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K.A. Mooijman

The fourteenth CRL-*Salmonella* workshop

25 and 26 May 2009, Bilthoven, the Netherlands

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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/330604/09/CS Community Reference Laboratory for *Salmonella* (2009)

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Abstract

The fourteenth CRL-*Salmonella* workshop
25 and 26 May 2009, Bilthoven, the Netherlands

This report contains the summaries of the presentations of the fourteenth annual workshop for the National Reference Laboratories (NRLs) for *Salmonella*, held in Bilthoven, the Netherlands on 25 and 26 May 2009. 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 NRLs of a few selected countries also described their activities and how they carried these out to meet their responsibilities.

Among the summaries are those of the presentations reporting the results of the annual baseline studies for *Salmonella*, in which each participating country determines the prevalence of *Salmonella* in certain products. In 2008 the products under study originated from breeding pigs and broiler carcasses. Different methods for detecting and typing of *Salmonella* were also discussed, including those that can be used for determining whether the *Salmonella* strain that has caused (an outbreak of) illness in humans is the same strain as that found in a product.

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.

Key words:
CRL-*Salmonella*, NRL-*Salmonella*, *Salmonella*, workshop 2009

Rapport in het kort

De veertiende CRL-*Salmonella* workshop
25 en 26 mei 2009, Bilthoven, Nederland

Dit rapport bevat verslagen van de presentaties die op 25 en 26 mei 2009 zijn gehouden tijdens de veertiende jaarlijkse workshop voor de Nationale Referentie Laboratoria (NRL's) voor *Salmonella*. Het doel van de workshop is informatie uitwisselen over activiteiten van zowel de NRL's als van het overkoepelend orgaan, het Communautair Referentie Laboratorium (CRL) *Salmonella*. 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. Ook presenteren de NRL's van enkele geselecteerde landen hoe zij hun taken en verplichtingen uitvoeren.

In de verslagen gaat veel aandacht uit naar de instrumenten om *Salmonella* aan te tonen. Onder andere komen de jaarlijkse *baseline* studies voor *Salmonella* aan de orde, waarin per deelnemend land wordt vastgesteld hoeveel *Salmonella* voorkomt bij bepaalde diergroepen. In 2008 betrof dit fokvarkens en karkassen van vleeskuikens. Vervolgens zijn meerdere methoden besproken die *Salmonella* aantonen en typeren. Bijvoorbeeld hoe kan worden vastgesteld of de *Salmonella* waarvan mensen ziek zijn geworden dezelfde is als in een product is aangetroffen.

De organisatie van deze workshop is in handen van het CRL voor *Salmonella*, die op het Rijksinstituut voor Volksgezondheid en Milieu is gevestigd. 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 Bilthoven, Nederland.

Trefwoorden:
CRL-*Salmonella*, NRL-*Salmonella*, *Salmonella*, workshop 2009

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Angelina Kuijpers is thanked for carefully presenting the activities of the CRL and for making minutes during the workshop.
Christiaan Veenman is thanked for making minutes during the workshop and for taking care of the publication of the presentations through the CRL-*Salmonella* website.

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List of abbreviations

A	Answer
AFNOR	Association Française de Normalisation
BAM	Bacteriological Analytical Manual
BPW	Buffered Peptone Water
CASCO	Committee on Conformity Assessment
CD	Committee Draft
CEN	European Committee for Standardisation
cfp	colony forming particle
CRL	Community Reference Laboratory
CSLI	Clinical and Laboratory Standards Institute
DG	Directorate General
DG-Sanco	Directorate General for Health and Consumer Protection
DNA	Deoxyribonucleic acid
DT	Definitive Type
EC	European Commission
EFSA	European Food Safety Authority
EFTA	European Free Trade Association
EU	European Union
FYROM	Former Yugoslav Republic of Macedonia
HACCP	Hazard Analysis and Critical Control Points
HPA	Health Protection Agency
ISO	International Standardisation Organisation
MKTTn	Mueller Kauffmann Tetrathionate broth with novobiocin
MLEE	Multi-Locus Enzyme Electrophoresis
MLST	Multi-Locus Sequence Typing
MLVA	Multiple-Locus Variable number tandem repeat Analysis
MPN	Most Probable Number
MS	Mass Spectrometry
MSRV	Modified Semi-solid Rappaport Vassiliadis
NRL	National Reference Laboratory
nt	not typable
NWIP	New Work Item Proposal
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
PG	Project Group
PT	Phage Type
PTS	Premi®Test Salmonella
Q	Question
QA	Quality Assurance
RDNC	React But Did Not Conform
RIVM	National Institute for Public Health and the Environment
RTE	Ready-To-Eat
RVS	Rappaport Vassiliadis broth with Soya
SC	Sub Committee
SE(20)	*Salmonella* Enteritidis (at a level of approximately 20 cfp/capsule)
SNP	Single-Nucleotide Polymorphism

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
TS	Technical Specification
UK	United Kingdom
US(A)	United States (of America)
USP	Unites States Pharmacopeia
VTEC	Verotoxigenic *Escherichia coli*
WG	Working Group
WHO	World Health Organisation
XLD	Xylose Lysine Deoxycholate

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Summary

On 25 and 26 May 2009 the Community Reference Laboratory for *Salmonella* (CRL-*Salmonella*) organised a workshop in Bilthoven, 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 52 participants were present at the two-days 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, on the baseline studies performed in 2008 and on European legislation concerning *Salmonella*. Furthermore the results of the interlaboratory comparison study on typing of *Salmonella* (serotyping and phage typing) as performed in 2008, were presented.
During the afternoon session of the first day, the results of the interlaboratory comparison studies on detection of *Salmonella* in a feed matrix (2008) and in a veterinary matrix (2009) were discussed. Also proposals for future interlaboratory comparison were discussed. The day was closed with presentations of three NRLs, dealing with (molecular) typing of *Salmonella*. These presentations were introduced by a presentation of EFSA on the outcome of a questionnaire on the availability of molecular typing methods in the EU countries.

On the second (half) day of the workshop, a DVD was presented on *Salmonella* control in laying farms. Next, 5 NRLs gave presentations, explaining their activities to fulfil the task and duties. Furthermore information was given on the standardization of methods at International (ISO) and European (CEN) level and on enumeration of *Salmonella*. The workshop was finished with a presentation on the work programme of the CRL-*Salmonella* for the next year.

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

1. Introduction

In this report the abstracts of the presentations given at the CRL-*Salmonella* workshop of 2009 are presented as well as a summary of the discussion that followed the presentations. The full presentations itself are not provided within this report, but can be found at the CRL-*Salmonella* website: http://www.rivm.nl/crlsalmonella/workshops/workshopXIV.jsp

The lay-out of the report is according to the programme of the workshop.
In chapter 2 all abstracts of the presentations of the first day are given.
In chapter 3 all abstracts of the presentations of the second day are given.
In Annex 1 the list of participants is given.
In Annex 2 the programme of the workshop is given.

2. Monday 25 May 2009: day 1 of the workshop

2.1 Opening and introduction

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

Kirsten Mooijman, head of CRL-*Salmonella*, opened the fourteenth workshop of CRL-*Salmonella* welcoming all participants in Bilthoven, the Netherlands. For the first time also NRLs from Iceland, Switzerland (both EFTA countries) and Croatia (Candidate country) participated.
After a roll call of the delegates, information was given on the changes at the CRL and other new aspects:

- Petra Berk has left the institute on 1 April 2009 and replacement is not yet allowed;
- Two third countries (Tunisia and Israel) participate in the interlaboratory comparison studies on detection of *Salmonella* spp. in a veterinary matrix, on request of DG-Sanco.

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 2007 Community summary report on Zoonoses – Overview on *Salmonella*

Pierre-Alexandre Beloeil, EFSA, Parma, Italy

The European Food Safety Authority (EFSA), in close collaboration with national authorities collects annually data on zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks from the Member States.
In the presentation an overview was given on what has been reported in the Community Summary Report on Zoonoses for 2007 in relation to *Salmonella*. Information was given on Salmonellosis cases in humans, *Salmonella* in poultry sectors and *Salmonella* in the pig sector.

Overall, the total case counts of Salmonellosis in humans have decreased since 2004. This decreasing Community trend is statistically significant. The reporting systems of the Member States (MS) showed different sensitivities and this may have influenced the reported rates; consequently, comparison between countries should be done with caution. Comparison between years within a country is, in general, more valid.
As in previous years, the two most common *Salmonella* serovars in 2007 were *S.* Enteritidis and *S.* Typhimurium, representing 81 % of all known types in human cases (7.2 % were unknown), compared to 86 % in 2006.

Summary of the presentation:
Salmonellosis in humans:

- second most often reported zoonotic disease in humans in the EU;
- decrease over time of the notification rate of Salmonellosis cases;

- *S.* Enteritidis: poultry meat, table eggs;
- *S.* Typhimurium: pigs, cattle, and products thereof – broilers and table eggs;
- *S.* Derby: turkey and pig production.

Breeding flocks of Gallus gallus:

- 15 MSs have already met the target;
- 9 MSs reported prevalence between 1.1 % and 26.3 %;
- improvements in the *Salmonella* status of breeding flocks from 2005.

Layer flocks:

- 4.3 % of layer flocks reported positive.

Broiler flocks:

- intensive monitoring of fresh broiler meat: overall 5.5 % tested positive;
- 6.8 % of non-ready-to-eat (non-RTE) products of broiler meat and 0.2 % of RTE products tested positive.

Turkey flocks:

- mean EU prevalence of 30.7 % in production turkey flocks in 2006-2007;
- routine monitoring reported 7.8 % prevalence in production turkey flocks;
- routine monitoring programmes less sensitive than the baseline survey protocol;
- 6.8 % in non-RTE turkey meat.

