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

The sixteenth EURL-Salmonella workshop

19 and 20 May 2011,
Zandvoort, the Netherlands

RIVM report 330604022/2011
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Colophon

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This investigation has been performed by order and for the account of the European Commission, Directorate-General for Health and Consumer Protection (DG-Sanco) and the Dutch Food and Consumer Product Safety Authority (VWA), within the framework of RIVM project V/330604/11/CS European Union Reference Laboratory for Salmonella (2011)

Abstract

The sixteenth EURL-*Salmonella* workshop
19 and 20 May 2011, Zandvoort, the Netherlands

This report contains the summaries of the presentations of the sixteenth annual workshop for the National Reference Laboratories (NRLs) for *Salmonella*, held in Zandvoort, the Netherlands on 19 and 20 May 2011. The aim of this workshop was 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 2009. It shows that, although the number of health problems caused by *Salmonella* is decreasing, it is still the second most important cause, after *Campylobacter*, of zoonotic diseases in Europe.

Three presentations dealt with the emerging '*Salmonella* Typhimurium-like' strains: the EFSA opinion on monitoring and assessment of the public health risk of this strain, a molecular technique to type the strain and two outbreaks in France caused by this type of strain.

In other summaries, the NRLs for *Salmonella* of a few selected countries describe their activities, the EURL-*Salmonella* gives information on standardisation of methods for detection of *Salmonella*, the validation of a molecular typing method of *Salmonella* Typhimurium is described and information is given on *Salmonella* in the pork slaughter chain.

The workshop was organised by the EURL-*Salmonella*, formerly called CRL-Salmonella, which 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 2011

Rapport in het kort

De zestiende EURL-*Salmonella* workshop
19 en 20 mei 2011, Zandvoort, Nederland

In dit rapport zijn de verslagen gebundeld van de presentaties die op 19 en 20 mei 2011 zijn gegeven tijdens de zestiende jaarlijkse workshop voor de Europese Nationale Referentie Laboratoria (NRL's) voor de bacterie *Salmonella*. Elk jaar wisselt het overkoepelende orgaan, het Europese Referentie Laboratorium (EURL) *Salmonella*, tijdens deze workshop informatie uit met de NRL's. Daarnaast worden de resultaten gepresenteerd van de ringonderzoeken van het EURL waarmee de kwaliteit van de NRL-laboratoria wordt gemeten. De resultaten hiervan worden uitgebreider in aparte RIVM-rapporten weergegeven. Een ander terugkerend onderwerp is het rapport van de European Food Safety Authority (EFSA) over zoönosen, oftewel ziekten die van dieren op mensen kunnen overgaan. Dit rapport geeft een overzicht van de aantallen en types zoönotische micro-organismen die in 2009 gezondheidsproblemen veroorzaakten in Europa. Hieruit blijkt dat *Salmonella* minder gezondheidsproblemen veroorzaakt, maar nog steeds, na de *Campylobacter*-bacterie, de tweede belangrijke veroorzaker is van zoönotische ziekten in Europa.

Drie presentaties behandelden een 'nieuwe' stam: '*Salmonella* Typhimurium-like'. Hierin is de mening van de EFSA uiteengezet over de wijze waarop de gezondheidsrisico's van deze stam het beste kunnen worden gemonitord en vastgesteld. Daarnaast is een moleculaire methode om deze stam te typeren toegelicht en ten slotte zijn twee uitbraken in Frankrijk beschreven die door deze stam veroorzaakt werden.

In andere verslagen beschrijven de NRL's voor *Salmonella* van enkele geselecteerde landen hun activiteiten. Verder geeft het EURL-*Salmonella* informatie over standaardisatie van methoden om *Salmonella* op te sporen en te typeren en wordt de validatie van een moleculaire typeringmethode voor *Salmonella* Typhimurium beschreven. Tenslotte wordt informatie gegeven over *Salmonella* in de slachtlijn van varkens.

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

Trefwoorden:
EURL-*Salmonella*, NRL-*Salmonella*, *Salmonella*, workshop 2011

Contents

Summary

On 19 and 20 May 2011 the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*), formerly called Community Reference Laboratory (CRL), organised her annual workshop in Zandvoort, the Netherlands. On both days representatives of the National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) were present, as well as representatives of the European Commission, Directorate-General for Health and Consumer Protection (DG-Sanco), of the European Food Safety Authority (EFSA) and several guest speakers. A total of 47 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 and DG-Sanco on trends and sources of Zoonoses in Europe and on European policy issues concerning *Salmonella*. Furthermore, information was given on the progress with the standardisation of methods on detection 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 food matrix as performed in 2010 were presented.
During the afternoon session of the first day, the results of the interlaboratory comparison studies on detection of *Salmonella* in a veterinary matrix (2011) and on serotyping and phage typing of *Salmonella* (2010) were discussed. Also proposals for future interlaboratory comparison studies and interpretation of results were discussed. The day was closed with presentations of two guest speakers: one presentation on the validation of a protocol for MLVA typing of *Salmonella* Typhimurium and another presentation on tracing of *Salmonella* in the pork slaughter chain.

On the second (half) day of the workshop, five NRLs for *Salmonella* gave presentations, explaining their activities to fulfil the task and duties of an NRL. On this second day of the workshop, also special attention was given to the emerging '*Salmonella* Typhimurium-like' *strain*. EFSA presented the scientific opinion on monitoring and assessment of the public health risk of this strain, the NRL of Italy explained a PCR-technique for typing of this type of strain and finally the NRL of France described two French outbreaks caused by this type of strain.
The workshop was finished with a presentation on the work programme of the EURL-*Salmonella* for the next year.

The full presentations given at the workshop can be found at:
http://www.rivm.nl/crlsalmonella/workshops/WorkshopXVI.jsp

1 Introduction

In this report the abstracts of the presentations given at the EURL-*Salmonella* workshop of 2011 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 EURL-*Salmonella* website: http://www.rivm.nl/crlsalmonella/workshops/WorkshopXVI.jsp

The lay-out of the report is according to 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 Thursday 19 May 2011: 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 sixteenth workshop of the EURL-*Salmonella*, welcoming all participants in Zandvoort, the Netherlands. From the EU Member States excuses were received from the NRLs of Spain (before the meeting) and from Cyprus (at the end of the meeting), due to health problems.
After a roll call of the delegates, information was given on the changes at the EURL and other informative aspects:

- Last year some changes in staff were introduced: Wendy van Overbeek (technician) and Irene Pol-Hofstad (researcher) have become member of the EURL-*Salmonella* team for part of their time. Christiaan Veenman has become more involved with other projects within the RIVM and less with the EURL-*Salmonella* activities. Hennie ter Hoeven (secretary) has taken over the management of the EURL-*Salmonella* website from April 2011.
- Last year the 5 years evaluation of the EURL has taken place. Shortly before the workshop the summary report was received, showing a very good result. More details were given by Klaus Kostenzer of DG-Sanco (see below).
- In March 2011 Regulation EC 208/2011 was published, by which the name 'Community Reference Laboratory (CRL)' has officially been changed into: 'European Union Reference Laboratory (EURL)'.
- 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 is currently in press.

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 2009 European Union summary report on Zoonoses – Overview on *Salmonella*

Giusi Amore, EFSA, Parma, Italy

The European Union (EU) system for the monitoring and collection of information on zoonoses is based on the Zoonoses Directive 2003/99/EC (EC, 1999), which obligates the EU Member States (MSs) to collect relevant and, where applicable, comparable data of zoonoses, zoonotic agents, antimicrobial resistance and food-borne outbreaks. The European Food Safety Authority (EFSA) has been assigned to analyse these data and publish the EU Summary Report (EUSR). Data on zoonotic infections in humans are reported via The European Surveillance System (TESSy) to the European Centre for Disease Prevention and Control (ECDC) that provides the data, as well as their analyses, for the EUSR. The 2009 EUSR was prepared by EFSA and ECDC with the assistance of EFSA's Zoonoses Collaboration Centre (ZCC) in the National Food Institute of the Technical University of Denmark (EFSA, 2011).

In 2009, salmonellosis was again the second most commonly reported zoonotic disease in humans in the EU, following campylobacteriosis. The number of salmonellosis cases in humans decreased by 17.4%, compared to 2008, and the statistically significant decreasing trend in the European Union continued for the fifth consecutive year. In total 108 614 confirmed human cases were reported in 2009 and, in particular, human cases caused by *Salmonella* Enteritidis decreased markedly. The case fatality rate was 0.08%. It is assumed that the observed reduction of salmonellosis cases is mainly attributed to successful implementation of national *Salmonella* control programmes in fowl populations; but also other control measures along the food chain may have contributed to the reduction.
In foodstuffs, the highest proportions of *Salmonella*-positive units were reported for fresh broiler meat and fresh turkey meat, on average at levels of 5.4% and 8.7%, respectively. In fresh pig meat, 0.7% of the tested units were found positive for *Salmonella* in the reporting MS group. *Salmonella* was rarely detected in other foodstuffs, such as dairy products, fruit and vegetables. Non-compliance with EU *Salmonella* criteria was most often observed in minced meat and meat preparations (8.7%) as well as in live molluscs (3.4%). Of particular risk for human health are the *Salmonella* findings from meat categories intended to be eaten raw, where *Salmonella* was detected in 1.2%-1.7% of the single units tested, which indicates a presence of a direct risk for consumers. The proportion of egg products not in compliance with the *Salmonella* criteria has fallen from 2.8% to 0.2% in single samples compared to 2008. In other food categories, the proportion of units in non-compliance with the criteria was very low.
All MSs reported data from the mandatory *Salmonella* control programmes in fowl (*Gallus gallus*) populations and also from other domestic animals and wildlife species. MSs had to meet EU *Salmonella* reduction target of ≤1% of breeding flocks of Gallus gallus infected with the five target serovars (*S.* Enteritidis, *S*. Typhimurium, *S*. Hadar, *S*. Infantis, *S*. Virchow) by the end of 2009. Together, 18 MSs (compared to 20 MSs in 2008) met this target in 2009. Overall, 1.2% (compared to 1.3% in 2008) of breeding flocks in EU were positive for the five target serovars during the production period. The seven MSs, not meeting the target, reported a prevalence of the five target serovars ranging from 1.2% to 7.0%. Together 2.7% of the breeding flocks in EU were positive for *Salmonella* (all serovars). Similarly, 17 MSs (compared to 21 MSs in 2008) met their relative reduction target for *S*. Enteritidis and *S*. Typhimurium in laying hen flocks of *Gallus gallus* set for 2009, while eight MSs (compared to two MSs in 2008) did not meet their target. Overall, during the production period, 6.7% and 3.2% of laying hen flocks in EU were positive for *Salmonella* (all serovars) and *S*. Enteritidis and/or *S*. Typhimurium in 2009, respectively. 2009 was the first year for MSs to implement the mandatory control programmes in broiler flocks, and already 18 MSs met the *Salmonella* reduction target of ≤1% for *S*. Enteritidis and/or *S*. Typhimurium, which is to be achieved by the end of 2011. In total, 5.0% and 0.7% of broiler flocks in EU were positive for *Salmonella* (all serovars) and *S*. Enteritidis and/or *S*. Typhimurium, respectively.

In most MSs, *S*. Enteritidis was the most frequently isolated serovar from table eggs and also frequently found in poultry meat. Therefore, the decrease observed in the number of *S*. Enteritidis cases in humans is supposed to be related to the decrease of this serovar in laying hen flocks reported for 2009. *S*. Typhimurium was the most frequently isolated serovar in pigs, cattle and meat thereof and it was also among the top ten serovars isolated from broilers and table eggs. It is important to underline that when interpreting results on

serovar distribution special attention should be given on specific serovars in some countries.
The number of food-borne outbreaks caused by *Salmonella* was at a lower level in 2009 than in previous years. However, *Salmonella* continued to be the most commonly reported causative agent in food-borne outbreaks in 2009, even though in decreasing numbers. In the reported *Salmonella* outbreaks, eggs and egg products as well as products containing raw eggs, continued to be the most important food vehicles. These outbreaks were mostly caused by *S*. Enteritidis.

In conclusion, as illustrated in the 2009 summary report, the numbers of human salmonellosis cases reported in EU continued to decline in 2009 as a part of a statistically significant trend since 2005. The reduction was particularly substantial for the most frequently reported serovar, *S*. Enteritidis. It is assumed that the observed reduction of salmonellosis cases is mainly due to successful *Salmonella* control programmes in fowl populations. The results from the control programmes in fowl populations are therefore promising and encourage taking into consideration broadening the intensified control efforts further to other animal populations, such as breeding and slaughter pigs.

