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

The fifteenth CRL-Salmonella workshop

27 June 2010, Saint Malo, France

Report 330604019/2010

K.A. Mooijman

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RIVM Report 330604019/2010

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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 V/330604/10/CS Community Reference Laboratory for *Salmonella* (2010)

Abstract

The fifteenth CRL-*Salmonella* workshop
27 June 2010, Saint Malo, France

This report contains the summaries of the presentations of the fifteenth annual workshop for the National Reference Laboratories (NRLs) for *Salmonella*, held on 27 June 2010. The aim of this workshop was to facilitate the exchange of information on the activities of the NRLs and the Community Reference Laboratory for *Salmonella* (CRL-*Salmonella*). An important item on the agenda was the presentation of the results of the annual ring trials organised by the CRL, which provide valuable information on the quality of the work carried out by the participating NRL laboratories. The results of these studies are also presented in separate RIVM reports.

Among the summaries is the presentation of the Community summary report on Zoonoses of 2008. This report of the European Food Safety Authority (EFSA) gives an overview of the number and types of zoonotic organisms causing health problems in 2008. Salmonellosis is the second most frequently reported zoonotic disease in the European Union, after Campylobacteriosis. An overview was also given of the annual baseline surveys for *Salmonella* as performed up to 2009. In these studies each participating country determined the prevalence of *Salmonella* in certain animal productions. Animal productions under review have been chicken laying hens (2005), chicken broilers (2006), turkeys (2007), slaughter pigs (2007), breeding pigs (2008) and chicken broiler carcasses (2008).

The workshop was organised by the CRL-*Salmonella*, which is located at the National Institute for Public Health and the Environment. The main task of the CRL-*Salmonella* is to evaluate the performance of the European NRLs in detecting and typing of *Salmonella* in different products. The workshop was organised in Saint Malo, France, in conjunction with the International Symposium on *Salmonella* and Salmonellosis (I3S).

Key words:
CRL-*Salmonella*, NRL-*Salmonella*, *Salmonella*, workshop 2010

Rapport in het kort

De vijftiende CRL-*Salmonella* workshop
27 juni 2010, Saint Malo, Frankrijk

In dit rapport zijn de verslagen gebundeld van de presentaties die op 27 juni 2010 zijn gehouden tijdens de vijftiende jaarlijkse workshop voor de Europese Nationale Referentie Laboratoria (NRL's) voor de bacterie *Salmonella*. Tijdens de workshop heeft het overkoepelende orgaan, het Communautair Referentie Laboratorium (CRL) *Salmonella*, informatie uitgewisseld met de NRL's. Een belangrijk onderdeel daarvan is de presentatie van de resultaten van de jaarlijks terugkerende ringonderzoeken van het CRL waarmee de kwaliteit van de NRL-laboratoria wordt gemeten. De resultaten hiervan worden ook in aparte RIVM-rapporten weergegeven.

Een van de verslagen betreft het rapport van de European Food Safety Authority (EFSA) van 2008 over zoönosen, oftewel ziekten die van dieren op mensen kunnen overgaan. Dit rapport geeft een overzicht van de aantallen en types zoönotische organismen. Hieruit blijkt onder meer dat de ziekte die door *Salmonella* wordt veroorzaakt na de ziekte die de bacterie *Campylobacter* veroorzaakt, de zoönose is die in de Europese Unie het vaakst worden gerapporteerd. Verder bevat het rapport een overzicht van de jaarlijkse zogeheten baselinestudies voor *Salmonella* die tot 2009 zijn uitgevoerd. Hierin is per deelnemend land vastgesteld hoeveel *Salmonella* voorkomt bij de diverse categorieën pluimvee en varkens. In 2005 is dit onderzoek gedaan bij leghennen, in 2006 bij vleeskuikens, in 2007 bij kalkoenen en slachtvarkens en in 2008 bij fokvarkens en vleeskuikenkarkassen.

De organisatie van de workshop is in handen van het CRL voor *Salmonella*, dat onderdeel is van het RIVM. De hoofdtaak van het CRL-*Salmonella* is toezien op de kwaliteit van de nationale referentielaboratoria voor deze bacterie in Europa. De workshop vond plaats in Saint Malo, Frankrijk, aansluitend bij het vierjaarlijkse internationale congres over *Salmonella* dat daar werd gehouden.

Trefwoorden:
CRL-*Salmonella*, NRL-*Salmonella*, *Salmonella*, workshop 2010

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The staff members of the organisation ISPAIA-ZOOPOLE développement are very much thanked for their help in organising the workshop. In this respect the author would especially like to thank Geneviève Clement. Without her help it would not have been possible to organise the workshop in Saint Malo!
Loes van Dijk and Jeanette van Essen of the secretariat of the Laboratory for Zoonoses and Environmental Microbiology (LZO) of the National Institute for Public Health and the Environment (RIVM) are thanked for the organisational part of the workshop in Bilthoven.
Angelina Kuijpers and Wilma Jacobs are thanked for carefully presenting the activities of the CRL and for making minutes during the workshop.
Christiaan Veenman is thanked for taking care of the publication of the presentations through the CRL-*Salmonella* website.

Summary

On 27 June 2010 the Community Reference Laboratory for *Salmonella* (CRL-*Salmonella*) organised the fifteenth annual workshop in Saint Malo, France. At this workshop 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) and of the European Food Safety Authority (EFSA). A total of 38 participants were present.

Several presentations were given on results of the interlaboratory comparison studies as organised by the CRL-*Salmonella.* These concerned studies on detection of *Salmonella* in a food matrix (2009) and in a veterinary matrix (2010), as well as on typing of *Salmonella* (serotyping and phage typing) as performed in 2009. Also proposals for future interlaboratory comparison studies were discussed.
Additional to the interlaboratory comparison studies, presentations were given by EFSA and DG-Sanco on trends and sources of Zoonoses in Europe (report 2008), on the baseline studies performed in relation with *Salmonella* and on European legislation concerning *Salmonella*.
Furthermore, information was given on the standardization of methods in relation to *Salmonella* at International (ISO) and European (CEN) level.
The workshop concluded with a presentation on the work programme of the CRL-*Salmonella* for the coming year.

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

1 Introduction

This report presents the abstracts of the presentations given at the CRL-*Salmonella* workshop of 2010 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 CRL-*Salmonella* website: http://www.rivm.nl/crlsalmonella/workshops/workshopXV.jsp

The lay-out of the report is according to the programme of the workshop.
All abstracts of the presentations are given in chapter 2.
The list of participants is given in Annex 1.
The programme of the workshop is given in Annex 2.

