. The NRL plays an important role in their coordination through the following activities:

- Harmonisation and updating of information for the correct implementation of the SNCP (samples, vaccines, reporting of results, authorised alternative methods);
- Updating information about the progress of SNCP;

- Technical assistance for accreditation purposes;
- Dissemination of information provided by the EURL;
- Organisation of annual meetings.

Organisation of interlaboratory comparison studies.
- Two studies per year on detection of *Salmonella* in avian faeces;
- One study per year on detection of *Salmonella* in feed;
- One study per year on *Salmonella* serotyping;
- Follow up of the laboratories with poor performance.

Training for laboratories working on SNCP
Since 2004, the LCV has organised training courses on the detection and identification of *Salmonella* for 5 laboratories as well as on serotyping for 34 laboratories (official and business operators).

Other activities on Salmonellosis
- *Salmonella* characterisation:
  - Serotyping of approximately 1500 *Salmonella* strains per year, coming from SNCP, other activities in official laboratories, universities etc. The methods used are agglutination according to the White-Kauffmann-Le Minor scheme (Grimont and Weill, 2007) and Multiplex PCR for detection of O, H1 and H2 antigens.
  - Performing PFGE typing
- Bacteriological and serological test for import and export (live animals);
- Maintenance of *Salmonella* collection;
- Collaboration with the Ministry of Agriculture, Food and Environment: registration of alternative *Salmonella* detection methods, implementation of databases for Salmonella, participation on Salmonella working groups with the Autonomous Communities;
- Monitoring and reporting of antimicrobial resistance on *Salmonella* strains (Commission Decision 2013/652 – EC, 2013);
- Collaboration in research activities with official institutions and universities: publications on *Salmonella*.

Discussion
Q: How many isolates do you receive for serotyping from private laboratories?
A: Not so many. Private laboratories either serotype their own isolates or send them to another official laboratory. We only receive some 'special' strains, e.g. for confirming monophasic *Salmonella* Typhimurium.
Q: Does this mean that the private laboratories do not report their positive samples? Is this a more general problem?
A: Several NRLs indicated that private laboratories do not report many positive samples and/or do not give all information with the samples or isolates. This latter may be done to protect the customers of the private laboratory. However, the Competent Authority should still have full access to the records of the private laboratories.
Q: What to do/how to act in case private laboratories have repeated poor performance in Proficiency Tests?

A: This may be decided at the national level with the Competent Authority. It can be decided to organise a follow-up study and/or a visit of the laboratory. In case the performance does not improve after these actions, the Competent Authority may decide to remove the laboratory from the list of official laboratories. In some Member States, the private laboratories only analyse the samples taken by the farmers and do not analyse official samples. The treatment of the different laboratories may therefore vary in the different countries.

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

Lubos Mikula, NRL-Salmonella*, Bratislava, Slovak Republic*

The surveillance of *Salmonella* in Slovakia is managed by two ministries, the Ministry of Agriculture and the Ministry of Public Health. At a second level are the State Veterinary and Food Administration and the Public Health Authority. At district level are 40 District Veterinary and Food Administrations and District Authorities of Public Health. In Slovakia there are four Veterinary and Food Institutes and nine Public Health Laboratories. The Veterinary administration supervise and send samples to Veterinary laboratories: samples from the primary production stage, foods of animal origin, foods of plant origin, from slaughterhouses, distribution of foods, retail and animal feed. The Public Health Administration supervises and sends samples to the Public Health Laboratories: samples from baby food primary production, restaurants, fast food, hospital catering, school catering, ice cream buffets, sandboxes and water.

Activities of the Slovakian NRL for *Salmonella*:

- Analysis of official samples for the National control programme for *Salmonella* in Slovakia;
- Serotyping of *Salmonella* isolates sent by the State Veterinary and Food Institutes;
- Storage of the *Salmonella* isolates;
- Organisation of interlaboratory comparison studies for *Salmonella* tests for four laboratories of the State Veterinary and Food Institutes;
- Antimicrobial susceptibility testing of *Salmonella* (using MIC);
- Drafting of a *Salmonella* report for the State Veterinary and Food Administration for EFSA;
- Reporting for the National contact point of the Ministry of Agriculture for cooperation with EFSA.

The NRL does not organise interlaboratory comparison studies for private laboratories; these laboratories are controlled by the Slovak National Accreditation Service.
At the institute in Bratislava, in total nine National Reference Laboratories are situated, including the NRL for *Salmonella*.

Discussion

Q: What caused the sharp drop in the presence of *Salmonella* Enteritidis in laying hens in 2012?
A: This information is not available at the laboratory.
Q: Do you organise Proficiency Tests in your country?

A: Yes, we organise studies in which four veterinary institutes participate. We make the samples ourselves.

3.5 Results 19th interlaboratory comparison study on typing of *Salmonella* (2014) – serotyping and PFGE

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

In November 2014, the 19th interlaboratory comparison study on serotyping, phage typing and PFGE typing of *Salmonella* was organised by the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*, Bilthoven, the Netherlands), in collaboration with Public Health England (London, United Kingdom) for the phage typing part. A total of 35 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), 3 NRLs of the EU-candidate-countries (Former Yugoslav Republic of Macedonia, Serbia, and Turkey), and 3 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 would be obtained.