Discussion
Q: Do the indicated percentages of *Salmonella* Typhimurium (STM) also include the '*Salmonella* Typhimurium-like' strains?
A: I am not sure about this. From the results as reported in 2009 it is not always possible to make a distinction between *Salmonella* Typhimurium and '*Salmonella* Typhimurium-like'. It was only by September 2010 that the EFSA opinion on '*Salmonella* Typhimurium-like' strains was published, resulting in a different way of reporting STM and 'STM-like' strains.
Q: Is it correct that 'STM-like' strains are more often found than in former years?
A: This might be the case, but again, it was not possible from the data of 2009 to make a distinction for this type of strains.
Q: When EU member states report the antigenic formula of a strain, will this be summarised in the group 'other *Salmonella* serovars'?
A: From this year on it is possible to report 'STM-like' with its antigenic formula separately. Before it was indeed summarised in the group 'other *Salmonella* serovars'.
Q: In the presentation it was indicated that the percentage of salmonellosis cases decreased in 2009 when compared to 2008. This has been explained by the fact that the EU control programme on *Salmonella* is indeed working. However, the percentage of cases caused by *Salmonella* Typhimurium increased in 2009, how can this be explained?
A: Indeed the percentage of cases caused by STM increased, but the total number of cases caused by STM decreased compared to 2008. Furthermore, the differences in percentages of STM cases in 2008 (22%) and in 2009 (23%) are small. The differences in percentages between cases caused by *Salmonella* Enteritidis are larger for the two years (58% in 2008 and 52% in 2009).
Q: Is there a correlation between the number of cases caused by STM in humans, compared to the numbers of STM found in breeding pigs?
A: We do not know, we have not looked at this. However, there is no direct link between the public health impacts caused by breeding pigs, as breeding pigs are in front of the line of primary production. Still pig meat is an important source for salmonellosis. It 'compensates' the decrease of the numbers caused by STM in poultry. A shift in sources seems to have taken place.
Q: Did you get much feed back from the press release concerning the reduction of *Salmonella* thanks to the success of the control programmes?

A: Yes we have received some feed back, but good news does not sell as well as bad news.

2.3 Recent policy issues on *Salmonella*

Klaus Kostenzer, European Commission, DG-Sanco, Brussels, Belgium

Regulation (EC) No 2160/2003 (EC, 2003) on the control of *Salmonella* and other specified food borne zoonotic agents is a framework legislation that provides for control of zoonoses all over the food chain, starting at the level of primary production. The aim of this Regulation is to ensure that effective measures are taken to decrease the occurrence of pathogens i.e. certain *Salmonella* serotypes that are of special significance for public health. One of the recent policy issues on *Salmonella* was to discuss the confirmation of the control target in laying hens in the EU. The Commissions working group on Zoonoses took into account an opinion of the European Food Safety Authority (EFSA) on a quantitative estimation of the public health impact of *Salmonella* in laying hens. Also the experiences of Member States in the implementation of the transitional target were taken on board. Thus, the target remains on *Salmonella* Enteritidis and *Salmonella* Typhimurium; as regards monophasic *S.* Typhimurium, strains with the antigenic formula 1,4,[5],12:i:- shall be included in the Union target.

Current discussions also touch upon the *Salmonella* criterion for fresh poultry meat as laid down in Annex E of the referred control Regulation stating "*Salmonella*: absence in 25 grams" from 2011 onwards. The details for the respective food safety criterion in Regulation (EC) No 2073/2005 (EC, 2005) were to be agreed upon from the Member States in order to grant a harmonised approach in the EU. A proposal has been discussed and was technically agreed upon by the Member States in March. A final adoption is foreseen after respecting the right of scrutiny of the European Parliament and the Council and after sanitary and phytosanitary measures of the World Trade Organisation were consulted with regards to the impact on world trade. The proposed criterion foresees the inclusion of *S*. Enteritidis and *S*. Typhimurium. EN ISO 6579 (Anonymous, 2002) plus serotyping is foreseen as the analytical method. None out of five samples of 25 g fresh poultry meat is allowed to test positive. Sampling rules and frequencies are the same as under the current process hygiene criterion for *Salmonella* in poultry carcases.

The Commission launched an evaluation of EURLs in 2010 including all 26 food and feed safety EURLs nominated in the period 2006-2010. The scope was to evaluate the functioning and performance of the laboratories, the obligations and duties laid down in Regulation 882/2004, the working programmes and to assess the relevance of tasks, possible overlaps or synergies and the appropriateness of current mandate. The EURL for *Salmonella* has performed excellently – partly adequately – over the evaluation period. One of the recommendations was that the feedback provided by participants in workshops could be summarised in a more systematic manner.

Discussion

Q: Have also EU control programmes for food been planned?
A: No. The current national programmes are based on EC Regulation 2160/2003 (EC, 2003), which only applies to animal populations as listed in the annex of this regulation. No control programmes are currently foreseen in regulations for food control.

Q: In the new legislation on poultry the same demands are given for STM and 'STM-like'. In UK often *Salmonella* 4,12:i:- is found. If it can be proven that this strain concerns a different serovar than STM, is it then still necessary to slaughter the flock?
A: No, for the naming of this type of strain please consult the EFSA opinion (see clause 3.6, ed.). If you can show that the isolated serovar does not belong to STM by using for example a PCR test, it does not have legal consequences. The legislation may not give guidance on all fields. It is important that experts in the member states would give advice for this kind of problems.
Q: What about the *Salmonella* targets in broilers and turkeys?
A: These will be in line with the target for layers. The target for broilers will soon be published, for turkeys it will be published in 2012. The number of 'STM-like' strains in turkeys seems to be limited up to now.
Q: Are the results of the cost benefit analysis in pigs available?
A: Currently a variety of control programmes in pigs exist in the EU member states. It is under discussion how this can be harmonised and what minimum demands can be set. The cost benefit analysis on primary production of pigs is available and sent to the contacts in the member states. However, no official conclusions are given yet. It is still under discussion where to set control points and what can be done best to protect human health.
Q: The testing of samples for EC Regulation 2073/2005, Microbiological criteria (EC, 2005), is poor. Will the control be improved?
A: It can be discussed to introduce changes to improve the procedure.

2.4 Technical issues on *Salmonella*

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

Kirsten Mooijman of the EURL-*Salmonella* presented an overview of activities in ISO and CEN in relation with *Salmonella*. Furthermore, she also informed the NRLs on the first results of research carried out at the EURL-*Salmonella* in relation to pooling of samples. This latter research has a relation with the revision of the CEN/ISO document on detection of *Salmonella* (EN ISO 6579; Anonymous, 2002) as well as with EU Regulation EC No 2073/2005 (EC, 2005).

Activities in ISO and CEN
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 Bournemouth, United Kingdom from 20 to 24 June 2011.

For *Salmonella* three procedures are under revision or preparation in CEN and ISO. The existing standard procedure for detection of *Salmonella* in food and animal feed is described in EN ISO 6579 (Anonymous, 2002) and in Annex D of this document for the detection of *Salmonella* in samples from primary production (Anonymous, 2007). After the five years review of EN ISO 6579 in 2007, it was decided to start the revision of this document. At the same time it was also agreed to start working on standard documents for enumeration of *Salmonella* and for serotyping of *Salmonella*. Therefore, it was agreed to split EN ISO 6579 (Anonymous, 2002) into 3 parts to deal with detection (part 1),

enumeration (part 2) and serotyping (part 3) of *Salmonella* spp. under one EN ISO number. The work for the three items is performed in three different working groups or ad hoc groups, of which Kirsten Mooijman is project leader. The progress of the work with the three documents was explained to the NRLs.

ISO 6579-1: Detection of Salmonella
Historical overview:
2007, July-December: Five years systematic review of ISO 6579:2002. Result of voting: 10 members confirmed, 2 members voted confirmation and correction, 4 members voted revision, 0 members voted withdrawal, 2 members abstained. Comments were included.
2008, May: Discussion of outcome systematic review at the meeting of ISO/TC34/SC9 in Helsinki.
2008, July-November: Enquiry launched to ask for specific comments and data.
2008/2009, December/January: an ad hoc group was raised.
2009, 6 April: meeting ad hoc group to review outcome enquiry. Fifteen decisions/proposals were made.
2009, May: Presentation (by Kirsten Mooijman) of the decisions/proposals at SC9 meeting in Valencia. SC9 agreed that revision of EN ISO 6579 (2002) was considered necessary. The 15 decisions were summarised by SC9 in resolution 395. It was also agreed to split EN ISO 6579 into 3 parts: part 1, detection; part 2, enumeration; part 3, serotyping of *Salmonella* spp.
2009, June-September: Call for experts to raise new Working Group (WG9), with Kirsten Mooijman (EURL-*Salmonella*) as convenor. 17 experts from 7 different countries were nominated.
2009, 15 December: First meeting WG9 (Paris). Several items and distribution of tasks agreed.
2010, January: Report of first meeting WG9 sent to the secretariat of SC9.
2010, May: First working draft of ISO 6579-1 prepared by Kirsten Mooijman and sent to the members of WG9.
2010, June: Summary of the progress presented at the meeting of SC9 in Buenos Aires. At this meeting it was agreed that for this work the lead should be at CEN level because of the fact that the method is part of validation studies under a mandate at CEN level.
2010, October: Second meeting WG9 (Brussels), discussion of outcome SC9 meeting and discussion of first working draft.
2010/2011, October-February: Contributions for update first working draft sent by members of WG9 to Kirsten.
2011, May: Second working draft prepared by Kirsten and sent to the members of WG9 for further comments. The final working draft (including the final comments of WG9) will be sent to the secretariat of ISO/TC34/SC9 and CEN/TC275/WG6 to send it around for a first official voting round (expected approximately September 2011).

The main changes in EN ISO 6579 part 1 compared to the version of 2002 are:
- Incorporation of ISO 6785 (milk and milk products);
- Samples for primary production added to the scope;
- Description of detection of *S*. Typhi and *S*. Paratyphi in normative annex: use of Selenite Cystine broth for selective enrichment
- Selective enrichment media:
    - First selective enrichment: choose either Rappaport Vassiliadis broth with Soya (RVS) or Modified Semi-solid Rappaport Vassiliadis (MSRV) agar;
    - Second selective enrichment: Mueller Kauffmann Tetrathionate broth with novobiocin (MKTTn).

- Primary production samples: only MSRV (like in the current Annex D of EN ISO 6579; Anonymous, 2007);
- Incubation time of selective enrichment media retained for 24 h, except for some specific food products (e.g. milk powder) and primary production on MSRV (48 h if necessary);
- In informative notes the possibility to refrigerate pre-, and selective enrichment cultures for a maximum of 72 h is added;
- Xylose Lysine Deoxycholate (XLD) is retained as mandatory isolation medium;
- The plating stage has been made less prescriptive (only indicate need to obtain isolated colonies);
- Tables are added in an annex to give a clearer direction for the choice of suitable second plating media;
- Confirmation on only one suspect colony (instead of one colony of each medium combination). If negative, 4 more suspect colonies from different media combinations have to be tested;
- Allowed to perform parallel biochemical testing and purity check;
- The non-selective medium for purification has been left for choice;
- Two confirmation tests have become optional: ß-Galactosidase test and indole reaction;
- One confirmation test has been deleted: Voges-Proskauer reaction;
- Details on serotyping have been moved to ISO 6579 part 3. In part 1 only serological confirmation (to serogroup level) is described;
- Performance testing for quality assurance of media is added;
- Validation data for analysing food samples on MSRV are added to an annex.

ISO 6579-2: Enumeration of Salmonella
Historical overview:
2007, April: At the plenary meeting the members of ISO/TC34/SC9 agreed to base the enumeration standard on a publication of Fravalo et al (2003): a Most Probable Number (MPN) technique in 12-well microtitre plates, with selective enrichment on MSRV (also in microtitre plates).
2007, December: First draft protocol for the mini-MSRV technique distributed to the members of SC9 for testing.
2008, fall: Information from the experiences of different SC9 members gathered and used to amend the document.
2009, January: Amended document launched for voting: positive outcome with some comments.
2009, May: Comments discussed in an ad hoc group. The finishing of the final draft document had to wait for an MPN calculation tool from the ISO working group on statistics
2010, February: Final draft document sent to the secretariat of SC9 to launch it for final voting.
2011, May: Final vote still not launched due to administrative problems at CEN level. However, it is expected that these administrative problems are solved by June 2011. Next the document needs to be translated in French and in German. Hence, the final voting is not expected before September 2011.

ISO 6579-3: Serotyping of Salmonella
Historical overview:
2008 and 2009: Enquiries were sent to the members of SC9 to ask for their interest in a standard document for serotyping of *Salmonella*.
2009, May: The outcome of the enquiries was presented at the plenary meeting of SC9 and it was agreed that there was a need for a standard document on

serotyping of *Salmonella.* Next it was agreed to raise an ISO ad hoc group to initiate the work.
2009, June: A call for experts for raising the ad hoc group was launched. Currently the ad hoc group exists of 9 experts from 7 countries (including a member of the WHO reference centre Paris) with EURL-*Salmonella* (Kirsten) being project leader.
2009, December: First meeting of the ad hoc group in Paris. At this meeting the ad hoc group indicated to prefer publication of the standards as an informative document, meaning an ISO/CEN 'Technical Report' (TR).
2010, March: Members of the ad hoc group sent comments/contributions to Kirsten.
2010, May: Kirsten made a first working draft and sent it to the ad hoc members.
2010, June: progress of the ad hoc group was reported at the plenary meeting of SC9 (Buenos Aires).
2010, June-fall 2010: Ad hoc group members gave comments/contributions to first working draft.
2011, April: Kirsten made the second working draft and sent it to the members of the ad hoc group for comments. The final working draft (including the final comments of the ad hoc group) will be sent to the secretariat of ISO/TC34/SC9 and CEN/TC275/WG6 to send it around for a first official voting round (expected approximately September 2011).

