



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

The 19th EURL-Salmonella workshop

26 and 27 May 2014, Zaandam,
the Netherlands

RIVM report 2014-0147

K.A. Mooijman



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

The 19th EURL-Salmonella workshop
26 and 27 May 2014, Zaandam, the Netherlands

RIVM Report 2014-0147

Colophon

© RIVM 2014

Parts of this publication may be reproduced, provided acknowledgement is given to: National Institute for Public Health and the Environment, along with the title and year of publication.

K.A. Mooijman

Contact:

Kirsten Mooijman

Centre for Zoonoses and Environmental Microbiology (Z&O)

kirsten.mooijman@rivm.nl

This investigation has been performed by order and for the account of the European Commission, Directorate-General for Health and Consumer Protection (DG-Sanco), within the framework of RIVM project E/114506/14/WO European Union Reference Laboratory for *Salmonella* (2014).

This is a publication of:

**National Institute for Public Health
and the Environment**

P.O. Box 1 | 3720 BA Bilthoven

The Netherlands

www.rivm.nl/en

Publiekssamenvatting

De negentiende EURL-*Salmonella* workshop

26 en 27 mei 2014, Zaandam, Nederland

Het RIVM heeft de verslagen gebundeld van de presentaties van de negentiende jaarlijkse workshop voor de Europese Nationale Referentie Laboratoria (NRL's) voor de bacterie *Salmonella* (26 en 27 mei 2014). 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.

***Campylobacter* en *Salmonella* blijven belangrijkste veroorzakers zoönosen**

Een terugkerend onderwerp is het jaarlijkse rapport van de European Food Safety Authority (EFSA) over zoönosen, oftewel ziekten die van dieren op mensen kunnen overgaan. Het verslag daarover bevat een overzicht van de aantallen en types zoönotische micro-organismen die in 2012 gezondheidsproblemen veroorzaakten in Europa. Hieruit blijkt dat *Salmonella* al een aantal jaren minder gezondheidsproblemen veroorzaakt, maar nog steeds, ná de *Campylobacter*-bacterie, de belangrijkste veroorzaker is van zoönotische ziekten in Europa.

Moleculaire typering steeds belangrijker

Andere verslagen geven informatie over nieuwe technieken die aantonen welk type *Salmonella* zich in voedsel of dieren bevindt. Diverse laboratoria maken hiervoor gebruik van moleculaire technieken, die erop zijn gebaseerd het DNA van de ziekmakende bacterie aan te tonen. Iedere bacteriestam heeft namelijk een eigen unieke moleculaire typering. Die informatie kan belangrijk zijn om na te gaan of een type *Salmonella* dat bij de mens wordt gevonden dezelfde is als in voedsel of bij dieren.

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.

Trefwoorden:

EURL-*Salmonella*, NRL-*Salmonella*, *Salmonella*, workshop 2014

Abstract

The 19th EURL-*Salmonella* workshop

26 and 27 May 2014, Zaandam, the Netherlands

The RIVM has summarised the presentations of the 19th annual workshop for the European National Reference Laboratories (NRLs) for *Salmonella* (26 and 27 May 2014). 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 yearly 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 NRLs. Detailed information on the results per ring trial is provided in separate RIVM reports.

***Campylobacter* and *Salmonella* still the most important zoonoses**

Another yearly item is the presentation of the most recent European summary report on zoonoses by the European Food Safety Authority (EFSA). The 2014 report gives an overview of the number and types of zoonotic micro-organisms that were causing health problems in Europe in 2012. For several years, the number of health problems caused by *Salmonella* has been decreasing, but in 2012 it was still the second most significant cause, after *Campylobacter*, of zoonotic diseases in Europe.

Molecular typing of increasing importance

Other presentations give information on new techniques to show the type of *Salmonella* present in food or animals. Several laboratories are now using molecular techniques, based on detection of DNA of the micro-organism. Each strain has a unique molecular typing pattern. This information is important to show whether a *Salmonella* type found in humans is similar to one found in food or animals.

The workshop is organised by the EURL-*Salmonella*. This EURL is located at 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 2014

Contents

1 Introduction – 11

2 Monday 26 May 2014: Day 1 of the workshop – 13

- 2.1 Opening and introduction – 13
- 2.2 EU *Salmonella* monitoring data (Summary report 2012) – 13
- 2.3 Looking at enhanced crisis preparedness and early detection of outbreaks in the EU – 15
- 2.4 Results of the interlaboratory comparison study on detection of *Salmonella* in food VI (2013) – 16
- 2.5 Activities in ISO and CEN – 18
- 2.6 Preliminary results of the interlaboratory comparison study on detection of *Salmonella* in chicken faeces – PPS XVII (2014) – 20
- 2.7 Activities of EFSA concerning molecular typing data collection from food and animals – 21
- 2.8 Molecular serotyping of *Salmonella* – Experiences of reference laboratory the Netherlands – 22
- 2.9 Molecular serotyping of *Salmonella* – Experiences NRL-*Salmonella* in Denmark using the CDC method – 23
- 2.10 Rapid detection and specific differentiation of *Salmonella* Enteritidis, *Salmonella* Typhimurium and its monophasic variant by multiplex real-time PCR – 24
- 2.11 Overview of the recent activities carried out by the Italian NRL related to *S.* 4,[5],12:i: – 24
- 2.12 *Salmonella* Typhimurium monophasic variant surveillance in France: molecular characterisation and guidelines from ANSES experts – 25

3 Tuesday 27 May 2014: Day 2 of the workshop – 29

- 3.1 Activities of the NRL-*Salmonella* to fulfil tasks and duties in Austria – 29
- 3.2 Activities of the NRL-*Salmonella* to fulfil tasks and duties in Bosnia and Herzegovina – 30
- 3.3 Activities of the NRL-*Salmonella* to fulfil tasks and duties in the United Kingdom – 31
- 3.4 Activities of the NRL-*Salmonella* to fulfil tasks and duties in Poland – 32
- 3.5 Activities of the NRL-*Salmonella* to fulfil tasks and duties in Germany – 33
- 3.6 Results of the interlaboratory comparison study on typing of *Salmonella* XVIII (2013) – serotyping and PFGE – 34
- 3.7 Results of the interlaboratory comparison study on typing of *Salmonella* XVIII (2013) – phage typing – 35
- 3.8 Work programme EURL-*Salmonella* (second half 2014, first half 2015), discussion on general items and closure – 36

4 Evaluation of the workshop – 39

- 4.1 Introduction – 39
- 4.3 Discussion and conclusions of the evaluation – 45

References – 47

Acknowledgements – 51

List of abbreviations – 53

Annex 1 Participants – 55

Annex 2 Programme of the workshop – 57

Annex 3 Evaluation form of the workshop – 61

Summary

On 26 and 27 May 2014, the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*) organised its annual workshop in Zaandam, the Netherlands. Participants of the workshop were representatives of the NRLs for *Salmonella* of 27 EU Member States, 2 European Free Trade Association (EFTA) countries and 3 EU candidate countries. Furthermore, representatives of the European Commission Directorate General for Health and Consumer Protection (EC DG-Sanco) and of the European Food Safety Authority (EFSA) were present. Excuses were received, due to illness of representatives of NRLs from two EU Member States (France and Luxembourg), one EFTA country (Switzerland) and one candidate country (Former Yugoslav Republic of Macedonia – FYROM). A total of 50 participants were present at the workshop.

During the workshop, presentations were given on several topics. The results of the interlaboratory comparison studies organised by the EURL-*Salmonella* in the past year were presented. These included studies on the detection of *Salmonella* in minced chicken meat (September 2013) and in samples from the primary production stage (March 2014) and the study on typing of *Salmonella* (November 2013).

A representative of EFSA gave a presentation on the most recent European summary report on zoonoses as published by EFSA and the European Centre for Disease Prevention and Control in February 2014. This latter report gives an overview on the number and types of zoonotic micro-organisms that were causing health problems in Europe in 2012. For several years, the number of health problems caused by *Salmonella* has been decreasing, but in 2012 it was still the second most important cause, after *Campylobacter*, of zoonotic diseases in Europe.

A representative of EC DG-Sanco gave an update on policy issues.

In six other presentations, information was given on the molecular typing of *Salmonella* and on the activities of EFSA concerning molecular typing data collection from food and animals.

Representatives of five NRLs for *Salmonella* gave presentations on their activities to fulfil the tasks and duties of being an NRL.

A staff member of the EURL-*Salmonella* gave a summary concerning standardisation of methods in ISO and CEN, related to the activities of the NRLs for *Salmonella*.

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

All the presentations given at the workshop can be found at:

http://www.eurlsalmonella.eu/Workshops/Workshop_2014

1 Introduction

In this report, the abstracts of the presentations given at the EURL-*Salmonella* workshop of 2014 are presented, as well as a summary of the discussions that followed the presentations. The full presentations are not provided within this report, but are available at the website of the EURL-*Salmonella*: http://www.eurlsalmonella.eu/Workshops/Workshop_2014

The layout of the report follows the programme of the workshop.
The abstracts of the presentations of the first day are given in chapter 2.
The abstracts of the presentations of the second day are given in chapter 3.
The participants' evaluation of the workshop is summarised in chapter 4 and the (blank) 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 Monday 26 May 2014: 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 19th workshop of the EURL-*Salmonella*, welcoming all participants to Zaandam, the Netherlands. There were 50 participants in this workshop, including representatives of the National Reference Laboratories (NRLs) for *Salmonella* of the EU Member States, candidate EU countries and member countries of the European Free Trade Association (EFTA). Furthermore, also representatives of the EC Directorate General for Health and Consumer Protection (EC DG-Sanco) and the European Food Safety Authority (EFSA) were present. Excuses were received of representatives of four NRLs, due to medical problems: those of the Former Yugoslav Republic of Macedonia (FYROM), France, Luxembourg and Switzerland. After a roll call of the delegates, the results of the evaluations of the last three workshops (2011, 2012 and 2013) were compared, showing variable results. The opinion on the scientific programme, however, was the same in all workshops: good to excellent.

Next, the participants were informed of a publication (by the ECDC) on the outbreak caused by *Salmonella* Stanley in the EU (2011–2013), to which several NRLs had contributed by providing PFGE profiles: P. Kinross, L. van Alphen, J. Martinez Urtaza, M. Struelens, J. Takkinen, D. Coulombier, P. Mäkelä, S. Bertrand, W. Mattheus, D. Schmid, E. Kanitz, V. Rucker, K. Krisztalovics, J. Pászti, Z. Szögyényi, Z. Lancz, W. Rabsch, B. Pfefferkorn, P. Hiller, K. Mooijman and C. Gossner. 'Multidisciplinary investigation of a multicountry outbreak of *Salmonella* Stanley infections associated with turkey meat in the European Union, August 2011 to January 2013'. *Eurosurveillance*, Volume 19, Issue 19, 15 May 2014:

<http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20801>

The workshop started after explaining the programme and after giving some general information concerning the workshop.

The programme of the workshop is presented in Annex 2.

2.2 EU *Salmonella* monitoring data (Summary report 2012)

Frank Boelaert, EFSA, Parma, Italy

Salmonellosis in humans continued to decrease in 2012 (EFSA and ECDC, 2014). Significant decreasing five-year trends were observed in 15 Member States (MSs) and two non-MSs as well as in the EU as a whole, representing a decrease of 43546 cases (32%) in 2012 when compared with the case numbers reported in 2008. Salmonellosis is nonetheless the second most common zoonosis in humans in the EU, with 1531 food-borne outbreaks reported in 2012 involving 12000 affected persons. The EU case-fatality rate was 0.14% and 61 deaths due to non-typhoidal salmonellosis were reported in the EU in 2012.

The salmonellosis notification rates for human cases of infection vary between the Member States, reflecting differences in, for example, disease prevalence in the domestic animal population, the proportion of travel-associated cases and the quality and coverage of the surveillance system. One example of the latter is that countries reporting the lowest notification rate for salmonellosis had the

highest proportion of hospitalisation, which may indicate that the surveillance systems in these countries are focusing on the diagnosis of the most severe cases.

No trend analysis for separate *Salmonella* serovars was included in the 2012 EU Summary report but the trends observed in 2007–2011 continued in 2012. The number of reported human cases of *Salmonella* Enteritidis decreased, but the number of cases of monophasic *Salmonella* Typhimurium 1,4,[5],12:i:- increased (possibly a reporting bias), as did the number of cases of *Salmonella* Infantis. A multi-country outbreak of *Salmonella* Stanley, affecting several MSs and linked to the turkey production chain, resulted in this serovar becoming the fifth most commonly reported in 2012. Large outbreaks in individual countries were also reflected in the top ten serovar list, e.g. *Salmonella* Thompson in the Netherlands.