All 35 laboratories performed serotyping. A total of 20 obligatory *Salmonella* strains plus 1 additional optional *Salmonella* strain from an uncommon source and subspecies 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 and phage typing, as well as an interim summary report on the general outcome, were emailed to the participants in February 2015.
The O-antigens were typed correctly by 29 of the 35 participants (83%). This corresponds to 97% of the total number of strains. The H-antigens were typed correctly by 22 of the 35 participants (63%), corresponding to 94% of the total number of strains. A total of 20 participants (57%) gave the correct serovar names, corresponding to 94% of all strains evaluated.
Apart from participant 22, who encountered many problems during the serotyping study, a completely correct identification by all other participants was obtained for eight strains: *S.* Arechavaleta (S8), *S.* Hadar (S9), *S.* Infantis (S10), *S.* Virchow (S11), *S.* Dublin (S12), *S*. Herston (S13), *S.* Typhimurium (S16), and *S.* Enteritidis (S18).
Most problems occurred with the serovar *S*. Bochum (S2). Eight laboratories had difficulties assigning the correct serovar name to this strain. The reported serovar names for strain S17 still show some variation of 'Typhimurium-like' names, but the example given in both the protocol and the electronic test report on how to preferably report this serovar name seems to be of help.
All but three participants actually did serotype the additional strain S21, being a *Salmonella enterica* subspecies *arizona*e 41:z_4,z_{23}:-. Twenty-eight laboratories correctly serotyped the O-antigens and the H-antigens for this strain.
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 two laboratories that did not achieve the level of good performance were offered a follow-up study consisting of ten additional strains for serotyping. This follow-up study is obligatory for NRLs of EU Member States, and the EU-NRL concerned obtained good scores in this follow-up study (April 2015).

The individual laboratory results on the PFGE typing part were reported to the 18 participants in May 2015. As in the first PFGE study, the participants were asked to test 10 *Salmonella* strains using their own routine PFGE method for digestion with XbaI. Results were evaluated on the quality of the PFGE gel images only. The PulseNet Guidelines (www.pulsenetinternational.org) were used for this quality grading, based on scoring 7 parameters with 1 (poor) point to 4 (excellent) points. As in the first study, quite some variation in the quality of the gel images was observed. General remarks to improve the quality of the gel images could be given by advising on the use of the *S*. Braenderup H9812 standard and on the capture of the image.
PFGE typing, concerning quality of PFGE gel image as well as optional gel analysis in Bionumerics, will be part of the 2015 interlaboratory comparison study on typing of *Salmonella*.

More details of the study can be found in the interim summary report (Jacobs-Reitsma et al., 2015).

Discussion
Q: Shall we continue including the 21st optional strain (being a serovar from another subspecies than *Salmonella enterica* subps. e*nterica*) in the EURL-*Salmonella* typing studies?
A: Yes!
Q: Shall we include optional evaluation of gel analysis by BioNumerics in the next interlaboratory comparison study on typing of *Salmonella*?
A: It may be difficult to compare the results if BioNumerics analysis is performed by different laboratories, as different interpretations may be given. However, for the molecular database of EFSA, the PFGE data should also be reported through BioNumerics. Therefore it will be a good experience for the NRLs as well as for the EURL to introduce interpretation with BioNumerics as an option in the next typing study.
Q: Is information available on when the new White-Kauffmann-Le Minor scheme will be published?
A: According to the latest information, the WHO reference laboratory in Paris is working hard on it. However, it seems that MLST data will also be added for all serovars, resulting in a delay in publication of the updated version.

3.6 Results 19th interlaboratory comparison study on typing of *Salmonella* (2014) – phage typing

Elizabeth de Pinna, PHE, London, United Kingdom

The *Salmonella* strains for phage typing in the nineteenth interlaboratory comparison study on the typing of *Salmonella* spp. organised for the National Reference Laboratories (NRL) were provided by the Gastrointestinal Bacteria Reference Unit (GBRU), of Public Health England (PHE), London, United Kingdom. Ten strains of *Salmonella* Enteritidis and

ten strains of *Salmonella* Typhimurium were selected from the PHE culture collection.
Seven NRLs took part in the phage typing of both the *S.* Enteritidis strains and the *S*. Typhimurium strains.
Four of the NRLs correctly phage typed nine of the *S*. Enteritidis strains. Two of the NRLs correctly typed eight of the *S.* Enteritidis strains and one NRL correctly phage typed six of the *S.* Enteritidis strains. Five of the *S*. Enteritidis strains were phage typed correctly by all the participating laboratories. Two strains, E7 (PT 35) and E8 (PT 13a), were incorrectly phage typed by one of the participating laboratories. Two strains, E4 (PT 3) and E10 (PT 56), were incorrectly phage typed by three laboratories. One strain, E6 (PT 59), was incorrectly phage typed by four of the participating laboratories.
Four NRLs correctly typed nine of the *S*. Typhimurium strains. One NRL correctly phage typed eight of the *S*. Typhimurium strains, and two NRLs correctly phage typed seven of the ten *S*. Typhimurium strains. Five of the *S*. Typhimurium strains were correctly phage typed by all the participating laboratories. Three strains, T2 (DT 104), T5 (DT 41) and T8 (DT 10), were incorrectly phage typed by one laboratory. One strain, T6 (DT 193), was incorrectly phage typed by two of the participating laboratories. One strain, T9 (DT 132), was incorrectly phage typed by all seven of the laboratories.
Overall, 83% of the *S*. Enteritidis strains and 83% of the *S*. Typhimurium strains were correctly phage typed.
When compared to the previous two studies, the results of the NRLs for the phage typing of *S*. Enteritidis were not as good as the studies conducted in 2012 and 2013, when 90% and 93% respectively of the strains were correctly phage typed. For the phage typing of S. Typhimurium, the results of this study were not as good as the study in 2012, when 92% of the strains were correctly phage typed. The results of this study were the same as in the 2013 study, when 83% of the strains were correctly phage typed.
This study showed there was no improvement in the phage typing of *S*. Typhimurium by the NRLs. The performance in the phage typing of *S*. Enteritidis was lower than in the two previous years.

More details of the study can be found in the interim summary report (Jacobs-Reitsma et al., 2015).