Pooling of samples
EU Regulation No 2073/2005 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 for *Salmonella*. The experiments are based on a draft protocol for pooling (compositing) 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 are included in the experimental design of the EURL. For dry pooling 25 g of meat is inoculated with a stressed *Salmonella* strain at a level of approximately 5 colony forming particles (cfp) per25 g. This sample is mixed with 4 x 25 g *Salmonella*-free meat and the 125 g pooled meat sample is 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) are followed. For the wet pooling also 25 g of meat is inoculated with a stressed *Salmonella* strain at a level of approximately 5 cfp per 25 g, but this is added to 225 ml BPW. Furthermore, four samples of 25 g of *Salmonella*-free meat are each added to 225 ml BPW. The BPW samples are incubated at 37 °C, like for the dry pooling. After incubation, 5 ml is taken from each BPW culture and mixed. From this mixture 0.5 ml is added to 50 ml Rappaport Vassiliadis with Soya (RVS), 5 ml is added to 50 ml Mueller Kauffmann Tetrathionate broth with novobiocin (MKTTn) and 0.1 ml is 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) are followed. Additionally, the inoculated sample of 25 g is 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 (*S.* Enteritidis, *S.* Typhimurium and 1,4,[5],12:i:-) are tested with different types of stress (cold, freezing, heating) on four types of poultry meat (chicken and turkey meat with and without skin). At least five different samples of each type of meat will be tested. Up to now different ways of stressing the strains have been tested and the pooling experimental design has been followed for four samples of chicken meat without skin, all showing comparable results for the dry pooling and the wet pooling. The experimental design will be further followed for the other type of samples as well.

Discussion
Q: Do the EN/ISO documents allow the use of commercial galleries for confirmation of the isolates?
A: Yes, this is allowed if the gallery contains at least the confirmation tests as described in the relevant EN/ISO procedure.

2.5 Results interlaboratory comparison study on bacteriological detection of *Salmonella* - FOOD IV - 2010

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

In September 2010, the Reference Laboratory of the European Union for *Salmonella* (EURL-*Salmonella*) organised the fourth interlaboratory comparison study on bacteriological detection of *Salmonella* in a food matrix: minced (pork and beef) meat. Participants were thirty-one National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) of the EU-Member States and of countries from the European Free Trade Association (EFTA): Norway, Switzerland and Iceland.

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 (capsules) containing either *Salmonella* (at various contamination levels) or sterile milk powder. 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 method (ISO 6579, 2002) and the additionally requested method (Annex D of ISO 6579, 2007). For the prescribed method, the selective enrichment media were Rappaport Vassiliadis Soya broth (RVS) and Mueller Kauffmann Tetrathionate novobiocin broth (MKTTn). For the requested method, the selective enrichment was Modified Semi-solid Rappaport Vassiliadis (MSRV) agar. Optionally, a laboratory could also use other, own media or procedures for the detection of *Salmonella*.

Twenty-nine individually numbered capsules had to be tested by the participants for the presence or absence of *Salmonella*. Twenty-four of the capsules had to be examined in combination with each 25 grams of *Salmonella* negative meat: 8 capsules contained approximately 5 colony forming particles (cfp) of *Salmonella* Typhimurium (STM5), 8 capsules contained approximately 50 cfp of *S*. Typhimurium (STM50) and 8 capsules did not contain any micro-organisms (blank capsules). The other five capsules, to which no meat had to be added, were control samples, comprising 3 capsules STM5, 1 capsule STM50 and 1 blank capsule.

On average, the laboratories found *Salmonella* in 99% of the (contaminated) samples either using the selective enrichment media prescribed for the food method (MKTTn and RVS) or the method for testing veterinary samples (MSRV).

Twenty-eight out of 31 laboratories achieved the level of good performance on the first attempt. One NRL scored a moderate performance because they made a transcription error during the transfer of raw data to the test report. Two laboratories needed a follow-up study conducted in January 2011 to reach the desired level. Cross-contamination of blank samples with other samples provided for testing and/or with samples from their own laboratory is the most likely explanation for the initial deviation of their results from the desired outcome.

Discussion
Q: It seems to be easier to detect *Salmonella* from artificially contaminated samples. Is it not possible to use naturally contaminated samples in the interlaboratory comparison studies?
A: It may indeed be the case that the recovery of *Salmonella* from artificially contaminated samples is easier than from naturally contaminated samples. However, it is hard to use naturally contaminated samples in a study for several reasons: i) difficult to find sufficient artificially contaminated material for one study; ii) most of the time, *Salmonella* is not homogeneously distributed in naturally contaminated samples which may cause problems in the interpretation of the results of the laboratories; iii) the level of contamination is not known and may vary a lot in the samples. To mimic the 'natural' situation as much as possible, reference materials with stressed *Salmonella* strains are used for the artificial contamination of the samples.

2.6 Results interlaboratory comparison study on bacteriological detection of *Salmonella* - Veterinary XIV - 2011

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

In March 2011 the Reference Laboratory of the European Union for *Salmonella* (EURL-*Salmonella*) organised the fourteenth veterinary interlaboratory comparison study on bacteriological detection of *Salmonella* in chicken faeces. Participants were 32 National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*): 28 NRLs from 27 EU Member States, three NRLs from member countries of the European Free Trade Association (EFTA), Switzerland, Norway and Iceland, and on request of DG-Sanco one non-Europe NRL from a third country, Israel.

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, chicken 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, 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*.

In this study for the first time lenticule discs were used as reference materials. The change from capsules (former studies) to lenticule discs was especially done

because of the easiness of handling of the lenticules. Furthermore, with lenticule discs it was easier to use the normal routine procedures for sample treatment and therefore to mimic the daily routine analyses better.

Thirty-two individually numbered lenticule discs had to be tested by the participants for the presence or absence of *Salmonella*. Twenty-five of the lenticule discs had to be examined in combination with each 25 gram of Salmonella-negative chicken faeces: 5 lenticule discs contained approximately 6 colony forming particles (cfp) of *Salmonella* Typhimurium (STM6), 5 lenticule discs contained approximately 61 cfp of *S.* Typhimurium (STM61), 5 lenticule discs contained approximately 6 cfp of *S*. Enteritidis (SE6), 5 lenticule discs contained approximately 57 cfp of *S.* Enteritidis (SE57) and 5 lenticule discs contained no Salmonella at all (blank lenticule discs). Six lenticule discs, to which no faeces had to be added, were control samples, existing of 2 lenticule discs STM6, 2 lenticule discs SE6, 1 lenticule disc SE57 and 2 blank lenticule discs.

On average the laboratories found *Salmonella* in 99% of the (contaminated) samples when using the prescribed veterinary method, with selective enrichment on MSRV.
Forty-eight hours of incubation of MSRV gave overall 10% more positive results than 24 h. This was most obvious for the low level contaminated SE samples which gave 30% more positive results after 48 h of incubation.

Twenty-nine NRLs fulfilled the criteria of good performance. Two laboratories had difficulty in detecting low levels of *Salmonella* (a sensitivity problem). One laboratory found a false positive blank sample (without matrix). A follow up study is planned after this workshop.

It was concluded that the first EURL-*Salmonella* study organised with lenticule discs as reference material was successful.

Discussion
Remark: Switzerland has also organised an interlaboratory comparison study in which animal faeces was artificially contaminated with lenticules. This study also showed the good applicability of the lenticules as well.

2.7 Proposal for interlaboratory comparison studies on detection of *Salmonella* – 2011/2012

Angelina Kuijpers and Kirsten Mooijman, EURL-Salmonella*, Bilthoven, the Netherlands*

The following interlaboratory comparison studies on detection of *Salmonella* spp. are planned for the coming year:
- September/October 2011: Detection of *Salmonella* spp. in a 'food' matrix;
- February/March 2012: Detection of *Salmonella* spp. in a 'veterinary' matrix.

Recent improvements made to the interlaboratory comparison studies:
- Analysis samples with each 25 gram of matrix (instead of 10 g);
- Use of lenticule discs (instead of capsules);
- Use of, as much as possible, the materials as used for routine analyses (e.g. plastic bags with pre-filled BPW);
- Treatment of the samples as in routine analyses (e.g. when applicable: mixing by using a stomacher).

With the use of lenticule discs as reference material the treatment of samples is as follows:

- Pre-warm BPW to at least room temperature;
- Addition of 25 g matrix to container with 225 ml BPW or Addition of 225 ml BPW to container with 25 g of matrix;
- Addition of lenticule discs to container (with 25 g matrix in 225 ml BPW);
- Leave at room temperature for 10-15 minutes (re-hydration of lenticule);
- Mix sample: by following normal routine procedures per type of matrix; e.g.:
  - Faeces: mix gently (shake/knead);
  - Food: mix by using a pulsifier or a stomacher.
- Place BPW sample at 37 ºC for 18 h;
- Analyse samples following ISO 6579 and Annex D of ISO 6579.

During the presentation, the advantages and disadvantages for the use of lenticule discs in interlaboratory comparison studies compared to the use of capsules was discussed.
The following advantages were indicated:

- Lenticules are easier to handle than capsules (dissolve easier);
- The treatment of the samples is more close to the normal routine procedures especially in relation to mixing of the sample (e.g. the use of a stomacher is possible with lenticules);
- There is a reduced risk of cross-contamination with the addition of the lenticule disc after the addition of matrix to the BPW.

One disadvantage could be indicated:

- SE lenticule discs gave atypical colonies on Rambach isolation medium.

For the food study in September/October 2011 it was suggested to use minced meat as matrix. This will be the first EURL-*Salmonella* food study with the use of lenticule discs as reference material. The number of samples will probably be comparable to the veterinary study of 2011. The prescribed method will be the reference method ISO 6579 and Annex D of ISO 6579 will be the (additional) requested method.

For the veterinary study it was suggested to use the same number and type of samples as used with the latest veterinary study in 2011. The prescribed method will be the reference method Annex D of ISO 6579. The choice for a suitable matrix for this study was discussed with the NRLs. Different matrices were suggested: pig faeces, cattle faeces, turkey faeces, but also other types of samples like boot socks and swabs. The pros and cons of the different samples were discussed. Samples like boot socks and swabs are complicated to prepare in large quantities by the EURL. For the detection of *Salmonella* in pig faeces and cattle faeces other serovars may be of interest than in poultry faeces. For cattle faeces *S.* Dublin may be of interest. But it was argued that this serovar may be difficult to detect in cattle faeces, which on the other hand is a good challenge to test the performance of the laboratories. Also eggs were suggested as matrix, but this is also complicated to prepare by the EURL. As alternative egg powder was suggested, but this was considered to be 'too easy' as this matrix contains in general no or very little background flora. It was agreed that the EURL will further explore the possibilities for using cattle faeces or pig faeces with *Salmonella* serovars most frequently found in these types of matrices.

The current criteria for testing the performance of the laboratories in interlaboratory comparison studies on detection of *Salmonella* were discussed. These criteria are summarised in Table 1. With the current criteria only good performance or poor performance can be determined. However, occasionally it

may also be needed to judge performances as 'moderate'. In a few studies some results of NRLs have already been judged as moderate. Reasons for judging these results as moderate were:

- Mixing up of reference materials where in the other results no deviations are seen;
- Problems with reconstitution of capsules;
- Electricity breakdown (matrix and reference materials stored at elevated temperatures);
- Transcription error from raw data into test report;
- Deviating results with control samples containing antibiotics.

In case of poor performance the following steps as a follow-up are taken:

- The participating laboratory is contacted to ask for possible (technical) explanations;
- In general a follow-up study is organised with a focus on the earlier problems;
- If good results are found in the follow-up study, no further actions are needed;
- If the three items as mentioned above are seen in three consecutive studies, then the follow-up study will be combined by a training/visit of EURL-*Salmonella* staff member(s) at the NRL to further explore possible reasons for the problems. The information concerning the performance of the NRL will be reported to DG-Sanco, independent of the outcome of the (third) follow-up/training.
- In case of poor performance in a follow-up study this will always be reported to DG-Sanco.

It was discussed whether further actions should also be taken in case of moderate performance. It was agreed that if moderate performance is seen in three consecutive studies, the NRL will be contacted by the EURL to discuss a proper follow-up. The type of follow-up will be considered on a case by case basis depending on the nature of the moderate performance. A visit of staff member(s) of the EURL-*Salmonella* to the NRL can be considered as a possible follow-up. Also in the case of repeated moderate performance DG-Sanco will be informed.

*Table 1 Current criteria for testing good performance of participating laboratories in EURL-*Salmonella *interlaboratory comparison studies*

Samples		Minimum result	
		Percentage positives	Examples No of positive samples/ total no of samples
Reference materials without matrix	STM/SE high	100%	1/1
	STM/SE low	50%	1/2
	Blank	0%	0/2
Reference materials with matrix	STM/SE high	80%	4/5
	STM/SE low	50%	2-3/5
	Blank	At max 20%	1/5

STM: *Salmonella* Typhimurium
SE: *Salmonella* Enteritidis
high: 'high' contamination level (e.g. 50-100 cfp/reference material)
low: 'low' contamination level (e.g.: 5-10 cfp/reference material)

Discussion
Q: Would it not be possible to use samples in which matrix and strain(s) are already mixed? There may be a risk in cheating when a laboratory needs to mix matrix and reference material in its laboratory before analyses.
A: We have a good knowledge on the stability of *Salmonella* in the reference materials we use for the interlaboratory comparison studies. However, this may be different for reference materials mixed with a matrix. Due to influence of the matrix and the background flora, the stability of the reference material may be influenced. This may also vary per type of matrix. Unfortunately, it is not possible to exclude the risk of cheating completely.