The continuing decrease in the numbers of salmonellosis cases in humans is likely to be mainly related to the successful *Salmonella* control programmes in fowl (*Gallus gallus*) populations that are in place in EU MSs and that have resulted, in particular, in a lower occurrence of *Salmonella* in eggs, though other control measures might also have contributed to the reduction. The majority of MSs met their *Salmonella* reduction targets for breeding flocks, laying hens and broilers of *Gallus gallus* and for turkey flocks in 2012. The EU-level prevalence of the target serovars, including *S. Enteritidis*, was further reduced in breeding flocks and laying hens of *Gallus gallus* and for turkey fattening flocks to 0.4%, 1.3% and 0.4%, respectively. In broiler flocks the EU-level prevalence remained at 0.3%, whereas in turkey breeding flocks the overall prevalence of the two target serovars in the two MSs with *Salmonella*-positive flocks was 0.5%. All these results indicate that MSs have continued to invest in *Salmonella* control and that this work is yielding further positive results. It is noteworthy that, compared to 2011, the 2012 EU level prevalence of flocks positive with *Salmonella* spp. decreased in laying hens, remained almost the same in breeding hens and broilers, but increased in breeding turkeys and fattening turkeys. In this context, the multi-country *S. Stanley* outbreak, which was highly likely due to contamination of the turkey production chain, serves as a reminder of the importance of acting upon any *Salmonella* serovar contamination in the food chain and monitoring to detect the emergence of new serovars or strains (ECDC and EFSA, 2012).

Reports on food-borne outbreaks caused by *Salmonella* within the EU have shown a reduction of 19% from 2008 to 2012, but slightly increased since 2011. Important sources of food-borne *Salmonella* outbreaks in 2012 were eggs and egg products, cheese, and mixed foods.

As in 2011, monophasic *S. Typhimurium* was in third place in the top 10 list of the most commonly reported serovars in human cases in 2012. The BIOHAZ Panel concluded in its opinion (EFSA, 2010) that monophasic *S. Typhimurium* appears to be of increasing importance in many MSs and has caused a substantial number of infections in both humans and animals bred for food. However, the reporting guidelines agreed in 2010, which aimed at more accurate identification of these strains, may have partly contributed to the increase in reports in 2011 and 2012 in some MSs.

Discussion

Q: In the last technical specification in relation to antimicrobial resistance testing, Streptomycin was removed for testing against *Salmonella*. However, this antimicrobial agent is important for monitoring trends, especially for monophasic *Salmonella* Typhimurium. What is the reason for removing this antimicrobial from the test list?

A: This has been a decision taken by experts, but the information does not seem to be very clear in the EFSA opinion. EFSA will have a closer look at this item.

Q: For the targets in breeding flocks of *Gallus gallus*, monitoring for *S. Virchow* and *S. Hadar* is still prescribed. However, these two serovars are no longer in the list of the ten most important health-related human serovars. Should these serovars still be monitored?

A: The monitoring was based on a microbiological risk assessment performed in 2009 (EFSA, 2009). The 'heavy measures' (eradication of flocks) are only needed in case *S. Enteritidis* or *S. Typhimurium* is present. The other three indicated *Salmonella* serovars (*S. Hadar*, *S. Virchow* and *S. Infantis*) are merely used for monitoring. The prevalence of *Salmonella* serovars varies per EU Member State. A serovar can be endemic in one MS, but absent in another MS. Therefore, the monitoring of specific serovars should be discussed at national level. This item will be taken forward to the next DG-Sanco working group on zoonoses.

Note: In the EFSA Opinion of 2009 the following recommendation is given on this subject: 'Further control policies for other Salmonella serovars in breeding hens should be guided by the level of their dissemination into production stock in individual EU MS, and may be considered in national control programs.'

2.3 Looking at enhanced crisis preparedness and early detection of outbreaks in the EU

Klaus Kostenzer, EC DG-Sanco, Brussels, Belgium

EC DG-Sanco policies may be involved in both sudden (e.g. bio-terrorism) and predictable (disease outbreaks) disruptions of public health in the Union. Therefore SANCO has a well-developed structure to address crisis management, but gives an even greater focus on preparedness and early detection of possible hazards. Rapid alert systems (e.g. the RASFF for food safety, the EWRS for public health and the ADNS for animal health) are used in a well-established routine for quickly sharing vital information on emerging problems. Since 2013, the management of serious cross border threats caused by communicable diseases is covered by EU Decision No 1082/2013/EU (EC, 2013) in terms of public health measures to be set.

DG SANCO arrangements are part of a broader structure across the Commission. It may be assisted by the European Centre for Disease Prevention and Control (ECDC), the European Food Safety Authority (EFSA), the European Medicines Agency (EMA) and a dense network of EU reference laboratories for the most relevant food-borne pathogens. The RASFF system plays a key role in ensuring a high level of food safety for EU citizens, and that information remains reliable, transparent, sound, robust and verified. It links directly with the WHO INFOSAN secretariat in case of major international food safety incidents. The development of a sound base of expertise in outbreak detection and management and other areas is supported by DG SANCO's 'Better training for safer food (BTSF)' programme.

This highly integrated approach ensures that e.g. in the management of disease incidents such as the *E. coli* outbreak in 2011 the necessary structures for emergency preparedness are at hand. Taking into account the lessons learned from previous challenges, the Commission will continuously improve the systems that are in place. This evolution goes hand in hand with a good performance of the Member States in addressing their mutual responsibilities and the collaboration of all the different services and stakeholders involved. Member States do have contingency plans in place to ensure crisis preparedness and to

allow a quick response to outbreaks of transmissible diseases that may or may not also pose a risk to human health in view of 'One Health'.

Discussion

Q: In several EU Regulations, the O:1 in the antigenic formula of monophasic *Salmonella* Typhimurium is not underlined (1,4,[5],12:i:-). In Belgium this has been interpreted as meaning that no measures are needed in case O:1 is not found (which happens often). Can the Regulation be corrected?

A: This was an editorial error. The legislation is intended for all variants of monophasic *Salmonella* Typhimurium (irrespective of the presence or absence of O:1). This was clarified in the working group on microbiological criteria some time ago. Scientifically it is clear that no distinction should be made between the variants with or without O:1. Thus, all variants of monophasic *Salmonella* Typhimurium, regardless of the presence of the O:1 antigen (so both 1,4,[5],12:i:- and 4,[5],12:i:-) are to be included in measures taken under Regulation (EC) No. 2160/2003 (EC, 2003b) and Regulation (EC) No. 2073/2005 (EC, 2005). The classification of these specific variants and the laboratory methods to be used for this purpose should follow the Scientific Opinion issued by EFSA in 2010 or more recent specific scientific publications as recommended by the EURL-*Salmonella*.

Q: Next Generation Sequencing (NGS)/Whole Genome Sequencing (WGS) may become more widely used for typing monophasic *Salmonella* Typhimurium. Will these data be included in the molecular database of the ECDC and/or of EFSA?

A: Indeed, NGS/WGS is being used more and more, but it is not yet a routine method. For the databases, it has been decided to start at first with data from a limited number of molecular methods and from methods that are used 'routinely' by a large number of laboratories. Data from NGS/WGS may be included at a later stage.

2.4 Results of the interlaboratory comparison study on detection of *Salmonella* in food VI (2013)

Angelina Kuijpers, EURL-Salmonella, Bilthoven, the Netherlands

In September 2013, the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*) organised the sixth interlaboratory comparison study on detection of *Salmonella* in a food matrix: minced chicken meat. Thirty-five National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) participated: 30 NRLs from 28 EU Member States (MS) and 5 NRLs from third countries: candidate EU-MSs or potential EU candidate MSs and member countries of the European Free Trade Association (EFTA).

The primary objective of the study was to test the performance of the participating laboratories for the detection of *Salmonella* at different contamination levels in a food matrix. To do so, the participants analysed 25 g samples of minced chicken meat artificially contaminated with *Salmonella* Infantis (SI) at various contamination levels. The performance of the laboratories was compared to criteria of good performance. In addition, a comparison was made between the prescribed method (ISO 6579: Anonymous, 2002) and the requested method (Annex D of ISO 6579: Anonymous, 2007). For the prescribed method, the selective enrichment media were Rappaport Vassiliadis Soya broth (RVS) and Mueller Kauffmann Tetrathionate novobiocin broth (MKTTn). For the requested method, the selective enrichment was Modified Semi-solid Rappaport Vassiliadis (MSRV) agar.

The samples consisted of minced chicken meat artificially contaminated with a diluted culture of *Salmonella* Infantis (SI) at low level (approximately 10 cfu/25 g meat) and at high level (approximately 100 cfu/25 g meat) and with no *Salmonella* at all (blank samples). The samples were artificially contaminated at the laboratory of the EURL, which was a new procedure for a food study. Before the start of the study, several tests 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, influence of amount of background flora).

Eighteen individually numbered blind samples with minced chicken meat had to be tested by the participants for the presence or absence of *Salmonella*. These samples consisted of six blank samples, six samples with low level *S. Infantis* (inoculum 11 cfu/sample, 5 MPN/sample) and six samples with high level *S. Infantis* (inoculum 104 cfu/sample, 55 MPN/sample). Additionally, three control samples had to be tested: two blank control samples (procedure control (BPW) and matrix control (minced chicken meat)) and one own (NRL) positive control (with *Salmonella*).

For the number of samples and the contamination levels of the samples, the information as given in CEN ISO/TS 22117 (Anonymous, 2010) was followed.

The laboratories found *Salmonella* in 61–78% of the (contaminated) samples depending on the selective enrichment medium used. The accuracy rates for the prescribed selective enrichment media for detection of *Salmonella* in food, MKTTn and RVS, were 73% and 83%, respectively. For the requested method (MSRV) the accuracy rate was 85%.

The number of competitive disturbing bacteria in the minced meat was high in this study and disturbed the detection of *Salmonella* in the low level contaminated minced chicken meat samples. Due to this fact, it was decided to slightly adjust the criteria of good performance for the low level contaminated samples.

A comparison between the different media was made. There was a significant higher change to find *Salmonella* after selective enrichment in RVS or on MSRV for SI contaminated minced chicken meat samples compared to selective enrichment in MKTTn. Longer incubation (two times 24 hours) of MSRV gave 10% more positive results.

For the positive control, the majority of the participants (21 laboratories) used a diluted culture of *Salmonella*. The *Salmonella* serovars used for the positive control sample were *S. Enteritidis* (17) and *S. Typhimurium* (8).

A PCR (real time) method was used by three participants as an own method in addition to the prescribed method. Two participants found the same results with the PCR method as with the bacteriological culture method.

Thirty-two out of 35 laboratories achieved the level of good performance. Two NRLs reported a positive result for a blank sample. However, in both cases this was due to a transcription error and the results of these NRLs were indicated as moderate. One participant (non-EU MS) had difficulties with the detection of *Salmonella* in all samples and also found a false positive blank result for the procedure control sample. For this NRL a follow-up study was organised in January 2014. The laboratory improved its performance significantly but still did not reach the desired level of good performance. The EC DG-Sanco was informed accordingly.

The samples in this food study, minced chicken meat artificially contaminated with a diluted culture of *Salmonella* Infantis (SI), mimicked 'real life' routine samples more closely and were easier to use than previously used mixtures of matrix and reference materials.

The use of a web-based template for reporting the results of the study by the participants was successful. The web based report was used for the first time in a food detection study.

More details of the study can be found in the interim summary report (Kuijpers and Mooijman, 2013).

2.5 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 for Microbial contaminants.

Both groups will hold their plenary meetings in Washington, DC, USA, from 23 to 27 June 2014. The progress on the *Salmonella* documents will be presented at these meetings by Kirsten Mooijman.

After an introduction on the different stages in the development of a standard (in ISO and in CEN), information was given on the status of the documents for detection, enumeration and serotyping of *Salmonella* in samples of the food chain. For this, EN ISO 6579 (Anonymous, 2002) is separated into three parts: Part 1: Horizontal method for the detection of *Salmonella*. The DIS (Draft International Standard) voting on this part was launched on 5 June 2014 and will end on 5 November 2014.

Part 2: Enumeration by a miniaturised Most Probable Number technique. This part was published in November 2012.

Part 3: Guidelines for serotyping of *Salmonella* spp. For this part an enquiry for acceptance of the final corrections was held between 10 March and 28 April 2014. The outcome was 100% positive, meaning that the document will be published soon (*editorial note: the final document was published in July 2014*).

The main changes in prEN DIS 6579 part 1, compared to EN ISO 6579: 2002 were presented in detail. The document will also contain the performance characteristics of the method for detection of *Salmonella* in the different matrices (food, animal feed and samples from the primary production stage).

Additional information was given on other ISO/CEN standards under development that are of possible interest to the NRLs-*Salmonella*:

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).

The documents were under DIS voting from 28 November 2013 to 28 April 2014. For all four documents the outcome was 'acceptance with comments'. The comments will be incorporated in an update of each document, after which the documents will be launched for the final voting stage (FDIS).

CEN-TAG 9 (Technical Advisory Group) 'Pre-enrichment step':

The aim of this group is to improve the pre-enrichment step to enhance recovery of Gram-negative bacteria like *Salmonella*, *Cronobacter*, *Enterobacteriaceae* and STEC. Chemically defined pre-enrichment broths have been tested, but this resulted in poor growth of the tested strains. Therefore, priority is given to improve the current peptone based formulation of buffered peptone water (BPW) and to define performance characteristics for BPW, to ensure reproducibility with stressed cells.

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.

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

The FDIS voting on the document took place between 19 December 2013 and 19 February 2014. The document was approved with some editorial comments and the final document was recently published as EN ISO standard.

