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

The 17th EURL-Salmonella workshop

14 and 15 May 2012, Chalkida, Greece

RIVM report 330604026/2012

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Colophon

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Abstract

The 17th EURL-*Salmonella* workshop
14 and 15 May 2012, Chalkida, Greece

This report contains the summaries of the presentations of the 17th annual workshop for the National Reference Laboratories (NRLs) for *Salmonella*, held in Chalkida, Greece on 14 and 15 May 2012. 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 NRL laboratories. Another yearly item is the presentation of the most recent European summary report on Zoonoses by the European Food Safety Authority (EFSA). This latter report gives an overview on the number and types of zoonotic microorganisms causing health problems in Europe in 2010. For several years the number of health problems caused by *Salmonella* is decreasing, but in 2010 it is still the second most important cause, after *Campylobacter*, of zoonotic diseases in Europe.

Another presentation gives an overview on the different methodologies for typing of *Salmonella*. The ('classical') golden standards are described, as well as new (molecular) alternatives. The conclusion is that still not one alternative method is available which can replace one golden standard method.

In other summaries, the NRLs for *Salmonella* of five selected countries describe their activities, for example on how they organise ring trials in their countries. Furthermore, the EURL-*Salmonella* gives information on international standardisation of methods for detection, enumeration and typing of *Salmonella.* Two other presentations give information on the validation of a new as well as of a 'traditional' method.

The workshop was organised by the EURL-*Salmonella*, with the help of the NRL-*Salmonella* in Greece. The EURL-*Salmonella*, formerly called Community Reference Laboratory for Salmonella (CRL-*Salmonella*), 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 of *Salmonella* in different products.

Keywords:
EURL-*Salmonella*, NRL-*Salmonella*, *Salmonella*, workshop 2012

Rapport in het kort

De zeventiende EURL-*Salmonella* workshop
14 en 15 mei 2012, Chalkida, Griekenland

In dit rapport zijn de verslagen gebundeld van de presentaties die op 14 en 15 mei 2012 zijn gegeven tijdens de zeventiende jaarlijkse workshop voor de Europese Nationale Referentie Laboratoria (NRL's) voor de bacterie *Salmonella*. Het doel van de workshop is dat het overkoepelende orgaan, het Europese Referentie Laboratorium (EURL) *Salmonella*, en de NRL's informatie met elkaar kunnen uitwisselen. Daarnaast worden de resultaten gepresenteerd van de ringonderzoeken van het EURL waarmee de kwaliteit van de NRL-laboratoria wordt gemeten. Een uitgebreidere weergave van de resultaten worden per ringonderzoek in aparte RIVM-rapporten opgenomen.

Campylobacter en Salmonella belangrijkste veroorzakers zoönosen
Een terugkerend onderwerp is het rapport van de European Food Safety Authority (EFSA) over zoönosen, oftewel ziekten die van dieren op mensen kunnen overgaan. Dit verslag bevat een overzicht van de aantallen en types zoönotische micro-organismen die in 2010 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.

Een ander verslag geeft een overzicht van de verschillende methoden waarmee *Salmonella* kan worden getypeerd. Zowel de ('klassieke') gouden standaards als de nieuwe (moleculaire) methoden worden besproken. Hieruit blijkt dat er nog niet één nieuwe methode voor handen is die een 'gouden standaard' methode kan vervangen.

Internationale standaardisering van methoden
Verder beschrijven de laboratoria van vijf geselecteerde landen hoe ze hun taak als NRL invullen, bijvoorbeeld over de wijze waarop zij ringonderzoeken in eigen land organiseren. Daarnaast geeft het EURL-*Salmonella* informatie over het proces waarmee de methoden om *Salmonella* respectievelijk op te sporen, te tellen en te typeren, wordt gestandaardiseerd op internationaal niveau. Twee andere verslagen bevatten informatie over de validaties van zowel een nieuwe als een traditionele methode.

De organisatie van de workshop is in handen van het EURL voor *Salmonella*, voorheen Community Reference Laboratory (CRL), dat onderdeel is van het RIVM. Bij de organisatie van de huidige workshop kreeg het EURL hulp van het NRL in Griekenland. 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 2012

Contents

Summary

On 14 and 15 May 2012, the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*) organised her annual workshop in Chalkida, Greece, with the help of the local National Reference Laboratory (NRL) for *Salmonella*. On both days representatives of the NRLs-*Salmonella* from the different European countries were present, as well as representatives of the European Food Safety Authority (EFSA) and several guest speakers. A total of 48 participants were present at the two-day workshop.

The programme of the workshop consisted of several parts.
During the morning session of the first day, presentations were given by EFSA on trends and sources of Zoonoses in Europe, on the estimation of the relative contribution of different food and animal sources to human *Salmonella* infections in the EU and on factors associated with *Salmonella* breeding pigs positivity. Furthermore, information was given on the progress with the standardisation of methods on detection, enumeration and typing of *Salmonella* at international (ISO) and at European (CEN) level. Also the results of the interlaboratory comparison study on detection of *Salmonella* in a matrix from primary production as performed in 2012 were presented.
During the afternoon session of the first day, the results of the interlaboratory comparison study on serotyping and phage typing of *Salmonella* (2011) were discussed, as well as a proposal for the typing study of 2012. The day was closed with presentations of two guest speakers. In one presentation an overview on different methodologies for *Salmonella* typing was given. The ('classical') gold standards as well as the (molecular) alternatives were presented. In the second presentation information was given on the occurrence of the multiresistant monophasic variant of *Salmonella* Typhimurium 1,4,[5],12:i:- in Greece, in the period 2006 to 2011.

During the morning session of the second day of the workshop, five NRLs for *Salmonella* gave presentations, explaining their activities to fulfil the task and duties of an NRL. Furthermore, the results of the interlaboratory comparison study on detection of *Salmonella* in a food matrix as performed in 2011 were presented. The morning session was finished with a discussion on future interlaboratory comparison studies on detection of *Salmonella* in different matrices, with a focus on the study to be organised in September/October 2012.
During the afternoon session of the second day, the validation of an alternative method through MicroVal was presented, as well as the validation of a 'traditional' method through the CEN mandate project. It was suggested to combine the latter study with the EURL-*Salmonella* study on detection of *Salmonella* in samples from primary production as to be organised early 2013.
The workshop was finished with a presentation on the work programme of the EURL-*Salmonella* for the next year.

All presentations given at the workshop can be found at:
http://www.rivm.nl/crlsalmonella/workshops/WorkshopXVII.jsp

1 Introduction

In this report, the abstracts of the presentations given at the EURL-*Salmonella* workshop of 2012 are presented as well as a summary of the discussion that followed the presentations. The full presentations are not provided within this report, but are available at the website of the EURL-*Salmonella* (formerly called CRL-*Salmonella*):
http://www.rivm.nl/crlsalmonella/workshops/WorkshopXVII.jsp

The lay-out of the report is consistent with the programme of the workshop.
All abstracts of the presentations of the first day are given in chapter 2.
All abstracts of the presentations of the second day are given in chapter 3.
The evaluation of the workshop is summarised in chapter 4.
The list of participants is given in Annex 1.
The programme of the workshop is given in Annex 2.

2 Monday 14 May 2012: 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 17th workshop of the EURL-*Salmonella*, welcoming all participants in Chalkida, Greece. This was the first time the EURL-*Salmonella* workshop was organised in Greece. The workshop could not have been organised without the help of the local NRL for *Salmonella.* Therefore, this NRL of Greece is very much thanked for their great help.
From the EU Member States excuses were received from the NRL of Malta. Furthermore, excuses were received from the NRLs of Iceland, Switzerland and Serbia. The EC, DG-Sanco was neither able to send a representative to the workshop.
After a roll call of the delegates, the results of the evaluation of the workshop of 2011 were presented. Remarks on the workshop of 2011 were, as much as possible, taken into account in trying to further improve the current workshop. Examples for this were: organisation of the workshop at another location, more microphones, different seating in the meeting room (U-shape instead of in rows) and hand-outs for most of the presentations. The results of the complete evaluation have been reported in the report on the workshop of 2011.
Next, information was given on the changes at the EURL and other informative aspects:

- Because of the new name (EURL-*Salmonella* instead of CRL-*Salmonella*) a new logo has been designed and was presented at the workshop for the first time. Furthermore, the website of the 'CRL-*Salmonella*' is under revision and it will also get a new name in the near future (www.eurlsalmonella.eu instead of www.rivm.nl/crlsalmonella). As soon as the amended website under its new name is functioning, the NRLs will be informed.
- By the end of 2010 the EURL had sent a manuscript entitled 'Detection of *Salmonella* in food, feed and veterinary samples by EU laboratories' (by Kuijpers and Mooijman) to the Journal 'Food Research International'. In April 2011 the manuscript was accepted and it took almost another year before the manuscript was published. It has finally been published in a special edition on *Salmonella* of the journal in March 2012.
- The EURL-*Salmonella* has been involved in the revision of a chapter on 'culture media for the isolation of *Salmonella*', which has been published as chapter 13 in the third edition of the Handbook of Culture media for food and water microbiology in January 2012 (Eds: J.E.L. Corry, G.D.W. Curtis and R.M. Baird. 2012. ISBN 9781847559166).
- In September 2011 a new EC Regulation (926) on the financial aid of the EURLs was published (EC, 2011). An important change in this Regulation is the fact that it is now possible to request a budget to reimburse the costs of a limited number of representatives of 'third countries' for participation in a EURL workshop. This makes it easier for e.g. candidate EU countries to participate in these workshops as well.

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 2010)

Frank Boelaert, EFSA, Parma, Italy

In 2010, salmonellosis was again the second most commonly reported zoonotic disease in humans in the EU, following campylobacteriosis (EFSA, 2012a). The number of salmonellosis cases in humans decreased by 8.8% compared to 2009, and the statistically significant decreasing trend in the European Union continued for the sixth consecutive year. In total, 99020 confirmed human cases were reported in 2010. *Salmonella* Enteritidis and *Salmonella* Typhimurium continued to be the most frequently reported *Salmonella* serovars in human cases. The overall decrease in salmonellosis is mostly attributed to the *Salmonella* Enteritidis serovar, which continued to decline for the fifth consecutive year. The reporting of *Salmonella* Typhimurium cases has also decreased but not to the same extent as *Salmonella* Enteritidis.
The continuing decrease in the numbers of salmonellosis cases in humans is likely to be mainly related to the successful *Salmonella* control programmes in poultry populations, particularly in laying hens. The majority of Member States (MSs) met their *Salmonella* reduction targets for laying hens, broilers, turkeys and breeding flocks in 2010, and the prevalence of the target serovars is clearly declining at EU level. A positive finding was that all except one reporting MS already met the targets set for turkey flocks, even though 2010 was the first year of implementation of these mandatory control programmes. All these results indicate that MSs have invested in *Salmonella* control and this work is giving positive results.
Reports on food-borne outbreaks caused by *Salmonella* within the EU have also shown a reduction in numbers, and there was a further decline in numbers of Salmonella food-borne outbreaks caused by eggs and egg products. However, the food-borne outbreak data still show that eggs are clearly the most important cause of food-borne *Salmonella* outbreaks. Other important sources of food-borne *Salmonella* outbreaks in 2010 were mixed and buffet meals, broiler meat, pig meat and bovine meat.
These results concur well with the latest source attribution estimation by the BIOHAZ Panel (EFSA, 2011a), according to which of all human salmonellosis cases in the EU (i.e. estimated true number of cases when accounting for underreporting) approximately 65% were attributed to laying hens (eggs) and 28%, 4.5% and 2.4% to pigs, turkeys and broilers, respectively. Furthermore, an external scientific report (EFSA, 2011d) which used serotyping data to investigate source attribution of human salmonellosis cases and used data from EU baseline surveys and EU Summary Reports estimated that the laying hen reservoir is the most important source in the EU, contributing to 43.8% of human cases, followed by 26.9% of cases attributed to pigs. Together 4.0% and 3.4% of human cases were attributed to turkeys and broilers.
An interesting development in 2010 was that the monophasic *Salmonella* Typhimurium appeared in fourth place on the top ten list of the most commonly reported serovars in human cases. These strains were also often detected in pigs, cattle and pig and bovine meat, but less often in poultry. The BIOHAZ Panel concluded in its recent opinion (EFSA, 2010) that monophasic *Salmonella* 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 recently agreed reporting guidelines for these strains may have partly contributed to these increased reports in 2010.
As regards findings in food, products in non-compliance with the European Union *Salmonella* criteria were mainly observed in minced meat and meat preparations

as well as live bivalve molluscs. *Salmonella* was most often detected in fresh broiler and turkey meat. Some decrease in the occurrence of *Salmonella* was apparent in products derived from poultry meat and table eggs. Otherwise no major developments in occurrence were observed compared with previous years.
Following a request from the European Commission, the Panels on Biological Hazards (BIOHAZ), on Contaminants in the Food Chain (CONTAM) and on Animal Health and Welfare (AHAW) were asked to deliver a series of Scientific Opinions on the public health hazards (biological and chemical) to be covered by inspection of meat for several animal species; the first scientific opinion dealt with swine (EFSA, 2011b). *Salmonella* was deemed to be of high relevance at present in the EU and one of the most relevant biological hazards in the context of meat inspection of swine, alongside *Yersinia enterocolitica*, *Toxoplasma gondii* and *Trichinella* spp.