2.8 Results on serotyping of *Salmonella* of the fifteenth interlaboratory comparison study on typing (2010)

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

The fifteenth 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), in cooperation with the Health Protection Agency (HPA, London, United Kingdom), in November 2010.

A total of 33 National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*), from all EU member states and some additional 'third countries', participated in this study. The main objectives of this study were 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.

Twenty different serovars of *Salmonella enterica* supsp. *enterica* were sent to the participants. 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 S1 was excluded from this year's evaluation, since it showed too many rough colonies.
The individual laboratory results were reported to the participants in January 2011. An interim summary report on the outcome of the study was prepared and sent to all participants in April 2011.

The serotyping results showed that the O-antigens were typed correctly by 29 of the 33 participating NRLs (88%). This corresponds to 98% of the total amount of strains. The H-antigens were typed correctly by 22 NRLs (67%), corresponding to 95% of the total amount of strains. Twenty NRLs (61%) identified all serovar names correctly, corresponding to 95% of all strains. A completely correct identification by all participants was obtained for four strains: *S*. Agona (S8), *S*. Enteritidis (S15), *S*. Virchow (S16), and *S*. Infantis (S19). Most problems occurred with the strains *S*. Liverpool (S5), *S*. Chester (S7), and *S*. Schwarzengrund (S17). The reported serovar name for strain S18 by the NRL laboratories showed a large variation of 'Typhimurium-like' names. The EFSA proposed (September 2010) to harmonise reporting of this serovar by asking the laboratories to report the antigenic formula as found by the laboratory.

Four participants did not meet the level of good performance at this stage of the study and three of these laboratories (the fourth laboratory being from a non-EU country) participated in the follow-up study which was organised in April 2011. In this follow-up study 10 additional strains had to be serotyped. All three participating laboratories achieved the level of good performance in this follow-up study.

Discussion
Q: How many participants had problems with serotyping strain S1?
A: Several participants indicated this strain to be rough, therefore it was decided to exclude the strain from the evaluation of the study.
Q: Will it be possible to use molecular techniques for serotyping in future studies?
A: We ask the NRLs to follow the reference method, which is currently still (traditional) serotyping following the White-Kauffmann-Le Minor scheme (Grimont and Weill, 2007). However, it is allowed to use other methods in addition to the reference method. We will consider adapting the test report to make the reporting of alternative methods in the typing studies more easy.
Q: For strain S18 ('STM-like') no PCR was performed, was this necessary?
A: No, this was not requested and the results of the participants were not evaluated for the use of the PCR.

2.9 Results on phage typing of *Salmonella* of the fifteenth interlaboratory comparison study on typing (2010)

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

The *Salmonella* strains for phage typing in the fifteenth interlaboratory comparison study on the typing of *Salmonella* spp., organised for the National Reference Laboratories (NRL), were provided by the Laboratory of Gastrointestinal Pathogens (LGP), of the Health Protection Agency (HPA), London, United Kingdom. Ten strains of *Salmonella* Enteritidis and ten strains of *Salmonella* Typhimurium were selected from the culture collection of the HPA. The selected strains were also used for phage typing in the third international External Quality Assurance (EQA) scheme on the typing of *Salmonella* spp. as organised by the Laboratory for Zoonoses and Environmental Microbiology (LZO) of the National Institute for Public Health and the Environment (RIVM). This latter study is performed in a project of the European Centre for Disease Prevention and Control (ECDC) for the laboratories of the Food and Waterborne Diseases (FWD) and zoonoses surveillance network.
Seven NRLs took part in the phage typing of the *S*. Enteritidis strains and six of these laboratories also took part in the phage typing of the *S*. Typhimurium strains.
Nineteen of the FWD laboratories participated in the phage typing of the *S.* Enteritidis strains and seventeen of these laboratories also participated in the phage typing of the *S*. Typhimurium strains.
Overall, the results of the study for the phage typing of *S*. Enteritidis by the NRLs were excellent. Six of the laboratories correctly phage typed all ten of the *S.* Enteritidis strains and one laboratory correctly phage typed nine of the ten strains. Of the FWD laboratories, seven laboratories correctly phage typed all ten strains of *S*. Enteritidis. Six of the FWD laboratories correctly typed nine of the *S*. Enteritidis strains and one FWD laboratory correctly typed eight of the ten *S*. Enteritidis strains. One FWD laboratory correctly phage typed seven of the strains and two FWD laboratories correctly typed five of the *S*. Enteritidis

strains. One FWD laboratory correctly phage typed four of the ten *S.* Enteritidis stains and the remaining laboratory phage typed only three of the strains correctly. One strain of *S*. Enteritidis – phage type 13 – caused problems for both the NRLs and the FWD laboratories.
Overall, the results of the phage typing of *S*. Typhimurium by the NRLs were also very good. The ten *S*. Typhimurium strains were correctly phage typed by five of the NRLs and one NRL typed nine of the ten *S.* Typhimurium strains correctly.
Five of the FWD laboratories correctly phage typed the ten *S*. Typhimurium strains. Three of the FWD laboratories correctly typed nine of the ten strains and two of the laboratories correctly phage typed eight of the strains. Three of the FWD laboratories correctly phage typed seven of the ten strains and three laboratories correctly phage typed six of the strains. The remaining laboratory correctly phage typed five of the ten *S*. Typhimurium strains. One strain – *S*. Typhimurium DT 7 – was incorrectly phage typed by the NRL and FWD laboratories.

When compared to the previous study the results of the NRLs for the phage typing of *S*. Enteritidis have improved from 94% correct results in 2009 to 98% correct results in 2010. For the phage typing of *S*. Typhimurium the results of this study were the same as the study in 2009 with 98% of the strains correctly phage typed.
For the FWD laboratories, the phage typing of *S*. Enteritidis was not as good as in the previous study when 85% of the strains were typed correctly. Only 82% of the strains were typed correctly in the current study. The phage typing of *S.* Typhimurium was also not as good as in the previous study. In 2009, 91% of the strains were correctly typed compared to 81% in this study.
These two studies show the NRLs continuing to perform phage typing at a high standard. The majority of the FWD laboratories also perform phage typing at a high standard but a few of these laboratories still need to show some further improvement.

Discussion
Q: Were typical reactions found for Phage Type 7 of *Salmonella* Typhimurium?
A: In general it is more difficult to phage type *Salmonella* Typhimurium than *Salmonella* Enteritidis. Especially the 'size' of the inoculum has a large influence on the results.

2.10 General aspects of the typing studies and proposal typing study 2011

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

The provisional planning of the sixteenth EURL-*Salmonellla* interlaboratory comparison study on typing of *Salmonella* was presented to the NRLs for *Salmonella*. The suggested dates are:

- Week 45 (7-11 November) 2011: mailing of the strains;
- Week 46 (14-18 November) 2011: starting with the identification of the strains.

On request of some NRLs last year, the two extensive tables for information on the background data of the serotyping results, became optional in the test report, though the majority of the participants still completed these tables. It was also noted that in case of deviating results a participant will be asked to fill in these tables retrospectively.

For the fifteenth typing study (2010) on *Salmonella*, reporting by electronically filling out the test-report (so not hand-written) and e-mailing was requested and all laboratories kindly cooperated in this. Therefore, a check-up of the result files by the laboratories was no longer needed and time was saved to be able to report the individual laboratory results sooner than in previous studies.

In 2010, information revealed that colonial form variation may occur with the expression of the O:6_1 antigen by some serogroup C_2 serovars (Hendriksen et al., 2009). As for the fourteenth study, also for the fifteenth study on serotyping it was decided to consider the serovar pairs concerned (e.g. *S.* Newport/ *S*. Bardo and *S*. Hadar/*S.* Istanbul) not as distinct serovars.
The WHO Collaborating Centre for Reference and Research on *Salmonella* (Institute Pasteur, Paris) has indicated that this subject will be dealt with in a next version of the White-Kauffmann-Le Minor scheme, but it is not yet known when this version is planned to be published.
For the time being, laboratories are requested to report strains in the EURL-*Salmonella* interlaboratory comparison studies on typing as either *S.* Hadar or as *S.* Istanbul (according to the O-antigens detected). Both serovar names will be evaluated as correct for a *S.* Hadar or a *S.* 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, a general remark for the people working in the laboratory and actually performing the serotyping tests was made: Please make sure that the instructions of the various manufacturers of the sera are followed in detail, because there may be small but essential differences between the different manufacturers (e.g. reading time and background for reading the reaction).

Discussion
Q: Is it possible to add a reptile strain in the next interlaboratory comparison study on typing?
A: Most of these isolates do not belong to *Salmonella enterica* subsp. *enterica*. However, if there is an interest to add such a strain to the study it may be considered to add it as an extra isolate to the study and to leave it up to the participant to type it or not (will not be part of the evaluation of the results).
Q: Is there any guidance/information how to perform quality control of antisera?
A: In part 3 of ISO 6579, on serotyping of *Salmonella*, which is currently under development, some suggestions are given for quality control. For instance, regularly test (new) antisera with known strains. For instance, the strains from the interlaboratory comparison studies can be kept in storage.
Q: What to do if antisera give strange/unexpected results?
A: Indicate this to the supplier of the antisera and if needed supply the manufacturer with the test strain showing the deviating results.

2.11 Validation of a protocol for MLVA typing of *Salmonella* Typhimurium

Eva Møller Nielsen, Statens Serum Institute, Kopenhagen, Denmark

Multi-locus variable number of tandem repeats analysis (MLVA) is increasingly being used for high-discriminatory typing of bacteria. For typing of *Salmonella* Typhimurium, the method developed by Lindstedt et al. (2004) is commonly used in Europe, e.g. in outbreak investigations. In general, MLVA is more discriminatory than Pulsed Field Gel Electrophoresis (PFGE) for typing of

S. Typhimurium. This is especially the case for common phage types as DT104, DT120 and DT12.
The *S.* Typhimurium MLVA method is based on PCR amplification of five variable number of tandem repeats (VNTR) loci followed by detection of the fragment sizes using capillary electrophoresis with an internal size standard in each sample. In principle, the five fragment sizes should be easily comparable between laboratories; however, the fragment analysis is not fully comparable when using different sequencers, polymers, size standards, fluorescent labels, etc. The exact fragment sizes and the actual number of repeat units of different alleles can be determined by sequencing of the loci. However, this is more expensive than performing a simple fragment analysis by capillary electrophoresis of five PCR products in one sample. Therefore, MLVA as a fast and cheap typing method should not involve sequencing. However, the raw fragment analysis data can be converted into the true fragment sizes by the use of a set of strains with known (sequenced) alleles. This procedure gives the possibility of a nomenclature that is independent of the equipment and materials used for fragment analysis and theoretically independent of the primers used (Larsson et al., 2009). This principle was tested with success in a study involving data from 17 laboratories in 2009.
In Spring 2011, ECDC funded a project with the aim of implementing MLVA for *S.* Typhimurium in more laboratories in Europe. For this project, Statens Serum Institute has developed a set of standardised strains and a detailed protocol for the laboratory work and data analysis. The protocol includes guidelines for how to use the standardised strains and how to convert raw data into normalised fragment sizes and number of repeat units according to the agreed common nomenclature. This protocol and panel of standardised strains were sent to 15 laboratories that wanted to set-up this method. In the implementation period, the laboratories have the possibility of getting assistance in trouble shooting with regards to the MLVA implementation in their laboratory.

Discussion
Q: Where do you collect your data?
A: In Bionumerics.
Q: How many times should the control strain be checked?
A: In our institute we have a set of strains of which the MLVA result is known and give immediate information on the validity of the result. It is important that laboratories use a standard set of strains, for instance the set as used in Denmark.

2.12 EU-project Biotracer, tracing *Salmonella* in the pork slaughter chain

Annemarie Pielaat, Laboratory for Zoonoses and Environmental Microbiology, National Institute for Public Health and the Environment, Bilthoven, the Netherlands

Salmonella causes around 30 000 cases of human illness per year in The Netherlands, of which an estimated 25% is caused by pork. *Salmonella* carrying pigs and resident flora on slaughter equipment are relevant sources of carcass contamination. Although recognized, the sources from which and the routes through which *Salmonella* is transmitted to the pig carcasses during slaughter are not well understood in a quantitative way.
Here, we present the application of a sampling scheme at predefined potential sources and at downstream sampled carcasses to get insight in the change in *Salmonella* numbers throughout a slaughter plant. The resulting data are

implemented in a biotracing system for *Salmonella* in the pork chain. This will result in a framework that:

- Gives insight in the most important source of *Salmonella* upon a contamination event;
- Becomes more powerful in source tracing each time new data is added;
- Can be used as a monitoring system on a day-to-day basis;
- Points to targeted intervention.