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

Microbiology of the food chain – Method validation -

- Part 1 'Vocabulary'. This part includes all definitions. The FDIS vote will probably be launched in July 2014.
- Part 2 'Protocol for the validation of alternative (proprietary) methods against a reference method'. The FDIS vote will be probably be launched in July 2014.
- Part 3 'Protocol for verification of reference methods and alternative methods in a single laboratory'. This document gives 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 methods'.

For parts 3-6 the voting for New Work Item Proposal (NWIP) has been launched on 10 March 2014 and will end on 11 June 2014.

EN ISO/TS 17728: 'Microbiology of food and animal feed – Sampling techniques for microbiological analysis of food and feed samples'

As this is a Technical Specification (TS), only one voting stage is needed, and this took place in 2013. The result of this voting stage in ISO was as follows: 22 approval and 3 disapproval. The result in CEN was: 19 approval and

3 disapproval. Due to the negative votes, the document was amended and a second vote is likely to take place during summer 2014.

Discussion

Q: If you want to pool samples before analysis, it is necessary to test whether this will give similar results to those obtained when testing individual samples. For this test do you always need to follow the procedure described in the new version of prEN ISO/DIS 6887-1 (Anonymous, 2013), or is it permitted to refer to publications as well?

A: If the pooling of samples in your laboratory is based on studies that have been published, this information can be used. However, if no (published) data are available, a laboratory should perform an own validation study to show that pooling does not affect the results. For this, the experimental design described in prEN ISO/DIS 6887-1 can be followed.

Q: Is it possible to store cultured BPW and cultured selective enrichment media?

A: Yes, in several experiments (also published) it is shown that *Salmonella* can still be isolated from pre-enriched cultures and selective enriched cultures when stored at 5 °C for at maximum of 72 h.

2.6 Preliminary results of the interlaboratory comparison study on detection of *Salmonella* in chicken faeces – PPS XVII (2014)

Angelina Kuijpers, EURL-Salmonella, Bilthoven, the Netherlands

In March 2014, the EURL-*Salmonella* organised the 17th interlaboratory comparison study on detection of *Salmonella* in samples from the primary production stage (PPS). The matrix of concern was chicken faeces. The participants were 36 National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*): 29 NRLs from 28 EU Member States (MS) and 7 NRLs from third countries: candidate EU MSs or potential EU candidate MSs, member countries of the European Free Trade Association (EFTA) and, at the request of EC DG-Sanco, a country outside Europe.

The primary 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 the primary production stage. To do so, chicken faeces artificially contaminated with *Salmonella* Typhimurium (STM) at various contamination levels were analysed. The performance of the laboratories was compared to the criteria for good performance. The prescribed method was Annex D of ISO 6579 (Anonymous, 2007), with selective enrichment on Modified Semi-solid Rappaport Vassiliadis (MSRV) agar.

The samples consisted of chicken faeces artificially contaminated with a diluted culture of *Salmonella* Typhimurium (STM) at low level (approximately 10–15 cfu/25 g chicken faeces), at high level (approximately 50–100 cfu/25g chicken faeces) and with no *Salmonella* at all (blank samples). The samples were artificially contaminated at the laboratory of the EURL. 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. stability at different storage temperatures, influence of amount of background flora).

Eighteen individually numbered blind samples with chicken faeces had to be tested by the participants for the presence or absence of *Salmonella*. These samples consisted of six blank samples, six samples with low level STM

(inoculum 14 cfu/sample, level during the study 3 MPN/sample) and six samples with high level STM (inoculum 67 cfu/sample, level during the study 35 MPN/sample). Additionally, three control samples had to be tested: two blank control samples (procedure control (BPW) and matrix control (chicken faeces)) and one own (NRL) positive control (with *Salmonella*). The number of samples and the contamination levels of the samples were in accordance with CEN/ISO /TS 22117 (Anonymous, 2010).

On average, the laboratories found *Salmonella* in 99% of the (contaminated) samples with the prescribed method, selective enrichment on MSR.V. All high-level contaminated samples and blank samples were scored correctly. Five participants did not detect *Salmonella* in one of the six low-level contaminated samples. Thirty-one of the 36 participants (86%) tested all chicken faeces samples contaminated with *S. Typhimurium* positive. An additional 24 h incubation of MSR.V gave only 0.05% more positive results compared to 24 hours of incubation. Plating out on XLD gave 3–4% more positive results after selective enrichment on MSR.V compared to other isolation media (mostly BGA or Rambach).

For the positive control, the majority of participants (24 laboratories) used a diluted culture of *Salmonella*. The *Salmonella* serovars used for the positive control sample were *S. Enteritidis* (15) and *S. Typhimurium* (9).

A PCR method (mostly a real time PCR) was used by five participants as an own method in addition to the prescribed method.

Thirty-four of the 36 participating NRLs fulfilled the criteria of good performance. Two participants scored below the criteria of good performance, as they could not detect *Salmonella* in their own positive control sample. However, in both cases this failure was due to a misunderstanding in the way of reporting the positive control results, and ultimately their performance was marked as moderate.

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

2.7 Activities of EFSA concerning molecular typing data collection from food and animals

Frank Boelaert, EFSA, Parma, Italy

Molecular typing or microbial DNA fingerprinting has developed rapidly in recent years. Many typing methods, like PCR techniques and sequencing, have become part of routine strain characterisation in many laboratories. Molecular typing provides essential tools for different surveillance purposes, such as monitoring spread of clones and strains, early detection of dispersed (international) outbreaks, and prediction of epidemic potential.

Data on the molecular testing of food-borne pathogens such as *Salmonella*, *Listeria monocytogenes* and Shiga toxin-producing *Escherichia coli* (STEC) could substantially contribute to the epidemiological investigations of food-borne outbreaks and to the identification of emerging health threats. The molecular testing data could also be very useful in source attribution studies when estimating the contributions of different food categories or animal species as sources of human infections.

Molecular testing of food-borne pathogens from food, animal feed and animal samples is mainly carried out by official laboratories designated in accordance with the provisions of Regulation (EC) No. 882/2004 (EC, 2004a). The Regulation also lays down the designation of National Reference Laboratories (NRLs), which should coordinate the activities of the official laboratories, and the designation of the EU Reference Laboratories (EURLs), coordinating the application of analytical methods by NRLs. An EURL has been nominated for each important food-borne pathogen. Several EURLs have already consolidated the typing capacity of the NRL network by regularly organising typing training sessions and Proficiency Test trials.

The added value of the molecular typing approach to the surveillance of food-borne pathogens was strongly supported by the European Commission, which in 2012 asked EFSA for technical support regarding the collection of data on the molecular typing of food, animal feed and animal isolates of food-borne pathogens, and made a similar request to ECDC regarding molecular typing data of human isolates. EFSA is tasked to develop and manage a database on isolates from food, animal feed and animal samples. In the initial phase of the development of this data collection system, an isolate-based molecular typing database should be created for *Salmonella*, *Listeria monocytogenes* and Shiga toxin-producing *Escherichia coli* (STEC) isolates. ECDC is asked to manage a similar database for human isolates in accordance with article 9 of Regulation (EC) No. 851/2004 (EC, 2004b). The databases may later be extended to include other food-borne pathogens, such as *Campylobacter*, upon agreement between EFSA, ECDC, the relevant EURL and the European Commission. EFSA and ECDC have also been asked to develop molecular typing data sharing across food/animal feed/animal/human sources.

Discussion

Q: Who will be the owner of molecular typing data? Is it the laboratory that performs the tests or the national competent authority?

A: In principle, the national competent authority is the owner of the data and it should arrange further details on ownership at national level.

2.8 Molecular serotyping of *Salmonella* – Experiences of reference laboratory the Netherlands

Max Heck, RIVM, Bilthoven, the Netherlands

Serotyping of *Salmonella* has been an invaluable subtyping method for epidemiologic studies for more than 70 years. The technical difficulties of serotyping, primarily in antiserum production and quality control, can be overcome with modern molecular methods.

The idea was to develop a method that could replace the present first screening step with antisera in microtitertrays of Salmonellae and that could determine the most prevalent serovariations in the Netherlands.

Based on literature (Hong et al., 2008; Herrera-León et al., 2004; Herrera-León et al., 2007; Echeita et al., 2002), a DNA-based assay was developed, i.e. a multiplex PCR targeting the genes encoding for the somatic O-antigens gene cluster *rffB*, the flagellar, i.e. H1 and H2 antigens, *fliC* and *fljB* of the White-Kauffmann-Le Minor serotyping scheme (Grimont and Weill, 2007). The three PCRs yielded amplicons that were analysed by automated gel electrophoresis. On basis of their fragments sizes, O-, H1, and H2 were determined and consequently serotyped. Over 500 strains have been tested using both PCR and conventional serotyping, resulting in promising results. The PCRs were faster

and less labour-intensive and showed good sensitivity and specificity. However, a thorough validation is needed. The PCR method will reduce the large number of strains that need full slide agglutination and it is planned to use the method as a pre-screening test.

Discussion

Q: What is the breaking point, in terms of the number of isolates serotyped annually, that will prompt the switch from the agglutination procedure to molecular serotyping?

A: We currently use the PCR method as an alternative for the first screening step. After this first PCR screening, a serovar name can be given to approximately 50% of the tested isolates. If the multiplex PCR is amended, enabling to test for more genes, then it may be possible to serotype more/all strains. As antisera are relatively expensive, the point at which a multiplex PCR could be chosen for serotyping may lie at approximately 100 strains per year.

Q: Switching to molecular serotyping is not only a matter of costs, but also a matter of progress.

A: That is true. The agglutination technique is seldom educated, which can lead to problems with young staff. Hence, investments in new techniques are important. Nevertheless, each newly developed method needs to be validated against the 'gold standard' (serotyping according to the White-Kauffmann-Le Minor scheme – Grimont and Weill, 2007).

2.9 Molecular serotyping of *Salmonella* – Experiences NRL-*Salmonella* in Denmark using the CDC method

Gitte Sørensen, Technical University of Denmark, Søborg, Denmark

Serotyping is the standard method for subtyping *Salmonella*, and information about serotypes is essential for understanding the epidemiology of *Salmonella*. Until 2013, the serotyping of *Salmonella* isolates from food and animals in Denmark was done by slide agglutination, using specific antisera that detect differences in cell surface structures: Somatic (O) lipopolysaccharides and flagellar antigens (H).

All *Salmonella* isolates from food and animals in Denmark are serotyped at the National Food Institute. During 2011 and 2012, a molecular, DNA-based, serotyping method developed by CDC was implemented and from 1 January 2013 this method was accredited and used by the National Food Institute as a supplement to the traditional serotyping based on agglutination.

The DNA-based molecular method detects genes indicating the corresponding O and H antigens and thus it is based on the White-Kauffmann-Le Minor scheme (Grimont and Weill, 2007). The assay is a multiplex bead-based suspension array based on the Luminex xTAG technology and detects the major O groups and H antigens (Fitzgerald et al., 2007; McQuiston et al., 2011). It can directly identify the most important serovars of *Salmonella*, but in some cases there is a need to supplement the DNA-based data with data from slide agglutination tests.

The experiences of using this method at the National Food Institute were presented. The DNA-based molecular serotyping has proved to be a valuable tool in the routine typing of *Salmonella*, especially common serotypes in O groups B and C1 are typed directly. Furthermore, the method enables serotyping of rough isolates. A routine scheme is used where molecular serotyping is performed two or three times each week. In this scheme, most isolates are serotyped in less than 48 hours from the start of typing. However, if a faster

result is needed, and the antigens are covered by the method, it is possible to determine a serovar within five hours.

Discussion

Q: Did the mismatches in results between agglutination and molecular serotyping result in different serovar names?

A: No. In fact, the molecular typing resulted in 'untypable' for these mismatches and thus no name could be given to the isolates.

2.10 Rapid detection and specific differentiation of *Salmonella* Enteritidis, *Salmonella* Typhimurium and its monophasic variant by multiplex real-time PCR

Sven Maurischat, Federal Institute for Risk Assessment, Berlin, Germany

In 2011, *Salmonella* Enteritidis and *Salmonella* Typhimurium still contributed to 69.3% of all human infections in Europe that are often related to the consumption of chicken meat and pork. Targeting a reduction of *Salmonella* prevalence in poultry, the EU set up control programmes that prohibit, for example, the occurrence of these *Salmonella* serovars in fresh poultry meat according to regulation (EU) No. 1086/2011 (EC, 2011).

In order to make the detection of these *Salmonella* serovars faster than it would be using the current cultural method according to ISO 6597 (Anonymous, 2002), we developed a multiplex real-time PCR assay. This assay detects specifically *S. Typhimurium* and *S. Enteritidis* in pre-enriched chicken meat and neck skin samples within 24 hours after sampling. The assay was validated according to ISO 16140 (Anonymous, 2011) for detection of *Salmonella* in fresh poultry meat. Additionally, the assay enables differentiation between biphasic and monophasic *S. Typhimurium* of the serotype 1,4,[5],12:i:- that gain more and more importance worldwide.

2.11 Overview of the recent activities carried out by the Italian NRL related to *S. 4*,[5],12:i:-

Lisa Barco, Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy

The results of a series of studies related to *S. 4*,[5],12:i:- and *S. Typhimurium* conducted by the Italian National Reference Laboratory during the last few years were presented.