Discussion
Q: From your presentation it can be seen that the human cases caused by *Campylobacter* are increasing and that the cases caused by *Salmonella* are decreasing. On the other hand more outbreaks are caused by *Salmonella*, how can it still be the case that more infections are caused by *Campylobacter*?
A: This report gives only a descriptive overview. The results are now worked out further to find the reasons. It is also questioned whether this is really an increase, or whether it is a result of the reporting system. Furthermore, most of the *Campylobacter* cases are sporadic cases. The transmission of *Campylobacter* is different from the transmission of *Salmonella*.
Q: Will the EC introduce a programme in the EU to reduce the number of *Campylobacter* cases in humans?
A: The EC is still discussing what should be done in relation to *Campylobacter* (at primary level and/or at processing level). No decision has been taken yet.
Q: What is meant with strong evidence outbreaks?
A: This means that the Member State has a strong knowledge on the source of the outbreak. If a MS considers to have strong evidence, this is taken over by EFSA, although this may not always be the same of what EFSA considers as strong evidence.
Q: I would have expected an increase in the contribution of pig products in relation to the *Salmonella* cases, but this is not confirmed by the zoonoses report, in which eggs and egg products are still the major source of *Salmonella* infections.
A: The food-borne outbreak data are biased by Member States who have a 'good' and extensive surveillance system (like for example France).

2.3 ISO and CEN activities for *Salmonella*

*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 with *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 organise their plenary meetings in Brussels, Belgium from 25 to 29 June 2012. The progress on the *Salmonella* documents will be presented at these meetings by Kirsten Mooijman.

For *Salmonella* three procedures are under revision or preparation in CEN and ISO. For this EN ISO 6579 (Anonymous, 2002) will be separated into three parts, being:
Microbiology of food and animal feed – Horizontal method for the detection, enumeration and serotyping of *Salmonella* –
Part 1: Horizontal method for the detection of *Salmonella*;
Part 2: Enumeration by a miniaturized Most Probable Number technique;
Part 3: Guidelines for serotyping of *Salmonella* spp.

The work for the three items is performed in three different working groups, of which Kirsten Mooijman is project leader.
The progress of the work with the three documents since May 2011 was explained to the NRLs.

EN ISO 6579-1: Detection of Salmonella
- May 2011: Second working draft prepared by Kirsten and sent to the members of ISO working group (WG9) for further comments.
- May 2011: work moved from ISO to CEN, because of the fact that part of the work of EN ISO 6579, being the validation of the method for samples of primary production (Annex D of EN ISO 6579, Anonymous, 2007) falls under the so-called 'CEN mandate' (more information on the CEN mandate is given in 3.9).
- June 2011: Progress reported at the meeting of CEN/TC275/WG6 in Bournemouth, UK.
- September 2011 – February 2012: two updates of the draft document prepared after receiving comments from the members of CEN TAG-*Salmonella*.
- 8 February – 8 April 2012: informal voting launched of prEN 6579-1 in ISO/TC34/SC9 and CEN/TC275/WG6. The document was also sent for comments to the NRLs-*Salmonella.*
- 18 April 2012: results of voting received: positive with some comments.
- 5 June 2012: meeting of CEN TAG-*Salmonella* in Paris, France, to discuss the comments and update the document.

The main changes in EN ISO 6579 part 1 compared to the version of 2002 were presented at the workshop of 2011 and were again shown at the current workshop.

EN ISO/TS 6579-2: Enumeration of Salmonella
- May 2011: Final vote still not launched due to administrative problems at CEN level (the final draft version was sent to ISO/TC34/SC9 already in February 2010).
- June – August 2011: administrative problems solved.
- September 2011 – January 2012: Translation of the document in French and German.
- 14 March – 15 June 2012: voting launched of pr ISO/TS 6579-2 in ISO/TC34/SC9 and CEN/TC275/WG6. The document was also sent for comments to the NRLs-*Salmonella.*

EN ISO/TR 6579-3: Serotyping of Salmonella

- April 2011: Kirsten Mooijman made the second working draft and sent it to the members of the ad hoc group for comments.
- June 2011: Progress reported at the meeting of ISO/TC34/SC9 in Bournemouth, UK.
- June 2011: The ad hoc group received the 'official status' as an ISO working group, being WG10.
- September – December 2011: three updates of the draft document prepared after receiving comments from ISO WG10.
- 13 December 2011 – 15 March 2012: voting launched on the New Work Item Proposal (NWIP) of ISO/TR 6579-3 in ISO/TC34/SC9 and CEN/TC275/WG6. The document was also sent for comments to the NRLs-*Salmonella.*
- 20 March 2012: results of voting received: positive with some comments.
- 20 April 2012: meeting of ISO WG10 in Paris, France, to discuss the comments and update the document.

Pooling of samples

EU Regulation No 2073/2005 (EC, 2005) prescribes the absence of *Salmonella* in poultry meat. According to the (new) rules this concerns absence of *S.* Typhimurium (including 'monophasic *S.* Typhimurium' 1,4,[5],12:i:-) and *S.* Enteritidis in five samples of 25 g fresh poultry meat (chicken and turkey). Several requests were made by EU Member States (to DG-Sanco) whether the five samples could be pooled instead of analysing them individually. However, information on the effect of pooling poultry meat samples on the sensitivity of the detection of *Salmonella* is not available in the literature. Therefore, an experimental design was set up to test this at the laboratory of the EURL-*Salmonella*. The testing started in 2011 and was finished early 2012. The statistical analyses on the results still need to be performed.

The experiments were based on a draft protocol for pooling of samples of the ISO working group on statistics. In this protocol two ways of pooling are described: dry pooling (pooling of sample units) and wet pooling (pooling of pre-enriched cultures). Both ways of pooling were included in the experimental design of the EURL. For dry pooling 25 g of meat was inoculated with a stressed *Salmonella* strain at a level of approximately 5 colony forming units (cfu) per 25 g. This sample was mixed with 4 x 25 g *Salmonella*-free meat and the 125 g pooled meat sample was added to 1125 ml Buffered Peptone Water (BPW) and incubated at 37 °C ± 1 °C for 18 h ± 2 h. Next, the procedures as described in ISO 6579 (Anonymous, 2002) and in Annex D of ISO 6579 (Anonymous, 2007) were followed. For the wet pooling also 25 g of meat was inoculated with a stressed *Salmonella* strain at a level of approximately 5 cfu per 25 g, but this was added to 225 ml BPW. Furthermore, four samples of 25 g of *Salmonella*-free meat were each added to 225 ml BPW. The BPW samples were incubated at 37 °C, like for the dry pooling. After incubation, 5 ml was taken from each BPW culture and mixed. From this mixture 0.5 ml was added to 50 ml Rappaport Vassiliadis broth with Soya (RVS), 5 ml was added to 50 ml Mueller Kauffmann Tetrathionate broth with novobiocin (MKTTn) and 0.1 ml was added in three drops to a plate of Modified semi-solid Rappaport Vassiliadis (MSRV) agar. Next the procedures as described in ISO 6579 (Anonymous, 2002) and in Annex D of ISO 6579 (Anonymous, 2007) were followed. As a control, the inoculated sample of 25 g was also tested in the 'normal' way for the detection of *Salmonella* by following ISO 6579 and Annex D of ISO 6579. In the design two strains of three serovars (*Salmonella* Enteritidis, *Salmonella* Typhimurium and *Salmonella* 1,4,[5],12:i:-) were tested with three different types of stress in duplicate (cold, freezing, heating) on four types of poultry meat (chicken and

turkey meat with and without skin). At least six different samples of each type of meat were tested.
Although the results still need to be statistically analysed, a short summary of the results can be given:
The highest amount of stress to the different *Salmonella* strains was caused by heating at 50 °C for 15 minutes. The lowest amount of stress was caused by storage at 4 °C, even if this was done for several weeks (tested for up to eight weeks of storage).
The amount of background flora (total number of aerobic bacteria and the number of *Enterobacteriaceae*) varied per type of matrix, but was in general higher for the poultry meat with skin. A relation seemed to exist between the number of positive samples and the amount of background flora. The higher the amount of background flora, the more samples were tested negative for *Salmonella*.
For the effect of pooling on the sensitivity of the method, it was seen that with MSRV the highest number of samples were found positive, for the wet pooling as well as for the dry pooling. The number of positives found after pooling were close to the number of positives found without pooling (control samples). The lowest number of positive samples was found with RVS in combination with dry pooling. Whether the differences were significant still needs to be statistically analysed.

Discussion
Q: Will there be any guidance in the amended ISO 6579 on the sampling and detection of *Salmonella* Gallinarum (biovars Gallinarum and Pullorum)?
A: This information will be limited as *Salmonella* Gallinarum is related to animal health and not to human health and therefore not part of the work of CEN/TC275/WG6 or of ISO/TC34/SC9. Only in the new Annex D (on detection of *Salmonella* Typhi and *Salmonella* Paratyphi) an informative note is added, indicating that the procedure as described in Annex D (additional selective enrichment in Selenite Cystine broth) may be a good choice for the detection of *Salmonella* Gallinarum as well. For more details on methods for (sampling and) detection of *Salmonella* Gallinarum reference needs to be made to the World Organisation for Animal Health (OIE).
Q: Do you know when the new White-Kauffmann-Le Minor scheme will be published?
A: No, we have not yet received any information about this. The EURL-*Salmonella* will ask the WHO reference centre in Paris about the status of the new document.

2.4 Information from EFSA

Frank Boelaert, EFSA, Parma, Italy

2.4.1 Estimation of the relative contribution of different food and animal sources to human Salmonella *infections in the EU*

Recently, the quantitative contribution of different food-animal sources to human *Salmonella* infections in the European Union was estimated. The most recent source attribution estimation by EFSA's BIOHAZ Panel (EFSA, 2012b), using data from 2010 and before, was based on microbial-subtyping. Data from 25 EU Member States were included, four food-animal sources of *Salmonella* (broilers, laying hens, turkeys and pigs) and 23 *Salmonella* serovars. The model, named 'Turkey Target *Salmonella* Attribution Model' (TT-SAM model), employed 2010 EU harmonised statutory monitoring data (EFSA, 2012a) on *Salmonella* in

animal populations (2006-2007 EU baseline survey data for slaughter pigs), data on reported 2010 cases of human salmonellosis for both sporadic and outbreak-related cases as provided by the European Centre for Disease Prevention and Control (ECDC), and food availability data. Of all human salmonellosis cases in the EU (i.e. estimated true number of cases when accounting for underreporting) 2.6%, 10.6%, 17.0% and 56.8% were attributable to turkeys, broilers, laying hens (eggs) and pigs, respectively.
In 2011, EFSA's BIOHAZ Panel (EFSA, 2011a), using the microbial-subtyping approach and data from 2009 and before, found that approximately 4.5%, 2.4%, 65% and 28% were attributable to turkeys, broilers, laying hens (eggs) and pigs, respectively. This model, named 'Broiler Target *Salmonella* Attribution Model' (BT-SAM model), considered 22 Member States, the four animal-food sources of *Salmonella* and also 23 *Salmonella* serovars. The animal population data underpinning these analyses were from the EU-wide *Salmonella* baseline surveys on broiler flocks (2005-2006), broiler carcasses (2008), turkey flocks (2006-2007) and slaughter pigs (2006-2007), and the 2009 EU harmonised statutory monitoring in broiler and laying hen flocks. The human salmonellosis data represented aggregated data (three years, 2007 to 2009) and was provided by the ECDC. These results concurred well with an external scientific report submitted to EFSA (EFSA, 2011d) in 2011 and using data from 2009 and before, estimating the laying hen reservoir to be the most important source in the EU contributing with 43.8% of cases attributed to this source, followed by pigs 26.9%. Turkeys and broilers were estimated to be less important sources of *Salmonella*, contributing with 4.0% and 3.4%, respectively. The results of this microbial-subtyping model, named 'EU-*Salmonella* Source Attribution' (EU-SSA), showed moreover that the relative contribution of food-animal sources varied between regions and countries. This report further attributed salmonellosis to 19 food sources and water based on an analysis of data from outbreak investigations. According to this modelling approach, eggs were estimated to be the most important source of disease in the study period, followed by pork, chicken, the general category 'meat and poultry', and dairy products. An analysis by year using data from 2007-2009 showed that the contribution of eggs decreased in 2009, and the proportion of disease attributed to other sources varied over the years and between regions.

Discussion
Q: The proportion of travel related cases is relatively high in the Northern part of Europe and less high in other regions of Europe. Do you have an explanation? Is it not possible to relate these cases to other sources?
A: It is indeed not straightforward how to deal with this type of analyses at national level.

2.4.2 *Analysis of the baseline survey on* Salmonella *in breeding pigs in the EU, 2008 – Part B: Factors associated with* Salmonella *pen positivity*

A European Union-wide *Salmonella* baseline survey was conducted in 2008 in holdings with breeding pigs (EFSA, 2011c). A total of 1609 randomly selected holdings housing and selling mainly breeding pigs (breeding holdings) and 3508 holdings housing commercial breeding pigs and mainly selling pigs for fattening or slaughter (production holdings) were sampled. In each selected holding, pooled fresh faecal samples were collected from ten randomly chosen pens of breeding pigs over six months of age, representing the different stages of the breeding herd, and examined for the presence of *Salmonella*. Analyses at country-level demonstrated a strong positive association between the

prevalence of *Salmonella*-positive breeding holdings and the prevalence of *Salmonella*-positive production holdings, suggesting a vertical dissemination of *Salmonella* between the holdings. Based on the combined results from breeding and production holdings, multivariable regression analysis showed that the odds of *Salmonella*-positive pens with pigs increased with the number of breeding pigs in the holding and with the following pen-level factors: flooring systems other than slatted floors or solid floors with straw, presence of maiden gilts, number of pigs per pen, feed of commercial compound origin or pelleted feed. A tendency towards some Member State group-specific *Salmonella* serovars was identified, but spatial distribution of other serovars was heterogeneous. *Salmonella* Typhimurium and *Salmonella* Derby were widespread and dominant in the EU, in both breeding and production holdings. However, many other serovars were relatively prevalent in Western EU Member States.
A complementary within-holding prevalence study indicated that, due to a non-perfect diagnostic test sensitivity, the observed EU-level prevalence of *Salmonella*-positive holdings with breeding pigs was roughly 80% of the estimated true EU-level prevalence. But this proportion varied between Member States.

