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K.A. Mooijman

The thirteenth CRL-Salmonella workshop

26 and 27 May 2008, Bilthoven, the Netherlands

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This investigation has been performed by order and for the account of 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/330604/08/CS Community Reference Laboratory for *Salmonella*

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Abstract

The thirteenth CRL-*Salmonella* workshop

The thirteenth annual workshop for the National Reference Laboratories (NRLs) for *Salmonella* was held on 26–27 May 2008. The aim of this workshop was to facilitate the exchange of information on the activities of the NRLs and the Community Reference Laboratory (CRL) for *Salmonella*. An important item on the agenda was the presentation of the results of the annual ring trials of the CRL, which provided valuable information on the quality of the participating NRL laboratories. The NRLs of selected countries also described their activities and how they carried these out to meet their responsibilities.

This report contains the summaries of the presentations. These included the presentations of the results of the annual baseline studies for *Salmonella*, in which each participating country determines the prevalence of *Salmonella* in certain products. Last year the products under study originated from turkeys and slaughter pigs, while this year the focus is on breeder pigs and broiler carcasses. Methods for detecting and typing of *Salmonella* were also discussed, including those that are of use in identifying whether the *Salmonella* that has caused (an outbreak of) illness in humans is the same as that found in a product. The different serological methods used to detect *Salmonella* have also been tested for their usefulness, and the results were reported at the workshop. Although different methods are used in the Member States, most laboratories obtained good results in the ring trials.

The workshop was organised by the CRL for *Salmonella*, which is situated at the RIVM. The main task of the CRL-*Salmonella* is to check the performance of the European National Reference Laboratories for the detection and typing of *Salmonella* in different products. The workshop was organised in Bilthoven, the Netherlands.

Key words:
CRL-*Salmonella*, NRL-*Salmonella*, *Salmonella*, workshop 2008

Rapport in het kort

De dertiende CRL-*Salmonella* workshop

Op 26 en 27 mei 2008 vond voor de dertiende keer de jaarlijkse workshop plaats 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 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.

Dit rapport bevat verslagen van de gehouden presentaties. Veel aandacht ging uit naar de instrumenten om *Salmonella* aan te tonen. Onder andere kwamen de jaarlijkse 'baseline' studies voor *Salmonella* aan de orde, waarin per deelnemend land wordt vastgesteld hoeveel *Salmonella* een bepaald product bevat. Vorig jaar betrof dit kalkoenen en slachtvarkens, dit jaar gaat het om fokvarkens en karkassen van kuikens. Verder zijn meedere methoden besproken die *Salmonella* aantonen en typeren. Bijvoorbeeld hoe vastgesteld kan worden of de *Salmonella* waarvan mensen ziek zijn geworden dezelfde is als die in een product is aangetroffen. Ook zijn verschillende serologische methoden om *Salmonella* op te sporen, die antistoffen in bloed meten, getoetst op hun werkzaamheid. Hoewel landen hiervoor verschillende methoden gebruiken, hebben de meeste goede resultaten behaald bij het ringonderzoek.

De organisatie van deze workshop is in handen van het CRL voor *Salmonella*, die op het RIVM 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 2008

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List of abbreviations

A	Answer
AOAC	Association of Analytical Communities
BPW	Buffered Peptone Water
CD	Committee Draft
CEN	European Committee for Standardisation
cfp	colony forming particle
CRL	Community Reference Laboratory
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
ELISA	Enzyme-linked immunosorbent assay
ENL	Enter-Net Laboratory
EU	European Union
FBO	Food Business Operators
FYROM	Former Yugoslav Republic of Macedonia
GD	Animal Health Service
HPA	Health Protection Agency
ISO	International Standardisation Organisation
LZO	Laboratory for Zoonoses and Environmental Microbiology
MKTTn	Mueller Kauffmann Tetrathionate broth with novobiocin
MLVA	Multiple-locus variable number tandem repeat analysis
MS	Mass Spectrometry
MSRV	Modified Semi-solid Rappaport Vassiliadis
NMKL	Nordic Committee on Food Analysis (Nordisk metodikkommitté för Livsmedel)
NRL	National Reference Laboratory
OD	Optical Density
PFGE	Pulsed Field Gel Electrophoresis
Q	Question
QA	Quality Assurance
RIVM	National Institute for Public Health and the Environment
ROC	Receiver Operating Characteristic
RVS	Rappaport Vassiliadis broth with Soya
SC	Sub Committee
SE(20)	*Salmonella* Enteritidis (at a level of approximately 20 cfu/capsule)
SPF	Specific Pathogens Free
STM(5)	*Salmonella* Typhimurium (at a level of approximately 5 cfu/capsule)
TC	Technical Committee
TS	Technical Specification
UK	United Kingdom
USA	United States of America
VTEC	Verotoxigenic *Escherichia coli*
WG	Working Group
WHO	World Health Organisation
XLD	Xylose lysine deoxycholate

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Summary

On 26 and 27 May 2007 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 46 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 and on the baseline studies and control programmes at primary production (past, present and future). Furthermore a presentation was given on the comparability of different serological (ELISA) methods on the detection of *Salmonella* spp. antibodies in meat juice and serum.
During the afternoon session of the first day, the results of the interlaboratory comparison studies on detection of *Salmonella* in a food matrix (2007) and in a veterinary matrix (2008) were discussed. Also proposals for future interlaboratory comparison were discussed. The day was closed with presentations of four NRLs, dealing with: monitoring methods for *Salmonella* in turkey flocks, epidemiology and biology of *Salmonella* Paratyphi B var. Java, phage typing of *Salmonella* Paratyphi B var. Java and molecular typing by using Multiple-locus variable number tandem repeat analysis (MLVA).

On the second (half) day of the workshop, an introduction was given on the task and duties of CRLs and NRLs, followed by presentations of NRLs from five different countries, explaining their activities to fulfil these task and duties. Furthermore information was given on the standardization of methods at International (ISO) and European (CEN) level. 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/workshopXIII.jsp.

1. Introduction

In this report the abstracts of the presentations given at the CRL-*Salmonella* workshop of 2008 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/workshopXIII.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 26 May 2008: 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 thirteenth workshop of CRL-*Salmonella* welcoming all participants in Bilthoven, the Netherlands.
After a roll call of the delegates, information was given on the changes at the CRL and other new aspects:

- The laboratory in which the CRL-*Salmonella* is situated (Laboratory for Zoonoses and Environmental Microbiology: LZO) has a new head: Yvonne van Duijnhoven;
- The new contact person for the CRL-*Salmonella* at DG-Sanco is Ari Hörman;
- The CRL-*Salmonella* website is amended and has replaced the former website in May 2007. The address remained the same (www.rivm.nl/crlsalmonella);
- On 14 May 2008 the CRL-*Salmonella* received a new issue for the antigenic formulae of the *Salmonella* serovars. This new version of the 'White-Kauffmann-Le Minor scheme' was prepared by the WHO Collaborating Centre for Reference and Research on *Salmonella*, Institute Pasteur, Paris, France. Copies of the new scheme were distributed to the participants. According to the French representative, the scheme should also be available at the website of the WHO collaborating centre. The exact path will be provided by the French delegation and forwarded to the NRLs by the CRL (http://www.pasteur.fr/sante/clre/cadrecnr/salmoms/WKLM_2007.pdf).

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 2006 Community trends and sources report on zoonoses

Frank Boelaert, 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 2006, twenty-four countries submitted information to the European Commission and EFSA. Further information on zoonotic cases in humans was acquired from the European Centre for Disease Prevention and Control (ECDC). Assisted by its Zoonoses Collaboration Centre, EFSA and ECDC jointly analysed the information and published the results in the 2006 Community Summary Report. Data collection and analysis helps identifying sources and trends of zoonoses and zoonotic agents and provides the bases to develop evidence-informed health policy. These analyses help in identifying appropriate priority-setting goals, such as reduction targets, and provide the means to evaluate the adequate development of control programmes in the Community.

In 2006, the most frequently reported zoonotic diseases in humans in the European Union were campylobacteriosis and salmonellosis with 175 561 and 160 649 cases respectively. Yersiniosis and VTEC infections accounted for 8979 and 4916 human cases respectively, listeriosis with a total of 1583 human cases is an important zoonosis due to the severity of the disease, and 14.2 % of the reported mortality is foodborne related. Echinococcosis and trichinellosis caused 458 and 231 human cases each (EFSA, 2007). Animals play an essential role in maintaining these human infections of European public health importance. Therefore, surveillance of zoonotic infectious agents in food and animal populations is pivotal for the prevention of human infection and to asses the efficacy of current control measures. This presentation describes the main findings of the 2006 report concerning prevalences of the ten of the most important zoonotic agents (excluding transmissible spongiform encephalopathies) in animal populations and food listed in decreasing number of human cases.