The main aim of the first study was to gain insight into the epidemiology of *S. 4*,[5],12:i:- and *S. Typhimurium* in order to identify similarities and differences between these related serovars. For this, the methodology of Random Forest (RF) was used to analyse a dataset of almost 900 strains of the two serovars isolated in the northeast of Italy from foodstuffs, animals and the environment. Both descriptive analysis and RF revealed that in spite of the similarities and close relationship between the two serovars, *S. 4*,[5],12:i:- is less heterogeneous than *S. Typhimurium*. Moreover, RF highlighted that phage type was the most important variable to differentiate the two serovars.

In the second study, a collection of isolates of *S. 4*,[5],12:i:- DT193 resistant to ampicillin, streptomycin, sulphonamides and tetracycline (R-typeASSuT), which has been recognised as a European emergent clone, was characterised. The

strains investigated were obtained from two unrelated outbreaks in Italy. Strains were characterised by pulsed-field gel electrophoresis (PFGE), performed with three different restriction enzymes (*XbaI*, *BlnI*, and *SpeI*), and multiple-locus variable-number tandem repeat analysis (MLVA). The study represented the first report of outbreaks occurring in Italy due to this clone. Subtyping analysis of the isolates demonstrated the high level of similarities documented by the fact that *XbaI*-PFGE showed strains related to the two outbreaks as indistinguishable. However, both *BlnI*-PFGE and MLVA characterised the strains related to the two outbreaks as different.

S. 4,[5],12:i:- is a variant of *S.* Typhimurium, which lacks the expression of phase-2 flagellar antigen generally associated with the deletion of the *fljB* gene. However, additional mechanisms involving the *fljAB* operon (*fljA*, *fljB* and *hin* genes) lead to the lack of expression of phase-2 flagellar antigens also in *Salmonella* strains harbouring the *fljB* gene. The aim of the third study was to investigate the reasons for the lack of expression of phase two flagellar antigen for a selection of *S.* 4,[5],12:i:- isolates (20) conserving the *fljB* gene. These strains were defined as 'inconsistent' *Salmonella* Typhimurium variants since they had phenotypically behaved as monophasic, even though the *fljB* gene was conserved. For these isolates the monophasic phenotype was partly due to the absence of the *hin* gene of the *fljAB* operon, or due to point non-conservative mutations in two genes of the operon.

The main results of a review carried out in order to define the state of the art concerning *Salmonella* source attribution through a microbial subtyping approach were presented. In particular, it has been briefly discussed the most commonly used molecular typing methods in the European Union (EU) laboratories in the context of their potential applicability to *Salmonella* source attribution studies in order to identify the methodologies that could be most promising in this context. The analysis revealed that the molecular methods that could be most useful for conducting source attribution studies based on microbial subtyping are PFGE and MLVA.

Finally, some data on *Salmonella* source attribution studies carried out so far were presented. It was pointed out that such studies confirmed pork as the main source of human salmonellosis in Italy. Further investigations will be conducted, using molecular subtyping data for *S.* 4,[5],12:i:- and *S.* Typhimurium isolates, in order to gain insight into the effective role of pork as a potential source of human salmonellosis in Italy.

Discussion

Q: Why are pigs an important source of monophasic *Salmonella* Typhimurium in Italy?

A: This may be related to Italian traditions. In Italy several pork products are eaten raw or semi-raw, which may cause problems. Additionally, there is little control of these products.

2.12 *Salmonella* Typhimurium monophasic variant surveillance in France: molecular characterisation and guidelines from ANSES experts

Renaud Lailler, ANSES, Maisons-Alfort, France

The French *Salmonella* monitoring system is supported by several actors all along the agri-food chain (from farm to fork). In the human sector, the National

Institute for Public Health Surveillance registers notifiable diseases. The National Reference Centre at the Pasteur Institute (Paris) manages a laboratory-based surveillance of *Salmonella* detection from human samples, allowing the collection of data on sporadic cases too. Around 1400 voluntary clinical laboratories, representing 30% to 40% of all French clinical laboratories involved in human medicine, send either strains or reports on the strains isolated and serotyped on a daily basis (N=10 000 strains/y). As no change in the reporting system for *Salmonella* surveillance has been made for decades, the network of National Reference Centre (NRC) laboratories allowed seeing the significant emergence of the monophasic variant of *Salmonella* Typhimurium in humans in France since 2008. Analysis of simple epidemiological data collected by the French NRC for *Salmonella* in 2011 found no distribution differences in age, sex, site of isolation or hospitalisation status between biphasic and monophasic *S. Typhimurium* cases.

In the non-human sector, the French competent authority implements programmes within the framework of the EU Regulation, in order to reduce the prevalence of *Salmonella* in primary production. The institutional national surveillance relies on annual surveillance or control plans in the agri-food sector, in association with the NRL. ANSES teams coordinate several networks on *Salmonella*, either specific to the animal sector or more generic, like the *Salmonella* Network. This large network centralises strains from 140 public or private veterinary laboratories, isolated from food, at slaughterhouses or at retail level (N=18000_strains/year). Several French ministerial decrees define the required control measures on breeding flocks of *Gallus gallus* and turkeys contaminated with *Salmonella*. Currently, detection of *S. 1,4,[5],12:i:-*, *S. 1,4,[5],12:-:1,2* and *S. 1,4,[5],12:-:-* generates the same control measures as for *S. Typhimurium*, i.e. the eradication of the batch of animals, resulting in costs for the stakeholders. Official laboratories must submit all variant isolates to the NRL-*Salmonella* for confirmation of the complete antigenic formulae and for characterisation.

In 2012, the French ministries addressed a formal request to ANSES in relation to the public health risk management of monophasic variants of *Salmonella* Typhimurium. In response, ANSES led a collective expertise group and a final opinion was published in July 2013. In particular, this opinion clarifies the definition of variant strains according to the EFSA guidelines and summarises the available data from human and non-human surveillance. Several *Salmonella* outbreaks have been linked to variants of monophasic *S. Typhimurium*. In 2010, a large outbreak was associated with the consumption of beefburgers and two other outbreaks in 2010 and 2011 were associated with the consumption of dried pork sausage. A smaller outbreak in 2011 was associated with the consumption of goat cheese made from raw milk. Three lineages of monophasic *S. Typhimurium* are circulating at international level but the great majority of French isolates correspond to the European lineage (ASSuT-R). In conclusion, ANSES recommends to maintain control pressure on the avian sector and to enlarge the surveillance on pigs and cattle. A multiplex PCR method was developed by the French NRL to differentiate variant strains from *S. Typhimurium* or other serovars. The opinion of ANSES highlights that the determinants of serovar and the variability of peptide sequence of *FliC* and *FljB* are not involved in the expression of the *Salmonella* virulence. A regulation based on the monitoring of a limited number of specific serotypes should not be considered as a monitoring of the most virulent strains. Thus, regulation should be regularly adjusted for a positive impact on public health.

Discussion

Q: There is much development in PCR methods used for typing monophasic *Salmonella* Typhimurium. What is the 'standard' PCR method to be used?

A: Currently there is no 'standard PCR method' for this, although some activities started within ISO for the development of such standard method. For the moment, the method described in the EFSA Opinion (EFSA, 2010) can be chosen, with reference to the publication of Tennant et al., 2010. Furthermore, additional information on the method was distributed to the NRLs (by the EURL) in September 2013.

3 Tuesday 27 May 2014: Day 2 of the workshop

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

Heimo Lassnig, NRL-Salmonella, Graz, Austria

The NRL-*Salmonella* in Austria is called the NRC (National Reference Centre) *Salmonella*. It deals with all *Salmonella* strains coming from animal feed, animals, food, the environment and humans. The NRC-*Salmonella* is located in AGES, the Austrian Agency for Health and Food Safety, mainly in the division of Public Health. AGES consists of five divisions: Austrian Medicines & Medical Devices, Public Health, Animal Health, Food Safety, and Food Security (animal feed and agriculture).

The NRC-*Salmonella* Austria consists of a main laboratory and three so-called outposts:

The main laboratory is located in the department for reference centres and reference laboratories of the Institute for Medical Microbiology and Hygiene/Centre of Infectious Food-borne Diseases in Graz. It deals with the subtyping of all *Salmonella* strains that have to be sent to this laboratory from all over Austria. In outbreak investigations it undertakes the part of the work relating to humans, including molecular fingerprinting. It participates in the EURL-*Salmonella* interlaboratory comparison subtyping studies. Furthermore, it organises workshops for other *Salmonella* laboratories to keep them up to date and to inform them of news from the EURL-*Salmonella*.

The food outpost is situated in the same institute as the main laboratory, but is in the department for food microbiology. In outbreak investigations, it undertakes the part of the work relating to food. It participates in the EURL-*Salmonella* interlaboratory comparison studies of food samples. Ring trials for other *Salmonella* laboratories in the AGES, or laboratories that are owned by county authorities, are organised by the food outpost.

The veterinary outpost is also located in this institute and is part of the department for veterinary microbiology but is in a different building (there is a plan to combine the two departments). In outbreak investigations, it undertakes the part of the work relating to animals. This laboratory participates in the EURL-*Salmonella* interlaboratory comparison studies on samples from the primary production stage. Every year or two, it organises ring trials for the five official veterinary *Salmonella* laboratories.

The feeding stuffs outpost has its seat in Linz, one of the six cities where AGES laboratories can be found (the others are Vienna, Graz, Innsbruck, Mödling and Salzburg). This part of the NRC-*Salmonella* is located in the Institute for animal nutrition and feed in the department of micro- and molecular microbiology. In outbreak investigations, it undertakes the part of the work relating to feeding stuffs. It participates in the EURL-*Salmonella* interlaboratory comparison studies on animal feeding stuffs.

The following figures indicate the amount of work undertaken by the NRC-*Salmonella* each year: serotyping and biochemical identification: approx. 4500 strains. Phage-typing of *S. Enteritidis*, *S. Typhimurium*, *S. Hadar*, *S. Virchow*, *S. Typhi*, *S. Paratyphi*: approx. 1000 strains. Antimicrobial susceptibility testing with disc diffusion: approx. 4500 strains.

The Austrian *Salmonella* control programme has managed to reduce the number of human primary isolates to the same level as occurred before the *S. Enteritidis* epidemic in the late 1980s.

A comparison of MSR/V batches from different suppliers showed differences in the number of suspect plates in routine work, although they all performed well in EURL interlaboratory comparison studies on the detection of *Salmonella* in samples from the primary production stage.

The testing of additional voluntary samples from commercial laying flocks where *S. Enteritidis* or *S. Typhimurium* has been found is performed by the veterinary outpost by analysing 4000 eggs in pools of 40 eggs. There are two ways this investigation is carried out: either whole eggs are pooled, or parts of the eggs (by pooling part of the shell, 1 ml of egg white and 1 ml of yolk). These two methods were compared in 22 analyses, showing no significant differences.

Discussion

Q: You tested MSR/V agar plates from different manufacturers and also used MSR/V with a higher concentration of novobiocin than prescribed (20 mg/L instead of 10 mg/L). Did you see differences in growth on the plates with the higher concentration of novobiocin?

A: No, we did not see any differences. *Salmonella* gave good growth on MSR/V plates with different concentrations of novobiocin, and also on plates without novobiocin.

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

Sead Hadziabdic, NRL-Salmonella, Sarajevo, Bosnia and Herzegovina

The Veterinary Faculty Sarajevo, Bosnia and Herzegovina (BiH), was established in 1949. Its main function, the education of future veterinarians, has been accomplished by evolution into an institution of leading importance in diagnostics, research and knowledge transfer. The Veterinary Institute itself is an organisational unit within the faculty and serves as a veterinarian institution of central importance in BiH with regard to the prevention and diagnosis of certain diseases of importance to animal and public health.

The laboratory for bacterial disease in poultry is one of several accredited laboratories within the Institute, its main aim being the prevention and diagnosis of bacterial pathogens in commercial poultry and wild birds. Since 2012, a quality assurance system based on ISO 17025 (Anonymous, 2005) has been implemented in the Institute and the scope of accreditation for the laboratory of bacterial disease of poultry includes the method based on ISO 6579:2002/Amd 1:2007. The laboratory's activities include monitoring of *Salmonella* and *Campylobacter* in commercial and backyard flocks, Proficiency Testing for regional authorized laboratories and participation in the EURL comparison studies on primary production.

As for the *Salmonella* control programme in BiH for 2012, the prevalence of *Salmonella* spp. in broiler flocks, laying hens and parent flocks was 10.0%, 4.0% and 6.7%, respectively. During 2013, a decrease in the prevalence of *Salmonella* in broiler flocks (0.8%) and laying hens (1.13%) was noticed. In 2012, the laboratory started molecular typing with PFGE, as a result of a project undertaken in collaboration with the Veterinary Institute of Slovenia. The main aim to start with molecular typing by PFGE is to ensure food safety by intervention in *Salmonella* epidemiology within primary poultry production.

Discussion

Q: You use chicken faeces as samples in your national interlaboratory comparison studies. Do you use naturally contaminated faeces or artificially contaminated faeces?

A: We use faeces artificially contaminated with a diluted culture at low level and at high level.