This work was supported by the European Union-funded Integrated Project BIOTRACER (contract #036272) under the 6th RTD Framework (www.biotracer.org).

Discussion

Q: Were the first samples during slaughtering taken at 11 am? Is that not quite late, as the slaughter process generally starts early in the morning? In this way the amount of house flora may be overestimated?

A: In the morning, the machines were sampled before any pig was slaughtered and they were again sampled after the last pig was slaughtered on one sampling day. In between samples were, amongst others, taken at 11 am.

3 Friday 20 May 2011: day 2 of the workshop

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

Madara Streikisa, NRL-Salmonella*, Riga, Latvia*

The NRL for *Salmonella* in Latvia is situated in the Institute of Food Safety, Animal Health and Environment (BIOR). The institute is located in Riga and has a long history.
The laboratory was founded in 1944. At that time it was the first veterinary laboratory in the country which investigated animal diseases. In 1992, the laboratory also began to investigate food and environmental samples and changed its name to State Veterinary Medicine Diagnostic Centre (SVMDC). In 2006, the laboratory was again reorganised: the Medical clinical microbiology laboratory joined and thus the Scientific Institute as a part of the National Diagnostic Centre (NDC) was founded. From 1 January 2010 the National Diagnostic Centre was consolidated with the Latvian Fish Resources Agency and acquired a new status and name: Institute of Food Safety, Animal Health and Environment (BIOR). The institute has assumed all functions of the National Diagnostic Centre and part of the Latvian Fish Resources Agency functions. Currently the seven laboratories and one Fish Resources Research Department are working in the institute. Four of them located in Riga: Animal Disease Diagnostic Laboratory, Laboratory of Food and Environmental Investigations, Medical Microbiology Laboratory, Calibration Laboratory and three regional laboratories, which are located in Valmiera, Daugavpils and Liepaja. All laboratories of the institute BIOR are accredited according to the EN ISO/IEC 17025 standard (Anonymous, 2005).
The main tasks of the institute are: research in the area of food safety, animal health and the environment and research in the area of fisheries (fish resources).
The institute has been nominated as the National Reference Laboratory in the following areas:

- diagnostics of infectious diseases (34 diseases);
- food, materials and objects contacting with food, animal feed, residues, including residues of pesticides, pathogens and antimicrobial resistance (20 areas);
- control and surveillance of the diagnosis standards and methods (three diseases).

The institute has the following main functions in each of the nominated areas:

- cooperation with the European Union Reference Laboratory;
- coordination of the activities of the laboratories carrying out the official controls;
- organization of proficiency tests between official state laboratories and other laboratories;
- organization of training courses;
- cooperation with the Latvian competent authorities.

The tasks under NRL activities at the *Salmonella* investigations are:

- testing samples from the National *Salmonella* Surveillance program;
- collection of *Salmonella* strains isolated by official laboratories from different kinds of samples;
- serotyping all *Salmonella* isolates and perform the antimicrobial resistance.

In the presentation information was given about diagnostic methods and about results of *Salmonella* investigations in 2010.

Discussion
Q: The samples taken from pigs and cattle were they taken from an active or a passive surveillance programme? What matrices were of concern?
A: It concerns a passive surveillance programme and the samples were taken from animal feed, poultry and food.
Q: Did you detect monophasic *Salmonella* Typhimurium?
A: This strain was detected only once in the programme for pigs in 2011.

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

Joël Mossong, NRL-Salmonella*, Luxembourg, Luxembourg*

In Luxembourg, the 'Laboratoire National de Santé' was nominated by the Ministry of Health as the principal reference laboratory for *Salmonella* in February 2009. The main focus of the reference laboratory is on comparing human and non-human isolates to detect outbreaks and find sources. Methods used include serotyping, antibiotic resistance testing, pulsed-field gel electrophoresis (PFGE), MLVA (*S.* Typhimurium and *S.* Enteritidis) and MLST (although mainly for Campylobacter). Reference activities are done as collaboration between human microbiology laboratories sending human isolates, a veterinary laboratory handling animal and unprocessed food samples and a food laboratory handling processed food and a feed laboratory. In addition, an investigation to an unusual increase of *S*. Enteritidis phage type 14b in Luxembourg and Belgium during August-September 2010, initially detected by MLVA typing in Luxembourg, was also presented. Most epidemiological evidence points towards imported eggs as the likely source, although no isolates were found in tested eggs.

Discussion
Q: Did you ask other 'neighbouring' countries whether they had problems with SE phage type 14b?
A: We asked France, but did not get a reply.
Q: Was a case control study done for this strain?
A: Yes, this was done for 30 cases in Luxembourg, but this investigation was started quite late so that information was lost. In Belgium the cases were not contacted, as it took some time before it was realised that it concerned an outbreak. This resulted in the fact that actions were taken quite late after the real outbreak.

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

John Egan, NRL-Salmonella*, Kildare, Ireland*

The National Reference Laboratory (NRL) functions for *Salmonella* in Food, Feed and Animal Health are undertaken in Ireland by The Department of Agriculture, Fisheries and Food (DAFF) laboratories at Backweston. This and other NRL functions, required under European Regulation 882/2004 (EC, 2004), were assigned to this laboratory in 2006 by the Departments of Health and Children and the Department of Agriculture Fisheries and Food (DAFF), as the Irish Competent Authorities.

The Backweston laboratories play an important statutory role in supporting food safety controls and in surveillance, diagnosis and control of animal diseases. In this regard, the laboratories provide highly specialised expertise, including research, to support DAFF's animal health and food safety policy.

Activities of the NRL-*Salmonella* are published in a Quarterly Newsletter and in its Annual Report. The main summary points in its 2010 Annual report include:

- The primary poultry production sector in Ireland is virtually free of *Salmonella* Enteritidis and the prevalence of *S.* Typhimurium and other *Salmonella* serovars is very low. The national control plans are highly effective and Ireland exceeds all targets specified for the sector.
- Significant additional testing was undertaken in 2010, associated with investigations of duck farms following an outbreak of *S.* Typhimurium DT8 in humans. A *Salmonella* control programme for duck producers was introduced.
- *S.* Typhimurium was the serovar most frequently submitted to the NRL in 2010 from the various testing undertaken. This serovar was mainly associated with the porcine sector. A revised *Salmonella* control programme is in place in the pig sector.
- The NRL-*Salmonella* continues to expand its range of diagnostic and typing services to deliver a more comprehensive service to the regulatory sector and Food Business Operators.
- The NRL continues to work with the EURL, other NRLs including the Human *Salmonella* Reference Laboratory and regulatory and other stakeholders to enhance food safety monitoring systems along the food chain continuum.

Discussion

Q: Do you know where *S.* Kentucky in the Irish broiler flocks came from?
A: I do not know exactly. It seems to have been one integrated group which showed a drop in the numbers and re-integrated again in recent years. Ireland has some own breeding flocks for broilers, but it makes also use of breeders from e.g. England.
Q: As the number of *Salmonella*-positive eggs is decreasing in Ireland, does this also result in a decline in the number of egg related salmonellosis in Ireland?
A: This is not so clear, as some cases have been related to imported eggs and to travelling.

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

Bjarne Bergsjø, NRL-Salmonella*, Oslo, Norway*

The Norwegian Veterinary Institute is a research institute in the areas of animal health, fish health and food safety whose primary function is to supply research support to the authorities. The institute was established in 1891 and is located in Oslo with five regional laboratories in other parts of Norway.
Important tasks of the NRL–*Salmonella* are:

- verification and further characterisation to track down the source of outbreaks of salmonellas from foods, feeds and environmental samples;
- recording the epidemiological *Salmonella* status on a national level and to take part in the *Salmonella* surveillance programmes.

Salmonella surveillance and control in Norway comprise a mandatory control of raw feed materials and a HACCP based control at the feed factories in addition to the EU approved (1995) surveillance and control programme for *Salmonella* in live animals, eggs and meat. These programmes were established to secure the

low occurrence of *Salmonella* in Norwegian domestic animals and products thereof.

Discussion
Q: Are the low levels of *Salmonella* in food also reflected in wild and pet animals in Norway?
A: *Salmonella* is well established in wild animals. It was found that every tenth sample taken from red foxes was positive for *Salmonella*. Furthermore, *S.* Arizona was found in sheep, but does not seem to spread further. Also *S.* Typhimurium-like strains were found in dogs and in raw materials for the production of animal feed.

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

Jasna Micunovic, NRL-Salmonella*, Ljubljana, Slovenia*

Veterinary laboratories in Slovenia dealing with *Salmonella* are at the Veterinary Faculty (which also includes the National Veterinary Institute with NRLs), at the Ministry of Defence in Military Health Service and in private companies. Public health laboratories dealing with *Salmonella* are at the Medical Faculties in Ljubljana and Maribor, at the National Institute for Public Health of the Republic of Slovenia and at eight regional Public Health Institutes. There are also some laboratories at food business operators companies. The main activities for the NRL-*Salmonella* are:

- cooperation with the EURL;
- participation in the EURL annual workshops;
- participation in the EURL inter-laboratory trials;
- spreading information received from EURL;
- scientific and expertise support for the Ministry of Agriculture, Forestry and Food (MAFF) – Veterinary Administration of the Republic of Slovenia (VARS);
- coordination of official laboratory activities;
- enhancing cooperation between the veterinary and the public health sector;
- participation in the preparation of the EU annual Zoonoses reports (published by EFSA).

The laboratory work comprises: serotyping of all *Salmonella* isolates from veterinary laboratories (approximately 300 per year), monitoring *Salmonella* antimicrobial resistance (together with the NRL for antimicrobial resistance testing) and participation in Baseline studies on the prevalence of *Salmonella*. Scientific and expertise support for laboratories includes: help in validation of methods, additional training on *Salmonella* examinations for veterinary laboratories, planned visits to laboratories, ad hoc support in resolving problems. Recently, the laboratory also became involved in accreditation activities (as a member of the Slovene Accreditation working group for microbiology).
The NRL also organises proficiency tests (PT) for veterinary laboratories (since 2003), which include isolation and identification to serogroup. Yearly, four matrices are tested in these studies: milk and milk products (3 samples), meat and meat products (3 samples), feeding-stuffs (3 samples) and faeces (5 samples). Depending on the matrix, 8 to 12 laboratories participate in the studies. A *Salmonella*-free matrix is spiked on the distribution day with cfp numbers close to the limit of detection of serovars Enteritidis, Typhimurium and other serovars of public health importance or frequently isolated serovars. Each correct result is appointed 2 points, a partly correct result (e.g. mistake in

group-serotyping) is appointed 1 point and wrong results do not receive any points. The average score and the standard deviation are calculated and reported for each PT and each laboratory per year. In case of deviating results, the relevant laboratory evaluates its performance and asks for additional samples. When more than 1/3 of the participating laboratories fail, this particular sample is not scored and the NRL evaluates possible deviations in the sample preparation. On laboratory request, the NRL can provide additional training.

3.6 EFSA's Scientific Opinion on monitoring and assessment of the public health risk of *Salmonella* Typhimurium-like strains

Giusi Amore, EFSA, Parma, Italy

Following a request from the European Commission, the Panel on Biological Hazards (BIOHAZ) was asked to deliver a Scientific Opinion on the monitoring and assessment of the public health risk of *Salmonella* Typhimurium-like strains. In particular, the Panel was asked to evaluate the analytical methods currently used and to advise on the appropriateness for identifying these strains; to propose a harmonised terminology for reporting which allows trend-analyses, comparison between Member States and with humans isolates, as well as to indicate if these strains should be classified as variants of *Salmonella* Typhimurium or as a separate serotype. Finally, the Panel was asked to assess the public health risk posed by these emerging strains, in particular to advise whether the public health risk, when detecting these strains in animals or food, should be considered similar, more or less important than (other) *Salmonella* Typhimurium strains.

The BIOHAZ Panel concluded that, within *Salmonella* Typhimurium-like strains, monophasic variants lacking the second phase H antigen (1,4,[5],12:i:-) appear to be of increasing importance in many EU Member States (MSs) and have caused a substantive number of infections in both human and animals bred for food. Strains lacking expression of the phase one flagellar antigens or both are also possible, but have not commonly been reported to be associated with significant disease in animals or humans. Therefore, for the purposes of the Opinion, only the monophasic variants lacking second phase H antigens were considered. Such variants were referred to as 'monophasic *S*. Typhimurium' in the Opinion.

With regard to the analytical methods currently used and their appropriateness for identifying these strains, the current standard methods (ISO 6579 and Annex D of ISO 6579) were considered suitable for isolation of monophasic *Salmonella* Typhimurium strains. Moreover, for identification of the monophasic 1,4,[5],12:i:- variant, it is advisable to proceed with serotyping until a first negative result of agglutination after flagellar phase inversion, and then apply a PCR protocol in order to confirm the lack of the second phase antigen. Other methods such as phage typing and genotyping are used to confirm relatedness to *S.* Typhimurium and/or to further subtype these isolates. The accurate characterisation of monophasic strains is deemed important, since misidentification of a non-*S*. Typhimurium-related strain could result in unnecessary regulatory action. Similarly, failure to confirm identity of a *Salmonella* Typhimurium-like organism could have significant public health consequences.