Discussion
Q: Will the report be available on the EFSA website?
A: Yes, it is possible to download the report from www.efsa.europa.eu

2.3 EU-wide Baseline surveys on the *Salmonella* prevalence in turkey flocks and in slaughter pigs

Frank Boelaert, EFSA, Parma, Italy

EU-wide Baseline Surveys on the *Salmonella* prevalence in turkey flocks

Salmonella is an important cause of food-borne illnesses in humans. Farm animals and food of animal origin form an important source of human *Salmonella* infections. Therefore, in order to reduce the incidence of human salmonellosis in the European Union, the Community legislation foresees setting of *Salmonella* reduction targets for food-animal populations including turkey flocks. To underpin such a target, a European Union-wide baseline survey was carried out to determine the prevalence of *Salmonella* in commercial turkey holdings with at least 250 birds for breeding turkeys and with at least 500 birds for fattening turkeys. The survey was the third of several baseline surveys to be conducted in the Community.

The sampling of turkey flocks took place between October 2006 and September 2007. Five environmental faeces samples were taken from breeding turkey flocks within nine weeks of slaughter and from fattening turkey flocks within three weeks of slaughter. A total of 539 breeding turkey flocks and 3769 fattening turkey flocks with validated results, from the EU and Norway, were included in the survey analyses.

In each Member State, the number of reported holdings was combined with the number of birds annually reared in each holding (as evaluated from this survey) to estimate turkey population size. The geographical distribution of breeding turkeys in the European Union was highly heterogeneous. In fact, France accounted for 56.0 % of the breeding population, followed by Italy (11.9 %) and The United Kingdom (10.1 %). None of the remaining Member States reached 5 % of the total breeding population. The distribution of fattening turkeys was less heterogeneous. Still, five Member States accounted for 79.3 % of the fattening bird population, namely, France (18.7 %), Germany (16.4 %), Italy (16.0 %), Spain (14.7 %), and Poland (13.5 %).

Six of the 14 Member States isolated *Salmonella* spp. in their breeding flocks, which resulted in a Community observed prevalence of *Salmonella*-positive breeding flocks of 13.6 %. This means that in the European Union around one in seven breeding turkey flocks raised over the one year period of the baseline survey was *Salmonella*-positive. The *Salmonella* prevalence in these flocks varied widely amongst the Member States, from 0 % to 82.9 %. Three of those six Member States isolated *Salmonella* Enteritidis and/or *Salmonella* Typhimurium, the two most common serovars found in *Salmonella* infection cases in humans. This resulted in an estimated Community observed prevalence of 1.7 % for these two serovars, varying from 0 % to 8.3 % within the Member States.

The Community observed prevalence of *Salmonella*-positive fattening flocks was 30.7 %, meaning that approximately one in three fattening turkey flocks raised over the one year period of the baseline survey were *Salmonella*-positive. The *Salmonella* prevalence in these flocks also varied widely amongst the Member States, from 0 % to 78.5 %. Thirteen of the 22 Member States with fattening turkey flocks reported to have isolated *S.* Enteritidis and/or *S.* Typhimurium resulting in a Community observed prevalence of 3.8 % in the fattening turkey flocks. The Member State-specific observed flock prevalence of *S.* Enteritidis and/or *S.* Typhimurium varied from 0 % to 18.4 % in fattening turkeys.

In breeding flocks no single *Salmonella* serovar was isolated in more than three of the 14 reporting Member States. The five most frequently isolated *Salmonella* serovars from fattening turkey flocks in the European Union, in decreasing order, were: *S.* Bredeney, *S.* Hadar, *S.* Derby, *S.* Saintpaul and *S.* Kottbus. Out of these, only *S.* Hadar and *S.* Derby are frequent causes of *Salmonella* infections in humans within the European Union. The serovar distribution varied amongst the Member States, with serovars tending towards specific distribution patterns of their own.

Salmonella positive turkey flocks contribute to a consequent contamination of turkey meat. The risk for human health arises from accidental under-cooking of the meat or cross-contamination to other foods. Thorough cooking and strict kitchen hygiene will prevent or reduce the risk posed by *Salmonella* contaminated turkey meat.

While Community reduction target will most likely be set for a transitional period only for *S.* Enteritidis and *S.* Typhimurium, Member States may wish to consider addressing in their national *Salmonella* control programmes also other serovars when these serovars are of public health importance in their country.

EU-wide Baseline Surveys on the *Salmonella* prevalence in slaughter pigs

In order to reduce the incidence of human salmonellosis in the European Union, Community legislation foresees the setting of *Salmonella* reduction targets for food-animal populations including slaughter pigs. To underpin such a target, a European Union-wide baseline survey was carried out to determine, at the point of slaughter, the prevalence of pigs infected with *Salmonella*. The pigs were randomly selected from those slaughterhouses that together accounted for 80 % of pigs slaughtered within each Member State. This slaughterhouse survey was the fourth baseline survey to be conducted in the Community.

The sampling of slaughter pigs took place between October 2006 and September 2007. All participating Member States and Norway sampled ileocæcal lymph nodes from the selected slaughtered pigs. In total 18 751 slaughter pigs were sampled and 18 663 valid lymph node samples were collected.

Twenty-four of the 25 participating Member States isolated *Salmonella* spp. from the lymph node samples, which resulted in a Community observed prevalence of *Salmonella*-positive slaughter pigs of 10.3 %. This means that in the European Union at the point of slaughter around one in ten slaughter pigs were estimated to be infected with *Salmonella* in the lymph nodes. The *Salmonella* prevalence in these slaughter pigs varied widely amongst the Member States, from 0.0 % to 29.0 %. All 24 Member States reporting *Salmonella* positive findings isolated *Salmonella* Typhimurium and 20 Member States detected *Salmonella* Derby, which are two common serovars found in *Salmonella* infection cases in humans. This resulted in an estimated Community observed prevalence of 4.7 % for *Salmonella* Typhimurium, varying from 0.0 % to 16.1 % within the Member States, and of 2.1 % for *Salmonella* Derby, varying from 0.0 % to 6.5 %.

From the pigs that had already been selected for sampling of lymph nodes, 10 Member States additionally collected either meat juice or blood samples with the aim of investigating prevalence of slaughter pigs with antibodies against *Salmonella*, indicating past exposure of the pig to *Salmonella*. These Member States used different laboratory antibody detection test kits and a comparison study done by the Community Reference Laboratory for *Salmonella* showed that the results of these different test methods were not comparable between the Member States. Therefore no prevalence slaughter pigs with antibodies against *Salmonella* could be estimated overall for this group of Member States. At the Member States' level the prevalence of slaughter pigs with antibodies against *Salmonella* ranged from 3.5 % to 42.7 %.

Moreover 13 Member States additionally sampled the pigs' carcasses by swabbing in order to appreciate the external contamination of the carcasses. Data from this group of Member States showed that the observed prevalence of carcasses contaminated with *Salmonella* spp. was 8.3 % overall, meaning that around one in ten carcasses were contaminated with *Salmonella* for this group of Member States. At the Member States' level, the prevalence of contaminated carcasses ranged from 0.0 % to 20.0 %.

The diversity of isolated *Salmonella* serovars in slaughter pig lymph nodes was big and in total 94 different serovars were isolated in the European Union. The five most frequently isolated *Salmonella* serovars from lymph nodes in the European Union were respectively in decreasing order *S*. Typhimurium, *S.* Derby, *S.* Rissen, *S*. Enteritidis and *S.* 4,[5],12:i:-. All these serovars, with the exception of *S*. Rissen, are frequent causes of *Salmonella* infections in humans within the European Union. *Salmonella* Typhimurium and *Salmonella* Derby serovars were highly predominant in lymph nodes; *S*. Typhimurium being the most common serovar, detected in 40.0 % of the *Salmonella*-positive slaughter pigs and reported by all 24 *Salmonella*-positive Member States. *S.* Derby accounted also for an important proportion of positive lymph nodes (14.6 %) and was reported by 20 *Salmonella*-positive Member States.

Together, 32 different serovars were reported from the surface of the slaughter pig carcasses by the 13 Member States that carried out the test. The five most frequently isolated serovars from carcasses were respectively in decreasing order *S.* Typhimurium, *S*. Derby, *S*. Infantis, *S*. Bredeney and *S.* Brandenburg. The former three serovars are frequent causes of *Salmonella* infections in humans within the European Union. *S*. Typhimurium was the most common serovar isolated on the surface of the slaughter pigs' carcasses and detected in 49.4 % of the *Salmonella* positive carcasses. The second most common serovar was *S.* Derby (24.3 % of the positive carcasses). *S.* Typhimurium and *S*. Derby were also the most commonly reported ones in terms of the number of Member States, in total 10 of the 13 participating Member States.

Salmonella infection in slaughter pigs has the potential to translate into *Salmonella* contamination of pig meats and lead to human disease. Intervention to reduce the prevalence of infection in pigs may reduce the number of human salmonellosis cases. Safe handing of raw meat and thorough cooking are important measures to minimise human health risks from *Salmonella* contaminated pig meat.

The results of this baseline survey are suitable to be used for setting of targets for reduction of *Salmonella* in pigs. The Community legislation foresees setting of target for slaughter pigs regarding all *Salmonella* serovars with public health significance supported by a cost benefit analysis.