2 Sunday 27 June 2010

2.1 Opening and introduction

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

Kirsten Mooijman, head of CRL-*Salmonella*, opened the fifteenth workshop of CRL-*Salmonella* welcoming all participants in Saint Malo, France and apologising for the fact that the workshop was held on a Sunday. The location and day was chosen to organise the workshop in conjunction with the International Symposium on Salmonella and Salmonellosis (I3S), which was organised on 28-30 June in Saint Malo. Excuses were received from the NRLs for *Salmonella* from Cyprus (EU member state), Malta (EU member state), Iceland (EFTA country), Switzerland (EFTA country) and the Former Yugoslav Republic of Macedonia (FYROM; EU candidate country).

After a roll call of the delegates, information was given on the changes at the CRL and other new aspects:

- On 1 January 2010 seven persons from RIKILT Institute of Food Safety (Wageningen, the Netherlands) have become RIVM colleagues. With this change, Wilma Jacobs has officially become member of the CRL-*Salmonella* team (for part of her time);
- Since the last workshop, two new contacts started working at DG-Sanco: Leena Rasanen and Klaus Kostenzer;
- In December 2009 it has been agreed through the Lisbon Treaty that the word 'Community' should be replaced by 'European', meaning that the name 'Community Reference Laboratory (CRL)' will change into 'European Reference Laboratory (EU-RL)'. However, the legal basis for the change of the name is still discussed at DG-Sanco and the CRLs have not yet received an official letter indicating to change the name. Therefore, the name CRL will still be used as long as it is not indicated to do differently.

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 2008 Community summary report on Zoonoses – Overview on *Salmonella*

Giusi Amore, EFSA, Parma, Italy

Background on the Zoonoses data collection system

The Community system for the monitoring and collection of information on zoonoses is based on the Zoonoses Directive 2003/99/EC, which obligates the European Union (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) is assigned the tasks of analysing these data and publishing the Community Summary Report (CSR). 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 CSR.
The CSR 2008 was prepared by EFSA (EFSA, 2010) in collaboration with ECDC and the assistance of EFSA's Zoonoses Collaboration Centre (ZCC, in the National Food Institute of the Technical University of Denmark).

Overview of the main results on *Salmonella* included in the Community Summary Report 2008

In 2008, Salmonellosis was again the second most frequently reported zoonotic disease in humans accounting for 131,468 confirmed cases. The statistically significant decreasing trend in the EU notification rate of Salmonellosis cases continued for the fifth consecutive year. Variability in the MS level trends of Salmonellosis notification rates was observed across the EU. As in previous years, *S*. Enteritidis and *S*. Typhimurium were the most frequently reported serovar (79.9% of all known serovars in human cases). The human cases caused by *S*. Enteritidis decreased markedly in 2008, while an increase in *S*. Typhimurium cases were observed.
A wide range of foodstuffs was tested for *Salmonella* by MSs, but the majority of samples were from various types of meat and products thereof. The highest proportion of *Salmonella*-positive units was reported for fresh broiler meat, turkey meat and pig meat. When comparing the results regarding the compliance with the EU *Salmonella* criteria, the food categories most often exceeding the criteria were minced meat and meat preparations; at average levels of 2.2%-6.7% in single samples. Of particular risk for human health are the *Salmonella* findings from meat categories intended to be eaten raw, where *Salmonella* was detected in 1.0%-2.2% 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 increased compared to previous years (to 2.8%). In other food categories, the proportion of units in non-compliance with the criteria was very low. Altogether 26 MSs reported data on *Salmonella* in various animal species, including farm animals, pets, zoo animals and wildlife. 2008 was the first year when MSs implemented the new *Salmonella* control programmes in laying hens, and 20 MSs have already met their relative reduction target for *S.* Enteritidis and *S*. Typhimurium set for this year. The improved *Salmonella* status of the laying hen flocks may have been reflected in the lower levels of *S.* Enteritidis cases reported in humans. In addition, 19 MSs reported a lower *Salmonella* prevalence than the EU reduction target of 1% set for breeding flocks of *Gallus gallus*, even though the target only had to be met by the end of 2009. This target covers the five serovars *S*. Enteritidis, *S*. Typhimurium, *S*. Hadar, *S*. Infantis, and *S*. Virchow. No major changes in *Salmonella* prevalence in broiler, turkey or pig populations were apparent at Community level. *Salmonella* was the most commonly reported causative agent in the food-borne outbreaks in 2008. Data from food-borne outbreaks and serovar/phage type distribution in human cases, food and animals can provide initial information as to the significance of different sources of human infections. 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 are mostly caused by

S. Enteritidis. Pig meat and products thereof was the third known most important food vehicle in *Salmonella* outbreaks, mainly related to *S.* Typhimurium outbreaks.
In most MSs, *S*. Enteritidis was the most frequently isolated serovar in table eggs and also frequently found from poultry meat. It can therefore be supposed that the decrease observed in the number of *S*. Enteritidis cases in humans may be related to the decrease of this serovar in laying hen flocks reported for 2008. *S.* Typhimurium was the most frequently isolated serovar in pigs (and cattle) and meat thereof and it was also among the top ten serovars isolated from broilers and table eggs. The increase in *S.* Typhimurium human cases observed in 2008 appears to be related to food-borne outbreaks, especially to a very large outbreak of *S.* Typhimurium U292 in a MS. The main hypothesis remains that the outbreak originates from a pig reservoir in a series of different foodstuffs. When interpreting results on serovar distribution a special attention should be given on specific serovars in some countries.
The data for 2008 suggest that the new *Salmonella* control programmes in poultry have had a positive impact on public health by reducing the number of human Salmonellosis cases, particularly cases caused by the *S.* Enteritidis serovar. The results from the control programmes in breeding and laying hen flocks are promising and encourage taking into consideration broadening the intensified control efforts further to other animal populations, such as breeding and slaughter pigs.

Discussion
Q: In the report, monophasic *Salmonella* Typhimurium is not reported separately. Is this information not available?
A: EFSA is dependent on the input of the Member States. The type has been reported in many different ways, e.g.: by giving the antigenic formula, by naming it monophasic *Salmonella* Typhimurium, by indicating it as non-typable. However, some MS also report it as *S.* Typhimurium. It is possible that the increase in the reported cases of *S.* Typhimurium is a result of the increase in monophasic *S.* Typhimurium. To obtain more information on this, it may be necessary to have a closer look at the data on MS level. However, EFSA only looks at the data at EU level.
Q: In EFSA a working group is preparing an opinion on monophasic *S.* Typhimurium. Is it possible to obtain more information from this group?
A: For the moment the (draft) content of the opinion is still confidential. The opinion needs to be finalised in September 2010. The questions from DG-Sanco which need to be addressed are: i) what analytical methods have to be used across all Member States allowing the detection of the strain? ii) what is the best way to report this strain in order to be able to compare reporting data? iii) what is the public health impact of this emerging strain?
Q: How do I know that I also need to report serotyping results?
A: This is indicated in relevant legislation. Furthermore the competent authority and/or the reporting officer should know. EFSA will check the name of the responsible person and will inform the relevant NRL.