Discussion
Q: Will you continue supporting the NRLs with the phages?
A: As long as Public Health England will perform phage typing, we can supply phages.

3.7 Work programme EURL-*Salmonella* second half 2015, first half 2016, 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 2015 and for early 2016.

Interlaboratory comparison studies
Three interlaboratory comparison studies are planned in the coming year:

- Detection of *Salmonella* spp. in a food matrix: September/October 2015. Experiments have been performed at the laboratory of the EURL-*Salmonella* to test whether it is possible to prepare sufficient stable materials when inoculating whole liquid egg with a diluted culture of *Salmonella*. For this, two different *Salmonella* strains have been tested and the results are promising.
- Typing of *Salmonella* spp.: November/December 2015. As in former typing studies, this study will contain an obligatory part for serotyping of 20 different *Salmonella enterica* serovars (and additionally, one optional non-*enterica* isolate) and an optional part for PFGE testing of 10 different *Salmonella* serovars. Phage typing will no longer be included in the typing studies.
- Detection of *Salmonella* spp. in a sample from the primary production stage: February/March 2015. 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). If necessary, performing experiments for the revision of EN ISO 6579.
- Consider the organisation of a 'verification study' with a small group of laboratories and to set performance characteristics of the protocols for identification of monophasic *Salmonella* Typhimurium.
- Testing different matrices for use in interlaboratory comparison studies.

Assistance to the Commission and communication

- If necessary/requested experts of the EURL-*Salmonella* 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 for 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 no longer as printed versions.

Training
Trainings can be given by EURL-*Salmonella* at the EURL or at the laboratory of the NRL. Requests for training will be considered case by case.

Molecular typing
With the publication of the 'Vision paper on molecular typing data' by DG-Sanco in 2013, it is clear that the EURLs will be given an important role in judging the (quality) of molecular typing data to be entered in the new (pilot) database of EFSA. For this, a staff member of the EURL-*Salmonella* is member of the Steering Committee in relation to the (pilot) molecular typing database of EFSA (started January 2015).

Activities 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 of NRLs for Salmonella on molecular typing;
- Cooperation 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.

Workshop 2016
The NRLs were asked to indicate on the evaluation form of the current workshop whether they want the 2016 workshop to be organised in conjunction with the international *Salmonella* symposium, I3S, or not. This symposium will take place in Saint Malo, France from Monday 6 June to Wednesday 8 June 2016. Depending on the outcome of the evaluation, a decision will be made on the location and dates of the workshop of 2016.

Discussion
Q: If the isolate from a positive sample turns out to be a vaccine strain, how should this be reported?
A: The vaccine strains are not a target. To EFSA, the result could be reported as negative, but for the laboratory report, it may be best to give more information and report the details about the isolate (being a vaccine strain).
Q: Is it necessary to differentiate all strains of *Salmonella* Enteritidis and *Salmonella* Typhimurium for being a vaccine strain or a wild strain? How long is the shedding of vaccine strains?
A: This needs to be decided on a case by case basis and per country. There are no international rules for this. In some Member States, the testing for the isolate being a vaccine strain is always performed in case *S.* Enteritidis or *S.* Typhimurium is found, independent of the timeframe between vaccination and testing. Some vaccines are shed for a longer period of time than others. There are some real time PCR assays available to differentiate (in approx. 2 hours) between vaccine strains and wild strains.

4 Evaluation of the workshop

4.1 Introduction

At the end of the workshop, an evaluation form was given to all participants to ask for their opinion on the workshop (see Annex 3). A total of thirteen questions were posed. For ten of these questions, participants were asked to give a score ranging from 1 to 5 as an answer to the questions, with 5 as the highest score (excellent) and 1 as the lowest score (very poor). In addition, it was possible to add comments on the questions. Three questions were 'open' questions, in which the participants were asked to give their opinion. The last question (13) was added to get the participants' opinions on their preferences for the location of the workshop of 2016.
The evaluation form was handed to 47 participants of the workshop and 43 completed forms were returned, which is a response rate of 91.5%.

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 all respondents considered the information given in advance to the workshop as good or excellent (scores 4-5). One remark was given: 'Excellent choice to organise the workshop in different European cities'.

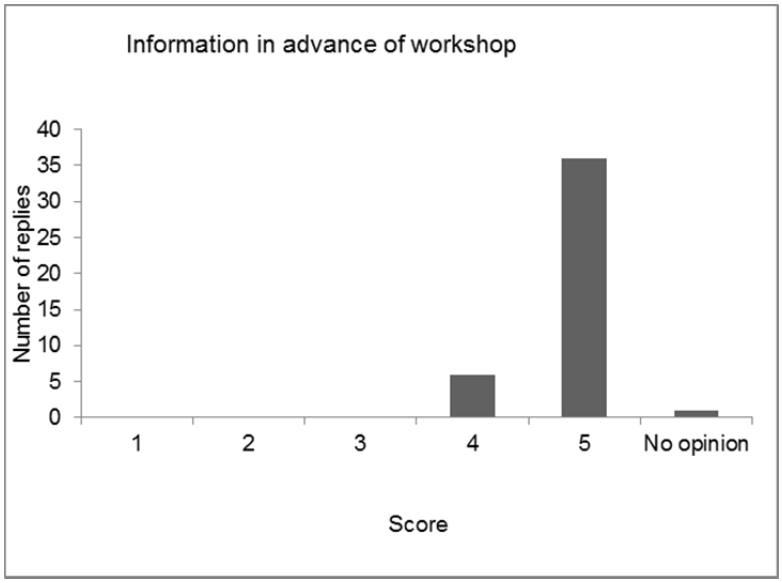

Figure 1 Scores given to question 1 'Opinion on information given in advance of the workshop'

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

The participants for whom tickets were arranged by the EURL were very satisfied. Participants who booked their own ticket indicated 'no opinion' (see Figure 2). One remark was given: 'Thank you so very much for making it convenient for me!'