Slaughter pigs and pig meat:

- EU prevalence in lymph nodes of slaughter pigs: 10.3 % (0 %-30 %);
- in 13 MS mean prevalence on pig carcasses: 8.3 % (0-20 %);
- routine monitoring reported 1.1 % of fresh pig meat positive in the EU;
- pig meat is one of the important sources of *Salmonella.*

Cattle and bovine meat:

- few MSs reported low prevalence of *Salmonella* in cattle and bovine meat.

Dairy products:

- low proportion of positive samples in the substantial number of dairy products tested.

Plant products:

- *Salmonella* seldom detected with in general < 0.1% at EU level;
- sprouted seeds.

Discussion

Q: What is meant with routine monitoring?
A: The analysis which is already performed in each country on the analyses in a certain sector. This is reported in the third level of the zoonoses report (EFSA, 2009).
Q: Are data on serology included?
A: Yes, but as different methods, antisera, etc. were used, comparison between countries is difficult. Within a country monitoring by serology may be useful.

2.3 Baseline surveys on *Salmonella* in broiler carcasses and on *Salmonella* in pig holdings with breeding pigs

Pierre-Alexandre Beloeil, EFSA, Parma, Italy

Preliminary results of the baseline surveys on *Salmonella* in broiler carcasses and on *Salmonella* in pig holdings with breeding pigs were presented. Publication of the results of these baseline surveys will wait for the publication of the EFSA reports. The timetable for the reports is as follows:

Salmonella in broiler meat:
- report part A: January 2010
- two reports part B: April 2010

Salmonella in breeding pigs:
- report part A: October 2009
- report part B: 2010

Discussion
Q: What is the status of the analyses of the data presently?
A: Recently the data for the presence of *Campylobacter* were analysed and sent to the Member States for a check-up. The cleaned dataset for *Salmonella* was recently received and the first statistical analyses will start soon. It is expected that the results of the statistical analyses will be sent to the MS by June 2009. MS are requested to check the results and to respond as quickly as possible so that the statistical analyses can be finalised.

2.4 Developments and perspectives for future legislation concerning *Salmonella*

Ari Hörman, European Commission, DG-Sanco, Brussels, Belgium

Annex II (E) of the European zoonoses control Regulation (2160/2003) lays down the following provisions on a *Salmonella* criterion for fresh poultry meat:
- From the end of 2010 on, fresh poultry meat from fowl (*Gallus gallus*) and turkeys may not be placed on the market for human consumption unless it meets the criterion '*Salmonella*: absence in 25 grams[1]'.
- Before the end of 2009, detailed rules for this criterion will be laid down in accordance with the Comitology procedure. These will specify, in particular, the sampling plan and analytical method.
- If the fresh poultry meat does not comply with this criterion it cannot be placed on the market and should be destined for industrial treatment or another treatment to eliminate *Salmonella*.

The food safety criterion 'absence of *Salmonella* in 25 grams of poultry meat' has already been adopted in the European zoonoses control regulation 2160/2003, being the frame work legislation, and will apply in any case from the end of 2010 on but detailed rules for the implementation must be laid down.

The objective of this legal initiative is to:
- ensure consistent implementation of the framework legislation and the overarching criterion of '*Salmonella*: absence in 25 grams' in fresh poultry meat;
- ensure Member States and food business operators testing regimes for the *Salmonella* are harmonised to allow internal and external trade in poultry throughout the Community;
- promote testing for *Salmonella* in foodstuffs to encourage reduction in *Salmonella* prevalence at prior stages of the food chain (flocks);
- ensure a high level of consumer protection and reduce human Salmonellosis by the consumption of fresh meat from fowl and turkeys;
- to guarantee consistency with existing *Salmonella* criteria in minced meat, meat preparations and meat products.

[1] Salmonella should not be detected at bacteriological investigation of samples containing 25 grams of fresh poultry meat

The proposed criterion is mainly intended to be used by food business operators in the context of their HACCP-procedures. It can also be used for the purpose of official controls to verify that the food business operators fulfil their obligations in line with the guidance document on official controls, under regulation (EC) No 882/2004, concerning microbiological sampling and testing of foodstuffs.

An overview of the Community strategy on *Salmonella* control in food derived from pigs and poultry is given in Figure 1.

It should be stressed that reduction of Salmonellosis through the consumption of food is not only the responsibility of the food business operators or competent authority but of each consumer by good kitchen practice (thorough cooking of meat and avoidance of cross-contamination).

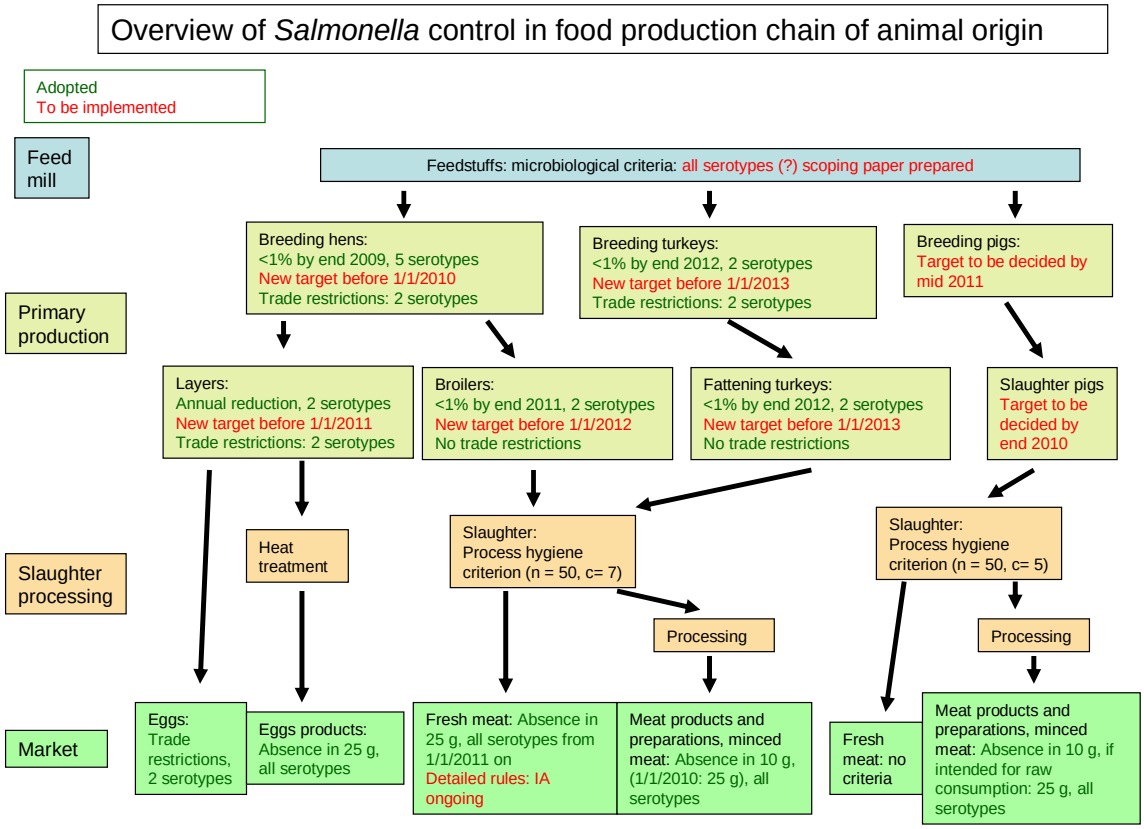

Figure 1 Overview of the Community strategy on *Salmonella* control in food derived from pigs and poultry

Discussion

Q: Is it expected that criteria will be set for *Salmonella* in cattle (e.g. *S.* Dublin)?
A: At the moment there is no specific discussion on *Salmonella* in cattle. The main purpose is to get a reduction of *Salmonella* in general. It is a step-by-step approach. First is started with the products which are most often contaminated with *Salmonella*, like poultry products.
Q: When will criteria be set for food and animal feed?
A: For several food products criteria are already implemented. For animal feed this may come in a few years time.

2.5 Results interlaboratory comparison study on typing of *Salmonella* - XIII – 2008: serotyping

Petra Berk, CRL-Salmonella, Bilthoven, the Netherlands

In 2008 the thirteenth interlaboratory comparison study on typing of *Salmonella* was organised by the EU Community Reference Laboratory for *Salmonella* (CRL-*Salmonella*, Bilthoven, the Netherlands) in collaboration with the Health Protection Agency (HPA, London, United Kingdom). The main objective of the study was to evaluate whether examination of samples by the National Reference Laboratories (NRLs-*Salmonella*) was carried out uniformly and whether comparable results were obtained.

28 NRLs-*Salmonella* of the Member States of the European Union participated, as well as NRL-Norway and NRL-Switzerland. All 30 NRLs performed serotyping. A total of 20 strains of the species *Salmonella enterica* subspecies *enterica* were selected for serotyping by the CRL-*Salmonella*. The strains had to be typed with the method routinely used in each laboratory, following the Kauffman-White scheme. The laboratories were allowed to send strains for serotyping to another specialised laboratory in their country. 95 % of the NRLs were able to correctly type the O-antigens and the H-antigens. 93 % of the NRLs indicated correct serovar names for the 20 serotyping strains. At the CRL-*Salmonella* workshop in 2007 (Mooijman, 2007) the CRL-*Salmonella* proposed a definition for good performance of the NRLs regarding the serotyping. Using this definition 26 NRLs achieved this level of good performance. The 4 NRLs which did not achieve the level of good performance received 10 extra strains for serotyping. All 4 NRLs achieved the level of good performance in this follow-up.