2.3 Summary of the EU baseline studies and ongoing activities on *Salmonella*

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

Regulation (EC) No 2160/2003 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. In particular Article 4 thereof requires that Community targets should be established. In order to set these EU-wide targets, comparable data on the prevalence of zoonotic agents in the poultry and pig populations throughout all the Member States had to be made available. Therefore baseline studies have been carried out in order to gather the relevant data.
From 2004 onwards, eight baseline studies have been performed both in the primary production and on slaughterhouse level. The European Commission and the European Food Safety Authority (EFSA) both assisted the Member States in the technical and scientific provisions. Prevalences for *Salmonella* were determined in holdings of laying hens, flocks of broilers, turkeys and breeding pigs and in slaughterhouses for pigs and broilers. The prevalence for *Campylobacter* was determined in slaughter batches and carcasses of broilers. Methicilline Resistant *Staphylococcus aureus* (MRSA) prevalence was studied in holdings of breeding pigs. Another survey will commence in 2010 in ready-to-eat food for *Listeria*.
The sampling material varied depending on the study population: in live poultry boot swabs and dust samples were taken, whereas in poultry carcasses samples of the neck skin, caeca and carcasses were looked at. For pigs faecal samples and dust swabs were collected at farm level, whereas lymph node samples, meat juice and carcass swabs were taken in the slaughterhouse.
The results have been analysed and published in detail by EFSA. Prevalences differed a lot in the various Member States throughout Europe. Furthermore risk factor analyses based on the obtained datasets complete the picture of some of the most relevant zoonotic agents and their patterns of infection, contamination, spatial and within-population distribution. Various more factors were analysed depending on the objectives of the mandates given to EFSA from the European Commission. In the case of *Salmonella* in poultry, targets have been set, control programmes and measures introduced and monitoring carried out to verify the achievement of the agreed Community targets of reduction in prevalence.
The tools that were available (data dictionaries, data validation; EU co-financing; CRL guidance, etc.) proved to be widely sufficient to carry out the studies. The gain of knowledge was not only determining the prevalences, but a full dataset on certain zoonotic agents containing also the occurrence and the distribution of serotypes, the underlying risk factors, antimicrobial resistance patterns, usefulness of methodology, etc.

Discussion

Q: There has not been a baseline survey for poultry breeders, is that a problem?
A: Many MS already had some programs in their countries and furthermore, results from other baseline studies can also give information on the situation at the breeding flocks. Also the pressure from the industry is high to keep the prevalence of *Salmonella* low.
Q: The official reduction targets are almost all already reached, how can this be explained?
A: The sensitivity of the protocols for the baseline surveys was higher than of the monitoring studies. Furthermore, the vaccination protocols may also have helped in the reduction of the prevalence. Additional to the targets set in animals it is good also to look at the trend in human cases.
Q: Can a baseline study for VTEC be expected?
A: The amount of resources is limited, so that no mandatory study can be expected now, only a monitoring on voluntary basis is possible.

2.4 ISO and CEN activities

Kirsten Mooijman, CRL-Salmonella, Bilthoven, the Netherlands

Kirsten Mooijman of the CRL-*Salmonella* presented an overview of activities in ISO and CEN in relation with *Salmonella*.
The relevant groups in ISO and CEN are:
ISO/TC34/SC9: International Standardisation Organisation, Technical Committee 34 on Food Products, Subcommittee 9 – Microbiology, and.
CEN/TC275/WG6: European Committee for Standardisation, Technical Committee 275 for Food Analysis – Horizontal methods, Working Group 6 for Microbial contaminants.
Both groups organised their last meeting in Buenos Aires, Argentina from 31 May – 4 June 2010.

At EN ISO level it was agreed to split EN ISO 6579 into three parts to deal with detection (part 1), enumeration (part 2) and serotyping (part 3) of *Salmonella* spp. under one EN ISO number.

ISO 6579-1: Detection of *Salmonella*

At the ISO/TC34/SC9 meeting in Valencia in May 2009, it was decided to raise a working group (WG9) to deal with the revision of ISO 6579-1. Kirsten Mooijman was appointed as convener of this working group. At this 2009 meeting, a Resolution was taken (no. 395) in which several items for revision of the ISO document for detection of *Salmonella* have been indicated.
The items of Resolution 395 were dealt with item by item during the meeting at Buenos Aires:

1. *Description of the detection of S. Typhi and S. Paratyphi in a normative annex of ISO 6579, considering the use of the SC enrichment broth*: A text proposal has been made for this annex and it is suggested by WG9 to include the detection of *S*. Gallinarum (biovars gallinarum and pullorum) as well. However, it was argued by SC9 that these latter *Salmonella* strains are not related to human health and are only pathogenic for animals. It was agreed that this will be checked with the WHO reference centre for

Salmonella. Additional, the secretariat of SC9 will ask the World Organisation for Animal Health (OIE) whether they would agree with inclusion of the detection of *S*. Gallinarum in an annex of the amended ISO 6579. The annex will become normative and in the full text of ISO 6579 it should be clearly indicated when this annex will be used.

2. *To launch a trial comparing selective enrichment in the BAM/USP formulation of tetrathionate broth and in MKTTn (ISO 6579 formulation). Kirsten Mooijman will prepare a protocol and will provide this to the SC9 secretariat. The secretariat will send this protocol to the SC9 members:* The protocol was prepared and sent to the members in August 2009. In total five datasets were received, all showing similar results. In summary MKTTn (ISO formulation) gave the best results. WG9 therefore suggested to retain MKTTn in the amended ISO 6579. SC9 agreed.
3. *The SC9 secretariat will launch an enquiry for data comparing the use of RVS and MSRV for food analysis. WG9 will also perform a literature review on this subject:* In total seven datasets were received in which MSRV was compared to ISO 6579 (or to only RVS). In one study the comparison was done between MSRV and ISO 6785 (for milk and milk products, SC and RVS). All studies showed similar results: MSRV shows equal or better results than ISO 6579. WG9 therefore suggests to allow the choice of subculturing to either RVS or MSRV (both to be incubated at 41.5 °C). It was discussed whether such a choice would be allowed. There were no strong feelings not to allow this as both media are very similar. Furthermore, the situation is comparable to the isolation step where one medium is prescribed and the second one is free for choice. It was agreed that WG9 will continue with this suggested choice and wait for further reactions as soon as a draft is distributed.
4. *Postpone the discussion about a further 24h incubation of the selective enrichment media until further information about the choice of the selective enrichment media is available:* this will be dealt with later.
5. *Retain XLD as the mandatory isolation medium. Clearer direction on suitable media for the second plate should be given in the document:* a draft proposal for this has been made in the first draft document. Furthermore, France suggested to replace or give the choice for XLT4 instead of XLD in case of analysing ‘dirty’ samples like primary production samples. This will be further discussed in a next meeting of WG9.
6. *The SC9 secretariat will launch an enquiry to collect data to support the possibility of refrigerating BPW and/or selective enrichment media before subculture:* call was launched in April 2010. Some data are available and it seems to be no problem to store cultured BPW for 72 h in the refrigerator. For the selective enrichment media this needs to be checked further.
7. *Make the plating stage less prescriptive:* a text proposal has been made for the first draft.
8. *Make the confirmation stage less prescriptive in terms of number of colonies to be confirmed:* a text proposal has been made for the first draft.
9. *The non-selective medium for purification of colonies should be left to choice:* a text proposal has been made for the first draft.