Discussion
Q: How did different tests correlate (serology and bacteriology)?
A: This has not yet been analysed and will be done in part B of the report.
Q: Does a relation exist between the Dutch scheme for phage typing and the scheme of the United Kingdom?
A: No, there is no uniform relationship. In some cases the Dutch scheme is more detailed (several Dutch phage types for one UK phage type), but it can also be the other way around.
Q: When the time between sampling and analysis is > 7 days, the results of the relevant samples are excluded from the dataset. Is this time limit of 7 days arbitrarily chosen?
A: This time limit was discussed in the EFSA working group. The number of data on survival of *Salmonella* in samples is only limited. The limit of 7 days was set in consensus with the EU Member States.
Q: What would happen in the time between sampling and analysis?
A: It was shown in the laying hen study that the change of detecting *Salmonella* decreased 3 days after sampling. The effect is influenced by the temperature during transport and storage. *Salmonella* may die during transport and storage and/or the presence of *Salmonella* in the sample may be masked by the presence of high amounts of background flora (which may have grown during storage). Samples containing high amounts of *Salmonella* (highest risk for human health) would in general still be found positive, but the low contaminated samples might become negative for *Salmonella* when tested several days after sampling. The effect may be serovar dependent, some *Salmonella* serovars can survive better than others.

2.4 Ongoing and possible future baseline studies on *Salmonella*, and control programmes at primary production

Kris de Smet, European Commission, DG-Sanco, Brussels, Belgium

Baseline surveys
In 2008, baseline studies on *Salmonella* in herds of breeding pigs and on broiler carcasses are ongoing in all Member States, Norway and Switzerland, Discussions are ongoing on a possible new baseline survey in 2009 as regards *Salmonella* in broiler meat at retail level. All baseline surveys are based on technical specifications provided by the EFSA which will also analyse and report the results. The ongoing baseline surveys are co-financed by the European Commission.

2008 Baseline survey in breeding pigs.
In accordance with European Decision 2008/55/EC, Member States are sampling up to 170 production holdings and up to 170 elite and multiplier holdings of breeding pigs. 10 pooled faecal samples are taken from each holding together with one dust sample for analysis on methicillin resistant

Staphylococcus aureus (MRSA).
In a number of Member States 100 individual samples are additionally collected on 10 holdings for the estimation of the within holding prevalence of *Salmonella*.
Information is collected on the holding in order to assess possible *Salmonella* risk factors. Analysis involves detection and serotyping, while phage typing and antimicrobial susceptibility testing is optional. Isolates must be stored for at least 2 years and 16 typable and 16 non-typable isolates must be sent to the CRL for quality assurance.
The results will be published in 2009. They will be used for a quantitative risk assessment on *Salmonella* in pigs by the EFSA, following by a cost/benefit analysis of measures to reduce *Salmonella*. The prevalence will also provide reference values for setting a target for reduction.

2008 Baseline survey in broiler carcasses
One carcass is collected from 384 randomly selected slaughter batches in all Member States during the 12 months of 2008 in accordance with European Decision 2007/516/EC. Neck skin samples are collected for analysis on *Salmonella* and *Campylobacter*. *Salmonella* analysis involves detection and serotyping while phage typing and antimicrobial susceptibility testing are optional. Isolates must be stored for at least 2 years and 16 non-typable isolates must be sent to the CRL for quality assurance.

Possible baseline survey for Salmonella in broiler meat at retail in 2009
The EFSA has published in 2006 technical specifications for a survey of fresh broiler meat and meat preparations at retail on *Salmonella* and *Campylobacter*. The proposed sampling size is 384, however discussions are still ongoing with experts of EFSA to evaluate if such survey could result in an assessment of the implementation of the existing *Salmonella* food safety criterion in meat preparations and provide guidance on the impact of a future criterion on poultry meat. A decision will be taken in September.

State of play of *Salmonella* control programmes in animals
Between 2007 and 2013, *Salmonella* control programmes are gradually introduced in all Member States in breeding hens, laying hens, broilers turkeys, slaughter pigs and breeding pigs. By the application of these control programmes, Member States should achieve the target for reduction of *Salmonella* already set for the poultry populations and to be set for pigs.
Within each control programme, harmonised minimum requirements for monitoring by food business operators and competent authorities apply in order to allow an evaluation of the progress towards the target for reduction. The control programmes, including the monitoring are mandatory in breeding hens and laying hens respectively since 1 January 2007 and 1 February 2008. They will become mandatory in broilers at the latest from 1 January 2009 on and in turkeys from 1 January 2010 on. Minimum requirements for the monitoring in all animal populations include similar provisions on the storage and transport of samples, methods to be used for detection and serotyping, and storage of isolates.

Breeding hens
Food business operators (FBO) must sample flocks 3 times during rearing and every 2 weeks in the hatchery or at the holding during production. Official sampling is required 3 times during production at the holding or every 16 weeks in the hatchery.
Details on sampling schemes and sampling procedures in hatcheries or at farms are laid down in European Regulation (EC) No 1003/2005. A revision is under discussion.

Laying hens
Since the beginning of this year, FBO must sample twice during rearing and every 15 weeks during laying. The minimum requirements for official samples and all sampling procedures are laid down in European Regulation (EC) No 1168/2006.

Broilers
At the latest from 1 January 2009 on, FBO must sample in principle all flocks within 3 weeks before birds move to the slaughterhouse.
A flock must be sampled by the competent authority on 10 % of all holdings with > 5000 birds each year. Two pairs of moistened boot swabs or socks, or one pair of boot swabs/socks + one dust sample must be collected. Details are in European Regulation (EC) No 646/2007. An amendment to this regulation will soon be published.

Turkeys
A target for the reduction of *Salmonella* in flocks of breeding and slaughter turkeys has been agreed on and will be published before 1 July 2008. Consequently, all Member States should have a control programme, including harmonised monitoring, at the latest from 1 January 2010 on.
The sampling requirements for breeding turkeys are similar to breeding hens and for slaughter turkeys similar to broilers. The result is only valid until 6 weeks after sampling.
The details and sampling requirements for the official controls (about 10% of all flocks per year) will be published before 1 July 2008 (European Regulation (EC) No 584/2008).

Pigs
EFSA has been requested to quantitatively assess the risk factors and mitigation options for *Salmonella* in breeding and slaughter pigs production.
This information will be used to carry out a cost/benefit analysis of potential targets for reduction. It is expected that control programmes, including harmonised monitoring, will apply in all Member States at the latest from 2011-2012 on.

Discussion
Q: Is there any discussion on baseline studies in cattle?
A: This was not yet discussed. In the discussion on what should be performed in baseline studies, *Salmonella* in cattle was not given high priority. A higher priority was given to *Listeria* at retail level and VTEC in cattle. It is not possible to organise baseline studies for all subjects. For some subjects it may be discussed whether a baseline study should be organised or whether priority should be given to harmonisation of (the sampling for) monitoring.
Q: In many of the baseline studies information is asked on the use of antimicrobial agents. Is there more information available on how often these agents are used in the Member States?
A: No, at the moment information of only 5 Member States is available. However, the use of antibiotics at farms is a very important subject and discussion is going on on the item.
Q: What happens if a Member State does not meet the aim of a control programme?
A: I do not believe in target setting on its own. All parties involved (Member State, farmer, industry) have to be aware to take the necessary restrictions accordingly to the target set. If a Member State does not meet a target it may be possible to set trade restrictions for this country.

2.5 Comparability of different ELISA's on the detection of *Salmonella* spp. antibodies in meat juice and serum

Petra Berk, CRL-Salmonella, Bilthoven, the Netherlands

From 1 October 2006 – 1 October 2007 a Community-wide baseline survey on the prevalence of *Salmonella* in slaughter pigs was carried out in the Member States' (2006/668/EC). New in this study,

when compared to former baseline studies, was the possibility to use a serological method in addition to the bacteriological method for the detection of *Salmonella* spp. antibodies in pigs sampled in the slaughterhouse. Ten NRLs-*Salmonella* agreed to perform serology based on meat juice samples, while one NRL used blood samples in addition. As no standard method existed for serology, the NRLs were allowed to use their own methods. In order to enable comparison of serological results, CRL-*Salmonella* organised both a duplicate analysis study based on field samples as well as an interlaboratory comparison study using reference sera.

For the duplicate analyses study the participating NRLs had to send a selection of 60 meat juice samples from the baseline study to the CRL-*Salmonella* where these samples were tested with one 'reference method', the HerdCheck Swine *Salmonella* ELISA from IDEXX.
Four NRLs used the Salmotype PigScreen ELISA (Labor Diagnostik Leipzig), three NRLs used the HerdCheck Swine *Salmonella* ELISA (IDEXX), one laboratory used the VetSign Porcine *Salmonella* ELISA (Guildhay) and two NRLs used an in-house ELISA. Different cut-off values were used by different NRLs. The NRLs which used the Salmotype Pigscreen ELISA all used the same cut-off values (- = OD % <10, ± = OD % >10 and <20, + = OD % >20). The NRL which used the VetSign Porcine *Salmonella* ELISA used cut-off values based on the S/P ratio (- = S/P ratio <0.10, ± = S/P ratio >0.10 and <0.25, + = S/P ratio >0.25). The NRLs which used the HerdCheck Swine ELISA all used different cut-off values (OD % >10, OD % >15 and OD % >20) and expressed their results only in – or +. The two NRLs that used an in-house ELISA also expressed their results as – or +, both used different cut-off values (OD % >20 and OD % >40).