Discussion

Q: For this study detailed information was asked on suppliers of the antisera. Was it useful (it was a lot of work)?
A: The information will be summarised in the report. In some cases it gave extra information for interpretation of the results
Q: Are problems with serotyping person related?
A: This may be possible. If necessary the CRL can offer a training.
Q: *S.* Poona is often used as control strain in several laboratories. Is it possible that cross contamination has occurred in some laboratories?
A: This may be an explanation, but still should not have happened.

2.6 Results interlaboratory comparison study on typing of *Salmonella* - XIII – 2008: phage typing

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

Salmonella strains for phage typing in the thirteenth interlaboratory comparison study on the typing of *Salmonella* 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 *Salmonella* Typhimurium strains were selected from the culture collection of the HPA.
Eight National Reference Laboratories (NRLs) took part in the phage typing of the *S.* Enteritidis strains and seven NRLs took part in the phage typing of the *S.* Typhimurium.

The participating laboratories were sent a new *S*. Enteritidis phage, phage 17, and an updated version of the *S*. Enteritidis phage typing scheme. The scheme now has 96 recognised phage types. The laboratories were also sent a new *S*. Typhimurium phage, additional phage 10var3.
Overall, the results of this study were very good. Six of the laboratories correctly phage typed all ten of the *S*. Enteritidis strains. One laboratory correctly phage typed nine of the ten strains and one NRL correctly typed six of the strains.
The ten *S*. Typhimurium strains were correctly phage typed by five of the NRLs. Two of the laboratories correctly phage typed nine of the ten *S*. Typhimurium strains. The *S*. Typhimurium DT208, which caused problems in the previous study, was correctly phage typed by all the laboratories in this study.
All 20 strains in the study were correctly phage typed by four of the laboratories. Three of the laboratories correctly phage typed 19 of the 20 strains.
Overall the NRLs phage typed 94 % of the *Salmonella* Enteritidis strains correctly and 97 % of the *Salmonella* Typhimurium strains.
The results of this study show the NRLs continue to perform phage typing of *Salmonella* to a high standard.

Discussion
Q: It is good that the new scheme for phage typing of *Salmonella* Enteritidis has been distributed. When can we expect the new scheme for phage typing of *Salmonella* Typhimurium?
A: We are working on it, but it is a lot of work and it will take some time before it is finalised.

2.7 Proposal typing study 2009

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

It is foreseen to organise an interlaboratory comparison study on typing of *Salmonella* spp. in November/December 2009. 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.

For the evaluation of the study the following procedure will be followed:
Distinction between the ‘top 5 serovars’ and other strains:

- 4 penalty points in case of:
  - incorrect typing of *S*. Enteritidis, *S*. Typhimurium, *S*. Hadar, *S*. Infantis or *S*. Virchow;
  - assigning the serovar names of *S*. Enteritidis, *S*. Typhimurium, *S*. Hadar, *S*. Infantis or *S*. Virchow to another strain.
- 1 penalty point in case of:
  - incorrect typing of other strains.
- Determining total amount of penalty points per NRL; Good performance at: < 4 penalty points

2.8 Results interlaboratory comparison study on bacteriological detection of *Salmonella* - FEED I - 2008

Angelina Kuijpers, CRL-Salmonella, Bilthoven, the Netherlands

In October 2008 the Community Reference Laboratory for *Salmonella* (CRL-*Salmonella*) organised the first interlaboratory comparison study on bacteriological detection of *Salmonella* in an animal feed matrix (chicken feed). Participants were 30 National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) of the EU Member States and of Norway and one candidate country Former Yugoslav Republic of Macedonia (FYROM).
The first and most important objective of the study, was to see whether the participating laboratories could detect *Salmonella* at different contamination levels in an animal feed matrix. To do so, chicken feed 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 methods (ISO 6579, 2002) and the 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*.

Thirty five individually numbered capsules had to be tested by the participants for the presence or absence of *Salmonella.* Twenty five of the capsules had to be examined in combination with each 25 gram of *Salmonella* negative chicken feed. These 25 capsules were divided over the following groups: 5 capsules contained approximately 5 colony forming particles (cfp) of *Salmonella* Typhimurium (STM5), 5 capsules contained approximately 50 cfp of *S.* Typhimurium (STM50), 5 capsules contained approximately 20 cfp of *S.* Enteritidis (SE20), 5 capsules contained approximately 100 cfp of *S.* Enteritidis (SE100) and 5 blank capsules. The other 10 capsules, to which no feed had to be added, were control samples, existing of 3 capsules STM5, 2 capsules SE20, 1 capsule SE100, 2 capsules containing approximately 5 cfp of *S.* Panama (SPan5) and 2 blank capsules.

On average the laboratories found *Salmonella* in 92 % of the (contaminated) samples when using selective enrichment in MKTTn (prescribed food method). The method for testing veterinary samples (MSRV) gave the best results with 99 % of the positive samples, very closely followed by the other food method (RVS).

Twenty-eight out of 30 laboratories achieved the level of good performance for at least one of the prescribed methods (MKTTn or RVS). One NRL achieved the level of good performance when also the requested method (MSRV) was taken into account. One NRL was unable to reach the level of good performance, neither in the follow up test. The reasons for their failures are currently being investigated.

Discussion
Q: Was it considered to include other *Salmonella* serovars in stead of *S.* Typhimurium (STM) and *S.* Enteritidis (SE)?
A: We have been thinking about it, but we wanted to stick to the same set-up as used for earlier studies by using reference materials. This limits our choice of serovars, as we have only reference materials with *S.* Typhimurium, *S.* Enteritidis and *S.* Panama.

2.9 Preliminary results interlaboratory comparison study on bacteriological detection of *Salmonella* - Veterinary XII - 2009

Angelina Kuijpers, CRL-Salmonella, Bilthoven, the Netherlands

In March 2009 the Community Reference Laboratory for *Salmonella* (CRL-*Salmonella*) organised the twelfth veterinary interlaboratory comparison study on bacteriological detection of *Salmonella* in chicken faeces. Participants were 34 National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*): 28 NRLs from 27 EU Member States, one candidate country: Former Yugoslav Republic of Macedonia (FYROM), 3 NRLs from member countries of the European Free Trade Association State: Switzerland, Norway and Iceland and on request of DG-Sanco, 2 non-Europe NRLs from third countries Israel and Tunesia.

The most important objective of the study was to see whether the participating laboratories could detect *Salmonella* at different contamination levels in a veterinary matrix. To do so, chicken faeces samples of 10 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. 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*.

Thirty five individually numbered capsules had to be tested by the participants for the presence or absence of *Salmonella.* Twenty five of the capsules had to be examined in combination with each 10 gram of *Salmonella* negative chicken faeces. These 25 capsules were divided over the following groups: 5 capsules contained approximately 5 colony forming particles (cfp) of *Salmonella* Typhimurium (STM5), 5 capsules contained approximately 50 cfp of *S.* Typhimurium (STM50), 5 capsules contained approximately 20 cfp of *S.* Enteritidis (SE20), 5 capsules contained approximately 100 cfp of *S.* Enteritidis (SE100) and 5 blank capsules. The other 10 capsules, to which no faeces had to be added, were control samples, existing of 3 capsules STM5, 2 capsules SE20, 1 capsule SE100, 2 capsules containing approximately 5 cfp of *S.* Panama (SPan5) and 2 blank capsules.

On average the laboratories found *Salmonella* in 98% of the (contaminated) samples when using the prescribed veterinary method, selective enrichment on MSRV.

Thirty-three out of 34 laboratories achieved the level of good performance. One NRL was unable to reach the level of good performance in the main study. The reason for their failure is currently being investigated and a follow up test with some additional material will be organised.

Discussion

Q: Would it be possible to use Annex D of ISO 6579 (MSRV) in stead of ISO 6579 for the detection of *Salmonella* spp. in animal feed? The results of the ring trial show good results with MSRV.
A. At the moment the scope of Annex D does not include animal feed (only samples from primary production). However, ISO 6579 is under revision and if sufficient data are available showing good results with MSRV it may perhaps be possible to introduce MSRV for detection of *Salmonella* spp. in food and feed as well. However, this will take some time (years).

2.10 Proposal for interlaboratory comparison studies on detection of *Salmonella* – 2009/2010

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 2009: Detection of *Salmonella* spp. in a food matrix;
- February/March 2010: Detection of *Salmonella* spp. in a 'veterinary' matrix

For the **food** study it was suggested to use the same set-up as used for earlier food studies:

- addition of *Salmonella* reference materials to a *Salmonella*-free matrix;
- the reference materials being: *S.* Typhiumurium at low level (approximately 5 cfp/capsule) and high level (approximately 50 cfp/capsule); *S.* Enteritidis at low level (approximately 20 cfp/capsule) and high level (approximately 100 cfp/capsule);
- prescribed method: ISO 6579 (Anonymous, 2002); requested method: Annex D of ISO 6579 (Anonymous, 2007).

For the choice of the matrix it was planned to use minced chicken meat. However, first studies performed at CRL-*Salmonella* with minced chicken meat obtained from the retail showed the meat to be suspect for *Salmonella*.
From the discussion it was clear that the matrix should be a reflection of what is regularly analysed by the laboratories. This is certainly the case for chicken meat, but less for a matrix like milk powder or egg powder. It was suggested to irradiate the chicken meat, but this has the disadvantage that the background flora will be killed as well. It was agreed that CRL-*Salmonella* will further investigate the availability of *Salmonella*-free chicken meat, e.g. by requesting separate slaughter of a *Salmonella*-free flock or by ordering the meat in a low prevalence country (like Finland or Sweden).