10. *Include a note to allow parallel biochemical testing and purity check:* a text proposal has been made for the first draft. Furthermore, it was mentioned that it is important that the text is clarified on serological confirmation in ISO 6579-1 and serotyping in ISO 6579-3. The text in ISO 6579-1 on serological confirmation should be in line with the text in ISO 7218. Additional, Resolution 214 as taken at the CEN/TC275/WG6 meeting was taken over by SC9, which stated that it should not be prescribed in a standardized method that isolates have to be further typed at a reference laboratory. This text need to be amended accordingly in ISO 6579-1.
11. *Investigate the usefulness of some biochemical tests:* recently some data has been received but they need to be studied further. The members of SC9 were asked to send more data if available.

A first amended draft text of ISO 6579-1 was recently sent to WG9. It was suggested to further discuss this draft and additional information at a meeting to be organised in autumn 2010. Furthermore it was agreed that Kirsten would draft a report in which all information and research results collected for revision of ISO 6579-1 will be summarised. As soon as this report will be finished it will be made available to SC9 and also the NRLs for *Salmonella* will be informed.
Finally it was indicated that the work of WG9 will be moved from ISO/TC34/SC9 to CEN/TC275/WG6.

ISO 6579-2: NWIP/TS Enumeration of *Salmonella* by a mini-MPN technique

The finalisation of the draft ISO TS 6579-2 document was delayed due to the fact that it was necessary to wait for the information from the ISO working group on statistics (WG2) on the MPN tool. In February 2010 the amended document was sent to the secretariat of SC9. The voting of the document has not been launched yet as it is necessary to have a resolution of CEN to publish the document as a 'Technical Specification' (TS). For this it is necessary to write a justification letter why the document should be published as a TS and not as a full standard. At ISO level it has been discussed whether it would be easier/quicker still to publish the document as a full standard. However, if the document will be published as a full standard it will be necessary to include validation data. Some data are available, but it will take time to collect them and to check whether they fulfil the criteria for inclusion in a standard. If the data do not fulfil the criteria it will further delay the publication of the standard. Therefore, it was decided still to publish the document as a TS and administrative details will be sorted out with CEN.

ISO 6579-3: Serotyping method for *Salmonella*

The ISO ad hoc group on serotyping met for the first time on 14 December 2010. At this meeting it was agreed to prepare a guidance document for serotyping and therefore to publish the document as a Technical Report (TR). At the SC9 meeting it has been asked whether publication of a TR would not cause problems for CEN. As this was not fully clear, it was agreed that it first needs to be checked at CEN.
The draft guidance document which was distributed with the second enquiry was taken as a basis and at the ad hoc group meeting some

amendments and additions were agreed. Members of the ad hoc group provided Kirsten with text proposals by March 2010, after which Kirsten prepared an amended document. This latter document was sent to the members of the ad hoc group in the first week of May 2010 and comments are asked before mid July 2010. It will depend on the nature of the comments how will be proceeded. If the comments are minor, Kirsten will amend the document, confirm it with the ad hoc group and send it to the secretariat of ISO/TC34/SC9 for launching the 'New Work Item Proposal (NWIP)'. If the comments are major a meeting will be planned in fall 2010 to discuss the comments.

Discussion

Q: Is MSRV also compared to the full ISO 6579? Would it be possible to use only MSRV for the analyses of food samples?
A: In several validation studies MSRV has indeed been compared to ISO 6579, showing good results for the detection of *Salmonella* in food samples. However, the working group in ISO prefers to retain a selective enrichment broth beside a semi-solid agar medium (MSRV) for the detection of non-motile *Salmonella*.
Q: Will the ISO ad hoc group for serotyping also take alternative methods for serotyping into account (e.g. PCR methods)?
A: The intention of the ad hoc group for serotyping is to draft a reference method for serotyping, based on the 'classical method' (White-Kauffmann-Le Minor scheme). Alternative methods should then be validated against this reference method. However, at the moment no protocol exists for the validation of confirmation or typing tests. It is requested to another working group in ISO to draft such a validation protocol, based on ISO 16140 (Anonymous, 2003).
Remark: A NRL found yellow colonies on XLD which were confirmed as *Salmonella* (lactose positive and H_2S negative). It was requested how to deal with these results. As a reply it was indicated that *S.* Mbandaka may often be lactose positive and can best be detected on Bismuth Sulphide agar.

2.5 Results interlaboratory comparison study on bacteriological detection of *Salmonella* - FOOD III - 2009

Angelina Kuijpers, CRL-Salmonella, Bilthoven, the Netherlands

Of the 32 National Reference Laboratories (NRLs) in the European Union which participated in a mandatory comparison study in 2009, 31 were able to detect both high and low levels of *Salmonella* in minced chicken meat, thereby achieving the desired outcome on the first attempt. During the follow-up study, the CRL-*Salmonella* staff visited the one NRL that had underperformed, with the aim of providing expert advice. This NRL obtained the desired outcome in the follow-up study. Cross-contamination of samples is the most likely explanation for the initial failure.

These results were presented in the third interlaboratory comparison study on food, organized by the Community Reference Laboratory (CRL) for *Salmonella*. The comparison study was conducted in October 2009, with the follow-up study in January 2010. The NRLs responsible

for *Salmonella* detection from all European Member States were obliged to participate in this study.

Three selective enrichment media for demonstrating the presence of *Salmonella* in chicken meat were used during the study. Two of these are part of the international standardized method for the detection of *Salmonella* in food (ISO 6579), and the third is part of an internationally prescribed method for the detection of *Salmonella* in veterinary samples (Annex D of ISO 6579). The application of this latter method in the study was not obligatory but requested by the CRL. Using the two methods for testing food, 96% of the samples were found to be positive for *Salmonella*. The best results were obtained using the method for veterinary samples, with *Salmonella* detected in 98% of the samples.

To perform the study, the laboratories had to follow the instructions given. Each laboratory received a package containing minced chicken meat and 35 gelatine capsules containing powdered milk artificially contaminated with different levels of *Salmonella* spp. The laboratories were instructed to spike the minced chicken meat with the capsules and then test the samples for the presence of *Salmonella*.