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

Shona Neal, NRL-Salmonella, London, United Kingdom

The UK NRL for Food Microbiology is situated at Public Health England – Colindale (PHE), under a contract with the competent authority, the Food Standards Agency (FSA). Other laboratories that the UK NRL closely interacts with include the EURL-*Salmonella*, the *Salmonella* Reference Service (within the Gastrointestinal Bacteria Reference Unit (GBRU), PHE), the Animal Health and Veterinary Laboratories Agency (AHVLA, which is the UK NRL for animals and feed), the 16 official control laboratories (OCLs) in the UK (of which five are within PHE) and the Scottish *Salmonella* Reference Laboratory (SSCDRL). Whilst the UK NRL has participated in the EURL Proficiency Tests (PT) on detection of *Salmonella* in food since 2006, GBRU provides both phages and selects the strains for the phage typing PT, and therefore cannot ensure unbiased participation to this PT. However, last year the UK NRL participated in the serotyping part of the PT, and received good performance.

An audit in 2013 revealed that most UK OCLs participate in various PT schemes, but does not allow performance to be compared. Therefore, the Food and Environment Proficiency Test Unit (FEPTU) at PHE organises the European Food Microbiology Legislation Scheme, which covers all agents within Regulation (EC) 2073/2005 and requires participants to select the tests to be performed, directed by the description of the sample sent. Although the PT does not use contaminated food, the lenticultured mixtures are highly stable and homogeneous, allowing repeat samples where necessary and easier interlaboratory comparisons. Twelve OCLs have registered for 2014–2015 schemes, where they will receive four distributions in the year, with three samples in each distribution. The NRL will receive a consolidated, anonymised report from the results of each distribution and this will allow comparisons between most of the OCLs, joint learning from systematic failures and the identification of areas where the NRL will be able to provide support if there is successive poor performance by any OCL.

The UK NRL also forwards the EURL-*Salmonella* newsletters to the FSA and the OCLs, arranges annual OCL user day meetings to report activities from the EURLs and the NRL, and forwards any other relevant information from the EURLs, where necessary. In autumn 2013, the UK NRL hosted a practical workshop for detection of VTEC in food using PCR; PHE has facilities to hold a *Salmonella* workshop, if warranted.

Other activities include the monitoring and characterisation of *Salmonella* in humans and food. A recent outbreak was described, which used whole genome sequencing to determine the closeness of the human isolates with those from foods.

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

Magdalena Skarzynska and Kinga Wieczorek, NRL-Salmonella, Pulawy, Poland

The National Veterinary Research Institute (NVRI) was established in 1945 as a scientific institution of the Ministry of Agriculture and Rural Development.

The NVRI conducts research within the scope of veterinary medicine, in particular controlling animal infectious diseases, zoonoses, food of animal origin and animal feeding stuffs.

The NVRI provides advisory and expert support to the Veterinary Administration, supervises regional veterinary laboratories and participates in medicine and vaccine licensing processes. It also runs postgraduate training for DVMs and laboratory personnel. The NVRI is also the National Reference Laboratory for several animal infectious diseases, zoonoses and safety of animal-origin food and animal feeding stuffs.

The National Reference Laboratory for *Salmonella* was established at the NVRI by the regulation of the Ministry of Agriculture and Rural Development of 13 February 2003. Since 2005, the NRL has been accredited for detection of *Salmonella* in food according to ISO 6579 (Anonymous, 2002) and since 2008 also for detection of *Salmonella* in samples from the primary production stage according to ISO 6579:2003/A1:2007 (Anonymous, 2007).

One of the main NRL-*Salmonella* tasks is the organisation of Proficiency Testing on detection and identification of *Salmonella* for regional veterinary laboratories and private laboratories approved by the Chief Veterinary Officer. Moreover, the NRL-*Salmonella* offers theoretical and practical training for personnel of diagnostic laboratories.

The NRL-*Salmonella* cooperates with the EURL *Salmonella* (by participating in the EURL annual workshops and Proficiency Tests) and transfers received information to competent authorities and supervised regional laboratories. The NRL is also responsible for the collection and confirmatory testing of *Salmonella* strains isolated by official laboratories from samples originating from animals, food of animal origin and feeding stuffs in accordance with European Regulations 2160/2003 (EC, 2003b) and 2073/2005 (EC, 2005).

The NRL's scope of work covers isolation of *Salmonella* from animals (including pet reptiles and game animals), detection of *Salmonella* in food of animal origin, and identification and susceptibility testing of isolates originating from animals, food and feeding stuffs. The NRL uses several techniques, including PFGE, MLST, PCR, antimicrobial resistance testing and plasmid profiling, in epidemiological studies of *Salmonella* infections.

Discussion

Q: How do you improve the performance of the veterinary laboratories?

A: By individual training.

Q: What do you do with laboratories that show repeated poor performance?

A: First, we organise a follow-up and/or training. If this still does not result in improvement of the performance, the laboratory may lose its official status.

Q: Is your NRL accredited for the organisation of Proficiency Test (PT) schemes?

A: No. We are accredited for individual tests but not for organising PT schemes.

Q: In my country, the NRL is required to be accredited for the organisation of PT schemes. If it is not accredited, it is not allowed to call such studies PT schemes. Is this also the case in other countries?

A: Regulation 882/2004 (EC, 2004a) does not prescribe that NRLs have to be accredited for the organisation of comparative tests. Even the revision of this regulation does not prescribe accreditation for the organisation of these studies for EURLs or for NRLs. It seems quite likely that accreditation bodies prescribe laboratories to participate in accredited PT schemes, resulting in the 'automatic' demand that the NRL has to be accredited for the organisation of the studies they organise as well. However, it is not the task of an NRL to organise PT schemes for the accreditation of official laboratories. The task of an NRL is to control the performance of national official laboratories, which can be done by organising comparative tests.

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

Istavan Szabo, NRL-Salmonella, Berlin, Germany

In Germany the federal system divides tasks between the federal government on the one hand and the individual federal states on the other hand. This also applies to the health system and therefore also to the field of salmonellosis. Two governmental institutes – for human isolates (Robert Koch-Institute [RKI]) and for isolates from animals, food, animal feed and the environment (Federal Institute for Risk Assessment [BfR]) – obtain isolated *Salmonella* strains from human and veterinary institutions of each federal state, medical and veterinary centres, universities, zoos, private companies, etc. in order to confirm serotyping and for further differentiation of isolates.

The focus of the work of the National Reference Laboratory for *Salmonella* is on the serological differentiation of *Salmonella* isolates and antibiotic resistance determination. A number of molecular-biological methods have been established for outbreak and epidemiological studies.

The NRL carries out the following tasks in accordance with the Zoonoses Directive 2003/99/EC (EC, 2003a) and with the Zoonoses Regulation (EC) No. 2160/2003 (EC, 2003b):

- Serotyping of *Salmonella* according to the White-Kauffmann-Le Minor scheme (Grimont and Weill, 2007);
- Antibiotic resistance testing of *Salmonella*;
- Epidemiological surveys on the incidence of *Salmonella* lysotypes and their resistance;
- Early warning system for the occurrence of *Salmonella*;
- Molecular-biological fine typing of *Salmonella*;
- Identification of vaccination strains;
- Elucidation of chains of infection and fine differentiation studies;
- Molecular biology of *Salmonella* resistance;
- Preparation of results and maintenance of the internal laboratory database;
- Rapid detection of *Salmonella* using PCR and other DNA-based methods;
- Multilaboratory studies;
- Participation in national and international research projects;
- Organisation of and participation in interlaboratory tests within the framework of the EU and WHO;
- Policy advice for diverse institutions;
- Management and maintenance of strain collections;
- Participation in national/international committees.

3.6 Results of the interlaboratory comparison study on typing of *Salmonella* XVIII (2013) – serotyping and PFGE

Wilma Jacobs, EURL-Salmonella, Bilthoven, the Netherlands

In November 2013, the 18th interlaboratory comparison study on serotyping, phage typing and PFGE typing of *Salmonella* spp. was organised by the European Reference Laboratory for *Salmonella* (EURL-*Salmonella*, Bilthoven, the Netherlands), in cooperation with Public Health England (PHE, London, United Kingdom) for the phage typing part.

A total of 34 laboratories participated in this study. These included 29 National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) in the 28 EU Member States, two NRLs of EU candidate countries and three NRLs of EFTA countries. The main objective of this study was to check the performance of the NRLs for typing of *Salmonella* spp. and to compare the results of typing of *Salmonella* spp. among the NRLs-*Salmonella*. All NRLs performed serotyping of the strains. NRLs of the EU Member States that do not achieve the level of good performance for serotyping have to participate in a follow-up study.

A total of 20 *Salmonella* strains had to be serotyped by the participants. As discussed at the previous EURL-*Salmonella* workshop, one additional strain from an uncommon source and subspecies was included in the study. Serotyping of this strain was optional and the results were not included in the evaluation. The strains had to be typed with the method routinely used in the 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 2014. The serotyping results showed that the O-antigens were typed correctly by 32 of the 34 participants (94%). This corresponds to nearly 100% of the total number of strains. The H-antigens were typed correctly by 24 of the 34 participants (71%), corresponding to 98% of the total number of strains. A total of 23 participants (68%) gave the correct serovar names, corresponding to 97% of all strains evaluated.

A completely correct identification by all participants was obtained for 12 strains: *S. Telelkebir* (S1), *S. Sandiego* (S3), *S. Plymouth* (S4), *S. Kingston* (S6), *S. Havana* (S7), *S. Hadar* (S8), *S. Worthington* (S10), *S. Kentucky* (S14), *S. Infantis* (S17), *S. Enteritidis* (S18), 1,4,[5],12:i:-(S19), and *S. Typhimurium* (S20). Most problems occurred with the serovar *S. Thompson* (S9). Eight laboratories had difficulties in assigning the correct serovar name to this strain, though in two cases this was caused by the (partly) non-typable nature of the strain. All but two participants actually did serotype the additional strain S21, which was a *Salmonella enterica* subspecies *salamae* 42:g,t:-. Thirty laboratories correctly serotyped the O-antigens and the H-antigens for this strain.

Two participants did not meet the level of good performance at the first stage of the study and these laboratories participated in the follow-up study of March/April 2014, in which they had to serotype an additional ten strains. Both participating EU NRLs achieved a good performance on their results in the follow-up study.

The individual laboratory results on the PGFE typing part were reported to the 20 participants in May 2014. In this pilot study participants were asked to test ten *Salmonella* strains, using their own routine PFGE method for digestion with XbaI enzyme. Evaluation of the results was done on the quality of the PFGE gel images only, not on gel analysis in Bionumerics (yet). The PulseNet guidelines

(www.pulsenetinternational.org) were used for this quality grading, based on the scoring of seven parameters on a scale of 1 (poor) to 4 (excellent) points. Considerable variation in the quality of the gel images was observed, but in general the results looked promising. General remarks to improve the quality of gels could be given by advising on the use of the *S. Braenderup* H9812 standard and on the capture of the image (resolution).

PFGE typing will also be included in the 2014 interlaboratory comparison study on typing of *Salmonella*.

More details of the study can be found in the Interim summary report (Jacobs-Reitsma et al., 2014).

Discussion

Q: The PFGE typing of *Salmonella* Dublin causes problems in our laboratory (smears). What are the experiences of other laboratories?

A: No information was available from the audience. It was only remarked that as alternative, MLVA can be used for subtyping *S. Dublin*.

3.7 Results of the interlaboratory comparison study on typing of *Salmonella* XVIII (2013) – phage typing

Elizabeth de Pinna, PHE, London, United Kingdom

The *Salmonella* strains for phage typing in the 18th 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 culture collection of PHE.

Seven NRLs took part in the phage typing part of the study for both the *S. Enteritidis* strains and the *S. Typhimurium* strains.

Five of the NRLs correctly phage typed all ten strains of *S. Enteritidis*. One of the NRLs correctly typed nine of the *S. Enteritidis* strains and one NRL correctly phage typed six of the *S. Enteritidis* strains. Six of the *S. Enteritidis* strains were phage typed correctly by all the participating laboratories. Three strains, E2 (PT 60), E3 (PT 5a) and E6 (PT 21), were each incorrectly phage typed by one of the participating laboratories. One strain, E7 (PT 5), was incorrectly phage typed by two laboratories.

None of the participating NRLs phage typed all ten *Salmonella* Typhimurium strains correctly. 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. One strain, T5 (DT 120), was incorrectly phage typed by one laboratory. Two strains, T4 (DT 7a) and T9 (DT 15a), were incorrectly typed by two of the participating laboratories. One strain, T2 (DT 104), was incorrectly phage typed by three laboratories and one strain, T10 (DT 135), was incorrectly phage typed by four of the participating laboratories.

Overall, 93% 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 better than in 2011 and 2012, when

respectively 87% and 90% of the strains were typed correctly. For the phage typing of *S. Typhimurium*, the results of this study were not as good as the studies in 2011 and 2012, when respectively 98% and 92% of the strains were correctly phage typed. This was due to the problems with the phage typing of *S. Typhimurium* strains T2 (DT 104) and T10 (DT 135).

This study has shown that there has been an improvement in the performance of the phage typing of *S. Enteritidis* by the NRLs. The performance in the phage typing of *S. Typhimurium* was lower than in the previous two years.

More details of the study can be found in the Interim summary report (Jacobs-Reitsma et al., 2014).

3.8 Work programme EURL-Salmonella (second half 2014, first half 2015), discussion on general items and closure

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

Kirsten Mooijman summarised the work programme of the EURL-*Salmonella* for the rest of 2014 and for early 2015.