For the **veterinary** study also the same set-up as used in earlier studies will be performed. This is the same as described for the food study, with the exception that for the veterinary study only Annex D of ISO 6579 (Anonymous, 2007) will be prescribed. For the veterinary study it was also discussed what type of sample would be preferred. No clear preference was indicated. It was discussed that cattle faeces might sometimes be difficult to analyse because of high amounts of background flora. However, as no monitoring is performed for cattle, this might not be a representative matrix. Pig faeces might be more representative, but some countries indicated that it might be difficult to obtain *Salmonella*-free pig faeces. The CRL-*Salmonella* will have a closer look at the choice of the matrix, but it may be quite likely that (*Salmonella*-free) poultry faeces will be chosen again

*The **evaluation of the studies** was once more summarised.*
For the studies on **detection of *Salmonella*** good performance will be defined as follows:

- blank control capsules (no matrix added): all samples negative;
- positive control capsules (no matrix added):
  - High level: all samples positive;
  - Low level: 1 out of 2 samples may be negative;
- blank capsules + matrix: at least 80 % of the samples negative;
- low level capsules (STM5 and SE20) + matrix: at least 50 % of the samples positive;
- high level capsules (STM50 and SE100) + matrix: at least 80 % of the samples positive.

2.11 Phage types and their antimicrobial resistance in German *Salmonella* isolates from pigs

Andreas Schroeter, Federal Institute for Risk Assessment, Germany

The National Reference Laboratory for *Salmonella* in Germany receives *Salmonella* isolates from animals, food, feed and the environment, isolated in institutions from all Federal States in Germany for further differentiation and/or confirmation of results.
In this study 2303 isolates from pigs were included, which were isolated between 2004 and 2008. The number of isolates varies between 518 and 411 yearly. The dominating serovar was *S.* Typhimurium (STM) with 1406 isolates (61 %) followed by *Salmonella* group B (most of them with serovar formula 4,[5],12:i:-) with 318 isolates (14 %) and *S.* Derby with 204 isolates (9 %).
There is an arising number of *S.* group B from 29 in 2004 to 115 in 2008, which belongs to *S.* Typhimurium in respect to his genetic background (B. Malorny pers. communication, 2008).
S. Enteritidis (SE) was relatively seldom isolated from pigs and only 36 isolates (2 %) were investigated in this study.
For phage typing the systems according to Ward et al. (1987) for *S.* Enteritidis (SE), respectively Anderson et al. (1977) for *S.* Typhimurium (STM) were used.

Five different phage types for SE could be detected, whereas PT4 and PT8 were dominating with each 14 isolates (39 %). Further phage types found were PT21, PT11 and PT13a. Only two isolates from PT4 are multiresistant (against more than one antimicrobial).
Using the 1406 STM isolates, 34 different phage types could be detected with DT104 L (30 %) and DT104 B low (19 %) as the most prevalent ones. Fifty isolates show no reaction with the used phages and were therefore not typable (nt). Furthermore, 266 isolates show a phage pattern, but there is no type till now (RDNC – React but Did Not Conform).

The Minimal Inhibitory Concentration (MIC) was determined using the broth microdilution method following CSLI guidelines (M07-A8, M100-S19, 2009) and using microtitre plates with antimicrobials (From 2004-2007: NLMV1A:2004-2007 and in 2008: EUMVS, TREK Diagnostic Systems Ltd., UK) with 17 respectively 14 different antimicrobial substances.
Eighty six percent (1206 isolates) were multiresistant, 5 % (73) were single resistant and 9 % (127) were sensitive. From the 34 different phage types detected, only 5 (11 isolates) were sensitive, 12 phage types had sensitive and resistant isolates (1066) and 17 phage types covered resistant isolates (329) only.
All DT104 B low isolates (272) were resistant and 99.5 % of DT 104 L (416) isolates were multiresistant. The well known substances of pentaresistance of DT104 (mostly chromosomal coded) – sulfamethoxazole (86 %), streptomycin (79 %), tetracycline (81 %), ampicillin (80 %) and chloramphenicol (44.4 %) - had the highest percentage of resistance from the antimicrobial substances tested. Beside the DT 104 types, the pentaresistance could be detected in DT2, DT41, DT120, DT193, U302, RDNC, and in nt isolates. There were isolates from DT104 B low, DT120, DT193, RDNC and nt, which harboured the resistance to 12 different antimicrobials.
The high resistance of *Salmonella* isolates from pigs show the usage of antimicrobials in the pig production as well as their stabile heredity over a five year period.

Discussion
It was discussed how to handle when ‘monophasic *S.* Typhimurium (1,4,[5],12 : i:-)’ is found. Should action been taken? What is the meaning of this isolate?
It was not possible to find a single answer to this problem. Reactions given:

- In the United Kingdom this isolate is widely found and it may also be multi-resistant. Therefore it is important to treat the isolate as a 'normal' STM.
- In Luxembourg 'monophasic-STM' showed to be clinically the same as 'normal' STM. A person even died from an infection with 'monophasic-STM'.
- On serological basis it is not possible to call 'monophasic-STM' *S.* Typhimurium. However, genetically the serovars are generally the same.
- According to legislation monophasic-STM is not a STM, meaning that no formal action has to be taken when monophasic-STM is found. However, as this monophasic STM can also cause diseases in humans it is important that the EC take this information into consideration when legislation is reviewed.

Q: Is there more information on the underlying resistance mechanisms?
A: The CRL for antimicrobial resistance is trying to find this together with NRLs of several countries. It depends on the choice of the breakpoints what is called resistant or not.

2.12 Questionnaire survey on the availability of the molecular typing methods for food-borne pathogens

Pierre-Alexandre Beloeil, EFSA, Parma, Italy

A questionnaire survey on molecular typing was carried out in autumn 2008 by the EFSA. The relevant CRLs were consulted on the formulation of the questions. The questionnaire covered the molecular typing of *Salmonella*, *Campylobacter*, VTEC, *Listeria monocytogenes* and *Staphylococcus aureus* isolates from food, animals and animal feed. In total 26 MSs and 4 non-MS (Norway, Switzerland, Turkey and FYROM) replied.
In summary the outcome of the questionnaire was:

- Molecular typing is performed mainly occasionally for all the pathogens.
- Only few countries perform molecular typing on isolates on a routine basis.
- The molecular typing frequency of isolates from different sources is variable.

Discussion
Q: What was the aim of EFSA to make this questionnaire?
A: If many MS are able to perform molecular typing on isolates, it may be necessary to enhance the summary report for information on molecular typing results.

2.13 DNA multiplex evaluation of *Salmonella* molecular serotyping

Anne Brisabois, AFSSA, Maison-Alfort, France

Salmonella serotyping is an important tool for classification, identification of contamination sources and epidemiological purposes. In addition, EU and USA regulations require monitoring of some serovars most frequently prevalent. Traditional serotyping is based on the Kauffmann-White antigen-antibody scheme and requires a lot of different polyvalent and monovalent antisera allowing the detection of somatic and flagellar antigens. Application of this method is limited by the high costs of the sera, possible deviations in quality, time consumption and presence of non typable isolates. Therefore, Check-Points BV and DSM have developed a general fast functional bacterial typing system based on DNA chips from ClonDiag, for the molecular serotyping of *Salmonella*. This new procedure

Premi®Test *Salmonella* (PTS) can be performed directly on animal, food or environmental samples after the enrichment and isolation steps on various culture media. The aim of this study was to evaluate the performance of the PTS method on a large diversity of serovars of the *Salmonella enterica* subspecies mainly detected in animals and food and to define the sensitivity and the specificity of the test on a variety of *Salmonella* and non *Salmonella* isolates.

The Premi®Test *Salmonella* system uses a methodology called multiplex ligation detection reaction to generate a collection of circular DNA molecules that are subsequently PCR amplified by means of a single pair of amplimers (1,2). The PCR products are next sorted by hybridization to a low-density DNA microarray. Positive hybridization is detected using a biotin label incorporated in one of the PCR primers. A set of genetic markers has been selected with the purpose of yielding unique microarray hybridization profiles to identify and discriminate *S. enterica* subsp. *enterica* serovars and to recognize the *Salmonella* genus. The test allows single-tube processing, which simplifies the technical work associated with strain typing and can be applied directly after the enrichment and isolation steps of the Standard ISO method for *Salmonella* detection.
At the first step, 102 strains belonging to 68 different serovars including the 9 European and USA regulation serovars (Typhimurium, Enteritidis, Hadar, Virchow, Infantis, Heidelberg, Montevideo, Newport and monophasic Typhimurium S.I 4,12:i:-) and all others serovars known to be detected by the PTS were tested. Moreover, strains belonging to other *Salmonella* subspecies of *S.enterica* and *S.bongori* and a variety of non *Salmonella* isolates were analysed with this system.
Out of the 102 tested strains, 91 of them gave a molecular serovar or genovar code identical with the slide agglutination results, giving an agreement of 94 %. Eleven strains were detected as *Salmonella* and no mistakes of serovar assignment were observed. A total agreement for the top 20 most prevalent serovars was observed except for the monophasic *S.* Typhimurium strains, which were sometimes identified as *S.* Typhimurium. The PTS method was able to recognize all the subspecies of *S. enterica* as '*Salmonella'*. Moreover, the species *bongori* was also well detected. Finally, 36 strains belonging to 27 other species than *Salmonella* were all detected as 'non-*Salmonella'* by the PTS test.
The repeatability of the system was tested using a set of 6 strains of various serovars known to target all the spots. PTS assays were performed twice by two technicians. Results yielded a repeatability of 97.7 % for the tested serovars and of 99.5 % at spot level.
Finally, 205 isolates routinely collected at the French *Salmonella* network were tested in blind both with the PTS and the conventional methods. Results gave a globally sensitivity of 92 % for all tested isolates whatever the serovar, and up to 96 % for the top 20 isolates. Moreover, the genovars detected for the non agglutinable strains (rough) correspond perfectly to the expected serovar deduced by the PFGE pattern after XbaI macro-restriction. Most of the strains with incomplete antigenic formulae were assigned to a genovar corresponding to a serovar very close to the antigenic formulae (more often one flagellar phase was missing).