Discussion
Q: What will be done with the results of the baseline pig studies? Will there be control programmes introduced like for the poultry sector?
A: This is up to the risk managers, the European Commission and the Member States. EFSA can only give some pressure to look at the results and to do something. The risk managers need to consider the best options. The poultry sector has done a lot to reduce the number of *Salmonella* in their sector. The pig sector should follow this example.
Q: From the calculations of EFSA it seems that floor types with straw are a bit more at risk for *Salmonella* infections than other types of floors. In our country we had the impression that solid and straw give a lower risk. Can you explain this difference?
A: The EFSA calculations are at EU level. Member States should also check the situation at national level. This may be different from EU level.

2.5 Results interlaboratory comparison study on bacteriological detection of *Salmonella* – Veterinary XV-2012

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

In February 2012, the European Union Reference Laboratory for *Salmonella* (EURL-Salmonella) organised the 15th veterinary interlaboratory comparison study on the detection of *Salmonella* in pig faeces. Participants were 33 National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) of EU Member States, candidate EU Member State, member countries of the European Free Trade Association (EFTA) and, on request of DG-Sanco, one NRL from a third country outside Europe.

The most important objective of the study was to test the performance of the participating laboratories for the detection of *Salmonella* at different contamination levels in a veterinary matrix. To do so, pig faeces samples of 25 g each, were analysed in the presence of reference materials containing *Salmonella* (at various contamination levels). A proposal for good performance was made and the performance of the laboratories was compared to this proposal. The prescribed method was Annex D of ISO 6579 (Anonymous, 2007),

with selective enrichment on Modified Semi-solid Rappaport Vassiliadis (MSRV) agar. Optionally, a laboratory could also use other, own media or procedures for the detection of *Salmonella*.

32 individually numbered lenticule discs had to be tested by the participants for the presence or absence of *Salmonella*. 25 lenticule discs had to be examined in combination with each 25 g of *Salmonella*-negative pig faeces: 5 lenticule discs contained approximately 10 colony forming units (cfu) of *Salmonella* Typhimurium (STM10), 5 lenticule discs contained approximately 58 cfu of *Salmonella* Typhimurium (STM58), 5 lenticule discs contained approximately 6 cfu of *Salmonella* Derby (SD6), 5 lenticule discs contained approximately 37 cfu of *Salmonella* Derby (SD37) and 5 lenticule discs contained no *Salmonella* at all (blank lenticule discs). Seven lenticule discs, to which no faeces had to be added, were control samples, existing of 2 lenticule discs STM10, 2 lenticule discs SD6, 1 lenticule disc SD37 and 2 blank lenticule discs.

The laboratories could find *Salmonella* in 93% of the (artificially contaminated) samples, when using the prescribed veterinary method (MSRV) while 100% accuracy was found for the control samples without matrix. The sensitivity rate of the high level materials was 98% and the sensitivity rate of the low level materials was close to 89%. Nine laboratories could not detect *Salmonella* Derby in one or more out of five SD6 lenticules with matrix and eight laboratories could not detect *Salmonella* Typhimurium in one or more samples of STM10 with matrix.
The matrix used in this study (pig faeces) contained a high and stable level of disturbing background flora. A preliminary test at the laboratory of the EURL-*Salmonella* showed that the detection of *Salmonella* in pig faeces contaminated with low-level lenticule discs (SD6 and STM10) was more difficult than for matrices used in earlier studies. Furthermore, the consistence of the portions of pig faeces sent to the participants was not homogenous in terms of moisture content. Due to this combination of facts it was decided to slightly adjust the criteria of good performance for the low level artificially contaminated pig faeces (at least 40% of the samples positive, instead of at least 60%, as used in earlier studies).
When the MSRV plates were incubated for 48 hours, 5% more positive results were found compared to 24 h incubation. This was most clear for the artificially contaminated STM samples, which gave 8% more positive results after 48 h incubation compared to 24 h.

31 NRLs fulfilled the criteria of good performance. Two laboratories had difficulty in detecting *Salmonella*. A follow-up study is planned after the workshop in June 2012.

Discussion
Q: Is it possible that a false positive blank result is in fact an exchange with a real positive sample?
A: When we note deviating results, like false positive blank results, we ask the laboratory about the possible cause. We do not know whether this is an exchange of samples or cross-contamination or anything else.
Q: Do you have information on what (second) isolation media are used?
A: The isolation media as used by the NRLs will be listed in the final report.

2.6 Results on serotyping of *Salmonella* of the sixteenth interlaboratory comparison study on typing (2011)

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

The sixteenth interlaboratory comparison study on serotyping and phage typing of *Salmonella* spp. was organised by the European Reference Laboratory for *Salmonella* (EURL-*Salmonella*, Bilthoven, the Netherlands), and in cooperation with the Health Protection Agency (HPA, London, United Kingdom), in November 2011.

A total of 36 National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*), from all EU members states and some additional 'third countries', participated in this study. 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 which do not achieve the level of good performance for serotyping have to participate in a follow-up study.

A total number of 20 *Salmonella* strains had to be serotyped by the participants. As discussed at the previous EURL-*Salmonella* workshop (Mooijman, 2011), one additional strain from an uncommon source was included in the study and serotyping of this strain was optional and 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). Strain S2 was only evaluated on the O-antigens and H-antigens results, and not on the biochemical reactions concerning serovar 6,7:c:1,5 which finally result in the name. Because of the deviations in the biochemical reactions, strain S6 was only evaluated on the O-antigens and H-antigens results. Strain S16 was excluded from the evaluation, since it showed too many rough colonies.
The individual laboratory results were reported to the participants in January 2012. An interim summary report on the outcome of the study was prepared and sent to all participants in April 2012.

The serotyping results showed that the O-antigens were typed correctly by 31 of the 36 participants (88%). This corresponds to 98% of the total amount of strains. The H-antigens were typed correctly by 25 of the 36 participants (69%), corresponding to 96% of the total amount of strains. A total of 25 participants (69%) gave the correct serovar names, corresponding to 96% of all strains as evaluated.
A completely correct identification by all participants was obtained for four strains: *Salmonella* Hadar, *Salmonella* Enteritidis, *Salmonella* Abaetetuba, and *Salmonella* Typhimurium. Most problems (five laboratories) occurred with the serovar Krefeld.
All but two participants actually did serotype the additional strain, being a *Salmonella enterica* subspecies *diarizonae* 38:r:z originating from flax seeds. The majority of the participants (27) were able to serotype this strain correctly, though the exact naming might need some more harmonisation.

Four participants did not meet the level of good performance at the first stage of the study and three of these laboratories (the fourth laboratory being from a non-EU country) participated in the follow-up study in March 2012, by serotyping an additional ten strains. The two participating EU NRLs achieved a good performance on their results of the follow-up study.

Discussion
Q: Should a reference laboratory also perform biochemical typing?
A: Yes, but we did not ask for it during the interlaboratory comparison study. Therefore, we have not judged the results on the fact whether a specific isolate was typed biochemically or not.
Q: Can you inform us about antisera of poor quality?
A: The more general information will be given in the report of the interlaboratory comparison study. We cannot publish a list of manufacturers which may have produced antisera of poor quality. Firstly, because we have to remain independent as EURL and secondly, poor antisera can be related to a specific batch and the quality can vary within a manufacturer and between manufacturers over time.
Q: Can you give more information on the PCR methods used for typing of *Salmonella*? Like which PCR methods, and the related results?
A: This information will be listed in the final report.

2.7 Results on phage typing of *Salmonella* of the sixteenth interlaboratory comparison study on typing (2011)

Elizabeth de Pinna, Health Protection Agency, London, United Kingdom

The Laboratory of Gastrointestinal Pathogens (LGP), of the Health Protection Agency (HPA), London, United Kingdom, provided the *Salmonella* strains for phage typing in the sixteenth interlaboratory comparison study on typing of *Salmonella* spp. Ten strains of *Salmonella* Enteritidis and ten strains of *Salmonella* Typhimurium were selected from the culture collection of the HPA. Nine NRLs took part in the phage typing of the *Salmonella* Enteritidis strains and eight of these laboratories also took part in the phage typing of the *Salmonella* Typhimurium strains.
Five of the NRLs correctly phage typed all ten strains of *Salmonella* Enteritidis. One of the NRLs correctly typed nine of the *Salmonella* Enteritidis strains. One NRL correctly phage typed eight of the *Salmonella* Enteritidis strains and two NRLs correctly typed six of the ten *Salmonella* Enteritidis strains. Four of the *Salmonella* Enteritidis strains were phage typed correctly by all the participating laboratories. One strain E2 (PT 35), was incorrectly phage typed by three of the participating laboratories.
Overall, the results of the phage typing of *Salmonella* Typhimurium by the NRLs were very good. The ten *Salmonella* Typhimurium strains were correctly phage typed by six of the NRLs. Two NRLs correctly typed nine of the ten *Salmonella* Typhimurium strains.
When compared to the previous study the results of the NRLs for the phage typing of *Salmonella* Enteritidis were not as good, with 87% being correctly typed in 2011 and 98% correctly typed in 2010. For the phage typing of *Salmonella* Typhimurium the results of this study were comparable to the study in 2010, with 98% of the strains correctly phage typed in 2011 and 98% correctly typed in 2010.
This study shows the NRLs continue to perform phage typing of *Salmonella* Typhimurium to a high standard. The results for *Salmonella* Enteritidis were average in this study, indicating that some of the laboratories need to improve their performance.

Discussion
Q: Can you give guidance on how to get the titre of the phages correct?
A: You can test this in your own laboratory by using the reference strains for *Salmonella* Enteritidis (1b) and *Salmonella* Typhimurium (DT36) which will react with all phages. If you do not have these reference strains, we can help you with them. We distribute the phages to you at a 100 times the recommended dilutions. By checking with the reference strain you can decide whether it is necessary to adjust the amount of dilution of the phages.
Q: We have had problems with reactions of some phages. Do you know what could have been the problem?
A: This may also have been a titre problem of the phages. For this it would also be best to check with the relevant reference strains.

2.8 Proposal typing study 2012

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

The provisional planning of the seventeenth *Salmonella* serotyping study in 2012 is given below.

Week	Date	Topic
43	22-26 October	Mailing of the protocol and test report
45	5-9 November	Mailing of the parcels to the participants as diagnostic specimens by door-to-door courier service
46	12-16 November	Starting with the identification of the strains
49	**7 December 2012**	Deadline for sending the electronically completed test report by email to the RIVM
	January 2013	Reporting of individual laboratory results
	January 2013	Interim Summary Report
	Summer 2013	Final report issued

As usual, serotyping of 20 *Salmonella* strains will be compulsory to the EU NRLs. Participants may expect strains to be typed up to O21 (Group L). As for the sixteenth study, an optional strain from an uncommon source will be added to the study in 2012.

On request of some NRLs, the two extensive tables on the background data of the serotyping results are *optional* tables in the test report now, though the majority of the participants still completed these tables. It was indicated that in case of deviating results a participant will be asked to fill in these tables retrospectively.
As for the fifteenth typing study on *Salmonella*, reporting by electronically filling out the test report (so not hand-written) and emailing was requested for the sixteenth study and all laboratories kindly cooperated in this. Therefore, a check-up of the result files by the labs was no longer needed and time was saved to be able to report the individual laboratory results as soon as possible. Currently, the EURL-Salmonella is exploring the possibilities of a web-based reporting system, which may also speed up the elaboration of results, and moreover may reduce the discrepancies in the way of reporting the results by the participants.

As reported, colonial form variation may occur with the expression of the O:6_1 antigen by some serogroup C2 serovars. As for the previous two studies, also for the sixteenth study on serotyping it was decided to consider the serovar pairs concerned not as distinct serovars.
The WHO Collaborating Centre for Reference and Research on *Salmonella* (Institut Pasteur, Paris) informed us that this matter will be taken up in a next version of the White-Kauffmann-Le Minor scheme, but it is not known yet when this version is planned to be published. On request of the workshop participants, the Institute Pasteur will be contacted again to ask about their time-scheme.
For the time being, laboratories are requested to report strains in our interlaboratory *Salmonella* typing studies as either Hadar or Istanbul (according to the O-antigens detected) and both serovar names will be evaluated as correct for a Hadar or an Istanbul strain as sent.
Results from the questionnaire revealed that a variety of sera from different manufacturers are generally used by the participants, and that the majority of the laboratories also use sera from more than one manufacturer to perform the study. Therefore, the general remark for the people working in the laboratory and actually performing the serotyping tests was repeated: Please be very careful in exactly following the instructions of the various manufacturers of the sera, because there may be small but essential differences between the different manufacturers (e.g. reading time and background for reading the reaction).

Discussion
Q: Would it be possible not to focus too much on typos?
A: We do not focus too much on typos, but we may remark on it when we notice them. We may consider in the case of web-based forms to include a 'roll-down' menu, so that the correct name can be picked from a list.
Q: Would it be possible to include more than one strain of another subspecies than *enterica*?
A: We can consider to vary the subspecies of the '21st' strain with every study.