Interlaboratory comparison studies

Three interlaboratory comparison studies are planned for the coming year:

- Detection of *Salmonella* spp. in animal feed: September/October 2014. Experiments have been performed at the laboratory of the EURL-*Salmonella* to test whether it is possible to prepare sufficient stable materials when inoculating grains/flour with a diluted culture of *Salmonella*. For this, two different *Salmonella* serovars have been tested and the results are promising. Additional tests will be done with storage of the inoculated samples for a longer period of time at different temperatures (-20 °C, +5 °C and +10 °C). The results of the different experiments will be used to draft the final set-up of the study.
- Typing of *Salmonella* spp.: November/December 2014. Like former typing studies, this study will contain an obligatory part for serotyping of 20 different *Salmonella enterica* serovars (and one additional optional non-*enterica* isolate) and an optional part on phage typing of ten STM isolates and ten SE isolates. Furthermore, again an optional part, for PFGE testing of ten different *Salmonella* serovars, will be included in the study.
- Detection of *Salmonella* spp. in a sample from the primary production stage: February/March 2014. The choice of the matrix will be decided later.

An additional remark was made to the phage typing part of the typing study. As phage typing is performed by only a few laboratories, the EURL-*Salmonella* discussed with PHE (Elizabeth de Pinna) and DG-Sanco (Klaus Kostenzer) whether phage typing should still be included in the typing studies of the EURL-*Salmonella*. It is clear from the pilot study on PFGE testing in 2013 that a larger number of NRLs is interested in a study on molecular typing than on phage typing. This is one of the reasons for the decision to focus more on molecular typing as an additional part of the interlaboratory studies on typing (additional to serotyping) and to stop the studies for phage typing from 2015. For the few NRLs still performing phage typing, the PHE can still give support by supplying phages. Furthermore, additional support may be considered by PHE, such as confirmation of phage typing by PHE on a set of strains selected by an NRL-*Salmonella*. However, this type of support will need to be discussed internally at

PHE. As soon as more information is available, the (relevant) NRLs will be informed.

Supporting activities

The 'research' performed by the EURL-*Salmonella* always relates 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;
- Testing different matrices for use in interlaboratory comparison studies.

Assistance to the Commission and communication

- Experts from the EURL-*Salmonella* regularly participate in working groups of EFSA and of EC DG-Sanco.
- EURL-*Salmonella* will perform ad hoc activities (on its own initiative or on request) and, if needed, will support DG-Sanco or EFSA in the event of outbreaks.
- The EURL regularly receives requests for information or advice from NRLs, DG-Sanco and third parties. Replies are given as quickly as possible but is sometimes delayed due to the fact that literature 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 that may be of interest to the other NRLs for publication through the newsletter.
- The EURL-*Salmonella* website will be kept up to date with information on new activities/results.
- Results of interlaboratory comparison studies and of the workshops are summarised in (RIVM) reports. The EURL is considering to send future reports only in digital form and no longer in printed form.

Training

Training can be given by the 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. Currently, staff members of the EURL-*Salmonella* participate in an EFSA working group on molecular typing (started in April 2013).

Activities foreseen for the coming year are:

- Including (again) PFGE typing in the EURL-*Salmonella* interlaboratory comparison study on typing of *Salmonella*;
- Continuing participation in the EFSA working group on molecular typing;
- Contributing to the standardisation of protocols for molecular typing;
- Training NRLs for *Salmonella* in molecular typing;
- Cooperating with the Statens Serum Institute (SSI) in Denmark, the curator of the ECDC database;
- Curating molecular data (PFGE) for the EFSA (pilot) database.

Workshop 2015

The dates and location of the 2015 EURL-*Salmonella* workshop are not yet fixed. The possibility that the 2015 workshop will be held in Berlin is under discussion with the German NRL-*Salmonella*.

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 of the workshop (see Annex 3). A total of 12 questions were posed. In answer to ten of these questions, participants were asked to give a score ranging from 1 to 5, with 5 as the highest score (excellent) and 1 as the lowest score (very poor). Participants were also able to add remarks to these answers. Two questions were 'open' questions, in which the participants were asked to give their opinion.

The evaluation form was handed to 45 participants of the workshop and 39 completed forms were returned, which is a response rate of 87%.

In Section 4.2, the scores for each question are shown 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 to have been good or excellent (scores 4–5).

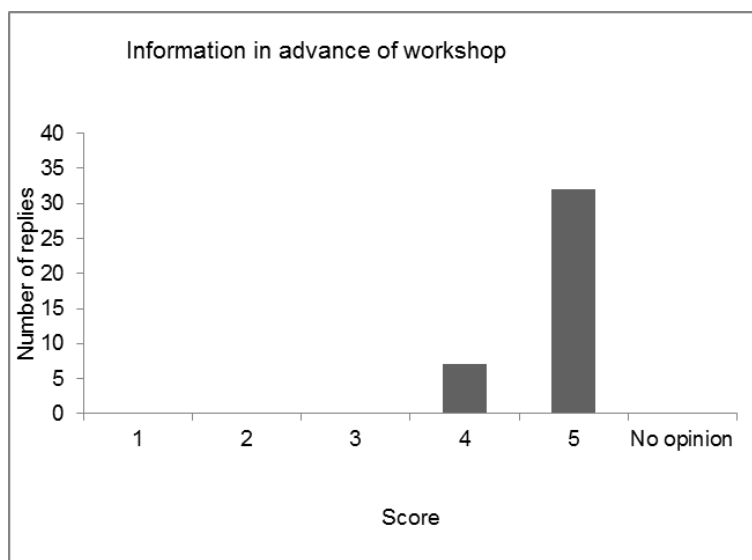


Figure 1. Scores given to question 1 'Opinion on information given in advance of the workshop'

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

The participants for whom tickets were arranged by the EURL were very satisfied. Participants who booked tickets themselves indicated 'no opinion' (see Figure 2). Remarks given were:

- 'Colleagues were very kind and organised the best way of my travel!'
- 'I have booked my tickets this time, but in previous years it was a very good experience. Staff were very helpful and, yes, that means excellent.'

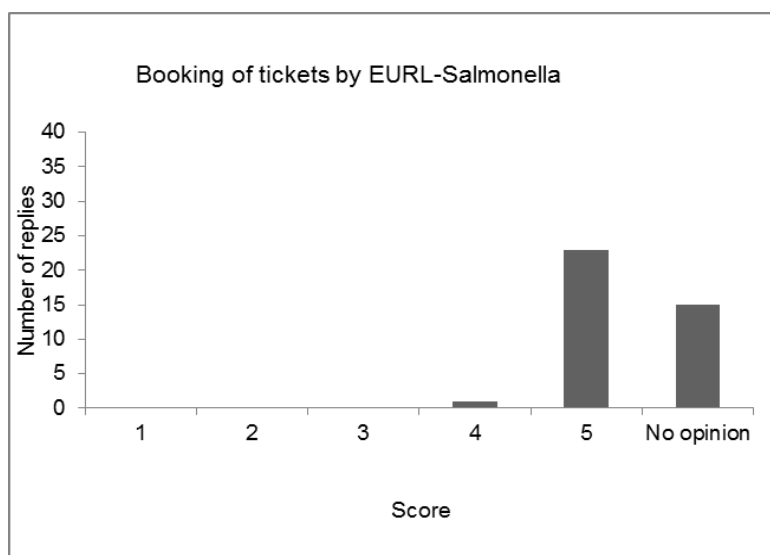


Figure 2. Scores given to question 2 'Opinion on the booking of the tickets by the EURL-Salmonella'

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

Opinions on the accessibility of the meeting venue were very positive this year. All participants considered the meeting venue easy to reach (Figure 3). Remarks given were:

- 'More than excellent this year!'
- 'Very happy with location, easy to reach.'

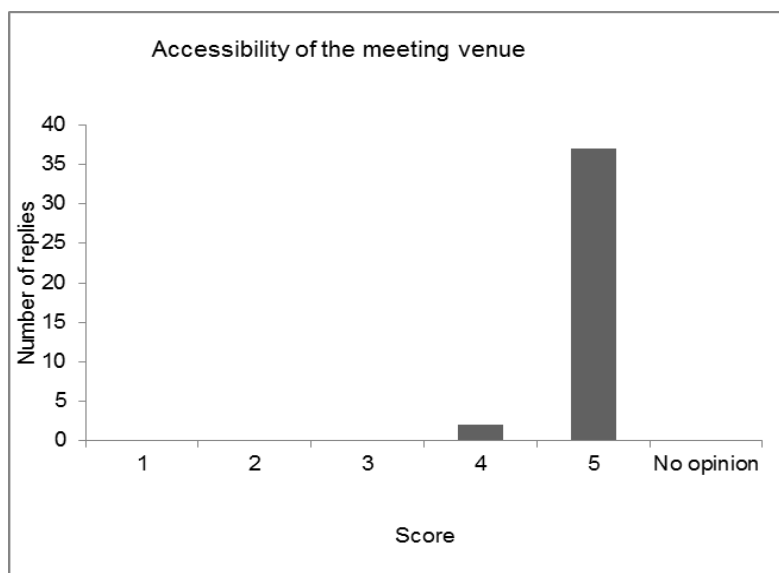


Figure 3. Scores given to question 3 'Opinion on the accessibility of the meeting venue'

4. What is your opinion of the hotel room?

The majority of the participants considered the hotel rooms good to excellent (scores 4-5, see Figure 4). The following remark was given:

- 'I have attending EURL meetings for 4 years now and this hotel is the best hotel; good room, nice hotel.'

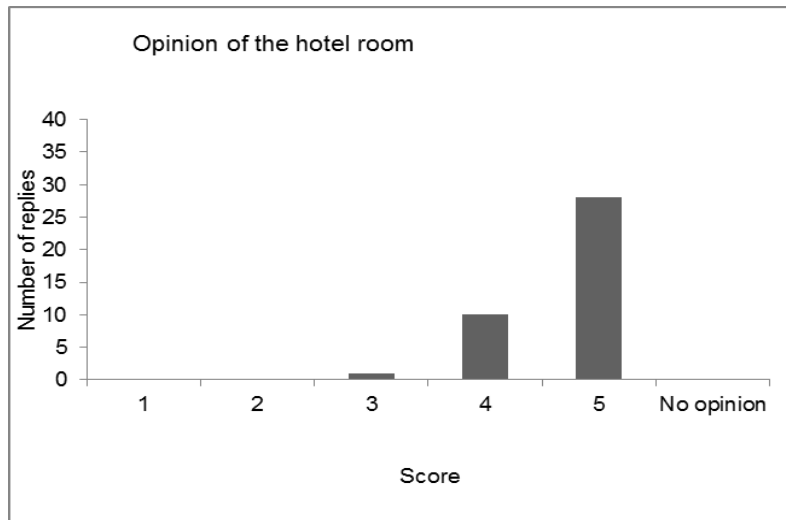


Figure 4. Scores given to question 4 'Opinion of the hotel room'

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

In general, the participants considered the meeting room as 'good' (Figure 5). Remarks given were:

- 'The air-conditioning was too strong on the first day.'
- 'The room was good and apart from few technical microphone issues, it was excellent.'

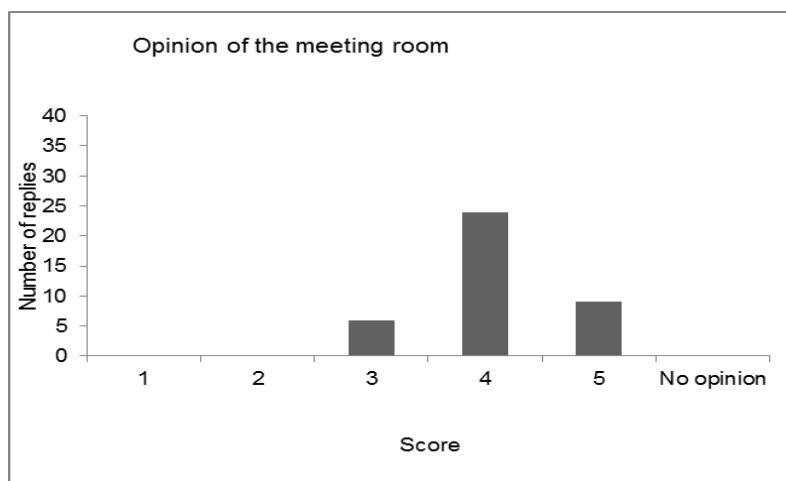


Figure 5. Scores given to question 5 'Opinion of the meeting room'

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

The readability of the presentations on the screen was on average considered as 'medium' (Figure 6). Remarks related to this question were:

- 'The information on the screen could not be seen properly from some seats, especially at the back of the room.' (5x),
- 'Classroom layout was fine, but at the back of the room it was hard to see the screen. Glad to have the hand-outs.'