Discussion

Q: The results of the interlaboratory comparison study looks very good, should it not be better to lower the contamination level of the reference materials?
A: When we changed from ‘matrix mixed with glycerol’ to ‘no mixed matrix’ we have also lowered the contamination level. For the reference materials containing *Salmonella* Typhimurium the low level material is already the lowest what is feasible to prepare (approximately 5 cfp/capsule). For the reference materials containing *Salmonella* Enteritidis a level of 10 cfp/capsules was too low when testing matrices with a high amount of background flora, but 20 cfp/capsule (current low level) seems again to be too high in case a matrix is tested with a low amount of background flora. The type of reference materials as well as the contamination level will be further reviewed.
Q: Perhaps useful to include a non-motile strain in a future study?
A: This will be further considered.

2.6 Results interlaboratory comparison study on bacteriological detection of *Salmonella* - Veterinary XIII - 2010

Angelina Kuijpers, CRL-Salmonella, Bilthoven, the Netherlands

Of the 33 National Reference Laboratories (NRLs) for *Salmonella* in the European Union which participated in a mandatory comparison study in 2010, 31 were able to detect both high and low levels of *Salmonella* in chicken faeces, thereby achieving the desired level of ‘good performance’ for the prescribed method. One laboratory mentioned to have problems with the reconstitution of the capsules and due to this they scored for the samples with a low level of *Salmonella* under the criteria of ‘good performance’. Another laboratory showed to have problems with an extra control included in this study. They may have had some problems with following the protocol and this gave doubts to

their results of the study. The performance of both laboratories was determined as 'moderate' and no follow-up was required.

These results were presented in the thirteenth veterinary interlaboratory comparison study organized by the Community Reference Laboratory (CRL) for *Salmonella*. The comparison study was conducted in March 2010. The NRLs responsible for *Salmonella* detection in veterinary samples from all European Member States were obliged to participate in this study.

The internationally prescribed selective enrichment medium (MSRV) for demonstrating the presence of *Salmonella* in veterinary samples was used during the study (Annex D of ISO 6579). Herewith 98% of the samples were found to be positive for *Salmonella*.

To perform the study, the laboratories had to follow the instructions given. Each laboratory received a package containing chicken faeces and 35 gelatine capsules containing powdered milk artificially contaminated with different levels of *Salmonella* spp. The laboratories were instructed to spike the chicken faeces with the capsules and then test the samples for the presence of *Salmonella*.
An extra control was included to check whether all participants added the faeces to the capsules. Four samples of chicken faeces were mixed with an antibiotic to which the *Salmonella* spp. present in the capsules is susceptible.

Discussion
Q: Is it possible to use again naturally contaminated samples in a future interlaboratory comparison study?
A: Indeed naturally contaminated samples would be more interesting to test, but the information from these samples can not be used to determine the performance of the laboratories, as the contamination level and homogeneity is not well known.

2.7 Proposal for interlaboratory comparison studies on detection of *Salmonella* – 2010/2011

Kirsten Mooijman, CRL-Salmonella, Bilthoven, the Netherlands

The following interlaboratory comparison studies on detection of *Salmonella* spp. are planned for the coming year:

- September/October 2010: Detection of *Salmonella* spp. in a food matrix;
- February/March 2011: Detection of *Salmonella* spp. in a 'veterinary' matrix.

Up to now the treatment of the samples for the interlaboratory comparison studies of the CRL-*Salmonella* deviates (largely) from the treatment of routine samples. It was suggested to improve this situation for future studies concerning the following aspects:

- Increase the amount of matrix from 10 g to 25 g;
- Optimise the procedure for artificial contamination of the samples;

- Make it possible to treat samples in a similar way as routine samples (e.g. using the stomacher if normally prescribed for the type of matrix).

The current treatment of the samples is the following:
- Pre-warming of the Buffered Peptone Water (BPW) at 37 °C overnight;
- Placing the capsules at room temperature one hour before starting the analysis;
- Addition of the capsules to pre-warmed BPW and placed at 37 °C for 45 min, to reconstitute the capsules;
- Addition of 10 g (occasionally 25 g) of matrix to the dissolved capsule in BPW (not shaken).

This procedure enhances the risk of cross contamination as matrix is added to BPW which already contains *Salmonella*. Furthermore, for the analyses of food samples it is normal practice to stomacher the food in the BPW before incubation. However, with the current ring trial samples (capsules) this is not possible.

At the CRL-*Salmonella* some tests were performed with lenticules in stead of capsules. For this purpose the following was tested:
- Tested matrices: minced meat (mixture of beef and pork) and chicken faeces;
- Tested lenticules: *Salmonella* Typhimurium at a level of approximately 9 cfp/lenticule (lenticules containing *Salmonella* Enteritidis at a level of 5-10 cfp/lenticule were at the time of the workshop under testing);
- To 225 ml BPW, 25 g matrix was added as well as one lenticule;
- Samples were mixed by using the stomacher or by using the pulsifier, or were not mixed at all;
- Further analyses was performed following ISO 6579 (Anonymous, 2002) and Annex D of ISO 6579 (Anonymous, 2007).

All tests showed positive results for *Salmonella*, indicating the usefulness of the lenticules to approach the treatment of routine samples for the interlaboratory comparison studies of the CRL-*Salmonella*.

Not all tests with the lenticules have already been finished. Furthermore, for an interlaboratory comparison study it is necessary to order ‘special’ batches of lenticules at the Health Protection Agency in the United Kingdom. This all will take some time, therefore it was proposed to organise the interlaboratory comparison study in September/October 2010 still with capsules, but to aim for the use of lenticules in the first study in 2011.
For the food study it was suggested to use minced pork as matrix of choice and to use 25 g samples in stead of 10 g. The number of samples will probably be comparable to earlier studies, but the type of capsules to be used may differ somewhat from earlier studies, but this will not be disclosed before the study. The prescribed method will again be the reference method ISO 6579 (Anonymous 2002) and Annex D of ISO 6579 (Anonymous, 2007) will again be the (additional) requested method.

Discussion

Q: Is it possible to add the capsules or lenticules to the matrix before sending to the NRLs?
A: For lenticules this may be possible, if the transport time and temperature is fully under control. However, this is not the case for all NRLs. Therefore it will better to ask each NRL to combine the matrix and the reference material in their laboratory.
Q: The matrix used in the ring trials is relatively old, resulting in a reduced number of *Enterobacteriaceae*. Would it be possible to add some extra background flora in an additional reference material?
A: This will be further considered.
Q: If the CRL will use lenticules for future interlaboratory comparison studies, will it then be possible for the NRLs to buy the same lenticules for a reduced price?
A: This will be asked at the Health Protection Agency (HPA) who produces the lenticules.
Remark: If lenticules will be used, which strain(s) will be used? Some strains of HPA (NCTC culture collection strains) may give atypical results on Rambach agar.
Remark: A NRL has some experiences with the use of lenticules in interlaboratory comparison studies. They found the best results if the lenticules were treated in the same way as the capsules (first dissolution of the lenticule for 45 minutes and next addition of the matrix).
Remark: A NRL inoculates faeces samples with a diluted broth (BPW) culture and next the samples are distributed to the participating laboratories. However, for this NRL all samples were delivered within the same day. It will not be possible for the CRL to organise a study in such a similar way.