2.9 Methodologies for *Salmonella* typing: gold standards and alternatives

Pierre Wattiau, CODA, CERVA, Brussels, Belgium

For about 80 years, typing of *Salmonella enterica* has been routinely performed by serotyping, a method in which surface antigens are identified based on agglutination reactions with specific antibodies. The serotyping scheme has generated over time a dataset of the utmost significance allowing for the long-term epidemiological surveillance of *Salmonella* in the food chain and in public health control. However, in epidemiological investigations, identification and tracking of salmonellosis outbreaks require the use of methodologies able to fingerprint the causative strains with a sensitivity far below the one achieved by serotyping. During the last two decades, alternative methods emerged that could successfully identify the serovar of a given strain by probing its DNA. Molecular-biology based methods were made available to address the phylogeny and fingerprinting issues. At the same time, accredited diagnostic became increasingly generalized, imposing strong methodological requirements in terms of traceability and measurability. In these new contexts, the hand-crafted character of classical serotyping is challenged although it is widely accepted that classification into serovars should be maintained. The presentation summarises and discusses modern typing methods with a particular focus on those having potential as alternatives for classical serotyping.

Discussion
Q: Do you consider whole genome sequencing as an alternative for the golden standards?
A: Whole genome sequencing is still very expensive, but it can be expected that the costs will go down in the near future. Currently, the main problem is the data analyses. With whole genome sequencing you obtain a lot of information, which may be too much for routine laboratories. For the treatment of these data no clear protocol currently exists, although this may also change in the (near) future.
Q: I would prefer to base an alternative typing method on (O,- and H-) antigens, to be able to compare historical data.
A: You may be right, but for every alternative method it is important that a validation is performed against the reference method.
Q: What method do you prefer as alternative method?
A: This depends on the equipment and the speed of analyses. Currently my preferred method is a hybridisation method as this type of method is by now the most rapid method.

2.10 Multiresistant monophasic *Salmonella* Typhimurium 1,4,[5],12:i:- serotype in Greece 2006-2011

Georgia Mandilara, National School of Public Health, National Reference Centre for Salmonella*, Vari, Greece*

Salmonella is one of the most common causes of bacterial food-borne diseases worldwide. Recently, *Salmonella enterica* serovar 1,4,[5],12:i:- emerged and is now among the most common serovars isolated from humans, poultry and pigs in many countries. This serovar is considered a monophasic variant of *Salmonella* Typhimurium (1,4,[5],12:i:1,2). In Greece, 1,4,[5],12:i:- serovar was recorded for the first time in human isolates in 2007 (0.3% of total isolates), increased sharply during the next four years and in 2011, was the third most frequent serovar (4.0% of total isolates). In the present study, *Salmonella enterica* 1,4,[5],12:i:- isolates of human, animal and food origin, isolated during 2006-2011, were examined. In order to determine if they were *Salmonella* Typhimurium monophasic variants, a specific for *Salmonella* Typhimurium IS200 fragment (1-kb) was investigated, using the PCR method. A second PCR, targeting the phase 2 (fljB) flagellar gene was also performed. From the 119 isolates initially serotyped as 1,4,[5],12:i:-, 22 (18.5%) were characterized as biphasic serovars (Typhimurium or other biphasic 1,4,[5],12:i:-), after applying the PCR method.
97 monophasic Typhimurium strains were examined using phenotypic (serotyping and phage typing) and molecular (PFGE) methods. Phage typing was performed to a subset of the isolates (16 of animal, 5 of food and 29 of human origin) by the Health Protection Agency (HPA), according to HPA protocols. Antimicrobial susceptibility testing was performed by the agar disk diffusion method according to the protocols and guidelines of the Clinical and Laboratory Standard Institute (www.clsi.org). PFGE was performed after digestion of genomic DNA with XbaI according to the Pulse-Net protocol. Fingerprints were analysed using GelCompar II v.4.1 software.
The most commonly identified phage types were DT120 (62%) and DT193 (24%), represented in all sources, animal, human, and food. DT120 and DT193 are the predominant phage types of monophasic Typhimurium in EU. Concerning the resistance of monophasic isolates to selected antibiotics, although several

multiresistant patterns were observed, the ASSuTSpTm pattern represented 56.7% of the isolates found in all sources, animal, human, and food. In EU, ASSuT is the most common R-type. PFGE analysis identified two unique profiles, Type A and Type B, that shared more than 94% similarity. Type A was divided in Subtype A1 and Subtype A2. The most common PFGE profile was Type A2 (53.7%). Combining PFGE, phage type and R-type, the most frequently occurring combination was DT120/ASSuSpTTm/Subtype A2 (approximately 40%), represented by isolates of human, pig and food origin. Many other combinations were observed, though at much lower frequencies.
According to our results, phenotypic and genotypic characteristics of animal isolates are also met in human isolates, demonstrating that common clones of *Salmonella* monophasic Typhimurium are circulating among animals and humans in Greece. Our results also indicate that in Greece, a particular clone of phage type DT120, R-type ASSuSpTTm and of a single PFGE profile is the most frequently represented in human, pig and food isolates.
Routine surveillance for *Salmonella*, including serotyping and standardised subtyping, would be an important advance in defining emerging serovars. It is also necessary to coordinate data reporting, considering the substantial increase of monophasic strains lately.

Discussion
Q: Do you see the same trend in Greece for certain phage types and antimicrobial resistance patterns as in the rest of Europe for *Salmonella* 1,4,[5],12:i:-?
A: We have done phage typing only on a subset of the isolates and for only two years. Therefore it is difficult to say if we see the same trends in Greece.
Q: Is it remarkable that of the 27% monophasic *Salmonella* Typhimurium isolates, still 18.5% are tested as biphasic with PCR?
A: We have done phase inversion two times instead of once. Furthermore, in France a similar pattern is seen.
Q: Did you look for the presence of the *Salmonella* genomic island, as it is linked to the resistance to six antibiotics?
A: No we did not.

3 Tuesday 15 May 2012: day 2 of the workshop

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

*Aphrodite Smpiraki, NRL-*Salmonella*, Chalkida, Greece*

The Veterinary Laboratories in Greece dealing with *Salmonella* are two State Veterinary Institutions and nine (out of 15) State Veterinary Laboratories of the Hellenic Ministry of Rural Development and Food. Two of the last ones have been accredited according to EN ISO 17025 (Anonymous, 2005a) to participate in the EU *Salmonella* control programmes. In addition, other laboratories dealing with *Salmonella* are the four Military Veterinary Microbiology Laboratories of the Ministry of Defence, the Laboratories of the two Greek Schools of Veterinary Medicine and, at least, 25 Private Laboratories.
The NRLs for *Salmonella* in Greece are the State Veterinary Laboratory of Chalkida and the human NRL-*Salmonella* Laboratory of the Ministry of Health and Social Solidarity. The SVL of Chalkida, was established in 1978 as a decentralised laboratory of the former Greek Ministry of Agriculture. It moved to its present facilities in 1993. The services provided had been focused on the needs of the Regional State Veterinary Services, contributing to the Diagnostics of animal and poultry diseases and conducting microbiological examinations in food, feeding stuffs and water samples. In 1997 it was assigned as the NRL for *Salmonella* and in 2007 as the NRL for *Salmonella* Antimicrobial Resistance. In 2008, the Laboratory was accredited according to EN ISO 17025 (Anonymous, 2005a) to conducts tests on the detection of *Salmonella* in food and feed of animal origin, animal faeces and samples of primary production, perform serotyping of *Salmonella* strains (five EU targeted serovars) and on the antimicrobial resistance of *Salmonella* by the disk diffusion method.
At present, the functions of the State Veterinary Laboratory (SVL) of Chalkida are focused on the laboratory diagnosis of *Salmonella* and antimicrobial resistance of *Salmonella* (as NRLs), of the *E. coli, Enterobacteriaceae, Listeria*, Avian Influenza and the determination of antimicrobial residues in animal origin food (via respective National Control Programmes) and on the parasitological examination of animal and avian origin samples.
According to article 33 of the EU Regulation 882/2004 (EC, 2004) the activities of NRL-*Salmonella* expand to three major areas of action. The first area of action concerns the provision of intra and inter-ministerial scientific and expertise support. Thus, appointing a scientific opinion and relevant legislative output, participating in meetings concerning the designation and implementation of National Control Programmes (NCPs), evaluating the Private Veterinary Laboratories (PVLs) in order to fulfil NCPs participating criteria, coordinating the actions of SVLs and PVLs contributing to NCPs, serotyping of all *Salmonella* isolates from SVLs and PVLs also collecting and summarising their data within the scope of NCPs, planning ordering and distributing the supplies and materials requested for *Salmonella* analysis to SVLs, and participating in the preparation of the EU annual zoonoses report. The second area of action concern the NRLs' international activities: collaboration with the EURL *Salmonella* and the EURL for antimicrobial resistance, participation in their interlaboratory comparison trials on a standard annual basis and participation in the EURLs' annual workshops. The third area of action concerns provision of scientific and technical support to SVLs and PVLs. Thus, spreading information received from the EURLs, issuing guidelines and questionnaires referring to PVLs involved in *Salmonella* NCPs, performing on site visits of the NRL personnel to SVLs and PVLs and

disseminating of updated information on sampling, methodology and legislation. Also organising meetings and trainings of Laboratories' personnel on detection and serotyping of *Salmonella* and providing technical assistance regarding validation of methods for accreditation purposes and organising comparative interlaboratory tests for SVLs and PVLs, evaluating the results and conducting of follow-up studies when needed.
The future prospects of the SVL of Chalkida -from the point of view of the NRL- focus on the extension of *Salmonella* serotyping accreditation to include a broader range of identifiable serovars, on the outspread of interlaboratory comparison tests to the SVLs on the area of *Salmonella* detection in food samples and, also on the promotion of *Salmonella* molecular typing methods. Other goals are to intensify the NRLs-*Salmonella* network and cooperation in Greece and of course, to intensify the cooperation with the relative EURLs. Additionally, it has been decided to proceed with the accreditation of the Donovan method, according EN ISO 16649-3 (Anonymous, 2005b) for detection and enumeration of *E. coli* on live bivalve molluscs and to the validation and verification of the Star Protocol for the determination of antimicrobial residues in animal origin food.

Discussion
Q: What measures have been taken in Greece to reduce the number of *Salmonella* in poultry?
A: Hygiene measures were taken as well as vaccination programmes. We are still working on it.
Q: Do you (as NRL) organise Proficiency Tests for the private laboratories in Greece?
A: Laboratories have to be accredited. As NRL we obtain the Proficiency Test samples from the Veterinary Laboratories Agency (VLA) in UK. Next we distribute these samples to the laboratories and the laboratories report their results to us.

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

Ruta Bubuliene, NRL-Salmonella, Vilnius, Lithuania

The National Food and Veterinary Risk Assessment Institute (NFVRAI) is the National Reference Laboratory (NRL) for *Salmonella* testing. At the institute the risk assessment in the field of food safety and animal health is performed. It ensures effective implementation of the state policy in the protection of consumers interests in the areas of food and veterinary safety.
The mission of the NFVRAI is to contribute scientific information, to give scientific and technical support in the implementation of state policy in the areas of food and veterinary safety and to communicate on risks.
NFVRAI was established on 1 July 2008 on the basis of a reorganisation of the National Veterinary Laboratory and Lithuanian State Inspection on Veterinary Preparations (medicines). It falls under the State Food and Veterinary Service (SFVS) and has territorial branches in Kaunas, Klaipėda, Panevėžys and Šiauliai. In total, it has 309 employees (181 in Vilnius, 52 in Kaunas, 27 in Klaipėda, 26 in Panevėžys, 23 in Šiauliai). Of these 309 employees, 12 have a scientific degree, 54 are veterinarians, 48 are chemists-engineers, 10 are biologists-microbiologists, 63 are laboratory technicians and 134 have another specialism.

Three NFVRAI departments are involved in the tasks and duties of the NRL for *salmonella*:

- Bacteriology department for serotyping, and testing of pathological material and environmental samples;
- Food Microbiology department for food and water testing;
- Molecular Biology and GMO department for PCR and genotyping.

The territorial branches perform *Salmonella* testing in food, pathological material and in environmental samples.
NFVRAI and its territorial branches are accredited according to EN ISO 17025 (Anonymous, 2005a). The accreditation was obtained from the German Accreditation Service, DakkS, in 2000. Since then, DakkS performs the annual supervising visits (external audits) of the NFVRAI. Additionally, the NFVRAI is accredited by the Federal Center of Hygiene and Epidemiology and GOST R systems in the Russian Federation. Since 2007 the NFVRAI is licensed to use the International Laboratory Accreditation Association Label – ILAC.

Activities of NFVRAI as NRL are:
- theoretical, practical training for the territorial branches and inspectors of the state inspection on Veterinary Service;
- giving advice to producers, farmers and new laboratories;
- organisation of annual Proficiency Tests for NFVRAI territorial branches, SFVS and poultry farms laboratories;
- providing the SFVS, EFSA and the EU Commission with data on *Salmonella* and its resistance to antimicrobial agents;
- collaboration with EURL-*Salmonella* and transfers information to other laboratories;
- collection of all *Salmonella* isolated in laboratory testing for official control, serotyping them and testing antimicrobial resistance;
- certification and control of veterinary and food-borne outbreak laboratories.

NFVRAI performs *Salmonella* detection by following reference (standard) methods. For antimicrobial resistance testing the MIC test is followed and as PCR method, the BAX Q7 system is used. Furthermore, the laboratory performs PFGE. NFVRAI participates in official *Salmonella* programmes: control programme of domestic poultry zoonoses, control of imported food of animal origin, state inspection control of food and wells water, feed control.

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

Katelijne Dierick, NRL-Salmonella, *Brussels, Belgium*

The Belgian NRL for *Salmonella* in food is situated in the Institute of Public Health (WIV-ISP) in Brussels (Ixelles). It is a part of the Scientific Service Food-borne pathogens, which belongs to the Operational Direction of Communicable and Infectious diseases.

