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The twelfth CRL-*Salmonella* workshop

7 and 8 May 2007, Bilthoven, the Netherlands

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Abstract

The twelfth CRL-*Salmonella* workshop

The Community Reference Laboratory (CRL) for *Salmonella* organised a successful meeting in Bilthoven, the Netherlands on 7 and 8 May 2007 for the National Reference Laboratories (NRLs) for *Salmonella* of all Member States of the European Union. This was the twelfth year that the workshop was organised. Each NRL was represented by, at least, one participant, resulting in a total attendance of 47 people, including several guest speakers.

The most important aim of the workshop was to bring the NRLs up to date on the activities of the CRL-*Salmonella*. Presentations by CRL-*Salmonella* staff members focused therefore on the results of the interlaboratory comparison studies (ring trials), in which the NRLs-*Salmonella* had participated. Plans for the coming year were also presented. Besides the activities of the CRL, other subjects included, zoonoses in Europe, the WHO Global Salm-Surv programme, *Salmonella* Dublin in cattle, *Salmonella* Typhimurium in cheese, the importance of environmental monitoring during outbreak investigations and the use of serological methods for the detection of *Salmonella* in pigs and poultry.

Participating for the first time with short presentations on the activities of their institutes were NRL delegates from the two new European Member States, Romania and Bulgaria, and the NRL from the Former Yugoslav Republic of Macedonia.

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

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Rapport in het kort

De twaalfde CRL-*Salmonella* workshop

Op 7 en 8 mei 2007 organiseerde het Communautair Referentie Laboratorium (CRL) voor *Salmonella* een succesvolle bijeenkomst voor de Nationale Referentie Laboratoria (NRL's) voor *Salmonella* van alle Europese lidstaten. De bijeenkomst vond plaats in Bilthoven, Nederland.

De workshop werd alweer voor het twaalfde jaar georganiseerd. Van ieder NRL was (minimaal) één deelnemer aanwezig. Verder waren er ook enkele gastsprekers aanwezig. In totaal namen 47 mensen deel aan de tweedaagse workshop.

Het belangrijkste doel van de workshop was om de NRL's te informeren over de activiteiten van het CRL-*Salmonella*. Hiertoe werden door medewerkers van het CRL-*Salmonella* presentaties gegeven over de resultaten van de rondzendoefeningen (ringonderzoeken) waar de NRL's aan hadden deelgenomen. Ook werden de plannen voor het komende jaar besproken.
Naast de activiteiten van het CRL, werden ook over diverse andere onderwerpen gediscussieerd. De volgende onderwerpen kwamen aan bod: zoönosen in Europa, WHO Global Salm-Surv programma, *Salmonella* Dublin in rundvee, *Salmonella* Typhimurium in kaas, onderzoek van de omgeving bij uitbraken, gebruik van serologische methoden voor het aantonen van *Salmonella* in varkens en pluimvee.

De twee nieuwe Europese lidstaten, Roemenië en Bulgarije, waren voor het eerst aanwezig bij de workshop. De deelnemers van de NRL's uit deze landen vertelden kort iets over de activiteiten van hun instituten. Ook het NRL van de Voormalige Joegoslavische Republiek van Macedonië was voor het eerst aanwezig en gaf een korte presentatie.

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

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Acknowledgements

An international workshop like the CRL-*Salmonella* workshop can not be organised without the help of several persons. The author is very grateful for this help and would like to thank all those who gave their contribution to the success of the workshop.

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Jeanette van Essen is thanked for arranging all the tickets and other organisational details, working together with Loes van Dijk.
Arjen van de Giessen is thanked for helping with the programme and chairing sessions of the workshop.
Angelina Kuijpers and Petra Berk are thanked for carefully presenting the activities of the CRL, for making minutes during the workshop and for critical reading of this report.

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Contents

List of abbreviations

A	Answer
AOAC	Association of Analytical Communities
BPW	Buffered Peptone Water
CEN	European Committee for Standardisation
cfp	colony forming particle
CRL	Community Reference Laboratory
DG	Directorate General
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
ENL	Enter-Net Laboratory
EQAS	External Quality Assurance System
EU	European Union
FDA	Food and Drug Administration
FEPAS	Food Examination Performance Assessment Scheme
FYROM	Former Yugoslav Republic of Macedonia
GC	Gas Chromatography
GD	Animal Health Service
GSS	Global Salm-Surv
HACCP	Hazard Analysis Critical Control Points
HPA	Health Protection Agency
HPLC	High Performance Liquid Chromatography
ISO	International Standardisation Organisation
LEP	Laboratory of Enteric Pathogens
LZO	Laboratory for Zoonoses and Environmental Microbiology
MKTTn	Mueller Kauffmann Tetrathionate broth with novobiocin
MS	Mass Spectrometry
MSRV	Modified Semi-solid Rappaport Vassiliadis
NRL	National Reference Laboratory
Q	Question
RIVM	National Institute for Public Health and the Environment
RVS	Rappaport Vassiliadis broth with Soya
SC	Sub Committee
SE	*Salmonella* Enteritidis
SPF	Specific Pathogens Free
STM	*Salmonella* Typhimurium
TC	Technical Committee
UK	United Kingdom
USA	United States of America
USDA	United States Department of Agriculture
VTEC	Verotoxigenic Escherichia coli
WHO	World Health Organisation
XLD	Xylose lysine deoxycholate

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Summary

On 7 and 8 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 Food Safety Authority (EFSA) and several guest speakers. A total of 47 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 on trends and sources of Zoonoses in Europe and on the baseline studies (past, present and future). Furthermore a presentation was given on the WHO Global Salm-Surv programme and possibilities on co-operation between Salm-Surv and CRL-*Salmonella* were discussed. At the end of the morning session the results of the interlaboratory comparison study on typing of *Salmonella*, organised early 2007, were discussed. During the afternoon session of the first day, the results of the interlaboratory comparison studies on detection of *Salmonella* in a food matrix (September 2006) and in a veterinary matrix (November 2006) were discussed. Also a proposal on the design of an interlaboratory comparison study on the detection of *Salmonella* in a food matrix to be organised in fall 2007 was discussed. The day was closed with presentations of three NRLs, dealing with: *Salmonella* Dublin in cattle, *Salmonella* Typhimurium in cheese and the importance of environmental monitoring during outbreak investigations.