To ensure complete consistency of reporting, all isolates of putative *Salmonella* should ideally be fully serotyped in accordance with the White-Kauffman-Le Minor scheme, and the full antigenic formula reported, as recommended by the

WHO Collaborating Centre for Reference and Research on *Salmonella* i.e., in the case of monophasic *S*. Typhimurium, 1,4,[5],12:i:-. It was suggested that, whenever possible, as much detail of the antigenic formula as determined by testing should be provided and reported. If the full antigenic formula is not available but a phage type that is consistent with *S*. Typhimurium lacking phase two flagellar antigens has been confirmed, and the lack of the second phase flagellar antigen has been verified by PCR, then the term 'monophasic *Salmonella* Typhimurium' is recommended for reporting purposes in the current situation.
It was further concluded that, on the basis of genetic similarity and ability to obtain a recognised *Salmonella* Typhimurium phage type, these emerging epidemic monophasic strains with the basic antigenic formula 1,4,[5],12:i:- are regarded as variants deriving from *S*. Typhimurium. Monophasic *S.* Typhimurium strains have been shown to have similar virulence and antimicrobial resistance characteristics to strains of *S*. Typhimurium.
The BIOHAZ Panel made a series of further recommendations on typing, molecular methods, antimicrobial susceptibility testing and on monitoring the spread of these strains in EU MSs. Specifically, it was recommended that in order to identify the emergence of new variants of *S.* Typhimurium, all *Salmonella* strains that could potentially be *S*. Typhimurium should be further typed by phage typing and /or molecular typing, referring the strains to a reference laboratory where necessary. Also, since new molecular methods of identification are continuously under development and they should be assessed in relation to the ability to characterise such strains as *S*. Typhimurium. Moreover, the antimicrobial resistance pattern should be determined and reported in a harmonised way for human, animal and food isolates, according to European guidelines. Finally, the importance of monitoring the further spread of these strains was underlined, particularly in poultry breeding flocks, where to date they do not appear to be established in EU MSs.

Discussion
Q: How should the 'STM-like' strains be reported?
A: Please report the antigenic formula you have found. For example, if you found 4,12:i:-, please report this and not 1,4,[5],12:i:-.
Q: If we should report what we have found, why does the EURL give the full formula of the White-Kauffmann-Le Minor scheme in the tables of the interlaboratory comparison studies?
A: Here the complete formula is given, to show what could possibly be found. The EURL will have a look at these tables again to make clear that here the full White-Kauffmann-Le Minor formulas are given and not the formulas which were found by the EURL.
Q: How discriminative is the PCR method which is mentioned in the EFSA opinion?
A: The PCR is able to discriminate the monophasic variants of the *Salmonella* Typhimurium strains, as well as the biphasic variant and *Salmonella* Typhimurium itself. This PCR method is given as an example in the EFSA opinion and it works fine after serotyping has shown that the isolate concerns an 'STM-like' strain.
Q: In the past we ordered a reference strain of *Salmonella* Typhimurium at a culture collection. After receipt it appeared to be a non-motile variant. Do other laboratories have experiences with this phenomenon?
A: Any strain can become non-motile for what ever reason. It may be good to order the strain again.
More generally, it was remarked that the use of *Salmonella* Typhimurium as positive control strain may not be the best choice because of the risk of cross

contamination of samples. It may be better to use a rare serovar for quality control purposes, so that cross contaminations are easier to detect.
Q: When antimicrobial resistance testing is done with the 'STM-like' strains, what antimicrobials should be used and what kind of break points should be used? Epidemiological cut-offs may deviate from some breakpoints. What should be used?
A: I am not sure, but I will take this question back to EFSA.
After the workshop the following answer to this question was received from EFSA: 'To answer this question we can refer to the conclusions in the Opinion (page 21), that is: Antimicrobial susceptibility testing is important for epidemiological investigations. The antimicrobial resistance pattern should be determined and reported in a harmonised way for human, animal and food isolates, according to European guidelines (EFSA, 2008; EUCAST, 2010).'

3.7 PCR technique for confirmation of monophasic *Salmonella* Typhimurium 1,4,[5],12:i:-

*Lisa Barco, NRL-*Salmonella*, Legnaro, Italy*

Salmonella 1,4,[5],12:i:- is an emerging serovar considered as a monophasic variant of *Salmonella* Typhimurium (STM). The antigenic and genetic similarity between *Salmonella* 1,4,[5],12:i:- and STM suggests that they may behave in a similar way and represent a comparable threat to public health. Serotyping is the reference method to identify *Salmonella* serovars, but it does not seem to be similarly efficacious for the identification of *Salmonella* 1,4,[5],12:i:- and its differentiation from *Salmonella* Typhimurium. In particular, there is not a general agreement on how many times the phase inversion should be repeated in order to ensure that the strain is truly monophasic, and that the inability to detect phase-2 antigen was not due to low-level expression of the antigen. The entire procedure for the identification of monophasic isolated based on traditional serotyping takes many days, hampering the timely application of consumers' protection measures. Therefore, a method that combines traditional serotyping and a multiplex PCR has been proposed and it could represent an appealing alternative to identify these strains, since phase inversion to detect phase-2 flagellar antigen is not necessary at the genetic level.
In the presentation this alternative method has been illustrated. The targets of the multiplex PCR have been considered, the protocol fully described, the tests performed and presented and the results discussed, paying particular attention to the cases where some discrepancies between the results of traditional serotyping and molecular analysis were evidenced.

Discussion
Q: Do the tested strains originate from Italy?
A: Some strains of the EURL studies were used, but indeed most of the strains were collected at national (Italian) level. However, most of the strains show phage types and antimicrobial resistance patterns which are common characteristics for 'STM-like' strains as found in other EU member states as well.
Q: Is the PCR validated and/or accredited?
A: No, we still wait for the results of the national ring trial.
Q: Is the protocol for the PCR method available?
A: Yes, this is published in the EFSA Opinion on 'STM-like' strains (ed: EFSA, 2010).
Q: Does the PCR method work fine in other countries as well?

A: In UK this PCR is also successfully used, also for other strains like the 'Copenhagen-variant'. In UK the PCR was, for instance, used to show that a breeder layer flock was infected with an 'STM-like' strain which was related to *Salmonella* Typhimurium. With this knowledge it was possible that the farmer obtained compensation for culling of the flock.
Q: If serotyping shows that a strain is an 'STM-like' strain, how often does the PCR technique then show that the strain does not belong to *Salmonella* Typhimurium, but may belong to one of the other six 'related' serovars (ed: *S.* Lagos, *S.* Agama, *S.* Farsta, *S.* Tsevie, *S.* Gloucester or *S.* Tumodi)? In other words is it always necessary to perform the PCR after serotyping an 'STM-like' strain?
A: Of the monophasic *S.* Typhimurium strains which were tested, 99.5% were related to *Salmonella* Typhimurium. However, the PCR method may still be needed for legal purposes, as specific measures have to be taken in case *Salmonella* Typhimurium is found in a flock.
Q: If the PCR is used for typing the 'STM-like' strain, is it then still necessary to perform phage typing as well?
A: No, this may not be necessary, as phage typing does not give additional information to the PCR result.

3.8 Outbreaks of *Salmonella enterica* 4,12:i:- and 4,12:-:-

Anne Brisabois, NRL-Salmonella*, Maison Alfort, France*

Food-related outbreaks of salmonellosis in humans in France are detected through the mandatory notification when two human cases are identified with digestive symptoms that can be linked to a common food source. Another way for the detection of clusters of cases is the use of statistical tests in order to identify an unusual increase on the routinely monitoring of human isolates at the National Reference Centre (NRC) in France. Here the investigation is summarised of two different outbreaks of *Salmonella enterica* variants of serovar Typhimurium, one with a non-motile 4,5,12:-:- strain in 2009 and the second one with the emerging monophasic variant 4,12:i:- in 2010. The investigations were managed at the French Institute of Public Health with the collaboration of the French Directorate General for Food and the participation of the National Reference Centre and the *Salmonella* network as a part of the National Reference Laboratory.

In May 2009, eight people were infected after consumption of a home-made Tiramisu prepared with raw eggs. Investigations at the suspected layer farm revealed the presence of positive samples for *Salmonella*. All strains isolated from humans, tiramisu and laying hens flocks yielded *Salmonella enterica* serovar 4,5,12,-:-. Molecular sub-typing of the isolates related to this outbreak revealed an indistinguishable PFGE profile commonly encountered and not exploitable. The multi-locus variable-number tandem-repeat analysis (MLVA) showed a unique type in the epidemic isolates. Investigations concluded that a non-motile variant of *Salmonella* serovar Typhimurium has been circulated in laying hens, whose contaminated eggs might result in food poisoning. This was the first report of food-borne outbreak due to a non-motile strain variant of serovar Typhimurium in France. Consequently, implications with regards to the detection method based on motility have to be considered. Food safety regulations in France added the monophasic and non-motile variants of *Salmonella* Typhimurium to the five other serovars targeted in European regulation in poultry.

In May 2010, a nationwide excess of infections with the emerging monophasic variant *Salmonella enterica* serovar 4,12:i:- was investigated. Molecular sub-typing methods with MLVA and with a newly developed method based on the polymorphism of the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) revealed a distinct epidemic strain within the excess of cases. A case-control study was conducted on the epidemic cases and again on the sporadic cases (as control) and revealed that cases have bought dried pork sausage in the supermarket chain A, with a high significant Odds Ratio. The investigation was further conducted using data recorded through loyalty card numbers of cases and identified a manufacturer of dried pork sausages. Quality control revealed that a *Salmonella* isolate was detected from a melee used to make a certain type and brand of sausages. Although *Salmonella* species were not isolated from a sample of the sausages, incriminated batches have been withdrawn and recalled. This outbreak occurred on the background of the emergence of monophasic *Salmonella* strains in Europe and future outbreaks due to this serovars are likely.

Discussion
Q: With the MSRV method the non-motile strains will not be detected. How was it possible still to detect this isolate in France?
A: In France also a selective enrichment broth is used additionally to MSRV. For this purpose Mueller Kauffmann Tetrathionate broth is used.
Q: How should these two different strains be reported to EFSA?
A: We reported the strains by their antigenic formulas.

3.9 Work programme EURL-*Salmonella* second half 2011, first half 2012 and closure

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

Work programme
Kirsten Mooijman gave information on the work programme of the EURL-*Salmonella* for the rest of 2011 and for early 2012.

Interlaboratory comparison studies
As indicated in earlier presentations, three interlaboratory comparison studies are planned in the coming year:
- Detection of *Salmonella* spp. in food: September/October 2011;
- Typing of *Salmonella* spp. (serotyping and phage typing): November/December 2011;
- Detection of *Salmonella* spp. in a 'veterinary' matrix: February/March 2012.

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). For more detailed information, see clause 2.4;
- Continuation of the pooling experiments (see clause 2.4);
- To test 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.
Experts of the EURL-*Salmonella* participate regularly 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 advise 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 2012
It was suggested to organise the workshop of 2012 in another country than the Netherlands (e.g. Greece). The feasibility of this suggestion will be further explored by the EURL.

CEN mandate on validation of methods (M/381)
In 2006, the European Commission (DG-Sanco) sent a mandate to CEN/TC275/WG6 for the validation of 15 microbiological methods as mentioned in Regulation EC 2073/2005, on Microbiological criteria. 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 in total 6 years. 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. Currently subcontracts are made between CEN and the (15) project leaders. It is hoped that the validation study of Annex D of EN ISO 6579 (detection of *Salmonella* in primary production samples) can be performed in 2012. The main part of the validation study is the organisation of an interlaboratory comparison study in which at least ten laboratories should produce acceptable results. To be sure that sufficient data will be available, it is the intention to ask at least 15 laboratories to participate. The EURL-*Salmonella* will invite NRLs for *Salmonella* to participate in the study. As soon as more information comes available, this will be communicated with the NRLs.

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

Discussion
Q: Is it possible to include animal feed and animal swabs in the validation study related to the CEN mandate?
A: For the detection of *Salmonella* in animal feed the full ISO 6579 (Anonymous, 2002) has to be followed and the validation study concerns Annex D of ISO 6579 (Anonymous, 2007). We can consider the use of animal swabs, but this type of samples may be complicated for use in an interlaboratory comparison study (complicated to contaminate a large batch of samples homogeneously with background flora as well as with the target strains).

4 Evaluation of the workshop

4.1 Introduction

One week after the workshop a questionnaire was sent to all participants to ask for their opinion on the workshop. In total 13 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 sent to 46 participants of the workshop and 22 completed forms were received, being a response of 48%. Furthermore, ten 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 are 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 as good or as excellent (scores 4-5).