History
Although microbiological analyses of food have been performed in the WIV-ISP since the beginning of the twentieth century, and became more intense in the nineties with the official controls in the framework of the official EU-zoonoses monitoring and the hygiene plans, a new challenge was given in 2003 with the dead of a 6-year old girl in a family food-borne outbreak (FBO). The Belgian authorities recognized the need of a centralized analyses and coordination of FBO and in 2005 the WIV-ISP was designated NRL for Food-borne outbreaks, *Salmonella* in food, antimicrobial resistance and Coagulase positive

Staphylococcus. The designation as NRL for the other food-borne pathogens followed in 2009 (*Listeria*, VTEC, *Campylobacter*) and in 2011 (Bivalve molluscs).

Activities
To keep in touch with routine analysis and to understand the daily problems of the 30 Belgian food microbiology laboratories recognised by the Belgian Food Safety Agency (FAVV-AFSCA), the NRL *Salmonella* performs a part of the analyses in the framework of the National zoonoses monitoring plan. In case of outbreaks, it performs all food analyses and the analyses of some human samples. Furthermore, it collects all human and food data for the food-borne outbreak reporting to the EU Commission. The laboratory is accredited according to EN ISO 17025 (Anonymous, 2005a) for almost all food-borne pathogens and hygiene parameters in food. It determines the antimicrobial resistance of the *Salmonella* isolates from food during the zoonoses monitoring. It organises Proficiency Tests (PTs) in different matrices (vegetables, carcass swabs, salmon, etc.) and performs technical audits for the Belgian accreditation body BELAC. Twice a year a communication meeting is organised for all recognized food microbiology laboratories to transfer information from the EURL's. Participation to the PTs and communication group meeting is mandatory for the food microbiology laboratories. Training courses for food microbiology laboratories are organised twice a year but laboratories can ask for individual assistance for specific analytical problems.

Collaborations
The NRL-*Salmonella* for food analyses collaborates with the NRL-*Salmonella* for animal health and animal feed, which is situated in the Institute for Veterinary and Agricultural Research (CODA-CERVA) in Brussels (Uccle). CODA-CERVA is involved in the *Salmonella* surveillance in live animals. It performs the serotyping of animal and feed isolates, serology for *Salmonella* in pigs, and it organises PT schemes for animal health laboratories.
The human National Reference Centre for *Salmonella*, which is situated in the WIV-ISP in the scientific service Bacterial diseases, performs the serotyping of human and food isolates, and the PFGE for comparison of strains.

Publications
The close collaboration between the three Belgian reference laboratories for *Salmonella* is reflected in several research papers:

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More information on the Institute of Public Health (WIV-ISP) can be found at the following website: www.wiv-isp.be/Programs/communicable-infectious-diseases/Pages/EN-foodpathogens.aspx

Discussion
Q: What methods do you use for the detection of *Salmonella*?
A: The official ISO/CEN methods.

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

Lennart Melin, NRL-Salmonella, *Uppsala, Sweden*

The National veterinary institute (Statens Veterinärmedicinska Anstalt, SVA) was founded in Stockholm in 1911. At that time there were five employees. Since then the institute have changed locations twice and the number of co-workers have increased to a little more than 400 people. Both technology and knowledge in Veterinary medicine have developed tremendously over time, but the basic philosophy to represent cutting edge knowledge about animal diseases and contaminants and how to prevent these to be transmitted to humans have remained the same.

Today SVA is an expert authority under the ministry of agriculture and one of the leading knowledge centres for veterinary medicine regarding animal contagious diseases in Sweden. In case of a disease outbreak, SVA should be able to diagnose the microbe and provide expert advice on how to combat the disease. This responsibility is specifically important regarding epizootic diseases and zoonoses. SVA is imposed to have the possibility and the ability to diagnose all epizootic diseases even if they have not been prevalent in the country for many years. SVA continuously develops new diagnostic methods and participates in several networks within the country as well as outside the country.

The goal for the institute is summarised in four words:
Healthy animals. Safe Humans.

Salmonellosis is a disease that has been in focus in Sweden for many years. Especially since a very large outbreak in 1953 in the city of Alvesta where 9000 people suffered from disease, 4200 hospitalised and 90 died. After this a quite rigorous and laborious national control programme has been in use. This programme includes a control from feed to food. This means that the feed provided for animal production shall be free from *Salmonella*. If *Salmonella* is found in one of the samplings of the production lines in a feed factory in Sweden

that line in the factory is closed and sanitised. After negative sampling post disinfection the production will start again.
If a positive lymph node is found during the regular sampling during slaughter the herd of origin is traced and then sampled for *Salmonella*. If positive, the farm will not be able to leave animals to slaughter until it is sanitised and has presented two negative samplings with two to three weeks in between.

The programme also includes random sampling of food to an unspecified extent.

At SVA the MSRV method as described in Annex D of EN ISO 6579 (Anonymous, 2007) is the standard method for samples from animals, the environment and feed. Due to current lack of space for incubators, enrichment in Selenite Cystine broth is performed in the BSL3 facilities. However, these facilities are to be extended and modernised.

In samples from animal feed, PCR is used as a screening method to test whether *Salmonella* is present in the feed. If this test is found positive, the sample will be tested with the MSRV method.

If *Salmonella* is detected the isolate will be serotyped and if necessary subtyped with PFGE, Phage typing, or MLVA. If a 'Monaphasic *Salmonella* Typhimurium' is found, it will be confirmed by a specific PCR for detection of the H2-flagella 1, 2.

For surveillance reasons, a number of the positive isolates will also be investigated for its antimicrobial resistance.

If a case of *Salmonella* is found in a human, an investigation to the source of the infection will be performed. If suspicion occurs that it might be due to a food-borne contamination of Swedish origin an effort to trace the herd will be done and the found herd/herds will be sampled for the presence of *Salmonella.*

Discussion
Q: You indicated to use MSRV agar as selective enrichment for the detection of *Salmonella* in animal feed. Do you have any information on the number of non-motile *Salmonella* you may miss because of using MSRV?
A: We started the use of MSRV only in April of this year. Currently we perform the analyses on MSRV in parallel with the 'old' method (selective enrichment in RV broth). It may take some time before we have more information on this subject. This parallel testing will be part of a validation study for the use of MSRV.
Q: The prevalence of *Salmonella* in Sweden is very low. How was this achieved?
A: Several aspects may play a role: Several years ago a control scheme has been introduced in Sweden which is still working. Furthermore, Sweden has little or no transport of animals over long distances and the distances between farms are large and therefore fewer chances of contamination between farms.
Q: Although the prevalence of *Salmonella* is low in Sweden, you still analyse a large number of samples. Can you explain this?
A: If a herd is found positive for *Salmonella* many samples have to be taken to try to eradicate *Salmonella* from the herd.
Q: In your presentation you give mainly information on the activities of your institute. Can you be more specific for the NRL?
A: The NRL performs the serotyping of *Salmonella*, gives guidance to two regional laboratories (e.g. by visiting them), and takes care of a good collaboration between veterinary and food laboratories.
Q: How is the control of *Salmonella* in animal feed production controlled?

A: Most of the positives come from imported animal feed or ingredients for animal feed. In general when material is ordered this is transported to Sweden by boat. Before the boat enters Sweden, samples are already taken for analyses. By the time the boat is in Sweden the result of the analysis is known. If the product in the boat happens to be positive for *Salmonella*, the material will be heat treated before further use.
Q: Do you as NRL prepare the samples for the Proficiency Testing schemes for the official laboratories in Sweden?
A: No, this is done by the Swedish Food Agency.

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

Gordan Kompes, NRL-Salmonella*, Zagreb, Croatia*

The NRL for *Salmonella* in Croatia is working as a part of the Croatian Veterinary Institute (CVI) which was established in 1901 in Križevci. In 1933 it was transferred to Zagreb. CVI consists of the Croatian Veterinary Institute in Zagreb and five regional departments. Basic activities of the CVI are diagnosis and control of the infectious diseases, veterinary public health (Ministry of Agriculture, Fisheries and Rural development), scientific work (Ministry of Science, Education and Sports) and market activities. In the period from 2007 to 2011, 28 laboratories were accredited according to the EN ISO 17025 (Anonymous, 2005a), for in total 146 methods.
The NRL-*Salmonella* in Croatia is consists of the four laboratories:

1. Laboratory for general bacteriology and mycology / Isolation of *Salmonella* spp. from mammals;
2. Laboratory for bacteriology of Centre for poultry farming/Isolation of *Salmonella* spp. from poultry;
3. Laboratory for food microbiology/Isolation *Salmonella* spp. from food;
4. Laboratory for animal feed microbiology/Isolation of *Salmonella* spp. from animal feed.

The main activities of the NRL-*Salmonella* are:

- participation in the EURL-*Salmonella* workshops;
- participation in the EURL-*Salmonella* inter-laboratory trials;
- organisation of training courses and proficiency testing for the official state laboratories;
- testing samples from the National *Salmonella* Control Programme;
- serotyping *Salmonella* isolates from other laboratories (official state laboratories);
- monitoring of resistance of *Salmonella* spp. isolated from poultry and swine.

Discussion
Q: What is the size of the poultry industry in Croatia?
A: This is relatively small. In fact we have only two large farms for poultry meat production and many small farms. Most of the poultry meat for consumption in Croatia comes from outside the country. Approximately only 20% of the meat consumed is produced in Croatia.
Q: Still you mentioned approximately 30 000 samples per year tested for the monitoring programme?
A: Yes, because of the large number of small farms we have many samples per year.
Q: You mentioned the presence of a multiresistant *Salmonella* Enteritidis strain. Do you know where it comes from?

A: We do not know yet. We received the strain in our laboratory only for antimicrobial resistance testing and did not (yet) receive additional information.
Q: What method do you use for antimicrobial susceptibility testing and what breakpoints or cut off values do you use?
A: All cut off values are derived from the CLSI (Clinical and Laboratory Standards Institute) manual. We use streak plates with microdilution.

3.6 Results interlaboratory comparison study on bacteriological detection of *Salmonella* – FOOD V - 2011

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

In September 2011, the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*) organised the fifth interlaboratory comparison study on detection of *Salmonella* in a food matrix, being minced (pork and beef) meat. Participants were 34 National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) of EU-Member States, EU candidate MSs and countries from the European Free Trade Association (EFTA).

The first and most important objective of the study was to see whether the participating laboratories could detect *Salmonella* at different contamination levels in a food matrix. To do so, minced meat samples of 25 g each, were analysed in the presence of reference materials (lenticule discs) containing either *Salmonella* (at various contamination levels) or no microorganisms at all (blank samples). A proposal for good performance was made and the performance of the laboratories was compared to this proposal. In addition to the performance testing of the laboratories, a comparison was made between the prescribed methods (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 medium was Modified Semi-solid Rappaport Vassiliadis (MSRV) agar. Optionally, a laboratory could also use other, own media or procedures for the detection of *Salmonella*.

32 individually numbered lenticule discs had to be tested by the participants for the presence or absence of *Salmonella*. 25 lenticule discs had to be examined in combination with 25 g of *Salmonella* negative meat each: 5 lenticule discs contained approximately 6 colony forming units (cfu) of *Salmonella* Typhimurium (STM6), 5 lenticule discs contained approximately 61 cfu of *Salmonella* Typhimurium (STM61), 5 lenticule discs contained approximately 8 cfu of *Salmonella* Enteritidis (SE8), 5 lenticule discs contained approximately 51 cfu of *Salmonella* Enteritidis (SE51) and 5 lenticule discs contained no *Salmonella* at all (blank lenticule discs). The other seven lenticule discs, to which no meat had to be added, were control samples, comprising 2 lenticule discs SE8, 1 lenticule disc SE51, 2 lenticule discs STM6 and 2 blank lenticule discs.

The laboratories found *Salmonella* in 96-98% of the (artificially contaminated) samples depending on the used selective enrichment medium. The accuracy rate for the prescribed method for food (MKTTn and RVS) was 96%. The accuracy rate for the requested method (MSRV) was 98%. A comparison between the different media did not show significant differences.

In general, the 'normal' procedures for pre-treatment of the samples did not seem to influence the results. However, one laboratory used an 'extreme' mixing time (20 minutes in stomacher) and found only 4 out of 20 positive results. Longer incubation (additional 24 hours) of selective enrichment media gave more positive results (5-13%), which was most clear for the low level SE contaminated samples.

29 out of 34 laboratories achieved the level of good performance on the first attempt. Two laboratories had difficulties with the detection of *Salmonella* with matrix and three laboratories found false positive results. One of the NRLs with false positive results scored a moderate performance because they made a transcription error during the transfer of raw data to the test report. For the remaining four laboratories a follow-up study was organised in January 2012; three laboratories reached the desired level and one laboratory (non-EU) did not return the results.

Discussion
Q: How do you obtain the lenticule discs?
A: We buy them at the Health Protection Agency (HPA) in England.
Q: What is the main objective of the interlaboratory comparison studies?
A: The main objective is that all participants show good performance for the detection and typing of *Salmonella* in different matrices.
Q: As NRL we do not always obtain sufficient funding to organise interlaboratory comparison studies for all laboratories at national level. Sometimes private laboratories are involved in the official monitoring and the performance of these laboratories should also be tested, but this is not always feasible. How should we deal with this?
A: The amount of funding per NRL may differ per country. A solution can be, for example, that the NRL selects a commercial Proficiency Testing Scheme and indicates that the national laboratories have to participate in this scheme, and have to pay the participation themselves. The results of each laboratory from the schemes should be made available to the NRL so that it is possible to check the performance of each laboratory.

3.7 Proposal interlaboratory comparison study for detection of *Salmonella* in food / animal feed 2012

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

General
In the current set-up of the EURL-*Salmonella* interlaboratory comparison studies on detection of *Salmonella*, most of the time 34 samples have to be analysed by each participant, consisting of:

- 25 artificially contaminated samples, being reference materials (lenticule disks) combined with a matrix;
- 7 control samples, being reference materials (lenticule disks) only;
- 2 procedure control samples, being BPW with matrix and BPW only.