Comparing the results from the CRL with those of the NRLs using a dependent t-test and a more complicated bivariate mixture fitting, statistically differences were found for most of the comparisons. For 5 NRLs the average OD % was statistically higher than that of the CRL, for 2 NRLs the average OD % was statistically lower, for 1 NRL the average S/P ratio was statistically lower than that of the CRL and for 2 NRLs no statistical differences were found. Four of the 5 NRLs which found higher OD % than the CRL used the same ELISA (Salmotype PigScreen), the other NRL used an in-house ELISA. The 2 NRLs which found lower OD % than the CRL used the HerdCheck Swine *Salmonella* ELISA and the NRL which found lower S/P ratios than the CRL used the VetSign Porcine *Salmonella* ELISA. The two NRLs which found no statistical different OD percentages from the CRL, used the HerdCheck Swine ELISA and the in-house ELISA. From the results of this study it was concluded that, it is difficult to compare the serological results of meat juice samples from different NRLs and different ELISA's. Even when the same ELISA (HerdCheck Swine *Salmonella*) was used by different laboratories, statistical differences were found.

At the end of the baseline study (September 2007) an interlaboratory comparison study on serological methods was organised by the CRL-*Salmonella*. In this study the same NRLs-*Salmonella* have participated as the ones participating in the method comparison study. The NRLs received a set of 'standard' sera to test with their own method. A total number of 40 sera had to be tested. Two sera were obtained from *Salmonella*-free pigs and two samples were obtained after inoculation of pigs with *Yersinia enterocolitica* O3-/O9-, which can possibly result in cross-reaction. All other 32 samples were obtained after experimental inoculation of pigs with different *Salmonella* strains (*S*. Typhimurium, *S*. Brandenburg, *S*. Panama, *S*. Goldcoast and *S*. Livingstone). Each NRL was asked to interpret their results by using the cut-off value that was routinely used in the baseline study.
A quantitative comparison of *Salmonella*-ELISA's was performed by computing Receiver Operating Characteristic (ROC) plots. The area below this curve is proportional to the diagnostic accuracy of a test. For all NRLs the ROC-area was very high, which indicates that all tests are able to detect the true status of the samples, although at different cut-off values.

The results from the 3 NRLs using the HerdCheck from IDEXX are comparable between the laboratories. On average labcode 8 showed the lowest OD % values and labcode 4 the highest, however this difference was mainly found for sera yielding high OD % values. Three of the 4 NRLs using the Salmotype ELISA showed comparable results for the different sera. However, the OD % values for labcode 10 were higher than for the other 3 NRLs (labcode 1, 2 and 6) for almost all sera. The results for the other 3 NRLs were almost identical, indicating a high interlaboratory reproducibility, especially in the more relevant low OD % range.

Discussion
The following items were discussed:

Used cut-off values:
Depending on the use of the ELISA method different cut-off values can be used. For categorising a herd, usually a cut-off value of 40 % is used. However, for other situations it may be better to use a lower cut-off value. The differences in results found in the present studies could partly be explained by the use of different cut-off values.

Choice of meat juice as matrix for analyses:
Meat juice is cheaper than serum, making it possible to analyse more meat juice samples.
The NRL of Denmark has good experiences with the stability of meat juice samples. They use frozen meat juice samples for day-to day quality control. Some meat juice samples are stored at -20 °C for already more than 10 years and still show similar results over a long period of time. This NRL also has sent many meat juice samples to different laboratories and did not notice any problems with the stability of the samples.

General
The NRL of Sweden remarked that they had tried different ELISA's and had to reject one batch of an ELISA test, because it gave only positive results. The NRL will provide the CRL with information on the supplier and batch number of this ELISA test.
No correlation was found between serology and bacteriology in the tested duplicate samples of the baseline study. In case of a recent contamination with *Salmonella* the bacteriology may give a positive result for the presence of *Salmonella*, but the serology may still be negative. However, in case of a contamination further back in the past, the serology may still give a positive result, while the bacteriology is negative. It was remarked that it need to be kept in mind that serological methods were developed to test the seroprevalence of *Salmonella* in herds. It is a fast method which makes it is possible to take measures to decrease *Salmonella* in high prevalence herds. The method is less optimal for use in herds with a low *Salmonella* prevalence. In these cases bacteriology can be used for 'fine-tuning'.
It was remarked that the serological tests are optimal for finding *Salmonella* Typhimurium. In theory the test can also detect other *Salmonella* serovars, but in practice this is more complicated.

2.6 Results interlaboratory comparison study FOOD II (2007) on bacteriological detection of *Salmonella* in minced beef

Angelina Kuijpers, CRL-Salmonella, Bilthoven, the Netherlands

The European National Reference Laboratories (NRLs) for *Salmonella* were able to detect high and low levels of *Salmonella* in minced beef. This was shown in the second interlaboratory comparison study on food, in which 30 laboratories participated.

The first and most important objective of the study, organized by the Community Reference Laboratory (CRL) for *Salmonella* in November 2007, was to see if the participating laboratories could detect *Salmonella* at different contamination levels in a food matrix. For a better testing of the performance of the laboratories the contamination levels in this study were lower than in earlier studies. The second objective was to compare the different methods for the detection of *Salmonella* in minced beef.

Each laboratory received a package containing minced beef and 35 gelatine capsules containing *Salmonella* spp. at different levels. The instructions to the laboratories were to spike the minced beef with the capsules and test these samples for the presence of *Salmonella*. The laboratories used three selective enrichment media for running this test: Rappaport Vassiliadis Soya broth (RVS), Mueller Kauffmann Tetrathionate novobiocin broth (MKTTn) and Modified Semi-solid Rappaport Vassiliadis (MSRV) agar. The first two media are internationally prescribed for the detection of *Salmonella* in food (Anonymous, 2002), while the third (MSRV) is prescribed for the detection of *Salmonella* in veterinary samples (Anonymous, 2007).

The laboratories found *Salmonella* in only 69 % of the samples using one of the food methods (MKTTn). The method for the veterinary samples (MSRV) gave the best results with 86 % positives, closely followed by the other food method (RVS) with in total 84 % positive samples. The MKTTn food method seems to be less optimal for detection of *Salmonella* spp. in minced beef.

Twenty four NRLs fulfilled the criteria of good performance, one NRL after they performed the study 3 months later. Six laboratories had difficulties with detecting *Salmonella* Enteritidis, especially at a low level with and without the addition of meat samples, and with the ‘blank meat ‘samples. The CRL-*Salmonella* contacted these laboratories to request an explanation for the deviating results: the CRL also offered the possibility of performing extra analyses. Five of these laboratories achieved a good performance in the follow-up study in February 2008. One laboratory still had deviating results with all the blank meat samples and the CRL is in contact with this laboratory to check whether their actions to prevent cross contamination are successful.

Discussion

Q: The medium MKTTn was introduced in ISO 6579 for the detection of *S.* Typhi and *S.* Paratyphi?
A. Not sure whether this medium is optimal for the detection of these *Salmonella* serovars. The medium does not seem to be very optimal.
Q: Will RVS be replaced by MSRV in the future version of ISO 6579?
A: This is presently discussed in ISO.
Q: Some samples have been at 15 °C during transport. Has this influenced the results?
A: No effect was found. These samples have been at 15 °C for only 1-2 h, which is too short to see an effect.

2.7 Results interlaboratory comparison study Veterinary XI (2008) on bacteriological detection of *Salmonella* in chicken faeces

Angelina Kuijpers, CRL-Salmonella, Bilthoven, the Netherlands

The European National Reference Laboratories (NRLs) for *Salmonella* were able to detect high and low levels of *Salmonella* in chicken faeces. This was shown in the 11th interlaboratory comparison study on veterinary samples, in which 32 laboratories participated (28 NRLs from 27 EU members, one NRL from a European Economic Area, one EU candidate member and 2 NRLs from third countries (non-EU)).

The most important objective of the study, organized by the Community Reference Laboratory (CRL) for *Salmonella* in March 2008, was to see if the participating laboratories could detect *Salmonella* at different contamination levels in a veterinary matrix. For a better testing of the performance of the laboratories the contamination levels in this study were lower than in earlier veterinary studies.

Each laboratory received a package containing chicken faeces and 35 gelatine capsules containing *Salmonella* spp. at different levels. The instructions to the laboratories were to spike the faeces with the capsules and test these samples for the presence of *Salmonella*. The laboratories used one prescribed method for running this test, namely, a selective culture step on Modified Semi-solid Rappaport Vassiliadis (MSRV).
The laboratories found *Salmonella* in 87 % of the samples using MSRV. Twenty-eight NRLs fulfilled the criteria of good performance. Two NRLs showed a moderate performance but a follow up study was not considered necessary. Two other NRLs (one EU member) showed a poor performance and the CRL *Salmonella* is in contact with both NRLs to discuss a follow up.