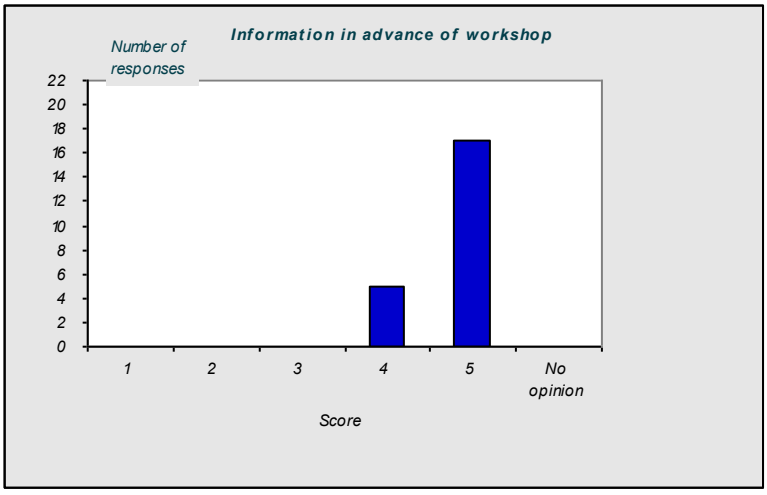

Figure 1 Scores given to question 1 'Opinion on information given in advance of the workshop'

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* as excellent (score 5) or had no opinion because they booked the tickets themselves.

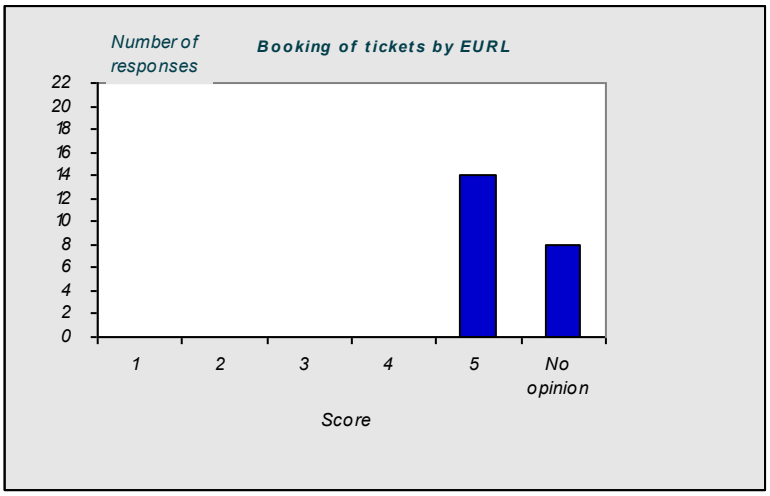

*Figure 2 Scores given to question 2 'Opinion on booking of the tickets by EURL-*Salmonella'

3. What is your opinion on the easiness to reach the meeting venue?
All respondents indicated that the meeting venue was good (score 4) or excellent (score 5) to reach (Figure 3).

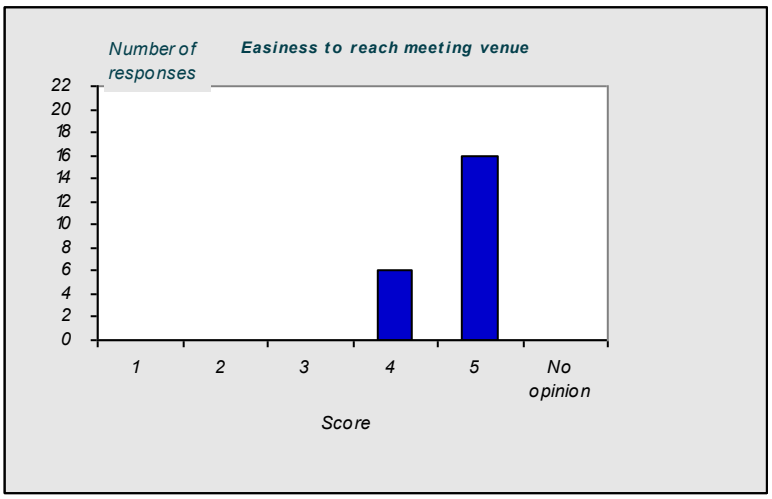

Figure 3 Scores given to question 3 'Opinion on easiness to reach meeting venue'

4. What is your opinion on the hotel room?
The opinions on the hotel room varied from moderate (score 2) to excellent (score 5), although the majority of the respondents indicated the hotel room as good or excellent (Figure 4). The following remarks were made to this question:

- 'Room sufficiently large and clean.'
- 'The hotel does not fulfil the criteria of a 4 star Best Western hotel, but it is sufficient for a normal meeting hotel. And it is very good to have the meeting in the same hotel.'
- 'The hotel and the breakfast were better in Bilthoven.'

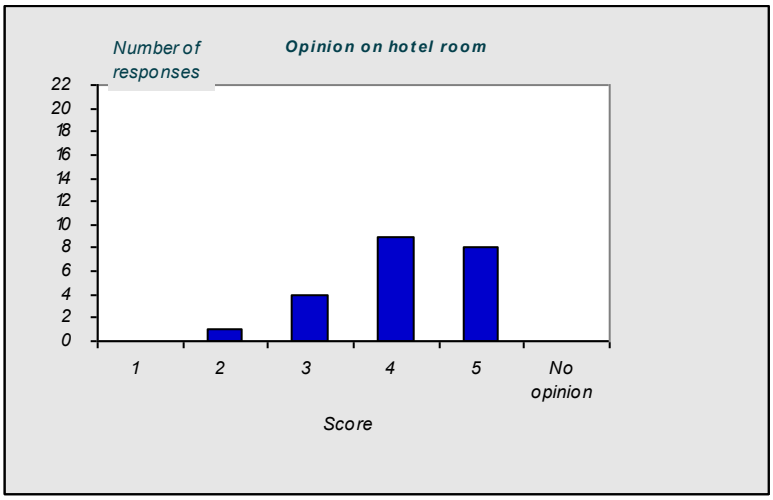

Figure 4 Scores given to question 4 'Opinion on the hotel room'

5. What is your opinion on the meeting room in general?
The majority of the respondents considered the meeting room as average (score 3) or good (score 4), see Figure 5. The following remarks were made to this question:

- 'The room was too small for the quantity of people and the quality of the beamer installation was poor (i. e. screen too small).'
- 'There were microphone problems.'
- 'Desk place for everyone.'
- 'A little bit too small.'
- 'It is very convenient to have the meeting room in the hotel.'
- 'Good to have tables in the room. The use of the microphones during the discussions could have been better.'
- 'It might have been better that we had a 'landscape' position instead of a 'portrait' position of the chairs.'

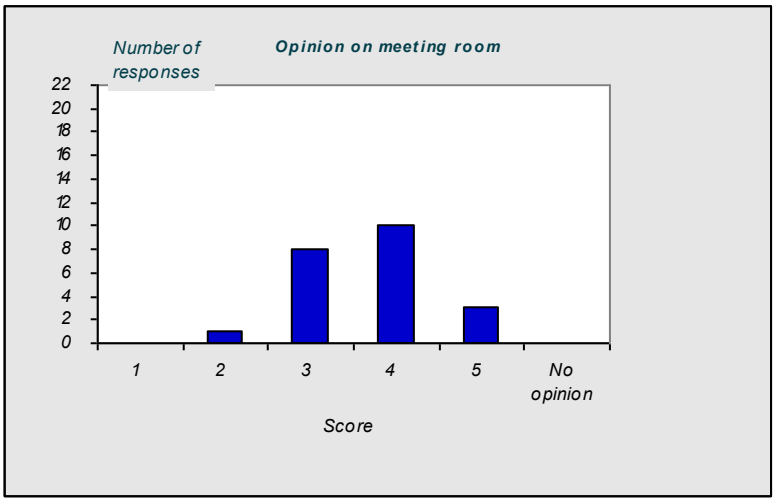

Figure 5 Scores given to question 5 'Opinion on the meeting room'

6. What is your opinion on the readability of the presentations on the screen?
In general the readability of the presentations was considered average (score 3), see Figure 6. The meeting room was narrow and deep and the PowerPoints were presented on a 'normal screen' resulting in the fact that the participants in the back of the room had some trouble reading the screen. This was also reflected in the remarks:

- 'The screen was too small' (remarked four times).
- 'Readability was also dependent on the presentations itself.'
- 'Difficult to read the presentations in the back of the room' (remarked three times).

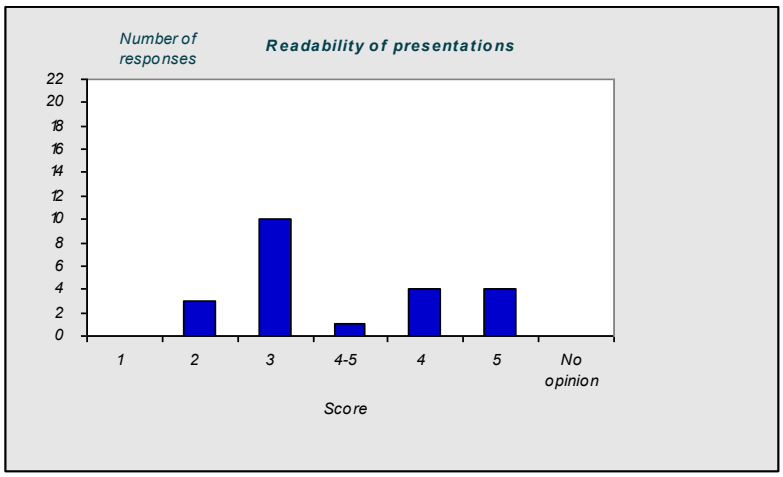

Figure 6 Scores given to question 6 'Opinion on the readability of the presentations'

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 as good (score 4), see Figure 7. Remarks given to this question were:

- 'Microphones were not always used in the proper way. One microphone for the auditorium is not enough.'
- 'A pointer was missing for the presentations. Furthermore, note blocks were missing.'
- 'The technical equipment was 'standard'. A higher screen would have been better.'
- 'A larger screen would have been better.'
- 'Hand-outs of the oral presentations were missing as well as paper to make notes.'

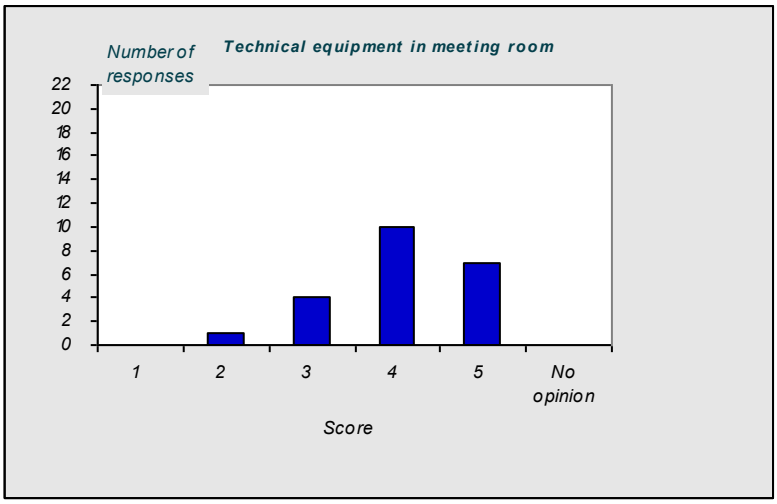

Figure 7 Scores given to question 7 'Opinion on the technical equipment'

8. What is your opinion on the catering during the workshop (breakfast, coffee, tea, lunch, dinner)?
The majority of the respondents considered the catering as good (score 4), see Figure 8. A few remarks were made:

- 'To use the restaurant of the hotel is easy, but was not always the nicest place. The quality of the food was minimal. It would have been nice to have dinner at a restaurant at the beach.'
- 'The food was ok, but it was not always sufficient. Sometimes the food was not sufficiently warm.'
- 'If we would go to Zandvoort again I would recommend having dinner in another restaurant.'

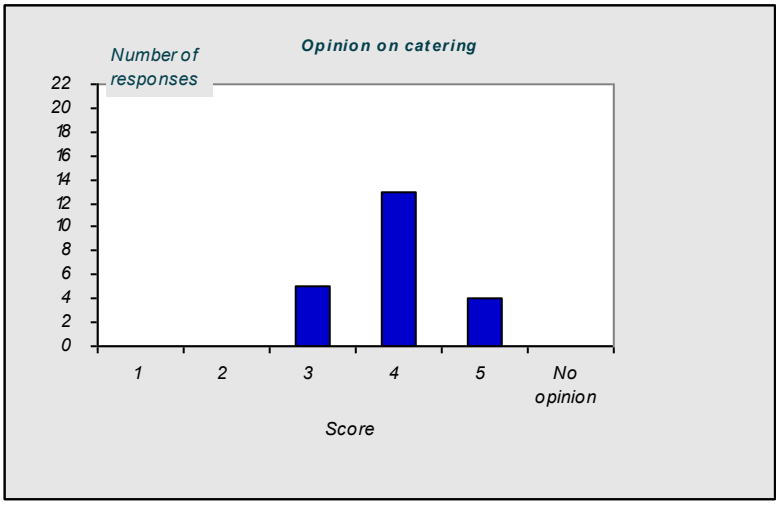

Figure 8 Scores given to question 8 'Opinion on the catering'

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).