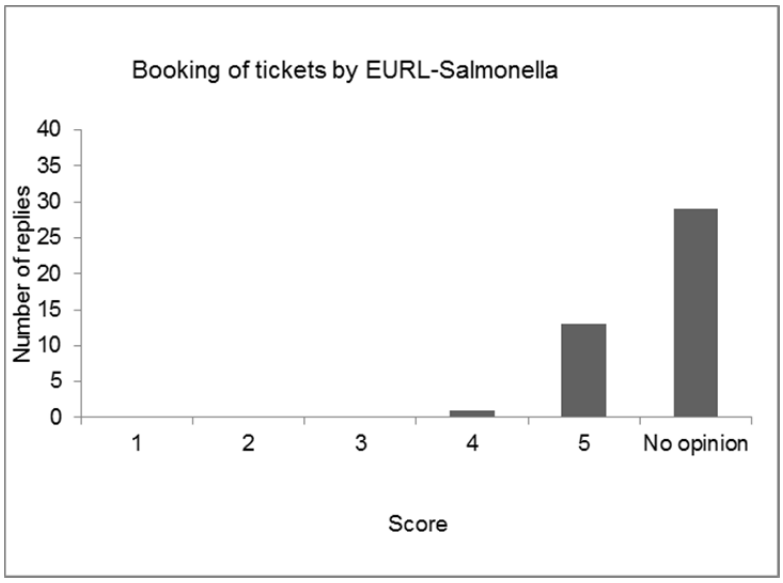

Figure 2 Scores given to question 2 'Opinion on the booking of the tickets by the EURL-Salmonella'

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

Most participants considered the meeting venue as 'easy to reach' (Figure 3). One remark was given: 'A lot of different transports to take between airport and hotel'.

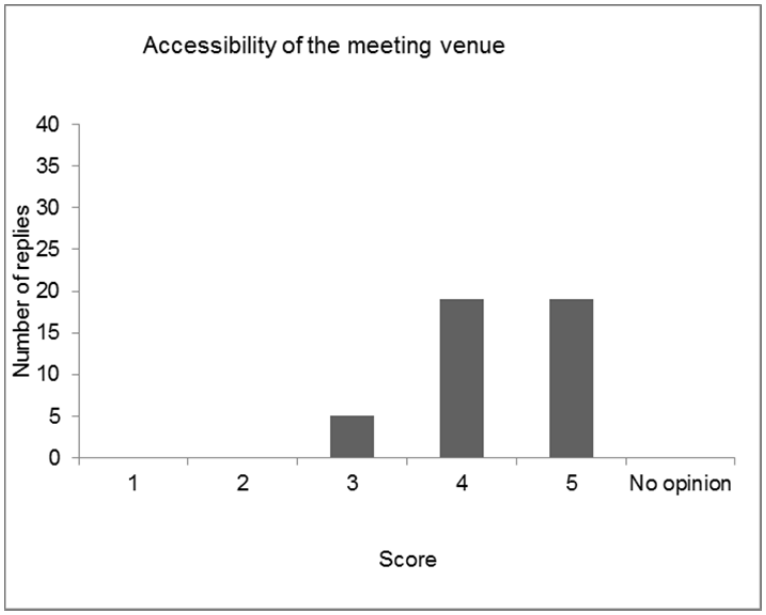

Figure 3 Scores given to question 3 'Opinion on the accessibility of the meeting venue'

4. What is your opinion on the hotel room?

The majority of the participants considered the hotel as good to excellent (scores 4-5, see Figure 4). In case 'no opinion' was indicated, this was because the participant used another hotel.

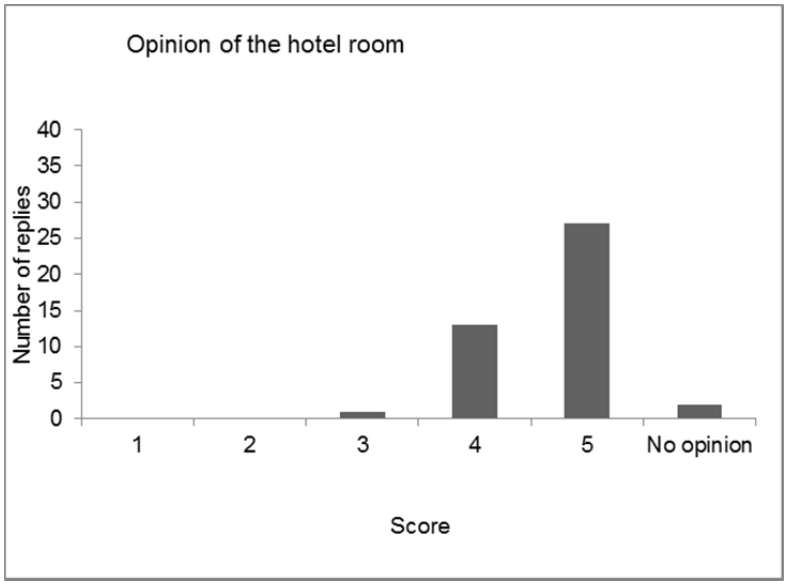

Figure 4 Scores given to question 4 'Opinion on the hotel room'

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

The participants considered the meeting room as good to excellent (Figure 5). Remarks made were:

- 'The sunlight made it sometimes hard to read the slides' (1x).
- 'The room was (a little) too large' (3x).