2.8 CRL-*Salmonella* interlaboratory comparison studies 2008 and 2009

Kirsten Mooijman, CRL-Salmonella, Bilthoven, the Netherlands

The following interlaboratory comparison studies are planned for the coming year:
- September/October 2008: Detection of *Salmonella* spp. In a 'food' matrix;
- November/December 2008: Typing of *Salmonella* spp.;
- February/March 2009: Detection of *Salmonella* spp. In a 'veterinary' matrix

For the **food** study the following was discussed:
DG-Sanco would prefer to have a study organised with animal feed as the research matrix. Several NRLs indicated to consider a ring trial with animal feed to be very useful. It was discussed what kind of feed should become the matrix of choice. It was indicated that feed ingredients (e.g. coconut, soya, rape seed, fish meal) would probably more of interest than complete animal feed. Also environmental samples (e.g. dust) from places where feed is prepared may be of interest. However, this latter sample would probably not be easy to collect in large amounts. The choice of the *Salmonella* serovars was also discussed. *Salmonella* Enteritidis and *Salmonella* Typhimurium are not often the main problems in feed, although it may happen that feed becomes (cross) contaminated when stored at the farm.
It was mentioned that EFSA will publish probably in July an opinion on animal feed. The CRL-*Salmonella* will inform at EFSA whether it is possible to get some information before the publication.

The NRL from Slovenia indicated to have organised several ring trials with (complete) animal feed as matrix, which was artificially contaminated with a low level concentration of *Salmonella* Typhimurium and with *Salmonella* Enteritidis. The studies were successful, but it was indicated that it was important to use low contamination levels, else *Salmonella* would be too easy to detect.
The following was agreed:

- CRL-*Salmonella* will investigate what matrix would be feasible for use in a ring trial in September/October;
- CRL-*Salmonella* will inform at EFSA to the draft opinion on animal feed;
- CRL-*Salmonella* will test one or more selected matrices by artificially contaminating it with (low level) *Salmonella* reference materials;
- The NRLs will indicate which laboratory in their country performs the feed analyses;
- The method of choice for analysing animal feed and its ingredients is ISO 6579 (Anonymous, 2002), with Rappaport-Vassiliadis medium with Soya (RVS) and Muller-Kauffmann tetrathionate-novobiocin broth (MKTTn) as selective enrichment media. However, it was considered worthwhile to also test animal feed with the procedure as described in Amendment 1 of ISO 6579 (Anonymous, 2007) with Modified semi-solid Rappaport-Vassiliadis medium (MSRV) as selective enrichment medium.

For the **veterinary** study it was discussed what type of sample would be preferred. Sheep faeces were considered, although this does not seem to be a problem in many countries. For cattle faeces *Salmonella* Dublin would be the serovar of interest. Also egg shells were indicated as a possible matrix. In conclusion no special preference was given for a certain matrix. The CRL-*Salmonella* will therefore investigate which matrix is feasible to be used in large amounts for the ring trial.

For the **typing** study the same set-up as for the earlier studies will be used:
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.

*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.

For the **serotyping**:
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

2.9 Evaluation and optimisation of monitoring methods for *Salmonella* in turkey flocks

Robert Davies, NRL-Salmonella Addlestone,United Kingdom

Recent EU legislations have introduced baseline surveys and harmonised monitoring plans for chicken and turkey flocks. Chicken breeding flocks are monitored by sampling each flock with five pairs of boot swabs every two weeks (European Regulation 1003/2005) and at the time the current study was proposed it was thought likely that monitoring for turkey breeding flocks would be similarly intensive and costly. There is also concern that pooling boot-swabs for culture may result in a loss of sensitivity such that the theoretical advantage of taking and testing five pairs is reduced. It was also not known what the effectiveness of a single pair of boot swabs from the whole of the house would be since mathematical simulations using data from the baseline survey cannot take into account the non-uniform distribution of detectable contamination in turkey houses. The study therefore aimed to compare several different sampling methods and investigate the potential for a dilution effect caused by pooling faeces samples. In addition randomly selected samples were tested by an ISO 6579 Annex D MSRV-based method (Anonymous, 2007) and by the NMKL-71 method (Anonymous, 1999). 106 flocks were sampled and the probability of detection of infection by each method was as follows: one pair of boot swabs from whole house plus a separate dust sample 0.73; single dust sample 0.67; five pairs of boot swabs cultured individually 0.67; single pair of boot swabs from whole house 0.59; two pairs of boot swabs pooled for culture 0.56; single litter sample (40 pinches per house) 0.53; small sponge drag swab 0.44. Collection of the single pair of boot swabs plus dust in one circuit of the house was straightforward and far easier than collecting two or five pairs of boot swabs. Dust was particularly sensitive in large controlled environment houses and breeding flocks and litter was a better sample when wood shavings rather than straw was used as bedding. Of 199 samples analysed by the MSRV or NMKL-71 method, 83.4 % of samples were positive with the former and 74.8 % with the latter, a statistically significant difference which was particularly pronounced when the within-flock *Salmonella* prevalence was low. Pooling of individual faeces in pools of five resulted in a small dilution effect when only one or two of the individual faeces were positive. However, mathematical modelling demonstrated that increasing the number of faeces in a pool (which is equivalent to what occurs during boot swabbing when hundreds of fresh faeces and dry material from litter contributes to the accumulated coating of the boot swabs) leads to improved detection due to the increased chance of including a faeces sample containing high numbers of *Salmonella* which can be easily identified against a background of competitor organisms. Information from a previous study demonstrated that in broiler flocks boot swab and dust samples could be cultured together in the same pre-enrichment with minimal interference and an enhanced overall chance of detection of positive flocks. Work in different types of commercial laying houses demonstrated the difficulty of detecting infection with large pooled faeces samples or boot swabs in this sector so it is recommended that any sampling programme is evaluated in the target population, as extrapolation from one situation to another may be inappropriate.

Discussion

Q: Does dust give information on an old contamination?

A: Dust is quite dynamic. If a dust sample is taken at an early stage it could still indicate an old contamination. However, if the flock is negative, the dust will also become negative. Only dust samples ‘far away’ from the flock (e.g. at the roof) may still indicate an old contamination.

2.10 Epidemiology and biology of d-Tartrate positive *Salmonella enterica* serovar Paratyphi B

Istvan Szabo, NRL-Salmonella, Berlin, Germany

The increasing incidence of *Salmonella enterica* subsp. *enterica* serovar Paratyphi B dextrorotatory tartrate-positive variant, formerly called *S. enterica* subsp. *enterica* serovar Java (*S.* Java) strains in Germany and neighbouring countries, especially in poultry and poultry products, highlights the growing importance of this pathogen.
The emergence of this serovar has been well documented. *S.* Java strains originating from the beginning of the 1960s to the beginning of the 1990s showed a high genetic diversity. Since the middle of the 90s a new clonal line of *S.* Java emerged and spreaded successfully. Today it represents the majority of contemporary strains and has replaced almost all other clones. Strains of this clonal line are multidrug-resistant and highly uniform with respect to molecular typing. Especially important is the development of resistance against critical important antimicrobials like fluoroquinolones and 3rd- 4th generation cephalosporins. Several mechanisms involving mobile genetic elements have been shown to contribute the spread of resistance.
S. Java is generally considered less virulent for humans. However, since human outbreaks occurred, *S.* Java strains are often described as potentially epidemic strains.
Recently a multinational *S.* Java outbreak has been reported in northern Europe, involving Denmark, Finland, UK, Norway and the Netherlands.
In a joint research project between BFR (Germany), CVI (the Netherlands), VLA (UK) and VAR (Belgium) on *S.* Java, its genetic relationship and pathogenic potential is elucidated by using a robust DNA-Microarray, targeting a total of 281 virulence, housekeeping, resistance and other marker genes.

Discussion
Q: What can be the reason that *S.* Paratyphi B var. Java causes problems in humans in Scotland and not in the Netherlands?
A: This is not known. The strains from Scotland and the Netherlands show the same PFGE profile (import of poultry from the Netherlands). The problems could be caused by a concentration effect.

2.11 Characterisation of a new multi-drug resistant strain of *Salmonella* Paratyphi B var. Java associated with poultry

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

In England and Wales in 1998 an increase was observed in a multi-drug resistant strain of *Salmonella enterica* serovar Paratyphi B var Java (*S.* Java) isolated from poultry and poultry products. Multi-drug resistant *S.* Java strains were also isolated from the human population. An increase in multi-drug resistant *S.* Java isolates associated with poultry has also been reported in other European countries.

To establish if the poultry-associated strains were related and if the human strains were the same as those isolated from the poultry, 153 multi-drug *S.* Java isolates were characterised. Phage typing and drug resistance screening were performed on all of the strains; 38 of the strains were further characterised by pulsed field gel electrophoresis (PFGE).

As the majority of the strains were not lysed by the 12 *Salmonella enterica* serovar Paratyphi B (*S.* Paratyphi B) typing phages a new phage was developed to differentiate these strains. Eighty four per cent of the strains were lysed by this phage to give a new provisional type, phage type (PT) Colindale.

Most of the strains that were fully characterised were typed as phage type Colindale and had the same PFGE profile, showing a close relationship between these strains and possibly a common source.

The addition of the new phage to the existing phage typing scheme has proved invaluable in identifying these new multi-drug resistant *S.* Java strains and in epidemiological studies.

Discussion
Q: Was the original source of *S.* Paratyphi B var. Java not reptiles (snakes)?
A: *S.* Paratyphi B var. Java is an example of what can go wrong. The strain has once been introduced in poultry and was reduced by the use of antibiotics, resulting in (multi)resistant clones. This clearly shows how a *Salmonella* problem should **not** be tackled.