This evaluation study of the PTS method presents clearly good agreement with the classical slide agglutination method for *Salmonella* serotyping and might be a valuable alternative method for the laboratories performing routine identification of *Salmonella* strains belonging to commonly encountered serovars. The method offers practical advantages as it reduces the delay in obtaining results. Furthermore, it can easily be performed without the need of complex devices. Moreover three samples can be identified in one single array tube and auto-agglutinable isolates can be identified. The discriminatory power of the PTS method is sometimes better than conventional serotyping. Nevertheless, some steps are critical for the result, and some serovar differ only in one spot. Consequently, depending on the reader and the background, dual results can be obtained. Serovars less commonly encountered can not yet be identified with the PTS method, but will be detected as '*Salmonella'*.

The results found with the PTS method are promising and the recognition by an approval organization will be the next step of the evaluation.

Discussion
Q: How should DNA be isolated, by boiling or by another method?
A: In the protocol of the method no procedure is given, but it is indeed important that the isolation of the DNA is performed in a proper way.
Q: How many laboratories will use this method?
A: In Belgium the presented method is also frequently and successfully used.
A: The use of this kind of methods may become a big issue in the coming years. The questions remain, how and when can these kinds of methods being used? It will be important to use genes which are related with serotyping, to be able to compare the results of molecular typing with the results found with the 'classical' way of typing (serotyping).
Q: How much does this method cost?
A: This may depend on how the analyses is organised. It is possible first to perform the DNA isolation per sample and to store these isolates, until sufficient samples are stored to perform 50 analyses on 1 day. In such a way it may cost approximately €30,- per sample.
Q: It has been indicated that the time of analysing is only 8 hours, is this correct?
A: In fact it is 2 days, as it is also necessary to perform a pre-enrichment step.
Q: Is it allowed, legally, to use genotyping in stead of serotyping?
A: In general it has been indicated in legislation that alternative methods can be used if validated following the procedure of ISO 16140 (Anonymous, 2003). However, for serotyping the procedures is less clear.
Q: Is it possible to differentiate 2 serovars?
A: Not known.

2.14 Multi Locus Sequence Typing (MLST) can replace serotyping of *Salmonella* enterica today!

Mark Achtman, University College Cork, Ireland

The application of Multi Locus Sequence Typing on *Salmonella enterica* was presented.
The advantages of MLST are:
- a single, curated global database per species;
- sequencing is unambiguous;
- data can be entered via the Internet;
- universal language;
- MLST is now the "Gold standard" for long-term epidemiology and conservative bacterial typing.

In several laboratories in different countries MLST is applied to *Salmonella* collections. So far the following can be concluded that MLST of *S. enterica:*
- can replace serotyping;
- allows inferences about the history of pandemic spread and country-wide changes in patterns;
- provides a common language;
- allows communication between scientists in different continents;
- has a link to Multi-locus enzyme electrophoresis (MLEE) reference strains and the Selander collection;
- is ready for use by the community.

The NRLs for *Salmonella* were asked to:

- implement MLST for own strain collections;
- send strains to Mark Achtman to do MLST and Single-nucleotide polymorphism (SNP) typing;
- plan to replace serotyping in Europe with an MLST-based version.

Discussion

Q: Why did you choose 7 household genes? Don't you miss household genes which are also important?
A: We started with 6 genes and end up with 7, showing good results.
Q: Could whole genome sequencing also be a good alternative for serotyping?
A: A lot of training is needed to perform full genome sequencing. You do not need a whole genome, MLST can be sufficient. MLST is easier to perform and it has advantages above serotyping, e.g. with MLST it is also possible to type rough strains.
Q: Is MSLT also useful in case of outbreaks?
A: MLST is not good for outbreaks. MLST can give information on serovar level. For more detail other methods are needed (like phage typing, MLVA).
Q: Is there a link between MLST and the Premi-test (former presentation)?
A: Premi-test is based on sequencing, MLST not.

3. Tuesday 26 May 2009: day 2 of the workshop

3.1 *Salmonella* control in laying farms

Robert Davies, Veterinary Laboratories Agency (VLA), Weybridge, United Kingdom

In the UK the Competent Authority (Defra) places great importance on effective communication and joint working with stakeholders from the poultry industry. Regular meetings are held to develop and maintain the effectiveness of National Control Programmes for *Salmonella* and practical research projects on the epidemiology and control of *Salmonella* are commissioned. An example of this is Defra-funded project OZ0325 which has focused on *Salmonella* control in the table egg industry. The extensive activities for dissemination of information from the project included input to revisions of Defra Codes of Practice on *Salmonella* and rodent control as well as the British Egg Industry Council (BEIC) Lion Code and a series of road-shows for the egg industry and contributions to articles in the poultry farming press. In addition to this, DVDs were produced to record the content of the road show presentations and to demonstrate farm footage of the implementation of practical monitoring and control measures. The farm DVD was scripted by the VLA Weybridge team and directed and produced by Copacetik Productions Ltd. Around 20 location shooting visits were carried out resulting in 40 hours of farm and green-screen footage. This was edited down to a one-hour DVD which focuses on between and within-farm biosecurity, including the importance of correct maintenance of disinfectant boot dips (chlorcresol based products are best for boot dips). Control of farm pests, especially rodents, is very important and if breeding rodent populations are fully controlled, *S.*Enteritidis (SE), and particularly *S.*Typhimurium (STM), can disappear from many vaccinated laying flocks within weeks. Total rodent control requires a much more intensive and expensive baiting and trapping programme than would usually be used in UK laying farms.

Cleaning and disinfection is another essential step for elimination of resident infection from laying farms. It is usually not possible to decontaminate effectively using dry cleaning followed by disinfection. Dry cleaning also allows a rapid increase in red mite levels in laying farms. Effective washing, using very high pressure jet washers followed by drying and power washing with 10 % formalin solution to run-off point is the most effective method, but a double disinfection using two passes of a formaldehyde/glutaraldehyde/quaternary ammonium compound (QAC) commercial compound disinfectant on consecutive days is also likely to be effective.

The DVD also considers control of other potential vectors such as flies, litter beetles and red mites. Timeliness and use of effective products is vital, so that large populations are not allowed to build up to uncontrollable levels. Red mites are a major welfare problem due to the unavailability of licensed products to control infestation during the laying period and high levels of resistance to acaricides. Another important issue is correct administration of vaccines to ensure that a high proportion of the vaccinated flock actually receives a full vaccine dose. There are issues relating to injection of killed vaccines due to the speed of administration and in delivery of live vaccines, because of problems with administration in long and complex drinker lines. As with most problems, care, adequate time and resources and a sense of responsibility amongst managers and staff is critical to vaccination and most other aspects of *Salmonella* control.

Finally the DVD demonstrates in detail the sampling methods required to conform to the current EC *Salmonella* control regulations. The sensitivity of detection is closely linked to the care and attention that is paid to sampling in a thorough and representative way. This is demonstrated for different production and housing systems in the DVD.

The DVD is complemented by a pictorial summary of the script. This combination of moving and static images is an effective way of delivering information to poultry farmers and risk managers.

Discussion
Q: Will you also make a similar DVD for pigs? What does it cost?
A: We would like to prepare a DVD for pigs, but we do not have a sponsor at the moment. The costs are approximately €15000,-.
Q: When you find positive dust, can you exclude it as an old infection?
A: Dust is a reliable and sensitive sample, but care should be taken not to cross contaminate dust with faeces. If dust is found positive, extra faeces samples should be taken to prove the positivity of the flock.
Q: You do not take samples outside the house?
A: Only samples inside the house are taken, as outside you can have contamination from wild birds which will not give reliable information on the status of the flock.
Q: Do you often find vaccine strains?
A: Yes, *S.* Enteritidis PT4 is sometimes found in the laying phase. This causes much extra work.

3.2 Activities NRL to fulfil tasks and duties in Italy

Lisa Barco, NRL-Salmonella, Legnaro, Italy

The Italian NRL for Salmonellosis is part of the Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), which is a veterinary public health institute located in Legnaro (Padua). IZSVe conducts laboratory controls and research activities in three main areas: animal health and welfare, food safety, and environmental protection, employing over 500 people (veterinarians, biologists, chemists, technicians and administration staff) in the three different regions in north-east Italy. The National Reference Laboratory (NRL) for *Salmonella* has been appointed in 1999 by the Ministry of Health, and was recognised in 2007 by the World Organisation for Animal Health (OIE) as Reference Laboratory for Salmonellosis. As such, the laboratory provides expertise, diagnostic services, training and support to OIE member countries.
The NRL performs phenotypic and genotypic characterisation of *Salmonella* strains isolated from samples of veterinary origin (animals, food, feed). The NRL performs sero- and phage-typing but also molecular typing for epidemiological investigations or subtyping of specific strains, and for the study of antimicrobial resistance. The laboratory has a rich collection of *Salmonella* strains and reference strains, which are provided upon request to laboratories asking for them.
Moreover, the NRL coordinates the Enter-vet net, which collects at the national level the information concerning *Salmonella* spp. typing results. The laboratory annually organises ring trials for national laboratories, as external quality control for *Salmonella* serotyping and detection.
The NRL collaborates with the Ministry of Health for the preparation of National *Salmonella* monitoring and control programmes in animal populations. Experts of the laboratory act as contact points for the Community Reference Laboratory of Bilthoven (NL), as well as for the EFSA Task Force on Zoonoses. The NRL provides expertise to the European Commission and EFSA in the framework of different working groups.