In the current studies, reference materials with two different *Salmonella* serovars are used at two different contamination levels (low and high). Additionally, blank reference materials are included in each study.

An amount of 34 samples is considered relatively high for an interlaboratory comparison study. Therefore, it was studied whether it would be possible to

lower the number of samples, still obtaining statistically valid results. For this, relevant information was found in a recently published CEN/ISO document: CEN ISO/TS 22117 (Anonymous, 2010). This document describes 'specific requirements and guidance for proficiency testing by interlaboratory comparison' in the field of microbiological analyses of food and animal feed. In the document, it is indicated that for the assessment of a qualitative method each participant should test at least 18 samples, consisting of:

- 6 negative samples, to check for the occurrence of false positive results;
- 6 low level samples, with a contamination level close to the detection limit of the method, so that ideally 50% of the samples are found positive and 50% negative;
- 6 high level samples, with a contamination level 10 times higher than the low level materials, representing the level at which all samples should be found positive.

The number of samples indicated in this CEN ISO document (18 samples) is considerable lower than the number of samples used in the current EURL-*Salmonella* studies (34 samples).
In the majority of the interlaboratory comparison studies organised by the EURL-*Salmonella*, two *Salmonella* serovars have been tested. To lower the number of samples to the ones as described in CEN ISO 22117, it can be considered to use samples with only one *Salmonella* serovar.

Other items which are considered by the EURL-*Salmonella* to make the interlaboratory comparison studies 'less heavy' are:

- Review of the test reports to lower the number of questions. Most questions currently mentioned in the test reports have been helpful to find a possible clarification in case of underperformance. However, NRLs are accredited according to EN ISO 17025 (Anonymous, 2005a), so that all relevant information should be available through their (quality) system. Hence, it may be possible to delete several questions from the test reports which are also well traceable through the quality system of the laboratory.
- Exploring the possibility to make the test reports web based. Web-based test reports may not only be easier to complete by the participants, but may also be helpful for the organiser to tabulate the data easier and faster in a more standardised way.

Food/feed study 2012

The interlaboratory comparison study on detection of *Salmonella* in food/animal feed is planned in September/October 2012. Although animal feed is part of the work field of the EURL and the NRLs, up to now only one EURL-*Salmonella* study on animal feed has been organised (in 2008). Therefore it was suggested to focus the 2012 study on animal feed, which may be either a feed ingredient (like soy meal) or complete animal feed. Another choice which needs to be made for this study is the type and number of *Salmonella* serovars to be tested. For this, information from literature was studied to get an idea on frequently reported serovars in animal feed. However, it was noticed that a variety of *Salmonella* serovars can be found in animal feed, which also varies per type of feed and its ingredients (EFSA, 2008 and EFSA, 2010).
The method of choice for detecting *Salmonella* in animal feed is ISO 6579 (Anonymous, 2002), which will therefore become the prescribed method in the study. However, like in former 'food' studies, it will also be requested to the participants to use Annex D of ISO 6579 (Anonymous, 2007) as an additional method.

Discussion
Q: Is it necessary to test reference materials (lenticules) without matrix?
A: These samples are added to get additional information in case of poor performance of a laboratory. When the control samples (lenticules without matrix) are all tested correctly, but the samples with matrix not, it is at least an indication that there was no problem with the reference materials which could have influenced the results.
Q: When in a future study the set-up of CEN ISO/TS 22117 is followed, would you then also follow the criteria for good performance of this CEN ISO document, especially with respect to blank samples?
A: We will stick to our own criteria, which are in fact closely related to the ones of CEN ISO/TS 22117. Only for the blank samples we may use some slightly deviating criteria from the ISO document, as we have no 100% guarantee on the *Salmonella*-negativity of the matrix.
Q: Is CEN ISO/TS 22117 also intended for primary production samples, like faeces and environmental samples?
A: Yes.

Discussion on matrix and serovar
During the workshop all NRLs were asked to give their opinion on the choice of the matrix. All participants agreed to organise a study with animal feed, but there was no clear preference whether this should be complete animal feed or an ingredient for animal feed. Of all participants, 18 voted for complete animal feed and 19 voted for an ingredient or indicated no opinion. It was agreed that the EURL-*Salmonella* will investigate what is feasible.
The same was the case for the choice of the *Salmonella* serovar to be tested. Although 'other' serovars, like Senftenberg and Tennessee may be of interest in relation to animal feed, also *Salmonella* Typhimurium and *Salmonella* Enteritidis are of importance with this type of matrix. Therefore, also the choice of the serovar will be left to the choice of the EURL-*salmonella*.

3.8 MicroVal validation of alternative methods

Wilma Jacobs, MicroVal Expert Laboratory, Bilthoven, the Netherlands

Various reasons can be given for the use of alternative methods. Compared to the reference methods for microbiological examinations, alternative methods often are less laborious, may need less consumables, have higher throughput and results will be available much faster, which all safes time and thereby also money.
Food Business Operators may use alternative methods as Commission Regulation (EC) No 2073/2005 (microbiological criteria for foodstuffs) states in article 5 on specific rules for testing and sampling: 'The use of alternative analytical methods is acceptable when the methods are validated against the reference method in Annex I and if a proprietary method, certified by a third party in accordance with the protocol set out in EN ISO standard 16140 or other internationally accepted similar protocols, is used.' Third parties as mentioned might be e.g. AFNOR, Nordval or MicroVal.
EN ISO 16140 'Microbiology of food and animal feeding stuffs - Protocol for the validation of alternative methods' (Anonymous, 2003) and
EN ISO 16140:2003/Amd 1 'Amendment 1: Interlaboratory study on quantitative methods' (Anonymous, 2011) are currently under revision and the new version is planned to consist of five separate parts in the future. Drafts for the new parts will be updated after a first round of comments word-wide in

2012. For the moment, validation studies still have to stick to the 2003 version of ISO 16140. Different objectives have to be met for either qualitative methods or quantitative methods, as given below.

Qualitative method validation (Detection):
Part A. Methods comparison study (MCS):
- Relative accuracy, relative specificity and relative sensitivity (60 samples per Food Category).
- Relative detection level (6 samples at 3-5 levels per Food Category).
- Inclusivity and exclusivity (50 target strains and 30 non-target strains).

Part B. Interlaboratory study (ILS):
- At least 10 collaborative laboratories within 3 countries (8 replicates at 3 levels = 24 samples by each laboratory).

Quantitative method validation (Enumeration):
Part A. Methods comparison study (MCS):
- Linearity and relative accuracy (Per Food Category at least 1 sample in duplicate at 5 different levels).
- Relative sensitivity and determination of unknown samples (Per Category at least 10 additional samples in duplicate).
- Detection and quantification limits (6 replicates of at least 3 levels).
- Specificity, inclusivity and exclusivity (30 target strains and 20 non-target strains).

Part B. Interlaboratory study (ILS):
- At least 8 collaborative laboratories within 3 countries (2 replicates at 4 levels = 8 samples by each laboratory).

The MicroVal Expert Laboratory (EL), to be chosen by the client, is in charge of the organisation and elaboration of the laboratory work in both the MCS and the ILS. Expert Laboratories have to be officially approved by MicroVal and have to work under accreditation (according to ISO 17025, Anonymous 2005a). Collaborative laboratories for participation in the interlaboratory study are usually contacted by the Expert Laboratory and preferably are working under a quality assurance system.

The Dutch Food Institute RIKILT (Wageningen, NL), nowadays at RIVM-LZO (Bilthoven, the Netherlands), finished the following alternative methods as an MicroVal Expert Laboratory:
Quantitative methods:
- Enumeration of *E. coli* (2007LR07).
- Enumeration of *Campylobacter* spp. (2008LR12).

Qualitative methods:
- Detection of *Salmonella* (2007LR06).
- Real-time PCR Detection of *Enterobacteriaceae* and/or *E. sakazakii* (2007LR08, 2007LR09, 2007LR19, 2007LR20).
- Real-time PCR detection of *Salmonella* (in preparation).

The numbers between brackets refer to the certificates as available at the MicroVal website: www.microval.org.

As an example of a validation study, details and data on the 2007LR06 study on detection of *Salmonella* were shown and discussed. Most of these data are also given as a summary in the final certificate for this alternative method (see www.microval.org).

Discussion
Q: During three interlaboratory comparisons studies of the EURL-*Salmonella*, I also used a PCR method. The PCR method gave different results in the different studies. In two studies similar results were found with the PCR method and the 'classical detection method', but in one study fewer positives were found with the PCR method. Which results can I use for the validation study?
A: These results may not be valid for an official validation (following EN ISO 16140), but may still be useful for an internal validation. Deletion of deviating data is only allowed in case these results are caused by technical problems.
Q: Is it true that validated methods are still not allowed to be used for official monitoring (as indicated in EU Regulations)?
A: This is not fully clear. The opinion of the competent authority of a member state is also of importance in this.

3.9 CEN mandate and proposal for veterinary interlaboratory comparison study 2013

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

In 2006, the European Commission (DG-Sanco) sent a mandate to CEN/TC275/WG6 for the validation of 15 microbiological methods (mandate M/381). The mandate 'falls within the rules to ensure food safety in the whole food chain in relation to biological hazards'. The mandate is related to several EC Regulations, like Regulation 882/2004 on food and animal feed control (EC, 2004) and Regulation EC 2073/2005, on Microbiological criteria (EC, 2005). Annex D of EN ISO 6579 (Anonymous, 2007) is one of the methods to become validated. By the end of 2007, the EURL-*Salmonella* was already assigned to become project leader for this validation study. Due to many administrative problems, it took up to December 2010 before the contract was signed between CEN and the EC. Officially the project started on 1 January 2011 and will last for six years in total. The final results of the project will be that validation studies of 15 microbiological methods are performed and that the validation data are published in the relevant CEN/ISO documents. As the publication of CEN/ISO documents lasts several years, it is the intention that the validation studies are all performed in the first half of the project, thus before the end of 2013.
In 2011 subcontracts were signed between CEN and most of the (15) project leaders.
A small group of project leaders has made a proposal for the general protocol for validation of qualitative and quantitative methods. This protocol is based on the procedures as described in EN ISO 16140 (Anonymous, 2003). It is the intention to perform the validation studies for the different methods in, as much as possible, a similar way. The validation studies are not intended to compare methods, but to set the performance characteristics of a method. For each validation study an interlaboratory study (ILS) needs to be organised. For qualitative methods this includes that at least ten laboratories should participate, obtaining at least ten valid data sets per contamination level. Samples with three different contamination levels have to be tested: blank, low level (at or slightly above the detection limit of the method) and high level (five to ten times above the detection limit of the method). Per level, eight blind replicates have to be tested. For a horizontal method (applicable for e.g. food and feed) at least five different categories of matrices have to be analysed. For a vertical method (like primary production), only one category of matrix needs to be analysed.

For the validation of Annex D of EN ISO 6579 (detection of *Salmonella* in primary production samples, Anonymous, 2007), the following is suggested:

- To use data of earlier organised EURL-*Salmonella* studies for the detection of *Salmonella* in animal faeces. This is currently under discussion with the coordinator and project leaders of the CEN mandate.
- To combine the study for the CEN mandate with the study of the EURL-*Salmonella* for detection of *Salmonella* in veterinary (environmental) samples in February/March 2013. The data will then be differently treated for the EURL study (testing performance of the laboratories) and for the CEN mandate (testing performance of the method).

The idea of combining the CEN mandate study with the EURL-*Salmonella* veterinary study of 2013 was discussed with the NRLs at the workshop. The advantage of combining both studies is that it is not necessary to organise an additional (fourth) interlaboratory study in 2013. Also the type of matrix was discussed. Dust has been suggested, but the majority of the NRLs were not in favour of this type of matrix as it is hardly ever analysed at the NRLs and additionally it can easily result in contamination of the laboratory. It was much more preferred to analyse boot swabs/socks as this is a type of matrix which is very often analysed at the NRLs.
It was agreed that the EURL-*Salmonella* will further explore the possibilities for the suggested ideas.

3.10 Work programme EURL-*Salmonella* second half 2012, first half 2013 and closure

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

Work programme
Kirsten Mooijman summarised the information on the work programme of the EURL-*Salmonella* for the rest of 2012 and for early 2013.

Interlaboratory comparison studies
As indicated in earlier presentations, three interlaboratory comparison studies are planned in the coming year:

- Detection of *Salmonella* spp. in animal feed (or an ingredient of animal feed): September/October 2012.
- Typing of *Salmonella* spp. (serotyping and phage typing): November/December 2012.
- Detection of *Salmonella* spp. in a 'veterinary' matrix, preferably boot swabs/socks (study in combination with the study for the CEN mandate): February/March 2013.

For the different interlaboratory comparison studies the following will be explored/considered:

- Testing fewer samples in the interlaboratory comparison studies on detection of *Salmonella,* following the rules of ISO/TS 22117 (Anonymous, 2010).
- Simplifying the test reports.
- Making use of web based test reports.

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

- Continuation of the activities for the standardisation organisations, ISO (at international level) and CEN (at European level).
- Statistical analyses of the results of the pooling experiments (see clause 2.3).
- Testing different matrices in combination with different/new reference materials for ring trials.

Communication and other activities
As before, the newsletter will be published four times a year through the EURL-*Salmonella* website. The NRLs are requested to provide any relevant information of interest for the other NRLs for publication through the newsletter.
The EURL-*Salmonella* website will be amended and linked to the new website address.
Experts of the EURL-*Salmonella* regularly participate in working groups of EFSA and of DG-Sanco.
EURL-*Salmonella* will perform ad hoc activities (on own initiative or on request) and may be of help by giving advice to NRLs to become accredited.
Furthermore, trainings can be given by EURL-*Salmonella* at the EURL or at the laboratory of the NRL. Requests for trainings will be considered case by case.