2.12 Multiple-locus variable number tandem repeat analysis (MLVA)

Daan Notermans, Laboratory for Infectious Diseases and Perinatal Screening, RIVM, Bilthoven, the Netherlands

Typing can be helpful when investigating *Salmonella* outbreaks.
A regional cluster of an unusual high number of cases with *Salmonella* Typhimurium phage type 561 (ST561: Dutch phage typing system; DT7 in the Colindale system) was detected early February 2006. This was a new phage type for the Netherlands. At the end of 2006 more than 200 laboratory-confirmed cases of ST561 were found. In the first 6 months of the outbreak 75 % of the cases came from the same region, expanding to other parts of the country afterwards. Trawling questionnaires incriminated a dairy farm with a production of 5000 litres of raw milk per day for the local production of cheese predominantly for regional retailers. Environmental samples, manure and dairy cattle of this farm were positive for ST561. However, no *Salmonella* could be detected in any product of the farm up to the end of October and no shortcomings could be shown in the hygiene control measures. Molecular typing with multi locus variable-number of tandem repeats (VNTR) analysis (MLVA) was introduced at the national public health laboratory. A pork sample from a supermarket from the region differed at two of the five loci from the most common found MLVA-type. All non-human samples related to the farm showed this MLVA-type. On this evidence no formal action was taken. In August-September a case-control study was performed enrolling 51 cases and 105 regional matched controls. This strongly implicated hard cheeses from raw milk, as well as the suspected dairy farm. Closer scrutiny of the cheeses demonstrated ST561, however in very low concentrations (4 bacteria per kg); legally, *Salmonella* should be absent in 25gr. The number of cases dropped considerably after it was decided that all batches that could be traced from the farm were to be screened and destroyed if found positive. However, in 2007 up to April, another 13 cases were found. A cluster of cases in another region showed an MLVA-typed differing by one locus from the main outbreak type. Patients in that region did not report a connection to cheese or the suspected farm.
Due to long shelf life, contaminated hard cheese may cause prolonged outbreaks. As shown for several other vehicles (soft cheese, chocolate) levels of contamination below legally accepted sampling levels may effectively cause a massive outbreak. This implies a necessary adaptation of the Food Act for

specific food products. MLVA has been shown to be a useful additional tool combined with sero- and phage typing to exclude non-outbreak related cases, farms and farm animals.

Discussion

Q: Did MLVA give additional information to the phage typing?
A: Yes, with MLVA we could make a distinction between isolates from pig/pork and cows.

3. Tuesday 27 May 2008: day 2 of the workshop

3.1 Tasks and duties of CRLs and NRLs

Kirsten Mooijman, CRL-Salmonella, Bilthoven, the Netherlands

In European Regulation 882/2004 the tasks and duties of the CRLs and NRLs are described in general terms. In short the tasks and duties of the CRLs are:

- Provide NRLs with details of analytical methods;
- Coordinate application of methods by NRLs, by organising comparative testing;
- Coordinate research on new methods (develop, validate and standardise methods);
- Conduct training for NRLs;
- Scientific and technical assistance to European Commission;
- Help NRLs to implement Quality Assurance.

In short the tasks and duties of the NRLs are:

- Collaborate with relevant CRL;
- Coordinate activities of official laboratories in MS;
- Organise comparative tests between official laboratories;
- Dissemination of info of CRL to competent authority and official laboratories;
- Scientific and technical assistance of competent authority;
- NRLs shall be accredited before 31/12/2009 (European Regulation 2076/2005);
- Communicate name and address of each NRL to Commission and to CRL;
- If a MS has more than one NRL per CRL, these NRLs should work closely together.

In February 2007 a protocol for management of underperformance/ lack of collaboration of NRLs, was published by DG-Sanco. In this protocol it is indicated that 'Appropriate action must be taken if the results of comparative tests reveal underperformance or if NRLs fail to collaborate with the CRL'.
In case of underperformance (i.e. failure in proficiency test), it is indicated that the CRL should contact the NRL and provide assistance. If possible a repetition of comparative test should be performed. If the relevant NRL performs well in this repetition study, no further action needs to be taken. If the results of the relevant NRL still indicate underperformance in the repetition study, the Commission shall be informed. Next the Commission shall inform the competent authority of the relevant Member State.
In case of lack of collaboration (proficiency tests or workshop) the CRL should contact the NRL and the justification for the lack of collaboration should be included in report to the Commission.
In case of repetitive lack of response of an NRL, the Commission shall be informed. Next the Commission shall inform the competent authority of the Member State.

For a 'smooth' collaboration between the CRL-*Salmonella* and the NRLs-*Salmonella* the NRLs are requested to remind:

- To regularly check their e-mail.
- To timely inform the CRL with changes in contact names, e-mail addresses, address, phone/fax numbers, etcetera.
- To respect deadlines, for sending in results of ring trials, for indicating participation in workshops, for providing additional information, etcetera.
- To consult the CRL in case deadlines can not be respected.

3.2 Activities NRL to fulfil tasks and duties in Cyprus

Contantinos Economides, NRL-Salmonella, Nicosia, Cyprus

The Laboratory for the Control of Foods of Animal Origin (LCFAO) belongs to the Division of Veterinary Laboratories of the Cyprus Veterinary Services and carries out microbiological examinations, both of general and specialized nature, in food and environmental samples. Each year, a number of more than 16 000 samples undergo multiple examinations by the Laboratory and more than 3000 are examined for the presence of *Salmonella*. The Laboratory consists of five different Departments and it is nominated as the NRL for *Salmonella*, *Listeria*, *Campylobacter*, *Staphylococcus*, VTEC, *T. spiralis*, Marine biotoxins and Milk. Since 2004, the Laboratory has been accredited by the Hellenic Accreditation Body (ESYD) and *Salmonella* detection (according to ISO 6579: Anonymous, 2002) is included in the scope of accreditation. NRL-*Salmonella* participates yearly in a number of ring tests organized by various providers, including all interlaboratory comparison studies organized by the CRL. In 2006, the NRL has organized a ring trial on the detection of *Salmonella* spp. in cheese, in which seven private and governmental laboratories have successfully participated. In the frame of European Baseline studies, the Laboratory closely collaborates with the Animal Health Laboratories of the Veterinary Services. A productive collaboration also exists with the Microbiology Department of the State General Laboratory and the Laboratory of the Nicosia Central Hospital, in spite of their different responsibilities. In 2008, a research project was loaded with the aim to asses the possible virulence of the *S*. Enteritidis isolated by animals, food and humans by determining their virulence gene profile of selected genes. Finally, in 2008, two study visits were organized together with the CRL on issues related to phenotypic and molecular typing of *Salmonella*.

Discussion
Q: What kind of water may be a source for *Salmonella*?
A: Water used in food industry. This water should have drinking water quality.
Q: Do you find *S.* Typhi in Cyprus
A: No.

3.3 Activities NRL to fulfil tasks and duties in Denmark

Dorte Lau Baggesen, NRL-Salmonella, Copenhagen, Denmark

The Danish Veterinary Institute and the Institute of Food Safety and Toxicology, first merged into the Danish Institute of Food and Veterinary Research and now into the National Food Institute (NFI) under the Technical University of Denmark has ‘always’ been the governmental reference laboratory for *Salmonella* and other food borne pathogens. In collaboration with the Danish Food and Veterinary Authorities, NFI has identified its tasks and fulfilled its obligations in order to improve the food safety and control the sources of human disease. However, since the implementation of the European Regulation 882/2004, the tasks and duties of NRL *Salmonella* and a number of other NRLs have been defined through the directive. Due to the implementation of the directive, there has been an increase in the number and scale of specific the tasks. There has, however, not been more resources – either financial or staff, and therefore it has been necessary to ‘think new thoughts’ and reorganize the NRL.

In Denmark, three regional official laboratories under the Food and Veterinary Authorities are responsible for the official analysis in relation to official control of *Salmonella*. These laboratories

perform accredited analysis for detection of *Salmonella* whereas characterization of isolated strains is performed by NFI. In 2008, a formal collaboration between the regional laboratories and NFI together with the central part of the Food and Veterinary Authorities is established as a 'Centre for Food analysis'. This centre will be responsible for the task and duties in relation to all the NRLs mentioned in European Regulation 882/2004.

The main benefit of the centre is the formal collaboration and sharing of experience and knowledge between partners and thereby the focusing on tasks that have to be fulfilled. The regional laboratories and the NFI have very different qualifications and professional profile and will therefore contribute to the activities in different ways. The regional laboratories perform a high number of routine investigations by accredited analysis and have a large experience in food control and risk management. In contrast, NFI serve the governmental system with research-based advice and perform risk analysis and trace of sources of human diseases as important parts of the work. In addition, research in relation to microbiological hazards in the food production chain, control of hazards and development of new methods for identification and characterisation of pathogens is carried out supporting the work as NRL.

Especially in relation to control of *Salmonella*, the staffs at NFI/NRL are responsible for the national strain collection, which includes several thousand of *Salmonella* isolates, collected through the comprehensive Danish surveillance programme in domestic animals and food. The strains are characterised bye epidemiological data, by serotyping and in different extent by phage typing, antimicrobial susceptibility typing and genotyping (PFGE/MLVA). The strain collection is together with the human strain collection one of the cornerstones in the Danish model for human illness attribution and for outbreak investigations.