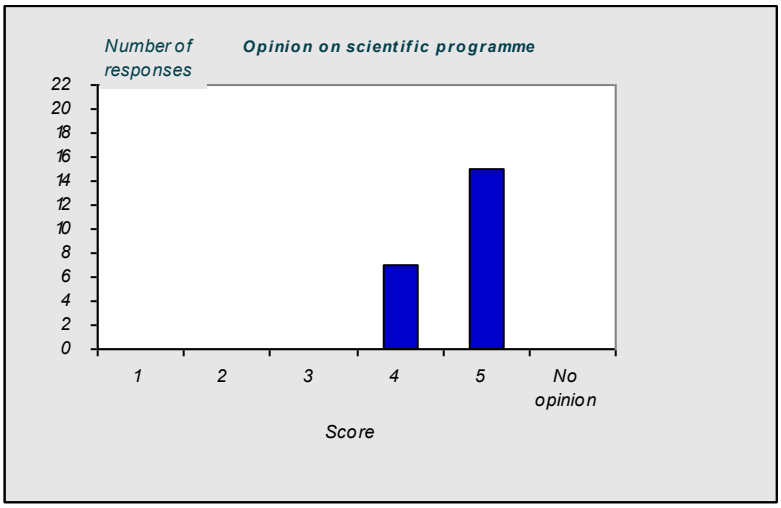

Figure 9 Scores given to question 9 'Opinion on the scientific programme'

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:

- 'It was very good to clarify the situation with the monophasic strains (kind of investigations, how to submit it, etc.).'
- 'I found all presentations very interesting, especially the presentations on the 'STM-like' strains in relation to the EFSA opinion and the PCR technique. I also enjoyed the presentations on the activities of the NRLs.'
- 'The presentations of the different NRLs were very interesting, but it might be good to give more guidance to the speakers, e.g. to ask them to focus on specific items of the NRL tasks and duties.'
- 'The presentations on the interlaboratory comparison studies as given by the colleagues of the EURL are, as always, well structured and explicit! I liked the presentations about the 'STM-like' strains.'

11. What is your opinion on the social programme (Casino)?
Most of the respondents enjoyed the short visit to the Casino (scores 4 and 5). However, a few did not join the group and had no opinion. Some others did not consider it very interesting (scores 2 and 3), see Figure 10. Remarks given to this question were:

- 'It was fun, but it was a pity that the time was limited' (remarked twice).
- 'It was quite boring.'

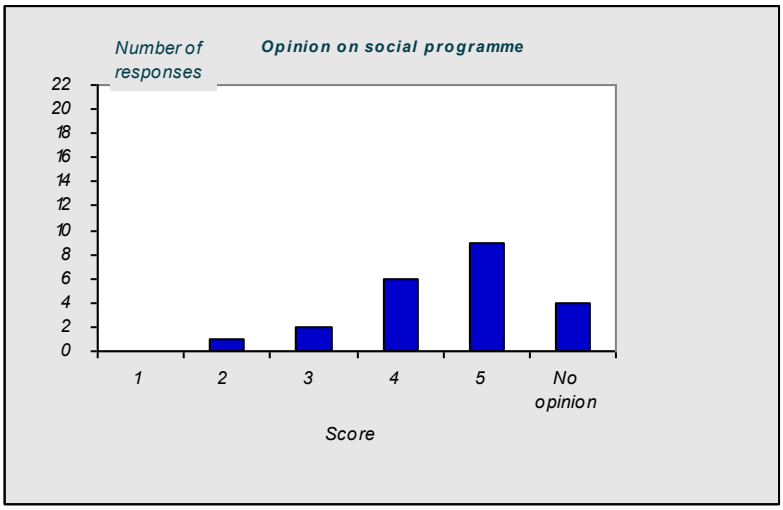

Figure 10 Scores given to question 11 'Opinion on the social programme'

12. 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 11.

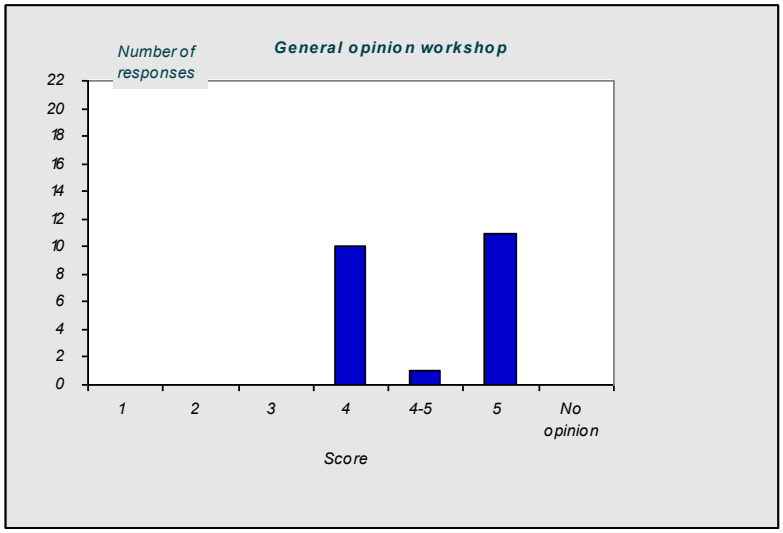

Figure 11 Scores given to question 12 'General opinion of the workshop'

13. 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:

- 'I enjoyed the stay in Zandvoort, as I think it is a wonderful location for this workshop. The atmosphere is very good because the number of persons is limited, this gave us a chance to contact almost all of the participants. Please continue in this way!'
- 'It is a good idea to change the place of the workshop from time to time despite the fact that this may be more work for the EURL-*Salmonella* team. In addition it could be useful for all participants to have all presentations as hand-outs at the beginning of the workshop, so that it is possible to write

the comments/remarks etc. directly to the presentation(s) or can read some conclusions again.'

- 'Thanks for the good organization. It was a very good idea to plan the workshop at this place by the sea. I appreciated all the presentations. It may be nice to change the location for every workshop. For the next one, why not Amsterdam?'
- 'The 'round table' (square actually!) positioning of the participants in the meeting room during last year's (XV) workshop in Saint Malo was much, much better. Have the next year's workshop in another country, e.g. Greece?'
- 'The workshop was well organised! It would be good to have more (free) time between the scientific programme and the social programme.'
- 'A very interesting workshop! Perhaps good to consider a kind of 'hostess' for the next workshop, who will take care of more general organisational aspects of the workshop. This may give more 'freedom' to the scientific staff of the EURL.'
- 'It would be nice to have a workshop in Amsterdam – or to rotate around NRLs from different countries – e.g. Slovenia – which looked very nice (ed: on the pictures in the presentation).'

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 in general. Especially the scientific programme was considered as interesting. However, the conditions under which the workshop was organised could, on some aspects, undergo some improvements. It was considered an advantage that the meeting venue was easy to reach and that the workshop was in the hotel itself, but the meeting room was not always considered as optimal. The room was narrow and deep and the screen relatively small, causing problems with the readability of the presentations at the back of the meeting room. Furthermore, some participants missed the hand-outs of the presentations. This was noticed and considered by the EURL, but the presentations were all received very late (most often on the day of the workshop itself) so that it was logistically not possible to make hand-outs. However, it will be considered again for the next workshop, but this will also need some cooperation of the speakers.
Some participants indicated it to be a good idea to change the location of the workshop from time to time, not only within one country, but also to consider different countries.

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List of abbreviations

A	Answer
BIOHAZ	EFSA Panel on Biological Hazards
BPW	Buffered Peptone Water
CEN	European Committee for Standardisation
cfp	colony forming particles
DAFF	Department of Agriculture, Fisheries and Food
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
EQA	External Quality Assurance
EU	European Union
EURL (CRL)	European Union (Community) Reference Laboratory
EUSR	European Union Summary Report
FWD	Food and Waterborne Diseases and Zoonoses surveillance network
HACCP	Hazard Analysis and Critical Control Points
HPA	Health Protection Agency
ISO	International Standardisation Organisation
LZO	Laboratory for Zoonoses and Environmental Microbiology
MKTTn	Mueller Kauffmann Tetrathionate broth with novobiocin
MLST	Multi-Locus Sequence Typing
MLVA	Multi-Locus Variable number of tandem repeats Analysis
MPN	Most Probable Number
MS	Member State
MSRV	Modified Semi-solid Rappaport Vassiliadis
NRL	National Reference Laboratory
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
PT	Proficiency Test
Q	Question
RIVM	National Institute for Public Health and the Environment
RVS	Rappaport Vassiliadis broth with Soya
SC	Sub Committee
SE(6)	*Salmonella* Enteritidis (at a level of approximately 6 cfp)
STM(5)	*Salmonella* Typhimurium (at a level of approximately 5 cfp)
SSI	Statens Serum Institute
TC	Technical Committee
Tessy	The European surveillance system
TR	Technical Report
TS	Technical Specification
UK	United Kingdom
VNTR	Variable Number of Tandem Repeats
WG	Working Group
XLD	Xylose Lysine Deoxycholate

Annex 1 Participants

European Commission	Klaus Kostenzer
European Food Safety Authority (EFSA)	Giusi Amore
EURL – *Salmonella*	Kirsten Mooijman Angelina Kuijpers Wilma Jacobs Henny Maas Anjo Verbruggen Arjen van de Giessen
Guest speaker (United Kingdom)	Elizabeth de Pinna (HPA, London)
Guest speaker (Denmark)	Eva Møller Nielsen (SSI, Kopenhagen)
Guest speaker (The Netherlands)	Annemarie Pielaat (RIVM, Bilthoven)

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PORTUGAL	Patricia Themudo
ROMANIA	Carmen Manea

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SLOVENIA	Jasna Micunovic
SPAIN	-
SWEDEN	Lennart Melin
SWITZERLAND	Gudrun Overesch
UNITED KINGDOM	Robert Davies

Annex 2 Programme of the workshop

Programme of the EURL-Salmonella workshop XVI
19 and 20 May 2011, Zandvoort, the Netherlands

General information

Hotel and location of the workshop
Best Western Palace Hotel,
Burgemeester van Fenemaplein 2, Zandvoort, the Netherlands
Tel: +31 (0)23 57 129 11
http://www.bestwestern.nl/en/palace

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 16 May 2011

Wednesday 18 May 2011
Arrival of most of the participants at the Palace hotel.
In case you arrive before dinner time and in case the costs of your travel and stay are paid from the budget of EURL-Salmonella, the EURL will also cover the expenses of a dinner with a maximum of € 30,- per person. A receipt will be needed in order to reimburse you for this meal. Alternatively, you can use a dinner in Palace hotel and ask to add the costs to the invoice of your room.

Thursday 19 May 2011

Morning chair: Wilma Jacobs

9:00 – 9:30	Opening and introduction (Kirsten Mooijman, EURL-Salmonella)
9:30 – 10:00	2009 European Summary Report on zoonoses – Overview on Salmonella (Giusi Amor, EFSA)
10:00 – 10:30	Recent policy issues on Salmonella (Klaus Kostenzer, DG-Sanco)
10:30 – 11:00	*Coffee/tea*
11:00 – 11:30	Technical issues on Salmonella (Kirsten Mooijman, EURL-Salmonella)
11:30 – 12:00	Results interlaboratory comparison study on bacteriological detection of Salmonella – FOOD IV – 2010 (Angelina Kuijpers, EURL-Salmonella)
12:00 – 13:00	*Lunch*

Afternoon chair: Kirsten Mooijman

13:00 – 13:30 Results interlaboratory comparison study on bacteriological detection of Salmonella-Veterinary XIV-2011 (Angelina Kuijpers, EURL-Salmonella)
13:30 – 14:00 Proposal on interlaboratory comparison studies on detection of Salmonella – 2011/2012 (Angelina Kuijpers, Kirsten Mooijman EURL-Salmonella)
14:00 – 14:30 Results typing study XV - 2010: serotyping (Wilma Jacobs, EURL-Salmonella)
14:30 – 15:00 Results typing study XV - 2010 : phagetyping (Elizabeth de Pinna, HPA, UK)

15.00 – 15.30 Coffee/tea

15:30 – 16:00 General aspects typing studies and proposal typing study 2011 (Wilma Jacobs, EURL-Salmonella)
16:00 –16:30 Validation of a protocol for MLVA typing of Salmonella Typhimurium (Eva Møller Nielsen, Statens Serum Institute, Denmark)
16:30 – 17:00 EU-project Biotracer, tracing Salmonella in the pork slaughter chain (Annemarie Pielaat, RIVM, the Netherlands)

17.30 and onwards Social programme and dinner

Friday 20 May 2011

Chair: Arjen van de Giessen

9.00 – 10:15 Activities NRLs to fulfill tasks and duties (including problems), 15 min each
Latvia (Madara Streikisa)
Luxembourg (Joël Mossong)
Ireland (John Egan)
Norway (Bjarne Bergsjo)
Slovenia (Jasna Micunovic)

10:15 – 10:45 Coffee/tea

10:45 – 11:15 EFSA's Scientific Opinion on monitoring and assessment of the public health risk of 'Salmonella Typhimurium-like' strains (Giusi Amore, EFSA)
11:15 – 11:45 PCR technique for confirmation of monophasic Salmonella Typhimurium 1,4,[5],12:i:- (Lisa Barco, Italy)
11:45 – 12:15 Outbreaks of Salmonella enterica 4,12:i:- and 4,12:-:- in France (Anne Brisabois, France)
12:15 – 12:45 Work programme EU-RL-Salmonella second half 2011, first half 2012 and closure (Kirsten Mooijman, EURL-Salmonella)

12:45 – 13:45 Lunch

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

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