Workshop 2013
In May 2013 (27-29 May) the I3S international symposium on *Salmonella* will again be organised in St. Malo, France (www.i3s2013.com). Like in former years it was suggested to organise the EURL-*Salmonella* workshop in conjunction with this I3S symposium. The NRLs agreed to do so. The EURL-*Salmonella* will therefore explore the possibilities to organise the workshop as a one day workshop in St. Malo on Thursday 30 May 2013.

Closure
Kirsten Mooijman closed the workshop, thanking all participants and guest speakers for their presence and contributions and thanking the staff members of the EURL-*Salmonella* and of the NRL-*Salmonella* of Greece for their help in organising the workshop.

4 Evaluation of the workshop

4.1 Introduction

At the end of the workshop a questionnaire was handed over to all participants to ask for their opinion on the workshop (see Annex 3). In total 12 questions were posed and it was requested to indicate a score from 1 to 5 as an answer to the questions, where 5 was the highest score (excellent) and 1 was the lowest score (very poor). If wanted, it was also possible to give remarks to the questions.
The questionnaire was handed over to 44 participants of the workshop and 30 completed forms were received, being a response of 68%. Furthermore, eleven respondents took the opportunity to give remarks to one or more questions.

In clause 4.2 the scores per question are indicated and also a summary of the remarks is given.

4.2 Questionnaire

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 be good or excellent (scores 4-5).

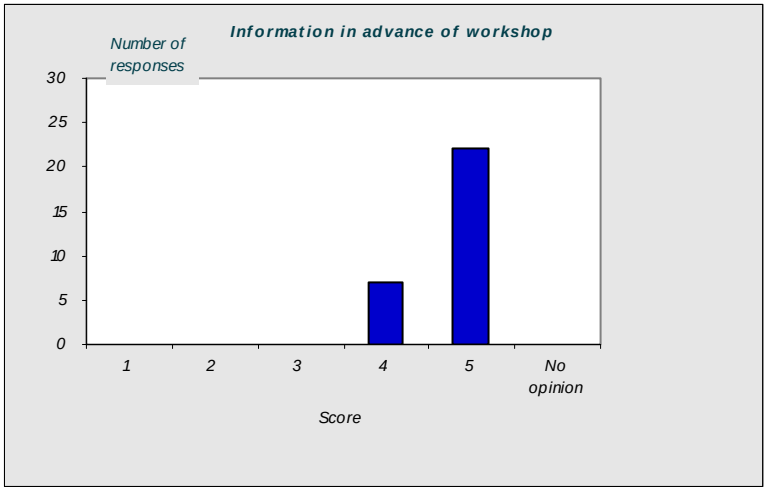

Figure 1 Scores given to question 1 'Opinion on information given in advance of the workshop'

*2. What is your opinion on the booking of the tickets by the EURL-*Salmonella*?*
Figure 2 shows that all respondents considered the booking of the tickets by the EURL-*Salmonella* to be good or excellent (scores 4-5) or had no opinion because they booked the tickets themselves. The following remark was made to this question:

- If you book a 'light' ticket, I cannot bring my computer, because only 6 kg hand luggage is allowed.

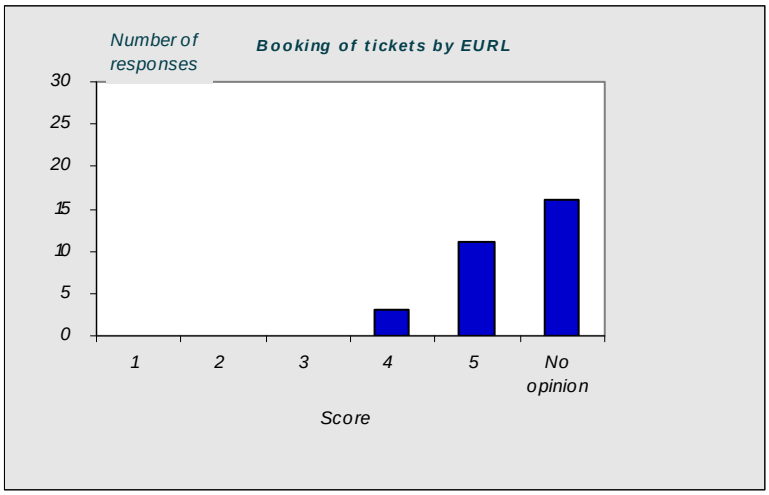

*Figure 2 Scores given to question 2 'Opinion on booking of the tickets by EURL-*Salmonella'

3. What is your opinion on the easiness to reach the meeting venue?
The majority of the respondents indicated that the meeting venue was good (score 4) or excellent (score 5) to reach (Figure 3). Only one respondent indicated that the meeting venue was 'poor' (score 2) to reach. No further comments were given.

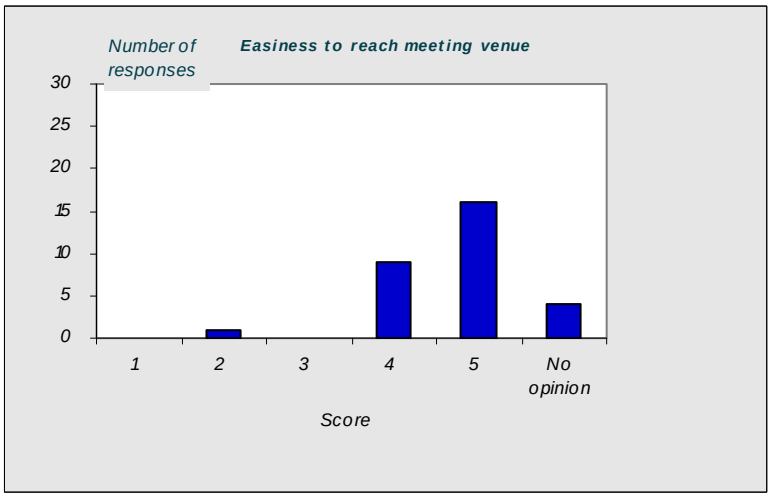

Figure 3 Scores given to question 3 'Opinion on easiness to reach meeting venue'

4. What is your opinion on the hotel room?
The majority of the respondents considered the hotel room to be good (score 4) or excellent (score 5), see Figure 4. Only one respondent indicated that the hotel room was 'poor' (score 2), without giving further comments. One respondent's comment was 'Smoker room', but still indicated the hotel room to be considered good (score 4).

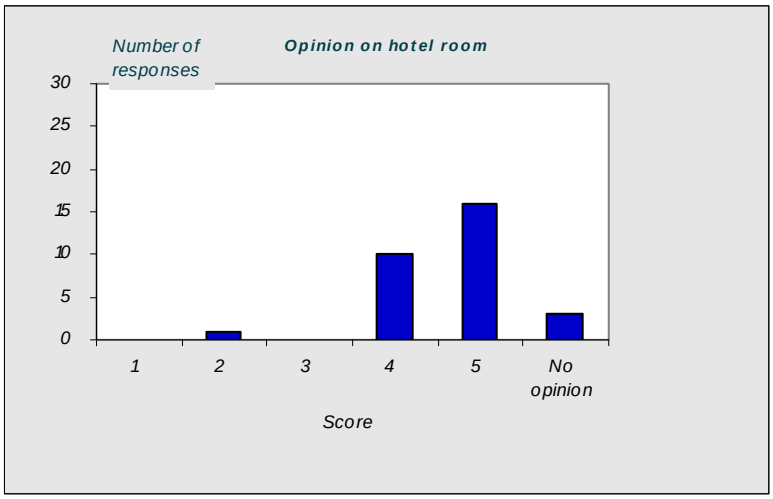

Figure 4 Scores given to question 4 'Opinion on the hotel room'

5. What is your opinion on the meeting room in general?
Figure 5 shows that all respondents considered the meeting room to be good or excellent (scores 4-5). Only one remark was given to this question being: 'Without sunlight'.

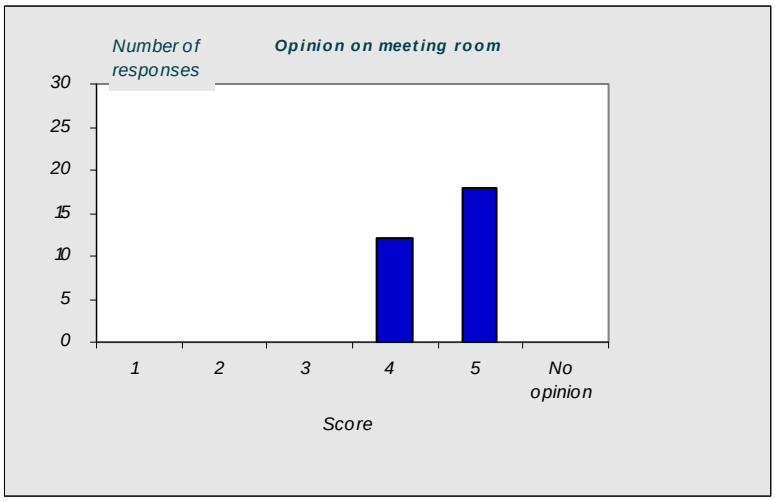

Figure 5 Scores given to question 5 'Opinion on the meeting room'

6. What is your opinion on the readability of the presentations on the screen?
The opinions on the readability of the presentations on the screen varied. 21 respondents considered the readability to be good or excellent (scores 4-5), but eight respondents indicated a moderate score (score 3) and one a poor score (score 2), see Figure 6. Remarks related to this question were:

- 'Many speakers had slides with too much information and thus too small letters'.
- 'The additional small screen blocked the vision on the large screen. It was not possible to read everything on the small screen'.

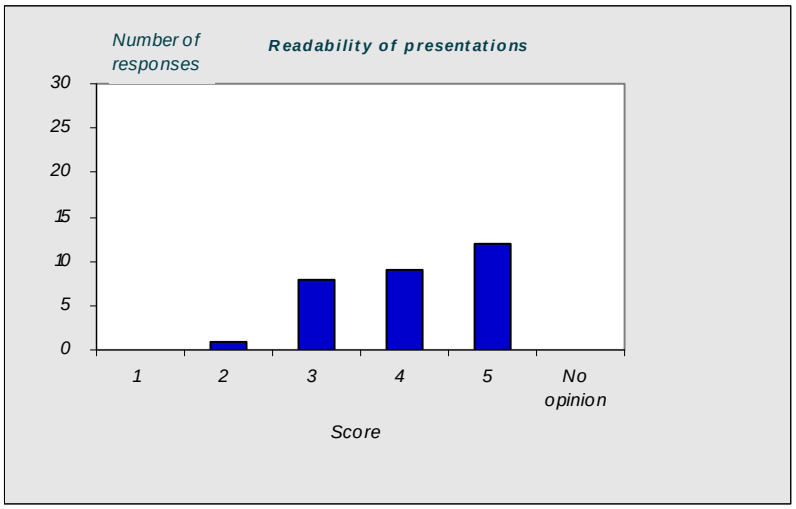

Figure 6 Scores given to question 6 'Opinion on the readability of the presentations'

7. What is your opinion on the technical equipment in the meeting room (computer, screen, microphones, etc.)?
The majority of the respondents considered the technical equipment to be good or excellent (scores 4-5), see Figure 7. Only one respondent considered the technical equipment to be moderate (score 3). One remark was given in relation to this question:

- 'The TV was not necessary. It could have been useful if it had been bigger'.

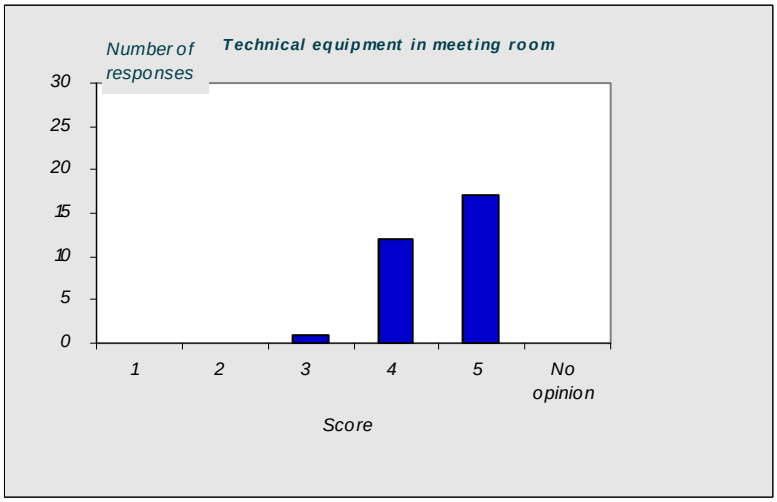

Figure 7 Scores given to question 7 'Opinion on the technical equipment'

8. What is your opinion on the catering 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. Only two respondents indicated a moderate score (score 3). One respondent remarked the breakfast to be poor.

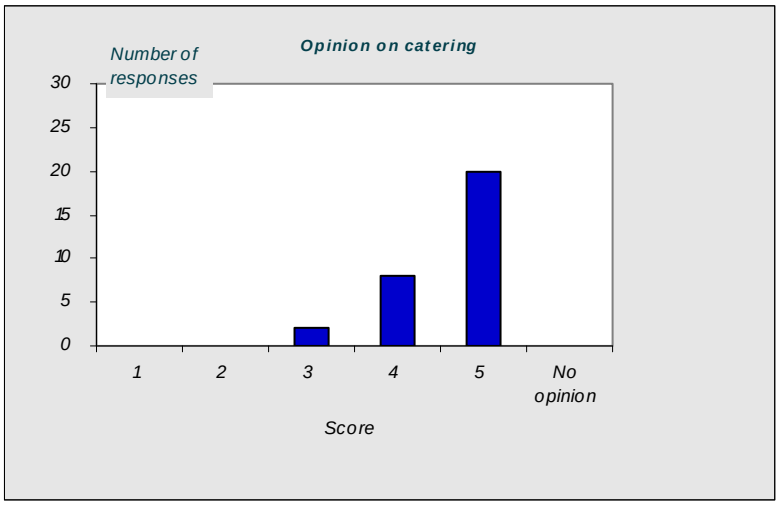

Figure 8 Scores given to question 8 'Opinion on the catering'

9. What is your opinion on the scientific programme of the workshop?
The respondents were very satisfied about the scientific programme of the workshop; only good (score 4) or excellent (score 5) scores were given (see Figure 9). No further comments were given to this question.