Since year 2000, the Danish NRL has regularly offered a ring trial for official and private laboratories performing isolation of *Salmonella* from samples collected in the primary animal production, as ring trials including faeces as matrix have not been commercial availably. Around 5-15 laboratories have participated in the ring trial where the performances of the official analysis were evaluated. In 2006, the Danish NRL began collaboration with the Swedish NRL, which in 2008 has been extended also to include the Norwegian NRL. The collaboration between the Scandinavian NRLs enables us to share the workload between the different NRLs and to perform the ring trails on a more cost-effective basic.

In the future I look forward to further collaboration in the network of NRLs together with the CRL. *Salmonella* does not 'accept borders' and food control and surveillance is more and more becoming a subject for international collaboration not only in EU but also all over the world. This development opens a need for further harmonization of methods and sharing of surveillance data and therefore strong and binding networks have to be established.

Discussion

Q: What is the optimal number of test samples to be used in ring trials?

A: Nobody seems to have the right answer for this. The number of samples could also be related to the number of studies organised per year (less samples in case of more studies). In studies organised in Belgium and in Denmark only 4-5 (food) samples per study are analysed. This number is concerned acceptable by the (national) board for accreditation. The set-up of proficiency tests is an item which is also thoroughly discussed in a working group at ISO level.

3.4 Activities NRL to fulfil tasks and duties in Estonia

Age Kärssin, NRL-Salmonella, Tartu, Estonia

The Estonian Food and Veterinary Laboratory (VFL) is responsible for priority statutory testing under various farm and wild animal disease surveillance and food safety control programs, also provides national authorities with relevant analytical support.
According to European Regulation (EC) 882/2004, VFL is nominated to be National Reference Laboratory for *Salmonella*.
Our work in the field of reference functions is communication and cooperation with CRL, cooperation with all relevant competent authorities concerning control programs and surveillance, coordination of the activities of official control laboratories, organization comparative tests between the official laboratories.
In the year 2007, VFL carried out approximately 12 000 analysis for *Salmonella* detection from food and samples from food industries. Analyses according to the national control program of animal diseases were 686 (pooled faecal samples) without diagnostics of clinical cases.

Discussion
Q: How do you spike sponge samples?
A: By bringing droplets of a culture onto a sponge.

3.5 Activities NRL to fulfil tasks and duties in Finland

Henry Kuronen, NRL-Salmonella, Kuopio, Finland

The Finnish Food Safety Authority Evira is the NRL-*Salmonella* in Finland. The Veterinary Bacteriology Unit in Kuopio is the NRL-*Salmonella* for sampling and analysing methods concerning samples from primary production, and the Microbiology Unit in Helsinki for food (Food Microbiology Section) and feed (Feed and Fertilizer Section). All isolates from non-human origin are confirmed and serotyped in Kuopio, and PFGE-typing is in continuous use. There are about 40 approved laboratories for *Salmonella* testing. NRL-*Salmonella* has close collaboration with them by meetings, courses, e-mail, phone and publications. NRL-*Salmonella* has also close collaboration with the National Public Health Institute KTL (reference laboratory for human samples and strains) for phage typing (performed only in KTL), genetic comparison of strains, scientific research and projects. There is a virtual Zoonoses Centre in Evira including experts from Evira and KTL. Additional to the activities concerning CRL-*Salmonella* and EU commission, NRL-*Salmonella* participates in other international activities like ISO and CEN, and in Nordic co-operation in revising *Salmonella* control programmes.

3.6 Activities NRL to fulfil tasks and duties in Hungary

Erzsebet Adriane, NRL-Salmonella, Budapest, Hungary

There was a reorganisation at the beginning of 2007 in the sphere of agriculture, animal health and food safety in Hungary. All the institutes related to this field are now under the supervision of the Ministry of Agriculture and Rural Development (MARD). The newly established Agricultural Administrative

Office which has central and regional institutes is also under the supervision of MARD and directly responsible for the control of the agricultural sphere.
The NRL-*Salmonella* is working in the Central Agricultural Office, Food and Feed Safety Directorate, Food Microbiological Diagnostic Department. The main subjects of this department are the food microbiological diagnostic activities as official laboratory and monitoring activities according to European Regulations 882/2004, 2073/2005 EC and European Directive 2003/99/EC. Different National Reference laboratories are working in this department besides *Salmonella: Listeria monocytogenes*, *Campylobacter*, *E. coli*, milk, *Staphylococcus*, GMO. This department is also responsible for the *Salmonella* baseline studies organized by the EU. The main tasks of the department are preparation of sampling plans, training on sampling methods, provide the sampling devices and detection, serotyping and phage-typing of *Salmonella*. The department is coordinating the activity of the six regional official laboratories. The staff consists of 29 members on an interdisciplinary approach: veterinarians, physicians, food technological engineers and technicians working together.
The department of NRL-*Salmonella* and all the six official laboratories are accredited according to ISO 17025 (Anonymous, 2005) for ISO 6579 (Anonymous, 2002).
The NRL-*Salmonella* participates on the yearly workshops and in the interlaboratory comparison studies for detection, serotyping and phage typing organized by the CRL-*Salmonella*.
The NRL-*Salmonella* organises proficiency tests for the official laboratories and for private laboratories using different matrices, like poultry faeces or environmental samples, meat or meat products, carcass swabs.
The NRL-*Salmonella* coordinates the activity of the official laboratories and shares all the information with them. Laboratory meetings are organized regularly on which there is a possibility to discuss the emerging problems, to get information about the implementation of monitoring plans, methods of analyses, etc. There is also a laboratory working group with laboratory experts. They discuss scientific or methodological problems and share the resolutions on the laboratory meetings.
Last year the department of the NRL-*Salmonella* organized a conference for the poultry sector and for all the interested parties like epidemiological and laboratory experts in order to increase the efficiency of the national *Salmonella* eradication program and to give an opportunity for the participants to share their experiences related to the subject. (Title: Introduction of new scientific results to achieve an effective *Salmonella* eradication program in Hungary, 2007)
The NRL-*Salmonella* gives practical training for the colleagues from the laboratory system if necessary to attain new methods or to train new colleagues.
Also an important task of this department is to collect and summarize the data of the food microbiological examinations performed by the regional laboratories and to prepare the yearly report for the Ministry of Agriculture and Rural Development.
On the department also a laboratory for typing *Salmonella* isolates, compulsory sent from the veterinary service, is present. Serotyping is done according to the Kauffmann-White scheme (Popoff, 2001) on strains isolated from food and feeding stuffs, animal health origin, breeding eggs, isolates from the national poultry *Salmonella* eradication program and from the EU baseline studies. The NRL-*Salmonella* serotypes 5-6000 strains yearly. The phage-typing is according to the HPA Colindale system from *S*. Enteritidis and *S*. Typhimurium serovars. Some strains are also tested for antimicrobial resistance.

Discussion
Q: How many laboratories participate in your ring trials?
A: Approximately 18 laboratories
Q: You see an increase in the prevalence of *S.* Infantis and a decrease in the prevalence of *S.* Enteritidis in poultry. Could this be caused by a vaccination programme against *S.* Enteritidis in your country?

A: Indeed we have a vaccination programme against *S.* Enteritidis, but the correlation between this programme and the increase in *S.* Infantis is not clear.
Q: You do not see problems of *S.* Infantis in humans despite the high prevalence in poultry. Do you have an explanation for this?
A: We have this still under research. Maybe there is a difference between virulence genes.

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 Helsinki, Finland from 5 to 9 May 2008.

Subjects of interest are:
- Annex D of ISO 6579: published in July 2007 (Anonymous, 2007)
- New Working Item: Enumeration of *Salmonella*
- Periodical (5 year) review of ISO 6579
- Standard or guide for serotyping *Salmonella* spp.?
- Working group (WG2) on statistics
- Working group (WG3) on validation of microbiological methods
- Working group (WG4) on Proficiency testing in microbiology
- Joint working group (WG 5) on QA of media
- Task group (TAG 5) Primary production
- Task group (TAG 6) sampling techniques
- CEN mandate on validation of microbiological methods

Enumeration of *Salmonella*
At the Cairo meeting in April 2007 it was agreed that the CRL-*Salmonella* would prepare a draft protocol for enumeration of *Salmonella* spp. by using the mini-MSRV method. This draft protocol was sent to the SC9 secretariat in November 2007 and distributed to the SC9 members as well as to the NRLs-*Salmonella* for further testing. The CRL performed some experiments with the protocol and received from one more Dutch laboratory some results. It was agreed to launch the draft protocol for a New Working Item Proposal to become published as an ISO Technical Specification (ISO/TS). In case the voting would result in many (technical) comments, this will be further discussed at the SC9 meeting in 2009, else the protocol might (after minor amendments) soon be published as an ISO/TS.

Periodical (5 year) review of ISO 6579 (Anonymous, 2002)
In the periodical review of this standard 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. It was agreed at the ISO meeting that an enquiry will be launched by the secretariat of SC9 for the review of implementation of ISO 6579 about:

- 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.