2.8 Results on serotyping of *Salmonella* of the fourteenth interlaboratory comparison study on typing (2009)

Wilma Jacobs, CRL-Salmonella, Bilthoven, the Netherlands

The fourteenth interlaboratory comparison study on serotyping and phage typing of *Salmonella* spp. was organised by the Community Reference Laboratory for Salmonella (CRL-Salmonella, Bilthoven, the Netherlands), in cooperation with the Health protection Agency (HPA, London, United Kingdom), in December 2009.

A total of 31 National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) 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* subsp. *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).

An interim summary report on the outcome of the study was prepared and sent to all participants in May 2010. Shortly after publication of this interim summary report, information became available on the possibility of colonial form variation which may occur with the expression of the O:61 antigen by some serogroup C2 serovars (Hendriksen et al., 2009). It was decided to allow, at least in this fourteenth interlaboratory comparison study, for this colonial form variation and not to consider the serovar pairs *S*. Newport/*S*. Bardo and *S*. Hadar/*S*. Istanbul as distinct serovars. The interim summary report will be revised by including the information as stated above. This presentation already gives the data as revised accordingly.

The serotyping results showed that the O-antigens were typed correctly by 23 of the 31 participating NRLs (74%). This corresponds to 97% of the total amount of strains. The H-antigens were typed correctly by 14 NRLs (45%), corresponding to 94% of the total amount of strains. Fifteen NRLs (48%) identified all serovar names correctly, corresponding to 93% of all strains.
A completely correct identification by all participants was obtained for four strains:
S. Stanley (S5), *S*. Enteritidis (S6), *S*. Agona (S11), and *S*. Brandenburg (S13).
Most problems occurred with the serovars *S*. Llandoff (S1), and *S*. Thompson (S18).

Five NRLs did not meet the level of good performance at this stage of the study and these laboratories participated to the follow-up study in April 2010, by serotyping an additional 10 strains.
All five laboratories achieved a good performance on their results of the follow-up study.

Discussion
Q: How can you distinguish *S*. Senftenberg from *S*. Dessau if no phage conversion is done?
A: We will ask the WHO reference centre in Paris what they advice for this. *Answer from the WHO reference centre, September 2010: This very rare serotype has been described by Kaufmann with the O-antigenic formula 1,3,15,19. After a search, I haven't been able to find Dessau with O:1,3,10,19. Therefore, I can't explain why 0:15 has been underlined in the KWL scheme. Maybe Prof. Le Minor anticipated that such strains existed but had not yet been identified. Anyway Dessau could be differentiated from Senftenberg by the presence of either O:10 (probably) or O:15 (certainly). Actually it is more complicated because it has been demonstrated that E1 and E4 strains have the same chromosomal rfb gene cluster (encoding the enzymes for O-antigen biosynthesis) and the difference between E1 and E4 were proposed to be due to the presence of gene(s) on a converting phage in E4, although the phage has not been observed (Xiang et al., 1993). By MLST the serotype Senftenberg has a large allele diversity and some Senftenberg share a same MLST type with Westhampton (ST14) or Dessau (ST185). All these results lead to the conclusion that the O-antigenic variation in these groups as assessed by serotyping does not reflect the reality of the bacterial populations. Therefore in the next KWL scheme (2012), we will consider the merger of E1 and E4 groups.*

Q: For this study it is indicated that *S*. Newport and *S*. Bardo, *S*. Hadar and *S*. Istanbul are not regarded as distinct serovars. Is this now also officially the case for isolates found in routine samples? What are the legal consequences as *S*. Hadar is mentioned in the 'top 5' of EU legislation?
A: Further information will be asked at the WHO reference centre in Paris. *Answer from the WHO reference centre, September 2010: This variation has been studied in depth by our colleagues from the Centre for Disease Control and Prevention (CDC) in the United States. This has also been confirmed at the WHO reference centre for different pairs by MLST (e.g., Haardt/Blockley, Pakistan/Litchfield) but not for all (e.g., Manhattan/Yovokome). The study of the remaining pairs is in progress. Regarding the Istanbul/Hadar pair that has legal consequences; it seems that they form a single population according to our US colleague. However, here the Istanbul reference strain (from Kauffmann) has a MLST type very different from other Hadar strains. I am rechecking this result as I think it is not consistent with my experience of such related groups. I will keep you informed. Anyway, here at the French Reference Centre for surveillance purpose I usually indicate Hadar or Newport even though there is no O:6. As soon as all pairs are studied, we will consider the merger of the majority of O:6+ and O:6- serotypes of C2 group that share the same antigenic formula.*
Q: How often should phase inversion be performed before you can decide that the isolate does not have (or does not express) the second phase?
A: This will also be asked at the WHO reference centre in Paris. *Answer from the WHO reference centre, September 2010: At the WHO reference centre the phase inversion is done systematically on all isolates. If the isolate is immobilized after an overnight incubation on Sven Guard agar containing an 'I' antiserum then we consider it as monophasic. We could test another colony but it is not compatible with the workload (4000 STM and 1000 monophasic last year).*
Remark: A NRL indicated to have problems to distinguish *S*. Llandoff from *S*. Senftenberg. It was suggested to summarise the problems and contact the CRL again on this problem. The typing department can then have a further look at possible reasons for the problems (e.g. quality of antisera).

2.9 Results on phage typing of *Salmonella* of the fourteenth interlaboratory comparison study on typing (2009)

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

The *Salmonella* strains for phage typing in the fourteenth 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 second international External Quality Assurance (EQA) scheme on the typing of *Salmonella* spp. organised under contract of the European Centre for

Disease 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.
Twenty of the FWD laboratories participated in the phage tying of the *S.* Enteritidis strains and eighteen 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 very good. Four of the laboratories correctly phage typed all ten of the *S.* Enteritidis strains. Two laboratories correctly phage typed nine of the ten strains. The remaining NRL correctly phage typed eight of the *S.* Enteritidis strains.
Six of the FWD laboratories correctly phage typed all ten strains of *S*. Enteritidis. Five of the FWD laboratories correctly typed nine of the *S*. Enteritidis strains and five FWD laboratories correctly typed eight of the ten *S*. Enteritidis strains. One FWD laboratory correctly phage typed seven of the strains, two FWD laboratories correctly typed six of the *S*. Enteritidis strains and the remaining laboratory correctly typed five of the ten strains.
Two strains of *S.* Enteritidis caused problems for both the NRLs and the FWD laboratories; these were phage type 1 and phage type 21. Phage type 21 has caused problems in previous studies.