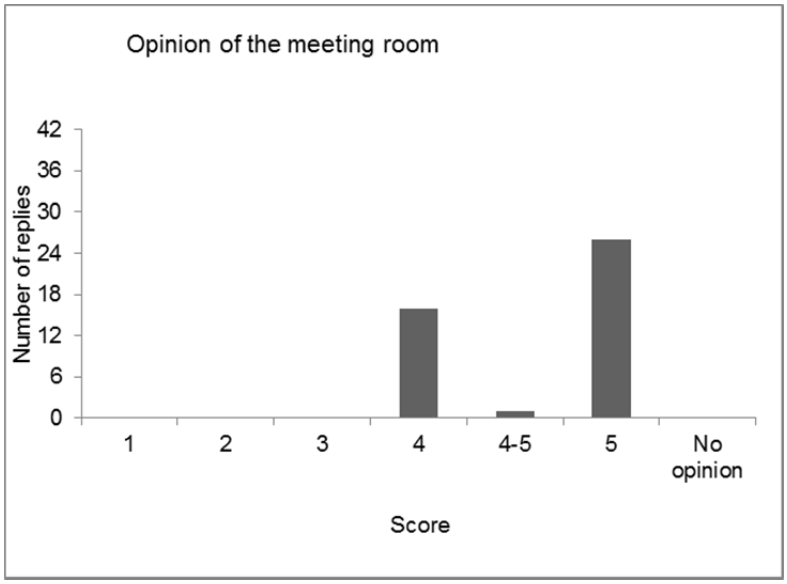

Figure 5 Scores given to question 5 'Opinion on the meeting room'

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

The readability of the presentations on the screen was considered good to excellent (Figure 6). One remark was given: 'Good screen size'.

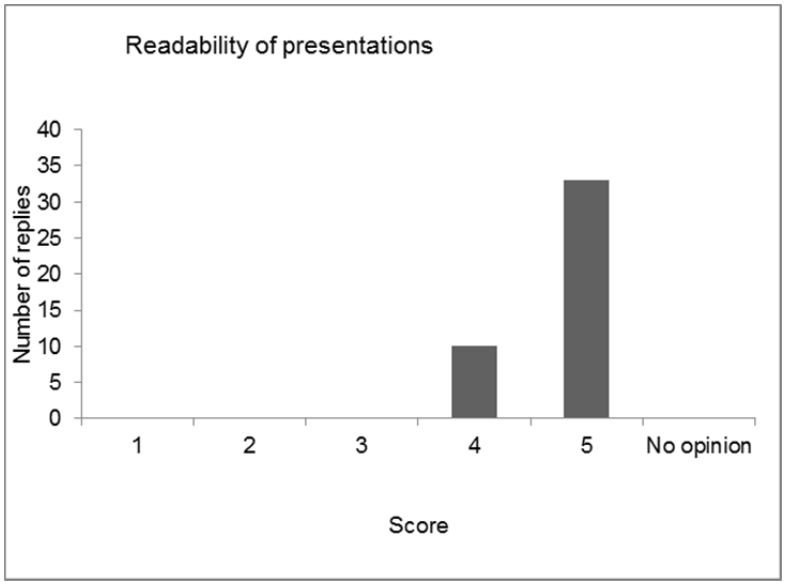

Figure 6 Scores given to question 6 'Opinion on the readability of the presentations on the screen'

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

No remarks were given in relation to the technical equipment, as this was in general considered as excellent (Figure 7).

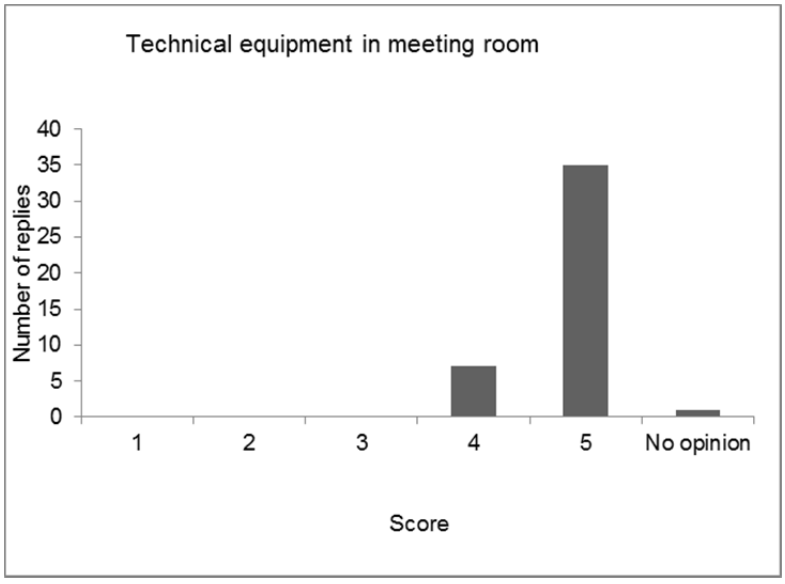

Figure 7 Scores given to question 7 'Opinion on the technical equipment'

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

The majority of the respondents considered the catering as good or excellent (scores 4-5), see Figure 8. Remarks given were:

- 'Excellent food, very high quality' (1x).
- 'Very quiet place, but it would be interesting to have the dinner at another place in Berlin' (1x).