All comments should be accompanied with supporting data or scientific publications. Positive as well as negative experiences will be requested.
An ad hoc group will be raised on the topic, to consider the outcome of the enquiry. The group will be convened by Kirsten Mooijman. The secretariat of ISO/TC34/SC9 will also launch a call for experts for this ad hoc group, together with the enquiry.

Standard or guide for serotyping *Salmonella* spp.?
Due to lack of time, the outcome of the short enquiry for the need of a standard for serotyping of *Salmonella* spp. could not be taken into account at the SC9 meeting. It was agreed that the secretariat of SC9 would launch a second enquiry on the subject (as the first enquiry was send around only very shortly before the meeting). To the second enquiry also a draft proposal for a guide on serotyping will be attached, to be provided by Kirsten Mooijman.

Working group (WG2) on statistics
This ISO working group reviews statistical aspects of (draft) ISO standards. Presently the group is preparing an amendment to ISO/TS 19036 (Anonymous, 2006), to cover also low counts for calculation of the measurement uncertainty. Furthermore, the group is highly involved in the statistical aspects of the revision of ISO 16140 on validation of microbiological methods (Anonymous, 2003).

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

It is planned that the first drafts of the different Project groups will become available at the next meeting of SC9 in 2009.

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.
At the SC9 meeting a short (ad-hoc) meeting was organised to discuss the following questions:
1. What should be removed from the text of draft CD 22117 now a draft version of ISO 17043 has been published?
2. Is there a conflict on information in draft CD 22117 compared to draft ISO 17043?
In general the WG considered draft CD22117 sufficient specific for microbiology to keep it as a separate standard beside ISO 17043. The microbiology document can only contain microbiological specific aspects. For the general aspects cross reference shall be made to ISO17043, when possible with

specific information for microbiology. It is planned that a new draft of ISO 22117 will be circulated for Committee Draft (CD) vote by the end of 2008.

Joint 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.

Task group (TAG 5) Primary production
The group is working on 3 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

Task group (TAG 6) sampling techniques
This group is planning to prepare a standard, divided in several parts to describe the different food products to be sampled.

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 projectleader 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.
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 projectleaders is very slow, due to bureaucratic problems and inexperience of AFNOR with such a large mandate.

Discussion
Remarks: If a document for serotyping is drafted this should be done in close cooperation with the WHO Collaborating Centre for Reference and Research on *Salmonella*. Furthermore, a procedure for controlling the quality of sera should be considered.
A: Indeed, advice will be asked at the WHO reference centre for drafting the document. Furthermore, it is considered to prepare a guidance document instead of a full standard as suppliers of sera all have their own prescriptions for use.
Q: Will molecular typing also be considered in the document?
A: Not sure. The problem is that in EU Regulation it is indicated that alternative methods may be used if validated against the reference method. However, for serotyping no such reference method does exist. Therefore first priority will be given to a guidance for (classical) serotyping.
Q: What is meant with validation of the MSRV method?
A: Setting performance characteristics. Validation may not be the best term here, as no comparison to another (reference) method will be performed.

Q: How is the interaction between ISO and CEN?
A: This interaction is very good. Most of the time the group of people working in CEN (European level) are the same as the ones working in ISO (international level). Furthermore, through a formal (Vienna) agreement CEN procedures may be adopted in ISO and vice versa.
Q: Why is there a European Standardisation Commission in addition to an International Standardisation Commission?
A: An ISO method can exist beside a (similar) national method. However, if a method is published at CEN level it overrules national methods on the same subject, meaning that the national method will be replaced by the CEN method.

3.8 Work programme CRL-*Salmonella* second half 2008, first half 2009 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 2008 and for early 2009.

Interlaboratory comparison studies
As indicated in earlier presentations, three interlaboratory comparison studies are planned in the coming year:
- Detection of *Salmonella* spp. in animal feed: September/October 2008;
- Typing of *Salmonella* spp. (serotyping and phage typing): November/December 2008;
- Detection of *Salmonella* spp. in a 'veterinary' matrix: February/March 2009.

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*:
  - Provide ISO/TC34/SC9 with the amended draft protocol for enumeration of *Salmonella* spp. to launch it as New Work Item Proposal for publication as ISO Technical Specification;
  - Collect comments on ISO 6579 (Anonymous, 2002) and convene an ad-hoc group to review the comments;
  - Work out the activities for the organisation of a validation study of Annex D of ISO 6579 (Anonymous, 2007) in relation to the CEN mandate.
- Use of molecular methods;
- Quality assurance activities in relation with the baseline studies:
  - Duplicate serotyping of typable and non-typable strains, isolated by the NRLs from the baseline survey on breeder pigs;
  - Duplicate serotyping of non-typable strains, isolated by the NRLs from the baseline survey on broiler carcasses;

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. For instance information on stability of cultured buffered peptone water (BPW), Modified semi-solid Rappaport-Vassiliadis medium (MSRV) and Xylose lysine deoxycholate (XLD) agar when stored at 5 °C can be published 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.

Closure
Kirsten Mooijman closed the workshop, thanking all participants, and guest speakers for their presence and contributions.

Discussion
Remark: The NRL-*Salmonella* in the United Kingdom has published some years ago a study on storage of MSRV. They found an increase in the number of *Salmonella* after storage. The publication will be sent by mail to the CRL.

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Annex 1 Participants

European Commission	Kris de Smet
European Food Safety Authority (EFSA)	Frank Boelaert
CRL – *Salmonella*	Kirsten Mooijman Petra Berk Angelina Kuijpers Christiaan Veenman Anjo Verbruggen
Guest speaker (United Kingdom)	Elizabeth de Pinna (HPA, London)
Guest speaker (Germany)	Istvan Szabo (NRL-*Salmonella*, Berlin, Germany)
Guest speaker (the Netherlands)	Daan Notermans (RIVM, Bilthoven)

National Reference Laboratories for *Salmonella*

AUSTRIA	Tanja Urbanka
BELGIUM	Katelijne Dierick
BULGARIA	Gergana Mateva
CYPRUS	Economides Constantinos
CZECH REPUBLIC	Tomas Cerny
DENMARK	Dorte Lau Baggesen Niels Feld
ESTONIA	Age Kärssin
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	Susanne Surman

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Annex 2 Programme of the workshop

Programme of the CRL-*Salmonella* workshop XIII
26 and 27 May 2008, 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 22 May 2008.

Sunday 25 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 26 May 2008

Morning chair: Arjen van de Giessen

9.00 - 9.15	Opening and introduction (Kirsten Mooijman)
9.15 - 9.30	2006 Community trends and sources report on zoonoses (Frank Boelaert)
9.30 - 10.00	EU-wide Baseline surveys on the *Salmonella* prevalence in turkey flocks and in slaughter pigs (Frank Boelaert)
10.00 - 10.30	Ongoing and possible future baseline studies on *Salmonella*, and control programmes at primary production (Kris de Smet)
10.30 - 11.00	*Coffee/tea*
11.00 - 11.15	Introduction general lay-out baseline survey slaughter pigs (Frank Boelaert)
11.15 - 12.00	Comparability of different ELISA's on the detection of *Salmonella* spp. antibodies in meat juice and serum (Petra Berk)
12.00 – 12.30	Discussion on the use of serological methods (Arjen van de Giessen and Frank Boelaert)
12.30 - 13.30	*Lunch*

Afternoon chair: Kirsten Mooijman

13.30 - 14.00	Results interlaboratory comparison study FOOD II (2007) on bacteriological detection of *Salmonella* in minced beef (Angelina Kuijpers)
14.00 - 14.30	Results interlaboratory comparison study Veterinary XI (2008) on bacteriological detection of *Salmonella* in chicken faeces (Angelina Kuijpers)
14.30 - 15.00	CRL-*Salmonella* interlaboratory comparison studies 2008 and 2009 (Kirsten Mooijman)
15.00 - 15.30	*Coffee/tea*
15.30 - 15.50	Evaluation and optimisation of monitoring methods for *Salmonella* in turkey flocks (Robert Davies)
15.50 - 16.20	Epidemiology and biology of d-Tatrate positive *Salmonella* enterica serovar Paratyphi B (Istvan Szabo)
16.20 - 16.40	Characterisation of a new multi-drug resistant strain of *Salmonella* Paratyphi B var. Java associated with poultry (Elizabeth de Pinna)
16.40 - 17.00	Multiple-locus variable number tandem repeat analysis (MLVA) (Daan Notermans)
17.30 and onwards	Social programme and dinner

Tuesday 27 May

Chair: Kirsten Mooijman

9.00 – 9.30	Tasks and Duties CRL and NRLs (Kirsten Mooijman)
9.30 – 10.45	Activities NRLs to fulfill tasks and duties in: Cyprus (Constantinos Economides) Denmark (Dorte Lau Baggesen) Estonia (Age Kärssin) Finland (Henry Kuronen) Hungary (Erzsebet Adriane)
10.45 - 11.15	*Coffee/tea*
11.15 – 11.45	ISO/CEN activities (Kirsten Mooijman)
11.45 – 12.15	Work programme CRL-*Salmonella* second half 2008, first half 2009 and closure (Kirsten Mooijman)
12.15 - 13.30	*Lunch*

13.45 Departure to train station Utrecht

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