Scientific and technical support is routinely provided to colleagues from different Institutes and countries, in the different fields, such as implementation and management of monitoring and control programmes, diagnostic laboratory methods, molecular methods, typing, antimicrobial resistance, cooperation with third countries (joint research projects, stages, training courses).

Discussion
Q: What type of reference materials do you use in the ring trials?
A: Lenticules from Health Protection Agency in UK. The *Salmonella* serovars and contamination level are similar to those used in the CRL-*Salmonella* studies.
Q: How many laboratories participate in the ring trials in Italy?
A: 11 laboratories for detection and 15 laboratories for typing of *Salmonella*.

3.3 Activities NRL to fulfil tasks and duties in Czech Republic

Tomas Cerny, NRL-Salmonella, Prague, Czech Republic

In the first part of the presentation information was given on the laboratory diagnostics system and organisation scheme of State Veterinary Administration of Czech Republic.
In the second part of the presentation, the results of the monitoring and eradication programmes which have processed in the Czech Republic in 2008 were discussed.
Furthermore, it was indicated that at the institute also risk analysis is performed and the importance of risk factors was stressed. Finally, present activities and aims of the NRL for 2009-2010 were presented.

Discussion
Q: You showed a large decrease in the prevalence of *Salmonella* in poultry flocks. Is this also seen in the human population?
A: Yes, but not as much as is seen in the flocks. The decrease is probably based on vaccination of the poultry flocks. The human outbreaks are mainly related to broiler meat. In broilers no vaccination is performed.
Q: Was vaccination in laying hens implemented after the baseline study?
A: No, during this study in 2007.

3.4 Activities NRL to fulfil tasks and duties in France

Marylène Bohnert and Anne Brisabois, NRL-Salmonella, Ploufragan and Maison-Alfort, France

The French Food Safety Agency (AFSSA) is in charge of the French NRL-*Salmonella*. In 2007 a reorganisation took place. The unit on Hygiene and Quality of avian and Pig Products (HQPAP) became the coordinator and the unit on Bacterial Epidemiology and Characterization (CEB) became the associated laboratory.
AFSSA is an administrative statutory body under three supervisory ministries, health, agriculture and consumer protection. The missions of AFSSA relate to the public health, the health and the welfare of the animals and the health of vegetables.
The HQPAP unit is situated at AFSSA- Ploufragan (LERAPP) in Brittany. The mission's of LERAPP relate to research, scientific and technical support activities in the fields of animal health and welfare, and food /feed safety for poultry and rabbit productions, pig production and further products, and fish pathology. Integration of problems is handled through various fields such as microbiology, virology,

parasitology, epidemiology, biotechnology, such as molecular biology, vaccinology, zootechnics and system analyses. Several epidemiological surveillance networks are managed.
The tasks of LERAPP in the field of NRL are (1) analyses of samples and data of epidemiology from the baseline studies (layers, broilers, turkeys, pig, broiler carcasses), (2) participation in interlaboratory comparison studies on detection of *Salmonella* of the CRL-*Salmonella* (veterinary and feed/food) , (3) organization of proficiency tests on the detection of *Salmonella* in animal faeces for the official French laboratories (about 70 public and private laboratories), (4) responsible for the collection of *Salmonella* strains isolated by officials laboratories from official samples under implementation of European Regulations 2160/2003.
The interlaboratory comparison studies on bacteriological detection of *Salmonella* in food and feed are organised by an association, the RAEMA, accredited for the PT trial organization.
The CEB unit is located at AFSSA-Maisons-Alfort, in the LERQAP laboratory (Food Quality and Agroalimentary Process Research). This unit manages a *Salmonella* network collecting strains of non-human sources and epidemiologic data from public and private laboratories, on voluntary basis. Strains received are routinely serotyped and on a selected panel molecular typing (PFGE) and antimicrobial resistance testing is performed. The tasks under NRL activities at the CEB unit are (1) serotyping reference laboratory for non-human sources and scientific and technical advice for serotyping, (2) analysis trends in the food chain and detection of unusual events, (3) participation in outbreak investigations in collaboration with the National Reference Centre, which is in charge of *Salmonella* and Salmonellosis in clinical human cases, (4) organization of a serotyping proficiency test trial at national level and of an annual meeting for the French *Salmonella* network.
The HQPAP and CEB units collaborate with the ministry of agriculture, the directory in charge of implementation of European Regulations on control of *Salmonella* (DGAl).
The two units are accredited according to the French accreditation body (COFRAC).

Discussion
Q: This early warning system is interesting. Is this early detection linked to prevention?
A: The system is a copy of the system running in Paris for humans. Interpretation is difficult. It is necessary to check everything very carefully before doing a warning.
Q: is it mandatory for food producers to send strains when they find *Salmonella*?
A: Strains are sent on voluntary basis. Therefore interpretation depends on which strains are obtained.
Q: Are ‘monophasic’ strains also found in France?
A: Yes, we also find ‘monophasic-STM’. We see an increase in ‘monophasic-STM’, but also an increase in STM without flagella. These strains can be detected by using the Premi-test.

3.5 Activities NRL to fulfil tasks and duties in Switzerland

Gudrun Overesch, NRL-Salmonella, Bern, Switzerland

In Switzerland three laboratories have the mandate of representing a reference function concerning *Salmonella*. The national centre for enteropathogenic bacteria (NENT) is located at the institute of food safety and hygiene, Vetsuisse Faculty of Zurich. Originally founded as a reference laboratory only for human Salmonellosis. In 1988 the spectrum of bacteria was extended to *Shigella* sp., *Campylobacter* spp, virulent *E. coli, Yersinia* sp. and *Vibrio cholerae*. Secondly, the national reference centre for poultry and rabbit diseases (NRKG) that is focussed on diagnostic and reference analyses of pathogens from two animal species: poultry and rabbits. This centre has the responsibility for *Salmonella* in poultry, and is located at the institute of veterinary bacteriology, Vetsuisse Faculty of Zurich. The third laboratory is the national centre for zoonoses, bacterial animal diseases and antimicrobial resistance

(ZOBA). It was founded in 2005 and is located at the institute of veterinary bacteriology, Vetsuisse Faculty of Bern. The ZOBA has the responsibility for *Salmonella* in all farm animals, excluding poultry. Within the network, ZOBA acts as the international contact address concerning *Salmonella*. The ZOBA constitutes the national reference laboratory for a broad variety of bacterial diseases including Salmonellosis and antimicrobial resistance. The tasks and duties follow the specifications laid down in the regulation (EC) 882/2004. This means especially confirmation of isolates from other laboratories, organization of ring trials, providing scientific and technical assistance to the federal veterinary office and collaboration and communication with the European reference laboratories.
An overview over the animal population and relevant data from Switzerland concerning *Salmonella* is given: In 2007 Switzerland had about 5 million broilers, 2.9 million laying hens, 1.6 million pigs, 1.3 million cattle, 160 000 breeding poultry and 110 000 turkeys. Specialities on Swiss farm sizes and types are shown. Switzerland has a total of 7.6 million inhabitants. In 2007 6113 cases of human Campylobacteriosis and 1796 cases of human Salmonellosis were registered. The predominant serovars were *S.* Enteritidis (>990 cases), *S.* Typhimurium (about 300 cases), *S.* Infantis, *S.* Virchow and *S.* Hadar.
Switzerland has close relations with the European Union on economic, scientific and trade levels. These relations are governed by a complex structure of bilateral agreements concluded over the last years. With the first bilateral agreements of 1999, for the first time special regulations within the agricultural sector were established, giving a reduction on tariffs and import quotas on agriculture products. The second bilateral agreements from 2004 went a big step further, as the relevant regulations and decisions from the EU hygiene package and on the monitoring of zoonoses and zoonotic agents were to be transferred into national legislation. As a consequence the European base line studies were conducted in Switzerland too. Results as far as available were presented.

3.6 Activities NRL to fulfil tasks and duties in Iceland

Eggert Gunnarsson, NRL-Salmonella, Reykjavik, Iceland

The NRL for *Salmonella* is situated in the Institute for experimental pathology, University of Iceland Keldur. Keldur was established in 1948 to combat serious infectious diseases brought to the country by import of animals. Research on animal diseases, production of vaccines and diagnostic service has since then been the main task of the institute. Since 2006 some test methods in Bacteriology are accredited according to ISO 17025 (Anonymous, 2005).
In 1995 the Icelandic *Salmonella* Surveillance Program for Pigs and Poultry was initiated. For this *Salmonella* has become notifiable. If *Salmonella* is found, appropriate measures should be taken to trace the source and protect consumers. Infected chicken meat has to be withdrawn from the market and destroyed. Infected swine meat should be heat treated. Infection should be eliminated. Furthermore, slaughterhouses and infected farms will be put under restriction, cleaned and disinfected.

Discussion
Q: For 3 years you did not find *Salmonella* in broilers. In one year you found 3 herds positive. Was it the same clone?
A: We think this infection comes from imported feed. Despite all actions it is still difficult to get rid of the infection.
Q: What kinds of antibiotics are used and what is the amount of resistance?
A: Antibiotics are in fact not allowed in poultry production. We do not know where the resistance comes from.

3.7 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 which may be of interest for the NRLs for *Salmonella*.
The relevant groups in ISO and CEN are:
ISO/TC34/SC9: International Standardisation Organisation, Technical Committee 34 on Food Products, Subcommittee 9 – Microbiology.
CEN/TC275/WG6: European Committee for Standardisation, Technical Committee 275 for Food Analysis – Horizontal methods, Working Group 6 for Microbial contaminants.
Both groups organised their last meeting in Valencia, Spain from 4 to 8 May 2009.