Overall, the results of the phage typing of *S.* Typhimurium by the NRLs were excellent. The ten *S.* Typhimurium strains were correctly phage typed by five of the NRLs. One NRL correctly typed nine of the ten *S.* Typhimurium strains.
Overall, the phage typing of *S*. Typhimurium by the FWD laboratories was better than the results for *S*. Enteritidis. Ten FWD laboratories correctly phage typed the ten *S.* Typhimurium strains. Four FWD laboratories correctly typed nine of the ten strains and three laboratories correctly phage typed eight of the strains. One FWD laboratory phage typed only three of the ten strains correctly.
Only one strain of *S.* Typhimurium was incorrectly typed by both NRLs and FWD laboratories; this was DT 208. This strain has also caused problems in previous studies.
When compared to the previous study the results of the NRLs for the phage typing of *S*. Enteritidis are the same, 94% correct. For the phage typing of *S.* Typhimurium there was an improvement in the results from 97% correct in the previous study to 98% correct in this study.
For the FWD laboratories the phage typing of *S*. Enteritidis was not as good as the last study when 89% of the strains were typed correctly. Only 85% were correct in this study. There was an improvement in the phage typing of *S.* Typhimurium by the FWD laboratories in this study with 91% of the strains being typed correctly compared to 80% in the previous study.
The results of these two studies show the NRLs continue 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: What is the reason for an incorrect titre of a phage?
A: Before the phages are sent to the users they are checked at a 100x higher titre than normally used. The user should also check the titre for correctness after receipt of the phages, as transport may be of influence on the titre.
Q: Only 7 NRLs, but 20 FWD laboratories perform phage typing. Does this cover all EU countries?
A: In the report it will be indicated in which countries phage typing is performed.

2.10 Proposal typing study 2009

Wilma Jacobs, CRL-Salmonella, Bilthoven, the Netherlands

It is foreseen to organise an interlaboratory comparison study on typing of *Salmonella* spp. in November/December 2010. The same set-up as for the earlier studies will be used, consisting of:
20 different *Salmonella* serovars for serotyping 10 *Salmonella* Enteritidis and 10 *Salmonella* Typhimurium strains for phage typing.
The phage typing will again be organised in cooperation with the Health Protection Agency in London, United Kingdom.

Some suggestions and remarks for the next study were made:

- The NRLs were asked to complete the tables on used antisera completely, including negative as well as positive results on tests per strain;
- For phage typing results, a separate Excel sheet will be used for the first time;
- Electronic reporting (by sending the completed test report by e-mail) is strongly recommended;
- A check-up by the NRLs of the submitted results is not longer needed when the results are sent by e-mail. This will save time, but NRLs need to be sure to fill out the right results at once.

Discussion

Q: Is it really necessary to always complete all tables on the antisera reactions of all strains? This is a lot of work and it is not clear whether the information is needed. Furthermore, it is also requested to give information on the producers of the antisera, why is this asked? Is it possible that CRL gives a list of acceptable producers?
A: The information is used in case of deviating results. An alternative could be that the CRL asks for further information afterwards in case deviating results are found. It will be checked with the typing department what would be best to do. The names of the producers are asked to find out whether specific problems are related to antisera of specific producers or specific batches. The CRL can not give a list of acceptable producers as it should remain independent.

2.11 Work programme CRL-*Salmonella* second half 2009, first half 2010 and closure

Kirsten Mooijman, CRL-Salmonella, Bilthoven, the Netherlands

Work programme

Kirsten Mooijman gave information on the work programme of the CRL-*Salmonella* for the rest of 2010 and for early 2011.

At first an update was given on the ***Salmonella* Goldcoast** study:

- In 2009 and ongoing in 2010 an outbreak took place of *S*. Goldcoast in 6 EU countries, with as possible source pork containing products;
- A high proportion of the human cases shared an indistinguishable PFGE profile and therefore ECDC wanted to check PFGE profiles from animals (especially pigs) and food in the EU;
- In March 2010: DG-Sanco/ECDC contacted CRL-*Salmonella* to ask for use of the NRL network to:
  - Collect *S.* Goldcoast isolates to perform PFGE at the CRL;
  - Collect PFGE profiles of *S.* Goldcoast from NRLs.
- On 12 March 2010 the CRL sent an e-mail to NRLs to ask for available strains/PFGE-profiles and by 30 March replies were received from 22 NRLs indicating an availability of in total 600 isolates of *S.* Goldcoast.
- In April/May 2010, 4 NRLs sent isolates (40) and 7 NRLs sent PFGE profiles (41).
- At the CRL-*Salmonella* several *S.* Goldcoast isolates were available in stock from, amongst others, baseline surveys. If PFGE profiles were not yet available this was tested on all isolates at the CRL.
- In May 2010: 118 PFGE profiles are available from 14 countries, from human, pig, cattle, dog
- The results from the different isolates show very similar PFGE profiles, with only minute differences. Therefore, the information from PFGE for *S*. Goldcoast seem to be limited
- By the end of May the results were sent to ECDC and to Italy to compare the results with the PFGE profiles from human isolates. The results still need to be further discussed.

Audit of the CRLs

All CRLs have been informed that their performance will be evaluated by DG-Sanco (through independent organisation) in 2010 and 2011. In 2009 already 12 CRLs have been evaluated.
In 2010/2011 the remaining 28 CRLs will be evaluated. The report of the evaluation is expected by the end of 2010 or by early 2011. If the outcome is not satisfactory, DG-Sanco can decide to tender for a specific CRL. If the outcome is positive, the CRL can continue for another 5 years from 2012.
The outcome of the audit will not yet have an influence on the work of the CRL for 2010 and 2011, meaning that the work programme for the coming year could be presented.

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 2010;
- Typing of *Salmonella* spp. (serotyping and phage typing): November/December 2010;
- Detection of *Salmonella* spp. in a 'veterinary' matrix: February/March 2011.

Research
The CRL-*Salmonella* has planned the following activities:

- Continuation of the activities for the standardization organisations, ISO (at international level) and CEN (at European level). Also see the earlier presentation on ISO/CEN activities:
  - Continue convenorship of the 3 ISO/CEN working groups/ ad hoc groups for drafting/amending documents on detection, enumeration and serotyping of *Salmonella.*
  - In relation to this: collect information from literature and/or perform laboratory experiments for possibility of refrigerating cultured enrichment media (pre-enrichment and selective enrichment), for optimising the incubation time of the selective enrichment, for testing the usefulness of some biochemical tests, for the option of pooling of samples.
  - Reporting of the information collected so far for revising ISO 6579-1.
  - Disseminate relevant information from ISO/CEN to NRLs.
- To test different samples for the interlaboratory comparison studies;
- The use of molecular methods.