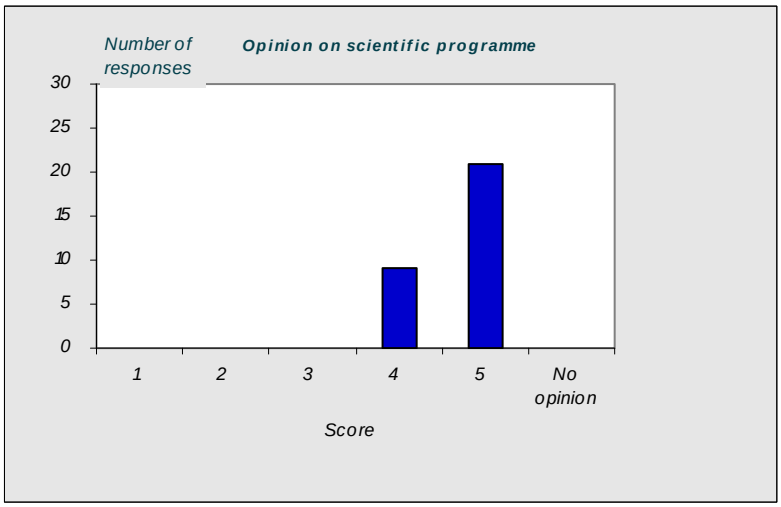

Figure 9 Scores given to question 9 'Opinion on the scientific programme'

10. Are there specific presentations you want to remark upon or did you miss information on certain subjects?
This concerned an 'open' question and the following responses were obtained:

- 'More information on statistical methods used in assessing performance of proficiency testing'.

11. What is your general opinion of the workshop?
The respondents indicated the workshop as a whole to be good (score 4) or excellent (score 5), see Figure 10. No further comments were given.

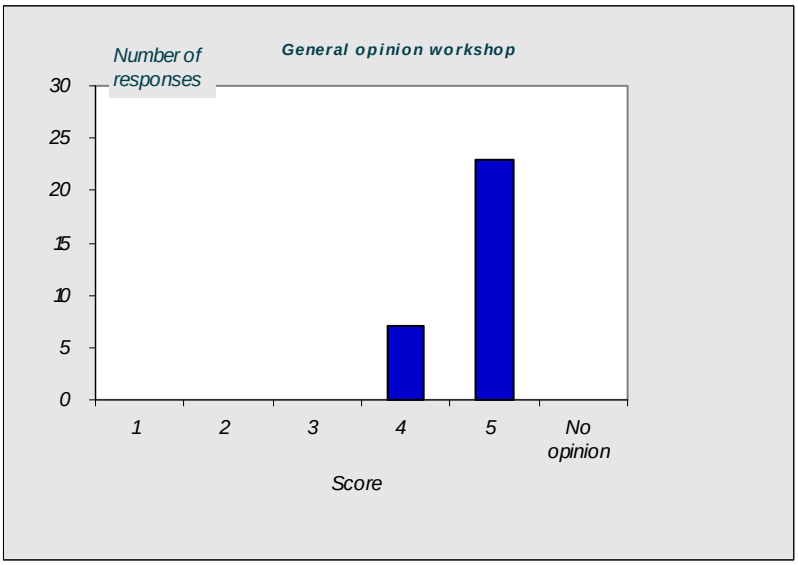

Figure 10 Scores given to question 11 'General opinion of the workshop'

12. Do you have any remarks or suggestions which we can use for future workshops?
This concerned an 'open' question and the following responses were obtained:

- 'Nice to pick another location for this year's workshop'.
- 'Continue discussion and experimental work on pooling of samples'.
- 'The hand-outs are a very good idea'.
- 'One day could have been enough for the workshop'.
- 'More details on molecular serotyping methods used by the NRLs on the panel of serotyping strains'.
- 'Information on new or (re-emerging) serovars or serovars growing in importance'.
- 'It would be nice to have one hour for general discussions, where everyone could say (if needed) something about his problem that he wants to discuss. This time I would have asked if other NRLs also have problems with rough strains'.

4.3 Discussion and conclusions of the evaluation

From the answers of the respondents to the questionnaire, it can be concluded that the participants were satisfied about the workshop. The scientific programme was considered interesting and also the conditions under which the workshop was organised was in general assessed as good. Only the readability of the presentations on the screen was not always considered sufficient. This was not only caused by technical aspects (like meeting room, size of screen), but also by the fact that some slides in some presentations contained too much information.
The remarks from the evaluation of the workshop of 2011 were taken into account as much as possible. Therefore, hand-outs of the majority of the presentations were organised and also the suggestion to change locations regularly was taken over. From the evaluation, it can be concluded that this was highly appreciated.

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List of abbreviations

A	Answer
BIOHAZ	EFSA Panel on Biological Hazards
BPW	Buffered Peptone Water
CEN	European Committee for Standardisation
cfu	colony forming units
DG	Directorate General
DG-Sanco	Directorate General for Health and Consumer Protection
DT	Definitive Type
EC	European Commission
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
EFTA	European Free Trade Association
EU	European Union
EURL (CRL)	European Union (Community) Reference Laboratory
FBO	Food-borne outbreak
HPA	Health Protection Agency
ILS	Interlaboratory study
ISO	International Standardisation Organisation
LZO	Laboratory for Zoonoses and Environmental Microbiology
MKTTn	Mueller Kauffmann Tetrathionate broth with novobiocin
MLVA	Multi-Locus Variable number of tandem repeats Analysis
MS	Member State
MSRV	Modified Semi-solid Rappaport Vassiliadis
NRL	National Reference Laboratory
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
PT	Proficiency Test
Q	Question
RIKILT	Institute of Food Safety
RIVM	National Institute for Public Health and the Environment
RVS	Rappaport Vassiliadis broth with Soya
SC	Sub Committee
SD(6)	*Salmonella* Derby (at a level of approximately 6 cfu)
SE(8)	*Salmonella* Enteritidis (at a level of approximately 8 cfu)
STM(10)	*Salmonella* Typhimurium (at a level of approximately 10 cfu)
TAG	Technical Advisory Group
TC	Technical Committee
TR	Technical Report
TS	Technical Specification
UK	United Kingdom
WG	Working Group

Annex 1 Participants

European Food Safety Authority (EFSA)	Frank Boelaert
EURL – *Salmonella*	Kirsten Mooijman Angelina Kuijpers Wilma Jacobs Wendy van Overbeek
Guest speaker (Belgium)	Pierre Wattiau (CODA, CERVA, Brussels, Belgium)
Guest speaker (Greece)	Georgia Mandilara, National School of Public Health, National Reference Centre for Salmonella, Vari, Greece
Guest speaker (United Kingdom)	Elizabeth de Pinna (HPA, London)

National Reference Laboratories for *Salmonella*

AUSTRIA	Heimo Lassnig
BELGIUM	Katelijne Dierick
BOSNIA HERZEGOVINA	Sead Hadziabdic
BULGARIA	Gergana Mateva
CROATIA	Gordan Kompes Borka Simpraga
CYPRUS	Maria Emmanuel
CZECH REPUBLIC	Tomas Cerny
DENMARK	Karl Pedersen Birgitte Nauerby
ESTONIA	Age Kärssin
FINLAND	Henry Kuronen
FRANCE	Anne Brisabois
FYROM	Dean Jankuloski
GERMANY	Istvan Szabo Andreas Schroeter
GREECE	Aphrodite Smpiraki Maria Passiotou-Gavala Athanassios Katsimpras Eleni Valkanou Nikki Mouttotou
HUNGARY	Erzsebet Andrian
IRELAND	John Egan
ITALY	Antonia Ricci
LATVIA	Madara Streikisa
LITHUANIA	Ruta Bubuliene
LUXEMBOURG	Joseph Schon
MALTA	-
NORTHERN IRELAND	Gintare Bagdonaite
NETHERLANDS	Anjo Verbruggen
NORWAY	Bjarne Bergsjø

POLAND	Magdalena Skarzynska
	Kinga Wieczorek
PORTUGAL	Patricia Themudo
ROMANIA	Luminita Monica Vanghele
SLOVAK REPUBLIC	Milan Sasik
SLOVENIA	Jasna Micunovic
SPAIN	Maria Christina de Frutos Escobar
SWEDEN	Lennart Melin
TURKEY	Elcin Gunaydin
UNITED KINGDOM	Samantha Chapell

Annex 2 Programme of the workshop

Programme of the EURL-*Salmonella* workshop XVII
14 and 15 May 2012, Chalkida, Greece

General information

Hotel and place of the workshop:
Best Western Lucy Hotel,
10 Voudouri ave.
34100 Evia – Chalkida
Greece
http://www.lucy-hotel.gr/

Presentations
For the ones who will give a presentation: please send your (PowerPoint) presentation and the abstract of your presentation to Kirsten Mooijman (*kirsten.mooijman@rivm.nl*) before 9 May 2012

Sunday 13 May 2012
Arrival of participants at Chalkida

18:30 – 19:30 Registration and get-together in hotel Best Western Lucy hotel
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 13 May, with a maximum of € 30,- per person. A receipt will be needed in order to reimburse you for this meal. You can choose either to use the dinner in the hotel and ask to add the costs to the invoice of your room, or to have dinner outside the hotel and ask the EURL for reimbursement of the costs afterwards.

Monday 14 May 2012

Morning chair: Wilma Jacobs

09:00 - 09:30	Opening and introduction	Kirsten Mooijman, EURL-*Salmonella*
09:30 - 10:00	EU *Salmonella* monitoring data (Summary report 2010)	Frank Boelaert, EFSA
10:00 - 10:30	ISO and CEN activities for *Salmonella*	Kirsten Mooijman, EURL-*Salmonella*
10:30 - 11:00	*Coffee/tea*	
11:00 - 11:45	Information from EFSA: • Estimation of the relative contribution of different food and animal sources to human *Salmonella* infections in the EU • Analysis of the baseline survey on *Salmonella* in breeding pigs in the EU, 2008 - Part B: Factors associated with *Salmonella* pen positivity	Frank Boelaert, EFSA
11:45 – 12:15	Results interlaboratory comparison study on bacteriological detection of *Salmonella*-Veterinary XV-2012	Angelina Kuijpers, EURL-*Salmonella*
12:15 – 13:45	*Lunch*	

Afternoon chair: Kirsten Mooijman

13:45 - 14:15	Results typing study XVI - 2011: serotyping	Wilma Jacobs, EURL-*Salmonella*
14:15 - 14:45	Results typing study XVI - 2011: phagetyping	Elizabeth de Pinna, HPA, UK
14:45 - 15:00	Proposal typing study 2012	Wilma Jacobs, EURL-*Salmonella*
15:00 – 15:30	*Coffee/tea*	
15:30 - 16:15	Methodologies for *Salmonella* typing: gold standards and alternatives	Pierre Wattiau, CODA, CERVA, Belgium
16:15 - 16:45	Multiresistant monophasic *Salmonella* Typhimurium 1,4,[5],12:i:- serotype in Greece 2006-2011	Georgia Mandilara, National School of Public Health, Greece
19:15 -	*Departure from hotel for dinner outside hotel*	

Tuesday 15 May 2012

Morning chair: Wilma Jacobs

	Activities NRLs to fulfil tasks and duties:	
09:00 - 09:20	NRL Greece	Aphrodite Smpiraki
09:20 - 09:40	NRL Lithuania	Ruta Bubuliene
09:40 - 10:00	NRL Belgium	Kathelijne Dierick
10:00 - 10:20	NRL Malta (Cancelled)	Albert Gambin
10:20 - 10:50	*Coffee/tea*	
10:50 - 11:10	NRL Sweden	Lennart Melin
11:10 - 11:30	NRL Croatia	Gordan Kompes
11:30 - 12:00	Results interlaboratory comparison study on bacteriological detection of *Salmonella* – FOOD V – 2011	Angelina Kuijpers, EURL-*Salmonella*
12:00 - 12:15	Proposal on interlaboratory comparison study on detection of *Salmonella* in food/animal feed 2012	Angelina Kuijpers, EURL-*Salmonella*
12:15 – 13:45	*Lunch*	

Afternoon chair: Kirsten Mooijman

13:45 - 14:15	MicroVal validation of alternative methods	Wilma Jacobs, RIVM-MicroVal expert lab
14:15 - 15:00	CEN mandate & Proposal for Veterinary interlaboratory comparison study 2013	Kirsten Mooijman, EURL-*Salmonella*
15:00 – 15:30	*Coffee/tea*	
15:30 - 16:00	Work programme EURL-*Salmonella* second half 2012, first half 2013 and closure	Kirsten Mooijman, EURL-*Salmonella*
19:00 -	*Dinner at hotel, including social programme*	

Annex 3 Evaluation form of the workshop

Evaluation of the XVIIth EURL-*Salmonella* workshop
14 and 15 May 2012, Chalkida, Greece

We would highly appreciate if you could give us your opinion on the 17th EURL-*Salmonella* workshop, organised in Chalkida, Greece on 14 and 15 May 2012. 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 the easiness to reach the meeting venue?

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

Remarks:

4. What is your opinion on the hotel room?

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

Remarks:

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

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 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 remark upon 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!

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