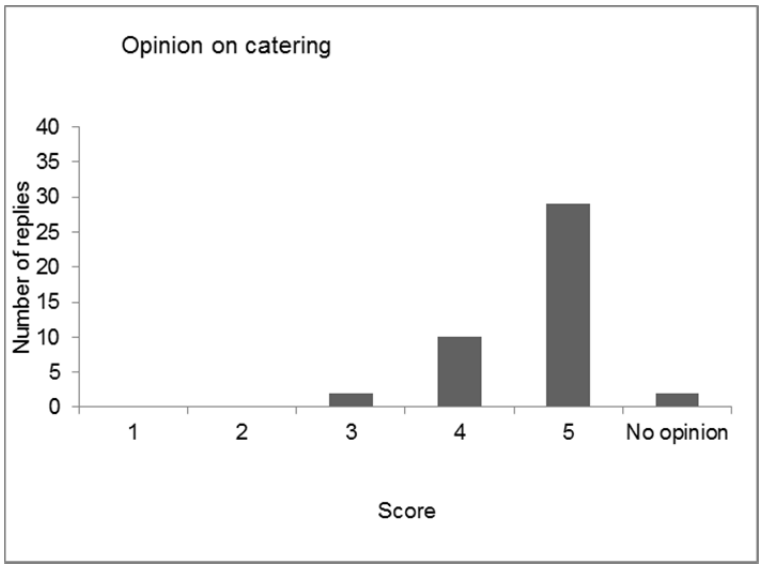

Figure 8 Scores given to question 8 'Opinion on the catering'

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 9). Remarks given were:

- 'More information about pre-tests done by the EURL is needed for organisation of ring trials by NRLs at national level'.
- 'The information is good, but I would like to see more new, different subjects to discuss. The scientific programme of the workshop is very repetitive'.

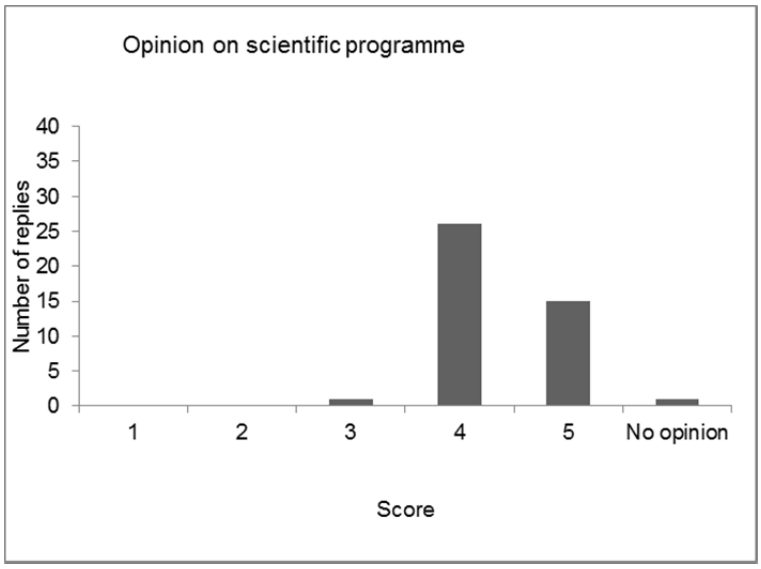

Figure 9 Scores given to question 9 'Opinion on the scientific programme'

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

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

- 'I would like to hear more about antimicrobial resistance'.
- 'Information on how to interpret Whole Genome Sequencing raw data'.
- 'We still seem to discuss about the same monophasic *S.* Typhimurium issues that have already been discussed two years ago'.
- 'Standardisation of a PCR method for identification monophasic *S.* Typhimurium was an important topic to discuss and it is needed to have an update on this issue so that the NRLs are guided on how to proceed with the identification of monophasic *S.* Typhimurium'.
- 'It could be useful to include some presentations about the activities that should be implemented by the NRLs to be able to upload good molecular data in the European Surveillance molecular database'.

11. What is your general opinion of the workshop?

The respondents indicated that the workshop as a whole had been good (score 4) or excellent (score 5), see Figure 10.

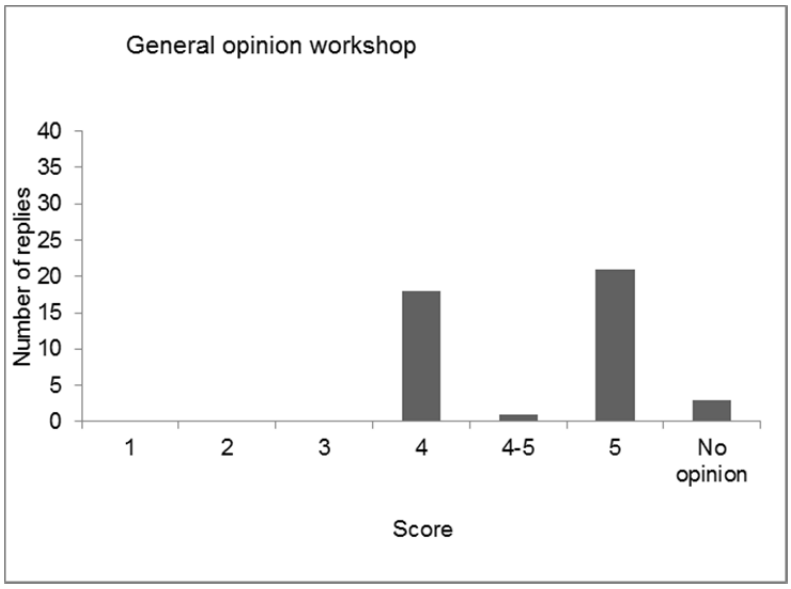

Figure 10 Scores given to question 11 'General opinion of the workshop'

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

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

- 'A city tour would have been nice'.
- 'I miss the free time programme you usually organised in the past'.
- 'I would like to thank you and the whole team, especially for the good atmosphere'.
- 'It was a nice conference room with good temperature and space'.
- 'Electronic copies of all presentations would have been helpful at the meeting'.
- 'Good job done by Istvan Szabo – great hospitality'.
- 'The NRL overviews given at the last morning is very informative'.
- 'I would like to get more detailed information about PFGE (technical details)'.
- 'It would be useful to include evaluation of PFGE data with BioNumerics during the interlaboratory comparison studies on typing of *Salmonella*, especially for the future EFSA database curation by the EURL-*Salmonella*'.
- 'Perhaps typing by using Maldi-Tof can be added to the next workshop?'
- 'It would be nice to get information on characterisation of *Salmonella* isolates by Next Generation Sequencing (NGS)'.
- 'Information on whether MLVA will replace phage typing'.
- 'I think it would be interesting to have a topic like how the *Salmonella* programme works in Member States'.
- 'One or two presentations of the head of the EURL-*Salmonella* should focus on questions and issues received during the year from individual NRLs, this could be beneficial for the participating members'.