Communication and other activities
As before, the newsletter will be published four times a year through the CRL-*Salmonella* website. The NRLs are requested to provide any relevant information of interest for the other NRLs for publication through the newsletter.
CRL-*Salmonella* participates in working groups of EFSA and of DG-Sanco.
CRL-*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 CRL-*Salmonella* at the CRL or at the laboratory of the NRL. Requests for trainings will be considered case by case. In 2010 (up to June 2010), the CRL has given five trainings (at the CRL or at the location of the NRL).

Workshop 2011
The 2011 workshop will most likely be organised in Bilthoven, the Netherlands in May/June.

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

Special thanks were given to Geneviève Clement of ISPAI for all her efforts to organise the workshop in Saint Malo.

Discussion

Q: Will CRL-*Salmonella* again organise interlaboratory comparison studies for immunological methods? What about standardization of these types of methods?

A: A few years ago the CRL has organised an interlaboratory comparison study on serological methods for the detection of *Salmonella* in pigs in cooperation with the Animal Health Service, Deventer, the Netherlands. This service also prepared reference sera which are most important to test the methods. Standardization of these types of methods is difficult as most methods are proprietary (commercial) methods. More information on the antisera can be found at the website of the Animal Health Service: http://www.gddeventer.com/templates/dispatcher.asp?page_id=25251246.

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List of abbreviations

A	Answer
BPW	Buffered Peptone Water
CEN	European Committee for Standardization
cfp	colony forming particle
CRL (EU-RL)	Community (European) Reference Laboratory
CSR	Community Summary Report
DG	Directorate General
DG-Sanco	Directorate General for Health and Consumer Protection
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
FWD	Food and Waterborne Diseases and Zoonoses surveillance network
FYROM	Former Yugoslav Republic of Macedonia
HPA	Health Protection Agency
ISO	International Standardisation Organisation
LZO	Laboratory for Zoonoses and Environmental Microbiology
MKTTn	Mueller Kauffmann Tetrathionate broth with novobiocin
MPN	Most Probable Number
MS	Member State
MSRV	Modified Semi-solid Rappaport Vassiliadis
NCTC	National Collection of Type Cultures
NRL	National Reference Laboratory
NWIP	New Work Item Proposal
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
PT	Phage Type
Q	Question
RIVM	National Institute for Public Health and the Environment
RVS	Rappaport Vassiliadis broth with Soya
SC	Sub Committee
SE(20)	*Salmonella* Enteritidis (at a level of approximately 20 cfp/capsule)
SPan(5)	*Salmonella* Panama (at a level of approximately 5 cfp/capsule)
STM(5)	*Salmonella* Typhimurium (at a level of approximately 5 cfp/capsule)
TC	Technical Committee
TR	Technical Report
TS	Technical Specification
UK	United Kingdom
USP	United States Pharmacopoeia
WG	Working Group
WHO	World Health Organisation
XLD	Xylose Lysine Deoxycholate
VTEC	Verotoxigenic *Escherichia coli*

Annex 1 Participants

European Commission	Klaus Kostenzer
European Food Safety Authority (EFSA)	Giusi Amore
CRL – *Salmonella*	Kirsten Mooijman Angelina Kuijpers Wilma Jacobs
Guest speaker (United Kingdom)	Elizabeth de Pinna (HPA, London)

National Reference Laboratories for *Salmonella*

AUSTRIA	Heimo Lassnig
BELGIUM	Hein Imberechts Katelijne Dierick
BULGARIA	Hristo Daskalov
CROATIA	Gordan Kompes
CYPRUS	-
CZECH REPUBLIC	Tomas Cerny
DENMARK	Dorte Lau Baggesen Karl Pedersen
ESTONIA	Age Kärssin
FINLAND	Henry Kuronen
FRANCE	Marylene Bohnert
GERMANY	Janine Beutlich
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SLOVENIA	Jasna Micunovic Vojislava Bole-Hribovsek
SPAIN	Maria Christina de Frutos Escobar
SWEDEN	Lennart Melin
UNITED KINGDOM	Robert Davies

Annex 2 Programme of the workshop

Programme of the CRL-Salmonella workshop XV,
Sunday 27 June 2010, St. Malo, France

General information

Place of the workshop:

Hotel France et Chateaubriand; 12 Place Chateaubriand; 35412 Saint-Malo, France
tel: +33 2 99 56 66 52
http://www.hotel-chateaubriand-st-malo.com/

Accomodation:

Hotel France et Chateaubriand (see above)
http://www.hotel-chateaubriand-st-malo.com/
or
Hotel de la Cité; Place Vauban; 35412 Saint-Malo, France
tel: +33 2 99 4 55 40
http://www.hotel-cite-st-malo-bretagne.com/

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 24 June 2010.

Saturday 26 June 2010

Arrival of representatives at St. Malo.

19.00 – 20.00 Registration and get-together in hotel France et Chateaubriand

- Final information concerning the programme
- Administrative aspects

Sunday 27 June 2010

Chair: Kirsten Mooijman

9:00 – 9:30	Opening and introduction (Kirsten Mooijman, CRL)
9:30 – 10:00	2008 Community Summary Report on Zoonoses – Overview on *Salmonella* (Giusi Amore, EFSA)
10:00 – 10:30	Summary of the EU baseline studies and ongoing activities on *Salmonella* (Klaus Kostenzer, DG-Sanco)
10:30 – 11:00	*Coffee/tea*
11:00 – 11:30	ISO/CEN activities (Kirsten Mooijman, CRL)
11:30 – 12:00	Results interlaboratory comparison study on bacteriological detection of Salmonella – FOOD III – October 2009 (Angelina Kuijpers, CRL)
12.00- 13.30	*Lunch*
13:30 – 14:00	Results interlaboratory comparison study on bacteriological detection of *Salmonella* – Veterinary XIII – March 2010 (Angelina Kuijpers, CRL)
14:00 – 14:30	Proposal on interlaboratory comparison studies on detection of *Salmonella* – 2010/2011 (Kirsten Mooijman, CRL)
14:30 – 15:00	Results typing study XIV - December 2009: serotyping (Wilma Jacobs, CRL)
15:00 - 15:30	*Coffee/tea*
15:30 – 16:00	Results typing study XIV – December 2009: phage typing (Elizabeth de Pinna, HPA)
16:00 – 16:30	Proposal typing study 2010 (Wilma Jacobs, CRL)
16:30 – 17:00	Work programme CRL 2010/2011 and closure (Kirsten Mooijman, CRL)
19.00	*Dinner*

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