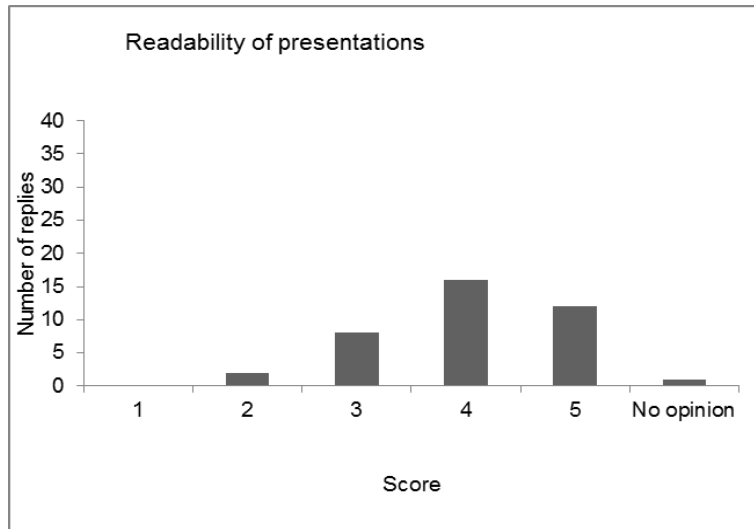


Figure 6. Scores given to question 6 'Opinion on the readability of the presentations'

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

The microphones in the meeting room frequently emitted disturbing noises, which influenced the scores on this item (Figure 7). Remarks related to this question were:

- 'Problems with the audio system were disturbing.' (9x)

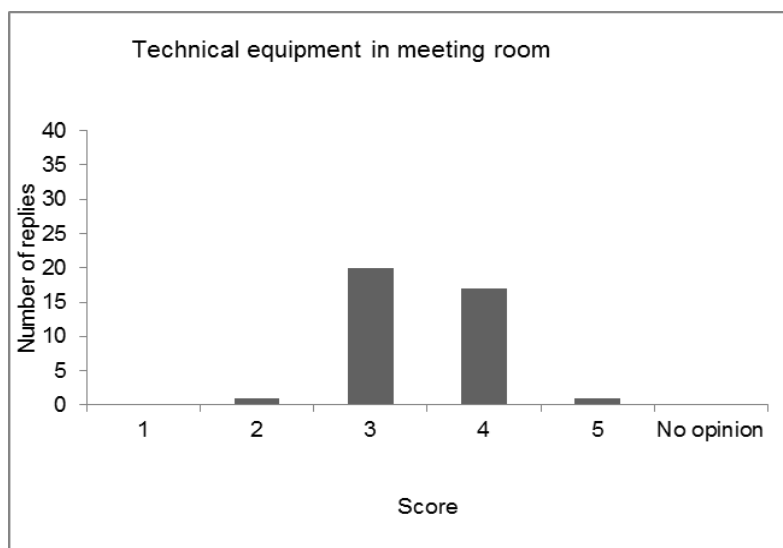


Figure 7. Scores given to question 7 'Opinion on the technical equipment'

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 to be good or excellent (scores 4–5); see Figure 8. Remarks given were:

- 'Some slow service, particularly at the dinner, but the food was lovely.'

- 'I thought this was the best breakfast and lunches in the past four years. There were plenty of healthy food options, which is rare in hotel catering. Thank you!'

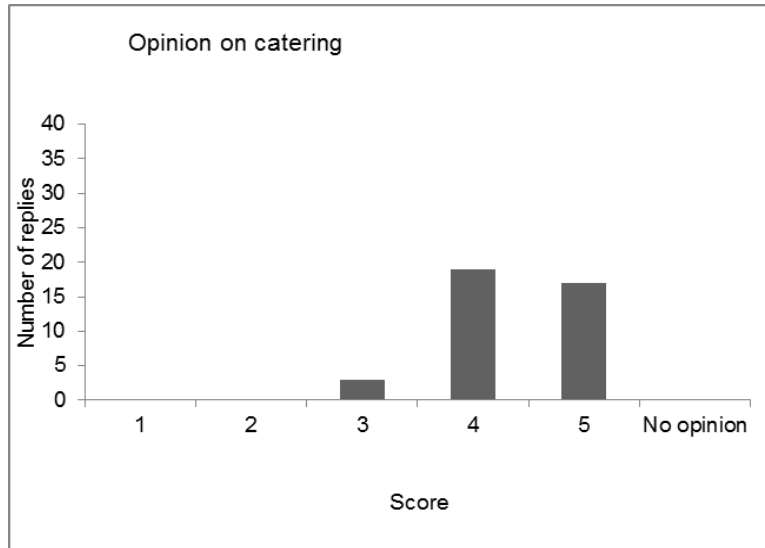


Figure 8. Scores given to question 8 'Opinion on the catering'

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:

- 'I found the meeting very useful!'
- 'The programme was fine, but I would be interested in having talks about overall *Salmonella* interventions and plans for controlling *Salmonella* in different animals.'

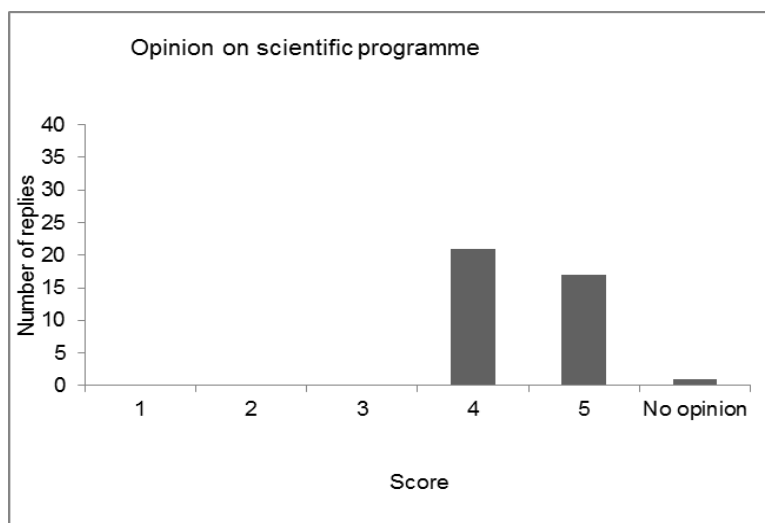


Figure 9. Scores given to question 9 'Opinion on the scientific programme'

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

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

- 'I would like to hear more about national control programmes and current challenges in poultry and pigs.'
- 'I would like to see more talks on antimicrobial resistance challenges and control methods for *Salmonella*.'
- 'I liked the "follow-up" on NRL-Italy's work with monophasic *Salmonella* Typhimurium.'
- 'From now on I am sure that molecular methods will be an issue of interest for EURL-*Salmonella*'s workshop every year.'
- 'An interesting topic was the discussion concerning the possible need for accreditation of NRLs for organising Proficiency Tests.'
- 'We had the opportunity to listen to very useful presentations about monophasic *Salmonella* Typhimurium.'
- 'The presentations on molecular typing were very interesting for me.'
- 'I would like to obtain more details on the implementation of the (EFSA) molecular typing database.'
- 'Too much information on the preliminary testing of ring trial samples. We know you work hard, but we are not interested in the details.'

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. One remark was given:

- 'It was a very good workshop. It allows us to meet people from all NRLs and to be aware of the challenges they face. It also gives the possibility to contact people in case of problems.'

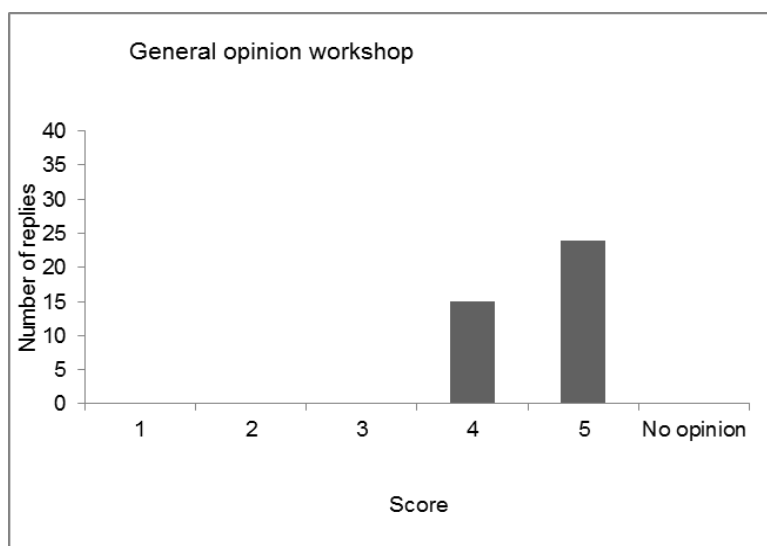


Figure 10. Scores given to question 11 'General opinion of the workshop'

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

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

- 'Thank you for this workshop and your great organisation!' (5x)

- 'I think it would be useful to ask NRLs before the meeting if they have specific areas that they would like to discuss at the workshop and somehow incorporate these questions in the programme so that we can have more interactive discussions.'
- 'To switch the focus of the middle session is a good idea. This time monophasic *Salmonella* Typhimurium, next year maybe Next Generation Sequencing or ring trials in general (tips, tricks when organising a PT).'
- 'Molecular typing seems to be very promising. It would be useful to include these methods in next year's workshop and give an overview of their applicability in accreditation.'
- 'Try to stimulate more discussions within certain topics, by maybe forming discussion groups on certain matters. Very often it seems that there are one or two questions after certain presentations.'
- 'Dust as a sample for the next interlaboratory comparison study on primary production?'
- 'I suggest that invited speakers speak first (in the morning). In that way it would be easier for the audience to focus on the issues presented, which are usually new to most people attending the workshop. EURL topics, such as Proficiency Testing results and ISO activities, are much easier topics to follow and to remain concentrated on in the afternoon.'
- 'It would be interesting to continue including information about molecular techniques (MLVA, PFGE, etc.).'
- 'I would like to have more information on rough strains.'
- 'I would like to have more information on PFGE troubleshooting.'
- 'I would like to have more information on Maldi-Tof applications and/or WGS/NGS.'
- 'More information on *Salmonella* outbreaks during the last year.'
- 'Future of the molecular typing database of EFSA and/or ECDC. Expansion to other typing methods, such as MLVA or WGS.'

4.3 Discussion and conclusions of the evaluation

In general, the participants were satisfied with the workshop. For several (general) items, scores were higher than for the 2013 workshop. However, a good organisation is not always a guarantee to be refrained from technical problems. Despite some technical problems with the microphones, the general opinion of the workshop was good to excellent.

Several participants made interesting suggestions for presentations for future workshops. These suggestions will be taken into consideration when organising the workshop of 2015 and later.

References

- Anonymous, 2002. EN ISO 6579. Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp. International Organization for Standardization, Geneva, Switzerland.
- Anonymous, 2003. EN ISO 16140. Microbiology of food and animal feeding stuffs – Protocol for the validation of alternative methods. International Organization for Standardization, Geneva, Switzerland.
- Anonymous, 2005. EN ISO/IEC 17025. General requirements for the competence of testing and calibration laboratories. International Organization for Standardization, Geneva, Switzerland.
- Anonymous, 2007. EN ISO 6579: 2002/Amd 1. Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp. – Annex D: Detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage. International Organization for Standardization, Geneva, Switzerland.
- Anonymous, 2010. CEN ISO/TS 22117. Microbiology of food and animal feeding stuffs – Specific requirements and guidance for Proficiency Testing (PT) by interlaboratory comparison. International Organization for Standardization, Geneva, Switzerland.
- Anonymous, 2011. EN ISO 16140:2003/Amd 1. Microbiology of food and animal feeding stuffs – Protocol for the validation of alternative methods. Amendment 1: Interlaboratory study on quantitative methods. International Organization for Standardization, Geneva, Switzerland.
- Anonymous, 2013. prEn ISO/DIS 6887-1. 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. International Organization for Standardization, Geneva, Switzerland.
- EC, 2003a. European Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC. Official Journal of the European Union L325: 12 December 2003. <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:325:0031:0040:EN:PDF> (accessed 02-09-2014).
- EC, 2003b. Regulation (EC) No. 2160/2003 of the European Parliament and of the Council of 17 November 2003 on the control of salmonella and other specified food-borne zoonotic agents. Official Journal of the European Union L325: 12 December 2003. <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:325:0001:0015:EN:PDF> (accessed 02-09-2014).

- EC, 2004a. European Regulation EC No. 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. Official Journal of the European Union L165: 30 April 2004. <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:165:0001:0141:EN:PDF> (accessed 15-07-2014).
- EC, 2004b. Regulation (EC) No 851/2004 of the European Parliament and of the Council of 21 April 2004 establishing a European centre for disease prevention and control. Official Journal of the European Union L142; 30 April 2004. <http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32004R0851&rid=5> (accessed 15-07-2014)
- EC, 2005. European Regulation EC No. 2073/2005 of the European Parliament and of the Council of 15 November 2005 on microbiological criteria for foodstuffs. Official Journal of the European Union L338: 22 December 2005. <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:338:0001:0026:EN:PDF> (accessed 02-09-2014).
- EC, 2011. Regulation (EU) No. 1086/2011 amending Annex II to Regulation (EC) No. 2160/2003 of the European Parliament and of the Council and Annex I to Commission Regulation (EC) No. 2073/2005 as regards *Salmonella* in fresh poultry meat. Official Journal of the European Union L281; 28 October 2011. <http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011R1086&qid=1409583296380&from=EN> (accessed 01-09-2014).
- EC, 2013. Commission Decision No. 1082/2013/EU of the European Parliament and of the Council of 22 October 2013 on serious cross-border threats to health and repealing Decision No. 2119/98/EC. Official Journal of the European Union L293: 5 November 2013. <http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32013D1082&rid=1> (accessed 15-07-2014).
- ECDC and EFSA, 2012. Multi-country outbreak of *Salmonella* Stanley infections Update. The EFSA Journal (2012);10(9): 2893, 16 pp. <http://www.efsa.europa.eu/en/efsajournal/pub/2893.htm> (accessed 15-07-2014).
- Echeita MA, Herrera S, Garaizar J and Usera MA, 2002. Multiplex PCR-based detection and identification of the most common *Salmonella* second-phase flagellar antigens. Res. Microbiol. Mar. 153(2): 107–113.
- EFSA, 2009. Scientific Opinion of the Panel on Biological Hazards on a request from European Commission on Quantitative estimation of the impact of setting a new target for the reduction of *Salmonella* in breeding hens of *Gallus gallus*. EFSA Journal 2009, 1036, 1–68. <http://www.efsa.europa.eu/en/search/doc/1036.pdf> (visited 28-08-2014).
- EFSA, 2010. Scientific Opinion on monitoring and assessment of the public health risk of '*Salmonella* Typhimurium-like' strains. EFSA Journal 2010; 8(10):1826. <http://www.efsa.europa.eu/en/efsajournal/pub/1826.htm> (accessed 15-07-2014).