- 'During the workshop I would like to be informed about more practical and relevant issues related to *Salmonella* control programmes and tests. Before the workshop, the NRLs could be asked what items they would like to discuss'.
- 'It could be very useful to include a presentation about procedures/requirements for organisation of Proficiency Tests and the analysis of the data'.

13. What is your preference for the workshop of 2016?

In 2016, the international *Salmonella* symposium I3S will be held in St. Malo, France. The participants were asked to indicate if they would prefer to organise (again) the EURL-*Salmonella* workshop in conjunction with this symposium or not. The majority indicated a preference for organising the workshop in conjunction with I3S in St. Malo (19 replies), 17 participants indicated that they had no preference, and seven preferred to organise the workshop separately from the I3S symposium, at another date and location (Figure 11). Remarks given were:

- 'Choose a location easy to reach'.
- 'If not St. Malo, perhaps Sweden can be chosen?'
- 'St. Malo is not an easy place to reach and the workshop is a different activity than I3S. It does not always have to be in conjunction with I3S'.

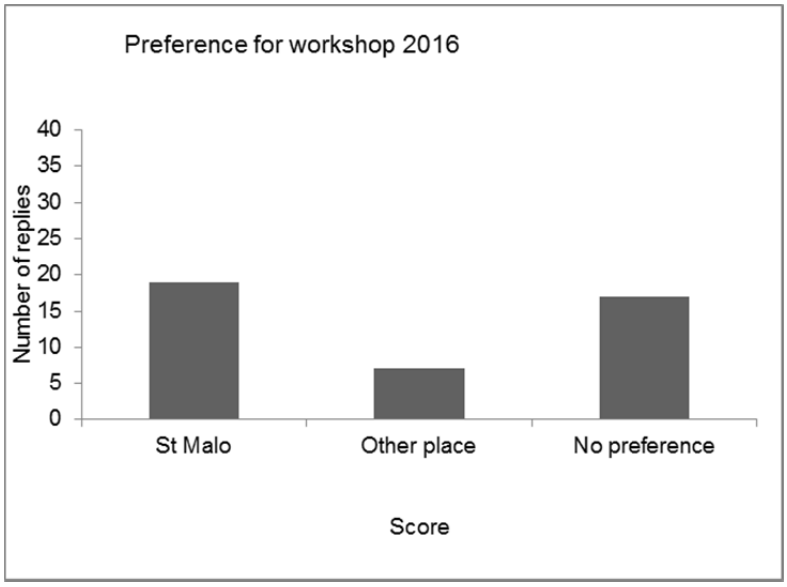

Figure 11 Scores given to question 13 'What is your preference for the workshop of 2016?'

4.3 Discussion and conclusions of the evaluation

In general, the participants were satisfied with the workshop and the scores were comparable to the workshop of 2014, with some small deviations depending on the subject.

Several participants made interesting suggestions for presentations for future workshops. These suggestions will be taken into consideration when organising the workshop of 2016 and later.

As the majority of the participants indicated having a preference for organisation of the workshop in conjunction with the I3S symposium in 2016, it will at first be explored if this is feasible again.

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The author would like to thank Elizabeth de Pinna of Public Health England (PHE), London, England for making excellent notes during the workshop, which have been very helpful when drafting the current report.

List of abbreviations

A	Answer
BGA	Brilliant Green Agar
BPW	Buffered Peptone Water
CEN	European Committee for Standardization
cfu	colony forming units
DG-Sante	Directorate-General for Health and Food Safety
DIS	Draft International Standard
DT	Definitive Type
EC	European Commission
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
EFTA	European Free Trade Association
EU	European Union
EURL	European Union Reference Laboratory
FBO	Food-borne Outbreak
FDIS	Final Draft International Standard
FYROM	Former Yugoslav Republic of Macedonia
ISO	International Organization for Standardization
MIC	Minimum Inhibitory Concentration
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
SC	Sub Committee
SSE(5)	*Salmonella* Senftenberg (at a level of approximately 5 cfu)
SOP	Standard Operating Procedure
SSI	Statens Serum Institute
STEC	Shiga toxin-producing *Escherichia coli*
STM(10)	*Salmonella* Typhimurium (at a level of approximately 10 cfu)
TAG	Technical Advisory Group
TC	Technical Committee
TR	Technical Report
TS	Technical Specification
USA	United States of America
WG	Working Group
WGS	Whole Genome Sequencing
WHO	World Health Organisation
XLD	Xylose Lysine Deoxycholate

Annex 1 Participants

European Food Safety Authority (EFSA)	Frank Boelaert
EC DG-Sante	Klaus Kostenzer
EURL – *Salmonella*	Kirsten Mooijman Angelina Kuijpers Wilma Jacobs
Guest speaker (France)	Renaud Lailler (ANSES, Paris)
Guest speaker (the Netherlands)	Anjo Verbruggen (RIVM, Bilthoven)
Guest speaker (United Kingdom)	Elizabeth de Pinna (PHE, London)