Subjects of interest are:
- New Working Item (NWI): Enumeration of *Salmonella* by mini-MPN;
- revision of ISO 6579 (detection of *Salmonella* in food & feed);
- New work item (NWI): ISO for serotyping *Salmonella* spp.;
- ISO-working group (WG2) on statistics;
- ISO-working group (WG3) on validation of microbiological methods;
- ISO-working group (WG4) on Proficiency testing in microbiology;
- Joint ISO-working group (JWG 5) on QA of media;
- CEN-Task group (TAG 5) on primary production;
- CEN-Task group (TAG 6) on sampling techniques;
- CEN mandate on validation of microbiological methods.

Enumeration of *Salmonella*
A New Working Item Proposal (NWIP) for publication of the document as an ISO Technical Specification (ISO/TS) was launched in January 2009. In total 16 members voted positive for addition of the NWIP to the SC9 programme, 2 members voted negative and 2 abstained. Some comments were included with the voting which were dealt with in a separate ad hoc group meeting in Valencia. Kirsten Mooijman will amend the document accordingly, after which it will be sent around for final vote for publication as a Technical Specification (TS), before the end of June 2009.

Revision of ISO 6579 (Anonymous, 2002)
In the periodical review of this standard (July-December 2007) there were 2 countries who voted for confirmation of the ISO standard, but with comments and 4 countries voted for revision of the standard (with many comments). Furthermore 12 countries voted for confirmation or abstained. In 2008 an enquiry was launched by the secretariat of SC9, to ask for information on the following:
- the enrichment in MKTTn at 37 ºC: the usefulness in addition to RVS enrichment, in particular for *S.* Typhi and *S.* Paratyphi;
- the usefulness of a further 24 h incubation of the selective enrichment broths;
- the possible replacement of RVS by MSRV;
- the choice of the mandatory isolation agar (XLD).

An ad hoc group was raised on the topic, to consider the outcome of the enquiry. The group met on 6 April 2009 (convened by Kirsten Mooijman) and made 15 decisions which were presented and discussed at the SC9 meeting in May 2009.

It was agreed that revision of EN ISO 6579 (2002) was considered necessary and that a Working Group (WG9) will need to be created. Kirsten Mooijman was nominated as convenor of the group. The SC9 secretariat will launch a call for experts for the new WG9.

During the meeting at Valencia, resolution number 395 was taken indicating that the members of SC9 agreed that WG9 would deal with the following items (proposals of the ad hoc group):

1. description of the detection of *S.* Typhi and *S.* Paratyphi in a normative annex of ISO 6579, considering the use of the Selenite Cystine enrichment broth;
2. to launch a trial comparing selective enrichment in the BAM/USP formulation of tetrathionate broth and in MKTTn (ISO6579 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;
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;
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;
5. retain XLD as the mandatory isolation medium. Clearer direction on suitable media for the second plate should be given in the document;
6. the SC9 secretariat will launch an enquiry to collect data to support the possibility of refrigerating cultured non-selective enrichment broth (BPW) and/or cultured selective enrichment media before subculture;
7. make the plating stage less prescriptive;
8. make the confirmation stage less prescriptive in terms of number of colonies to be confirmed;
9. the non-selective medium for purification of colonies should be left to choice;
10. include a note to allow parallel biochemical testing and purity check.;
11. investigate the usefulness of some biochemical tests.

It was also agreed to split EN ISO 6579 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 working group will need to discuss whether it would be necessary to have a fourth part of EN ISO6579 dealing with samples from the primary production.

New work item: ISO for serotyping *Salmonella* spp.

In 2008 and in 2009 enquiries were sent to the members of SC9 to ask for the need of a standard for serotyping of *Salmonella* spp. With the enquiry of 2009 also a draft proposal for a guidance document was sent as an example for a possible standard. At the meeting of 2008 the outcome of the enquiry could not be presented due to lack of time. At the 2009 meeting in Valencia, Kirsten Mooijman (CRL-*Salmonella*) presented the outcome of both enquiries. In 2008, 11 reactions were received (5 positive, 4 negative, 2 doubtful). In 2009, 12 reactions were received (11 positive, 1 abstention). The results of the enquiries showed a need for a standard document for serotyping. It was agreed to raise an ad hoc group which will deal with the subject and will discuss at least the following:

- The status of the document: Technical Report (TR –guidance document), Technical Specification (TS) or full ISO Standard;
- The main items to be included in the document;
- To include quality assurance.

The SC9 secretariat will launch a call for experts for the ad hoc group before June 2009. Kirsten Mooijman was nominated as convenor of the group. The WHO reference centre at Institute Pasteur in Paris (France) will be asked to become a member of the ad hoc group.

ISO Working group (WG2) on statistics

This ISO working group reviews statistical aspects of (draft) ISO standards. The group is highly involved in the statistical aspects of the revision of ISO 16140 on validation of microbiological methods (Anonymous, 2003).

ISO Working group (WG3) on validation of microbiological methods

This working group is revising ISO 16140 on validation of microbiological methods (Anonymous, 2003). The work is divided over 6 Project groups (PG):

- PG1 Terminology;
- PG2 Validation of proprietary methods (present ISO 16140);
- PG3 Intermediate validation (validation with small group of laboratories);
- PG4 Method verification (e.g. for accreditation);
- PG5 In-house method validation (with/without a reference method);
- PG6 Technical requirements for establishing and/or revising standard methods;

The first drafts of PG1 and PG2 became available in April 2009.

ISO Working group (WG4) on Proficiency testing in microbiology

The working group is preparing a document which describes the organisation of Proficiency tests for microbiological laboratories. The number of this document will become ISO 22117. Presently also ISO Guide 43, part 1 and 2 (Anonymous, 1997a and 1997b) is under revision (and will become ISO 17043), which describes the general items for organising Proficiency tests.
The last meeting of the working group was in Paris in March 2009. At this meeting the comments to the Committee draft version of ISO 22117 were discussed. Before this WG meeting, much discussion and negotiation took place with ISO CASCO about the status of the microbiological document in relation with ISO 17043. ISO CASCO strongly insists that for all general items cross reference shall be made to ISO 17043 and these parts should be deleted from ISO 22117. During the WG meeting it was agreed which parts of the document should be deleted and replaced by a cross reference to the appropriate clauses of ISO 17043. Sue Passmore (convenor of WG4) will amend the document and send it around for final publication as TS before the end of July 2009. The document will also be sent to ISO/TC147/SC4 (water microbiology) so that it can be discussed at the meeting of WG 12 of SC4 in September 2009. Hopefully there will be an agreement to integrate water in the scope of the document as well.

Joint ISO working group (WG 5) on QA of media

This working group is revising ISO/TS 11133-1 (Anonymous, 2000) and ISO/TS 11133-2 (Anonymous, 2004). The document will become a joint consolidated document of both parts for quality assurance of culture media for microbiological analyses in the area of water, food, animal feed and primary production. A first draft consolidated document was sent for comments to WG5 in November 2008. Comments were received and discussed in this WG. The document will be amended and send for CD vote by July 2009.

CEN Task group (TAG 5) Primary production

The group is working on three standards:

- sampling techniques:
  - taking samples at the farm;
  - taking samples at slaughter house;
  - taking samples at hatchery;
  - transport of samples.

- preparation of samples from primary production at the laboratory;
- detection and enumeration of *Campylobacter* spp. in samples from primary production.

Draft documents have been sent around for 'new work item proposal (NWIP)' vote till April 2009.

CEN Task group (TAG 6) sampling techniques
This group will prepare a standard, divided in several parts, to describe the sampling of different foods, depending on the physiological state of the sample (liquid, powder, etc.). Parts on sampling described in ISO 6887 series (preparation of test samples) will be extracted from ISO 6887, resulting in the fact that the ISO 6887 series also need to be revised. TAG 6 will meet in conjunction with the group revising ISO 6887 series in October 2009.

CEN mandate on validation of microbiological methods
In January 2006 the EC addressed a mandate to CEN/TC275/WG6 for the validation of 15 microbiological methods. CRL-*Salmonella* has been assigned as project leader for the validation of Annex D of ISO 6579 (Anonymous, 2007): Detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage. A draft proposal was sent to CEN in 2006. For the validation study, ISO 16140 (Anonymous, 2003) need to be followed, or the new draft of this ISO document. For the validation a ring trial shall be organised. The CRL-*Salmonella* will request some NRLs-*Salmonella* to participate in such a ring trial, as they are well experienced with the method. The progress with the full proposal of the 15 project leaders is very slow, due to bureaucratic problems and inexperience of AFNOR with such a large mandate. At the meeting in Valencia a first draft consolidated proposal was presented. The project leaders were asked to comment on the draft proposal before the end of May 2009. After amendment of the proposal it will be sent to the EC. The EC will review the proposal and can ask for further amendments. It is still not sure when validations for this mandate may start.

Discussion
Q: Is it possible to receive through CRL-*Salmonella* an overview on activities/new publications in ISO?
A: Yes, we will do that whenever possible. Information will be given at the workshop and when necessary through the newsletter.
Q: Will information on non-typable *Salmonella* isolates and on controls also be taken into consideration in the document for serotyping of *Salmonella*? The new document should give additional information to the present ISO 6579.
A: Yes, the content of the document will be further discussed in the ad hoc group.