- EFSA and ECDC, 2014. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2012; EFSA Journal 2014;12(2): 3547.
<http://www.efsa.europa.eu/en/efsajournal/pub/3547.htm> (accessed 15-07-2014).
- Fitzgerald C, Collins M, van Duyn S, Mikoleit M, Brown T and Fields P, 2007. Multiplex, bead-based suspension array for molecular determination of common salmonella serogroups. Journal of Clinical Microbiology 45(10): 3323–3334.
- Grimont PAD and Weill F-X, 2007. Antigenic formulae of the *Salmonella* serovars, 9th ed. WHO Collaborating Centre for Reference and Research on Salmonella. Institut Pasteur, Paris, France.
<http://www.pasteur.fr/ip/portal/action/WebdriveActionEvent/oid/01s-000036-089> (accessed 19-07-2013).
- Herrera-León S, McQuiston JR, Usera MA, Fields PI, Garaizar J and Echeita MA, 2004. Multiplex PCR for distinguishing the most common phase-1 flagellar antigens of *Salmonella* spp. J. Clin. Microbiol. 42(6): 2581–2586.
- Herrera-León S, Ramiro R, Arroyo M, Díez R, Usera MA and Echeita MA, 2007. Blind comparison of traditional serotyping with three multiplex PCRs for the identification of *Salmonella* serotypes. Res. Microbiol. 158(2): 122–127.
- Hong Y, Liu T, Lee MD, Hofacre CL, Maier M, White DG, Ayers S, Wang L, Berghaus R and Maurer JJ, 2008. Rapid screening of *Salmonella enterica* serovars Enteritidis, Hadar, Heidelberg and Typhimurium using a serologically correlative allelotyping PCR targeting the O and H antigen alleles. BMC Microbiol. 9(8): 178.
- Jacobs-Reitsma WF, Maas HME, de Pinna E and Mooijman KA, 2014. Interim summary report on the 18th EURL-*Salmonella* interlaboratory comparison study (2013) on typing of *Salmonella* spp.
<http://www.euralsalmonella.eu/dsresource?type=pdf&disposition=inline&objectid=rivmp:238245&versionid=&subobjectname=> (accessed 05-09-2014).
- Kuijpers AFA and Mooijman KA, 2013. Interim summary report EURL-*Salmonella* Interlaboratory Comparison study FOOD VI (2013). Detection of *Salmonella* in minced chicken meat. National Institute for Public Health and the Environment, Bilthoven, The Netherlands. RIVM Letter Report 130/2013 cZ&O ku/ak.
<http://www.euralsalmonella.eu/dsresource?type=pdf&disposition=inline&objectid=rivmp:228330&versionid=&subobjectname=> (accessed 05-09-2014)
- Kuijpers AFA and Mooijman KA, 2014. Interim summary report EURL-*Salmonella* Interlaboratory Comparison study Primary Production XVII (2014) on detection of *Salmonella* in chicken faeces. National Institute for Public Health and the Environment, Bilthoven, The Netherlands. RIVM Letter Report 045/2014 Z&O ku/ak.
<http://www.euralsalmonella.eu/dsresource?type=pdf&disposition=inline&objectid=rivmp:247023&versionid=&subobjectname=> (accessed 05-09-2014).

- McQuiston JR, Waters RJ, Dinsmore BA, Mikoleit ML and Fields PI, 2011.
Molecular determination of H antigens of *Salmonella* by use of a microsphere-based liquid array. *Journal of Clinical Microbiology* 49(2): 565–573.
- Tennant SM, Diallo S, Levy H, Livio S, Sow SO, Tapia M, Fields PI, Mikoleit M, Tamboura B, Kotloff KL, Nataro JP, Galen JE and Levine MM, 2010.
Identification by PCR of non-typhoidal *Salmonella* enterica serovars associated with invasive infections among febrile patients in Mali. *PLoS. Negl. Trop. Dis.* 4, e621.

Acknowledgements

The author would like to thank Elizabeth de Pinna of Public Health England (PHE), London, England for making clear notes during the workshop.

List of abbreviations

A	Answer
ADNS	Animal Disease Notification System
BGA	Brilliant Green Agar
BPW	Buffered Peptone Water
CEN	European Committee for Standardization
CDC	Centre for Disease Control and Prevention
cfu	colony forming units
DG-Sanco	Directorate General for Health and Consumer Protection
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
EQA	External Quality Assessment
EU	European Union
EURL	European Union Reference Laboratory
EWRS	Early Warning Response System
FDIS	Final Draft International Standard
FYROM	Former Yugoslav Republic of Macedonia
ISO	International Organization for Standardization
MKTTn	Mueller Kauffmann Tetrathionate broth with novobiocin
MLST	Multi-Locus Sequence Typing
MLVA	Multi-Locus Variable number of tandem repeats Analysis
MPN	Most Probable Number
MS	Member State
MSRV	Modified Semi-solid Rappaport Vassiliadis
NGS	Next Generation Sequencing
NRL	National Reference Laboratory
NWIP	New Work Item Proposal
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
PHE	Public Health England
PPS	Primary Production Stage
PT	Proficiency Test
Q	Question
RASFF	Rapid Alert System for Food and Feed
RIVM	National Institute for Public Health and the Environment
RVS	Rappaport Vassiliadis broth with Soya
SC	Sub-Committee
SI(10)	<i>Salmonella</i> Infantis (at a level of approximately 10 cfu)
SSI	Statens Serum Institute
STEC	Shiga toxin-producing <i>Escherichia coli</i>
STM(10)	<i>Salmonella</i> 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
XLD

World Health Organisation
Xylose Lysine Deoxycholate

Annex 1 Participants

European Food Safety Authority (EFSA)	Frank Boelaert
EC DG-Sanco	Klaus Kostenzer
EURL– <i>Salmonella</i>	Kirsten Mooijman Angelina Kuijpers Wilma Jacobs Wendy van Overbeek
Guest speaker (the Netherlands)	Max Heck (RIVM, Bilthoven)
Guest speaker (Germany)	Sven Maurischat (BFR, Berlin)
Guest speaker (Italy)	Lisa Barco (IZSVe, Legnaro)
Guest speaker (United Kingdom)	Elizabeth de Pinna (PHE, London)

National Reference Laboratories for *Salmonella*

AUSTRIA	Heimo Lassnig
BELGIUM	Katelijne Dierick Vicky Jasson
BOSNIA AND 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 Satu Hakola
FRANCE	Renaud Lailier
FYROM	-
GERMANY	Istvan Szabo
GREECE	Aphrodite Smpiraki
HUNGARY	Erzsebet Adrian
ICELAND	Franklin Georgsson
IRELAND	Rosemarie Slowey
ITALY	Antonia Ricci
LATVIA	Daina Camane
LITHUANIA	Tatjana Kutyrivova
LUXEMBOURG	-
MALTA	Albert Gambin
NETHERLANDS	Anjo Verbruggen Henny Maas Sjoerd Kuiling
NORTHERN IRELAND	Gintare Bagdonaite
NORWAY	Bjarne Bergsjø
POLAND	Magdalena Skarzynska Kinga Wiczorek

PORTUGAL
ROMANIA
SERBIA

SLOVAK REPUBLIC
SLOVENIA
SPAIN
SWEDEN
SWITZERLAND
TURKEY
UNITED KINGDOM

Patricia Themudo
Alexandra Tutoveanu
Zivka Ilic
Oliver Radanovic
Milan Sasik
Jasna Micunovic
Maria Christina de Frutos Escobar
Lennart Melin
-
Derya Karatas Yeni
Shona Neal
Nicola Elviss

Annex 2 Programme of the workshop

Programme of the EURL-*Salmonella* workshop XIX 26 and 27 May 2014, Zaandam, the Netherlands

General information

Place of accommodation and meeting venue:

Inntel hotel Zaandam
Provincialeweg 102
1506 MD Zaandam
Tel: +31 (0)75 631 1711
<http://www.inntelhotelsamsterdamzaandam.nl/>

Information for participants 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 2014**. 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 **at latest on 6 June 2014**

Sunday 25 May 2014

17.00 – 18.00 Registration in the lobby of hotel Inntel Zaandam

- Final information concerning the programme
- Administrative aspects

Dinner information

For participants for whom the costs of travel and stay are paid from the budget of EURL-*Salmonella*, the EURL will also cover the expenses of a dinner on Sunday 25 May, with a maximum of € 30,- per person. A receipt will be needed in order to be able to reimburse you for this meal.

Monday 26 May 2014

08:30–09:00 Registration

Morning chair: Wilma Jacobs

09:00–09:30	Opening and introduction	Kirsten Mooijman, EURL- <i>Salmonella</i>
09:30–10:00	EU <i>Salmonella</i> monitoring data (Summary report 2012)	Frank Boelaert, EFSA
10:00–10:30	Looking at enhanced crisis preparedness and early detection of outbreaks in the EU	Klaus Kostenzer, EC DG-Sanco
10:30–11:00	<i>Coffee/tea</i>	
11:00–11:30	Results of the interlaboratory comparison study on detection of <i>Salmonella</i> in food VI (2013)	Angelina Kuijpers, EURL- <i>Salmonella</i>
11:30–12:00	Activities in ISO and CEN	Kirsten Mooijman, EURL- <i>Salmonella</i>
12:00–13:15	<i>Lunch</i>	

Afternoon chair: Kirsten Mooijman

13:15–13:45	Preliminary results of the interlaboratory comparison study on detection of <i>Salmonella</i> in chicken faeces–PPS XVII (2014)	Angelina Kuijpers, EURL- <i>Salmonella</i>
13:45–14:15	Activities of EFSA concerning molecular typing data collection from food and animals	Frank Boelaert, EFSA
14:15–14:45	Molecular serotyping of <i>Salmonella</i> – Experiences of reference laboratory the Netherlands	Max Heck, the Netherlands
14:45–15:15	Molecular serotyping of <i>Salmonella</i> – Experiences NRL- <i>Salmonella</i> in Denmark using the CDC-method	Gitte Sørensen, Denmark
15:15–15:45	<i>Coffee/tea</i>	
15:45–16:15	Rapid detection and specific differentiation of <i>Salmonella</i> Enteritidis, <i>Salmonella</i> Typhimurium and its monophasic variant by multiplex real-time PCR	Sven Maurischat, Germany
16:15–16:45	Overview of the recent activities carried out by the Italian NRL related to <i>S.</i> 4,[5],12:i:-	Lisa Barco, Italy
16:45–17:15	<i>Salmonella</i> Typhimurium monophasic variant surveillance in France: molecular characterisation and guidelines from ANSES experts	Renaud Lailler, France
19:00–	<i>Dinner at hotel Inntel</i>	

Tuesday 27 May 2014

Morning chair: Kirsten Mooijman

- 09:00–10:15 Activities of the NRL-*Salmonella* to fulfill tasks and duties in (15 min. each):
Austria (Heimo Lassnig)
Bosnia and Herzegovina (Sead Hadziabdic)
United Kingdom (Shona Neal)
Poland (Magdalena Skarzynska, Kinga Wieczorek)
Germany (Istvan Szabo)
- 10:15–10:45 *Coffee/tea*
- 10:45–11:30 Results of the interlaboratory comparison study on typing of *Salmonella* XVIII (2013) – serotyping and PFGE Wilma Jacobs, EURL-*Salmonella*
- 11:30–12:00 Results of the interlaboratory comparison study on typing of *Salmonella* XVIII (2013) – phage typing Elizabeth de Pinna, PHE, UK
- 12:00–12:30 Work programme EURL-*Salmonella* (second half 2014, first half 2015), discussion on general items and closure Kirsten Mooijman, EURL-*Salmonella*
- 12:30–13:30 *Lunch*

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

Annex 3 Evaluation form of the workshop

**Evaluation of the XIXth EURL-*Salmonella* workshop
26 and 27 May 2014, Zaandam, the Netherlands**

We would highly appreciate if you could give us your opinion on the 19th EURL-*Salmonella* workshop, held in Zaandam, the Netherlands on 26 and 27 May 2014. 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?

Thank you very much!

RIVM

Committed to health and sustainability