National Reference Laboratories for *Salmonella*

AUSTRIA	Heimo Lassnig
BELGIUM	Vicky Jasson Katelijne Dierick
BOSNIA HERZEGOVINA	Sead Hadziabdic
BULGARIA	Hristo Daskalov
CROATIA	Gordan Kompes
CYPRUS	Maria Emmanuel
CZECH REPUBLIC	Tomas Cerny
DENMARK	Gitte Sørensen
ESTONIA	Age Kärssin
FINLAND	Henry Kuronen
FRANCE	Annaëlle Kerouanton
FYROM	Dean Jankuloski
GERMANY	Istvan Szabo Burkhard Malorny Petra Hiller Andreas Schroeter
GREECE	Aphrodite Smpiraki
HUNGARY	Sara Kostyak
ICELAND	Franklin Georgsson
IRELAND	Rosemarie Slowey
ITALY	Lisa Barco
LATVIA	Madara Streikisa
LITHUANIA	Indre Zigariene
LUXEMBOURG	-
MALTA	-
NETHERLANDS	Irene Pol
NORTHERN IRELAND	Gintare Bagdonaite
NORWAY	Bjarne Bergsjø
POLAND	Magdalena Skarzynska Elzbieta Kukier
PORTUGAL	Patricia Themudo
ROMANIA	Luminita Monica Vanghele

SERBIA	Jasna Kureljusic
SLOVAK REPUBLIC	Lubos Mikula
SLOVENIA	Jasna Micunovic
SPAIN	Maria Christina de Frutos Escobar
SWEDEN	Lennart Melin
SWITZERLAND	Gudrun Overesch
TURKEY	Derya Karatas Yeni
UNITED KINGDOM	Doris Mueller-Doblies
	Jim Mc Lauchlin

Annex 2 Programme of the workshop

Programme of the 20th EURL-*Salmonella* workshop
28 and 29 May 2015, Berlin, Germany

General information

Place of accommodation and Meeting venue:
Seminaris Campushotel Berlin
Takustraße 39
14195 Berlin
Germany
Tel: +49 (0)30 55 77 97-0
http://www.seminaris.de/en/hotels/seminaris-campushotel-berlin/hotel.html

Information for the ones giving a presentation:

Presentations: To be able to make hand-outs for all participants, please send your (Power Point) presentation to Kirsten Mooijman (kirsten.mooijman@rivm.nl) before 21 May 2015. Alternatively, bring your own hand-outs.

Abstract: For the preparation of the report of the workshop it is necessary to also 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 28 May 2015

Wednesday 27 May 2015

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

Thursday 28 May 2015

08:30 - 09:00Registration

Morning chair: Wilma Jacobs

09:00 - 09:30	Opening and introduction	Kirsten Mooijman, EURL-*Salmonella*
09:30 - 10:00	EU *Salmonella* monitoring data, food-borne outbreaks and antimicrobial resistance	Frank Boelaert, EFSA
10:00 - 10:30	Update of the European Commission	Klaus Kostenzer, DG-Sante
10:30 - 11:00	*Coffee/tea*	
11:00 - 11:30	Results interlaboratory comparison study on detection of *Salmonella* in animal feed III (2014)	Angelina Kuijpers, EURL-*Salmonella*
11:30 – 12:00	Preliminary results interlaboratory comparison study on detection of *Salmonella* in pig faeces – PPS XVIII (2015)	Irene Pol, EURL-*Salmonella*
12:00 – 13:15	*Lunch*	

Afternoon chair: Kirsten Mooijman

13:15 – 13:45	Update on EFSA's Molecular typing project	Frank Boelaert, EFSA
13:45 - 14:15	Comparison of a rapid molecular serotyping method (Check and Trace Test) to conventional serotyping	Doris Mueller-Doblies, UK
14:15 - 14:45	Input of sequencing data for Foodborne Outbreak investigations: the recent French experience	Renaud Lailler, France
14:45 - 15:15	A large outbreak of *Salmonella* Thompson related to smoked salmon in the Netherlands, 2012	Anjo Verbruggen, the Netherlands
15:15 – 15:45	*Coffee/tea*	

15:45 - 16:15	International *Salmonella* Newport outbreak in 2011-2012	Petra Hiller, Germany
16:15 - 16:45	Standardisation of a method for PCR identification of monophasic *Salmonella* Typhimurium: a status report	Burkhard Malorny, Germany
16:45 – 17:15	Update on activities in ISO and CEN	Kirsten Mooijman, EURL-*Salmonella*

19:00 - Dinner at hotel

Friday 29 May 2015

Morning chair: Kirsten Mooijman

09:00 - 10:15	Activities NRLs to fulfil tasks and duties, 15-20 min each North-Ireland (Gintare Bagdonaite) Portugal (Patricia Themudo) Spain (Cristina de Frutos Escobar) Slovak Republic (Lubos Mikula)	
10:15 - 10:45	*Coffee/tea*	
10:45 - 11:30	Results 19th interlaboratory comparison study on typing of *Salmonella* (2014) – serotyping and PFGE	Wilma Jacobs, EURL-*Salmonella*
11:30 – 12:00	Results 19th interlaboratory comparison study on typing of *Salmonella* (2014) - phagetyping	Elizabeth de Pinna, PHE, UK
12:00 – 12:30	Work programme EURL-*Salmonella* second half 2015, first half 2016, discussion on general items and closure	Kirsten Mooijman, EURL-*Salmonella*

12:30 – 13:30 Lunch

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

Annex 3 Workshop evaluation form

Evaluation of the 20th EURL-*Salmonella* workshop
28 and 29 May 2015, Berlin, Germany

We would highly appreciate if you could give us your opinion on the 20th EURL-*Salmonella* workshop, organised in Berlin, Germany on 28 and 29 May 2015. 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 5 is the highest score (excellent) and 1 is the lowest score (very poor).

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*?

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 (breakfast, coffee, tea, lunches, dinners)?

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?

13. What is your preference for the workshop of 2016?

	Organise in conjunction with I3S symposium (6-8 June 2016) in St. Malo
	Organise separate from I3S symposium at other dates and location
	No preference

Remarks:

Thank you very much!

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