3.8 Enumeration of *Salmonella* by MPN, mini-MPN and Real-time PCR

Nadine Krämer, Federal Institute for Risk Assessment (BFR), Berlin, Germany

For quantitative microbial risk assessment, control of hazard analysis and critical control point (HACCP) concepts and modelling of *Salmonella* in the meat chain, the generation of quantitative data is required.
The traditional microbiological standard Most Probable Number (MPN) method, based on a serial dilution test and a probability calculation, is labour-intensive, time-consuming and costly. Additionally, this method is mainly useful for low numbers of *Salmonella* and/or in the presence of a relatively high background flora and provides confirmed results within 3 to 6 days.

Currently, an ISO standard for the Mini-MPN technique is elaborated, offering a miniaturized version of the standard MPN method with benefits regarding procedural handling and consumption of reagents and consequently reduction of costs, but without improvements in process time.
For both methods it is possible to replace the traditional confirmation, performed by plating on XLD agar plates, biochemical and serological testing by a qualitative detection of *Salmonella* using PCR or real-time PCR, at least making the confirmation step quite easy and fast.
Enumeration by the molecular biological based real-time PCR method needs a standard curve, but offers several advantages in terms of speed, detection limit, costs and high-throughput. As a concept of the quantitative real-time PCR method a quantification assay has been developed using slaughterhouse cork borer samples taken from pig carcasses after chilling as matrix. This quantification assay comprises a short enrichment step to ensure the desired sensitivity to detect and quantify about 1 colony forming particles (cfp) per 10 g sample. DNA is extracted by thermal cell lyses with Chile 100 and used as template in a quantitative real-time PCR using the ttr-locus which is highly specific for *Salmonella* as target.
The standard curve with corresponding 95 % confidence intervals was calculated based on data of in total 118 samples artificially contaminated with cold stressed *Salmonella* cells in seven independent experiments, always in five different concentrations and three replicates using three different *Salmonella* serovars.
As quantitative process control a lyophilized *Salmonella* Typhimurium strain with a known number of cfp was used in two dilutions.
For validation on naturally contaminated samples, 200 cork bore samples were taken over a period of seven weeks and at first tested for the presence of *Salmonella*. Altogether 26 *Salmonella* positive samples were found and further quantified by real-time PCR and Mini-MPN as reference method.
For both, the lyophilized cells and the cells on naturally contaminated samples, the values were approximately 12-fold higher, when calculated by Mini-MPN compared to the results received by real-time PCR. Most likely this was due to cell stress, but from the statistical point of view this could not be denoted as a significant difference, because the confidence intervals of both methods still showed overlapping areas. An adjustment of both methods could be achieved by slight prolongation of the enrichment time to allow convalescence of the stressed cells.

Discussion
Q: From which producer do you use the microtiter plates? For instance, the plates of Falcon have wells with too small diameters.
A: We use Greiner plates.

3.9 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 2009 and for early 2010.

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 2009;
- typing of *Salmonella* spp. (serotyping and phage typing): November/December 2009;
- detection of *Salmonella* spp. in a 'veterinary' matrix: February/March 2010.

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

- continuation of the activities for the standardisation organisations, ISO (at international level) and CEN (at European level). For more detailed information, see former presentation. Specific for *Salmonella*:
    - organisation of an ad hoc group meeting to discuss the ISO document for serotyping;
    - revision of ISO 6579: Collect data, test alternative media, test stability cultured enrichment media stored at 5 °C, literature review– Organisation of an ISO-WG9 meeting;
    - disseminate relevant information from ISO/CEN to NRLs.
- to test different matrices for ring trials;
- 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.

Workshop 2010
It was discussed whether the workshop of 2010 should be held in conjunction with the I3S (*Salmonella*) symposium, which is organised in St. Malo, France from 28 to 30 June 2010. Several NRLs indicated a preference to do so. CRL-*Salmonella* will investigate the possibilities.

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 CRL for their help in organising the workshop.

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Annex 1 Participants

European Commission	Ari Hörman
European Food Safety Authority (EFSA)	Pierre-Alexandre Beloeil
CRL – *Salmonella*	Kirsten Mooijman Angelina Kuijpers Christiaan Veenman Henny Maas Anjo Verbruggen
Guest speaker (United Kingdom)	Elizabeth de Pinna (HPA, London)
Guest speaker (Germany)	Nadine Krämer (NRL-*Salmonella*, Berlin)
Guest speaker (the Netherlands)	Petra Berk (Before CRL-*Salmonella*)
Guest speaker (France)	Anne Brisabois
Guest speaker (Ireland)	Mark Achtman

National Reference Laboratories for *Salmonella*

AUSTRIA	Christian Kornschober
BELGIUM	Hein Imberechts Katelijne Dierick
BULGARIA	Hristo Daskalov
CROATIA	Boris Habrun
CYPRUS	Constantinos Economides
CZECH REPUBLIC	Tomas Cerny
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ESTONIA	Age Kärssin
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FRANCE	Marylene Bohnert
FYROM (Macedonia Republic)	Dean Jankulovski
GERMANY	Andreas Schroeter
GREECE	Maria Passioutou-Gavala
HUNGARY	Erzsebet Andrian
ICELAND	Eggert Gunnarsson
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LATVIA	Madara Streikisa
LITHUANIA	Tatjana Kutyriova
LUXEMBOURG	Joël Mossong
MALTA	Albert Gambin
NORTHERN IRELAND	Sam Strain
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SLOVENIA	Jasna Micunovic
SPAIN	Christina de Frutos Escobar
SWEDEN	Lennart Melin
SWITZERLAND	Gudrun Overesch
UNITED KINGDOM	Robert Davies

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Annex 2 Programme of the workshop

Programme of the CRL-Salmonella workshop XIV
25 and 26 May 2009, Bilthoven

General information

Hotel and place of the workshop:
Hotel Biltsche Hoek, De Holle Bilt 1, De Bilt, The Netherlands,
tel.: +31 30 2205811
http://www.valk.com/pages/?ID=3376&propertyCode=BIL&i=0

Presentations: For the ones who will give a presentation, please send your (Power Point) presentation and the abstract of your presentation to Kirsten Mooijman (kirsten.mooijman@rivm.nl) before 19 May 2009.

Sunday 24 May 2008

Arrival of most of the representatives of the NRLs at Hotel De Biltsche Hoek.
In case you still need a dinner after arrival, you can use your dinner at the Biltsche Hoek and add the costs to the bill of your room (only in case the costs of your travel and stay are paid from the budget of CRL-Salmonella). CRL-Salmonella will take care of these expenses directly with the Hotel. Unfortunately, CRL-Salmonella can not refund bills from other restaurants.

Monday 25 May 2009

Morning chair: Kirsten Mooijman

9.00 - 9.15	Opening and introduction (Kirsten Mooijman, CRL)
9.15 - 9.45	2007 Community Summary Report on zoonoses – Overview on Salmonella (Pierre-Alexandre Beloeil, EFSA)
9.45 - 10.15	Baseline surveys on Salmonella in broiler carcasses and on Salmonella in pig holdings with breeding pigs (Pierre-Alexandre Beloeil, EFSA)
10.15 - 10.45	Developments and perspectives for future legislation concerning Salmonella (Ari Hörman, DG-Sanco)
10.45 - 11.15	*Coffee/tea*
11.15 – 11.45	Results typing study XIII - 2008: serotyping (Petra Berk, CRL)
11.45 - 12.15	Results typing study XIII - 2008 : phagetyping (Elizabeth de Pinna, HPA, UK)
12.15 – 12.30	Proposal typing study 2009 (Kirsten Mooijman, CRL)
12.30 - 13.30	*Lunch*

Afternoon chair: Yvonne van Duijnhoven

13.30 - 14.00	Results interlaboratory comparison study on bacteriological detection of Salmonella – FEED I – 2008 (Angelina Kuijpers, CRL)
14.00 - 14.30	Preliminary results interlaboratory comparison study on bacteriological detection of Salmonella – Veterinary XII – 2009 (Angelina Kuijpers, CRL)
14.30 – 15.00	Proposal on interlaboratory comparison studies on detection of Salmonella – 2009/2010 (Kirsten Mooijman, CRL)
15.00 - 15.30	*Coffee/tea*
15.30 - 15.50	Phage types and their antimicrobial resistance in German Salmonella isolates from pigs (Andreas Schroeter, Federal Inst. For Risk assessment, DE)
15.50 – 16.10	Questionnaire Survey on the availability of the molecular typing methods for food-borne pathogens (Pierre-Alexandre Beloeil, EFSA)
16.10 - 16.30	DNA multiplex evaluation for Salmonella molecular serotyping (Anne Brisabois, AFSSA, FR)
16.30 – 17.00	Multi Locus Sequence Typing (MLST) can replace serotyping of Salmonella enterica today! (Mark Achtman, University College Cork, IE)
17.30 and onwards	Social programme and dinner

Tuesday 26 May

Chair: Arjen van de Giessen

9.00 – 9.30	Salmonella control in laying farms (Robert Davies, Vet. Lab. Agency, UK)
9.30 – 10.45	Activities NRLs to fulfill tasks and duties (including problems), 15 min each Italy (Lisa Barco) Czech Republic (Tomas Cerny) France (Marylène Bohnert) Switzerland (Gudrun Overesch) Iceland (Eggert Gunnarsson)
10.45 - 11.15	*Coffee/tea*
11.15 – 11.45	ISO/CEN activities (Kirsten Mooijman, CRL)
11.45 - 12.15	Enumeration of Salmonella by MPN, mini-MPN and Realtime PCR (Nadine Kraemer, Federal Inst. For Risk assessment, DE)
12.15 – 12.45	Work programme CRL-Salmonella second half 2009, first half 2010 and closure (Kirsten Mooijman, CRL)
12.15 - 13.30	*Lunch*
13.45	Departure to train station Utrecht

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