On the second (half) day of the workshop, the NRLs of the two 'new' EU Member States (Romania and Bulgaria) introduced themselves. Also Candidate Country Former Yugoslav Republic of Macedonia (FYROM) gave a presentation. The rest of the morning session was used to discuss the use of serology for the detection of *Salmonella* in pigs and poultry. 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/workshopXII.jsp.

1 Introduction

In this report the abstracts of the presentations given at the CRL-*Salmonella* workshop of 2007 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/workshopXII.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 7 May 2007: 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 twelfth workshop of CRL-*Salmonella* welcoming all participants in Bilthoven, the Netherlands. A very special welcome was given to the new Member States Romania and Bulgaria and to the NRL of the Candidate Country Former Yugoslav Republic of Macedonia (FYROM).
After a roll call of the delegates, information was given on the changes at the CRL, which were limited:

- The name of the laboratory in which the CRL-*Salmonella* is situated has changed into the Laboratory for Zoonoses and Environmental Microbiology (LZO);
- The head of LZO, Anne Mensink, has changed job;
- The CRL-*Salmonella* website is amended and will replace the present website very soon. The address will remain the same (www.rivm.nl/crlsalmonella).

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 EFSA report on trends and sources of Zoonoses in Europe

Frank Boelaert, EFSA, Parma, Italy

Zoonoses are diseases or infections, which are transmissible from animals to humans. The infection can be acquired directly from animals, or through ingestion of contaminated foodstuffs. In humans, the gravity of these diseases can vary from mild symptoms to life threatening conditions. The importance of a zoonosis as a human disease depends on several factors, such as severity of the disease, the case fatality, and number of cases (incidence) in the population.
In order to prevent these diseases from occurring, it is important to identify which animals and foodstuffs are the main sources of the infections. For this purpose, information is collected and analysed from all European Union (EU) Member States in order to help the Community to improve control measures in the food production chain and to protect human health.
In 2005, twenty-four Member States submitted information on the occurrence of zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks to the European Commission and the European Food Safety Authority (EFSA). Further information on zoonoses cases in humans was acquired from the European Centre for Disease Prevention and Control (ECDC). These data covered 16 zoonotic diseases. Assisted by its Zoonoses Collaboration Centre, EFSA and ECDC jointly analysed the information and published the results in this annual Community Summary Report (Anonymous, 2006a). In addition, three countries not belonging to EU provided information on zoonoses for the report.

The analysis of the 2005 data highlighted campylobacteriosis as the most frequently reported zoonotic disease in humans within the EU. Reported *Campylobacter* cases increased by 7.8 % compared to the previous year rising to an incidence rate of 51.6 cases per 100 000 people and to a total of 197 363 recorded cases. Salmonellosis remained the second most frequent zoonosis with 176 395 reported human cases, despite the fall of 9.5 % to an incidence rate of 38.2 % compared to 2004. Amongst foodstuffs, the highest proportion of *Campylobacter* positive samples was reported for fresh poultry meat, where up to 66 % samples were found positive. *Campylobacter* was also commonly detected from live poultry, pigs and cattle.
Salmonella was most often reported from fresh poultry and pig meat where proportions of positive samples up to 18 % were detected. In table eggs, findings of positive samples ranged from 0 % to 6 %, but over the past five years an overall decreasing trend in occurrence of *Salmonella* in eggs was observed. In animal populations, *Salmonella* was most frequently detected in poultry flocks.
Salmonella, *Campylobacter*, and viruses were the most important causes of reported foodborne outbreaks in 2005. Egg and bakery products were the most common sources of *Salmonella* outbreaks, whereas broiler meat was an important source for both *Salmonella* and *Campylobacter* outbreaks. Foodborne virus outbreaks were most often caused by drinking water, fruit and vegetables.
Relatively high proportions of *Campylobacter* and *Salmonella* isolates from animals and food were resistant to antimicrobials commonly used in treatment of human diseases. This is especially the case of resistance to fluoroquinolones in *Campylobacter* isolates from poultry, where up to 94 % of isolates were reported resistant to ciprofloxacin. Foodborne infections caused by these resistant bacteria pose a particular risk to humans due to possible treatment failure.
In 2005, a total of 9630 human yersiniosis cases were reported. Other bacterial zoonoses - listeriosis, infections caused by verotoxigenic *Escherichia coli* (VTEC) and brucellosis – accounted for approximately 1000 - 3000 reported human cases each, whereas a total of 119 tuberculosis cases caused by *Mycobacterium bovis* was registered.
Very few ready-to-eat foods contained *Listeria monocytogenes* bacteria at levels over a limit that poses a significant risk to human health. Samples exceeding this limit were most often found in fishery products. The lack of serotype and virulence factor information of the VTEC and *Yersinia* findings in food and animals prevented a proper assessment of the relevance of these findings to human disease cases.
Most of the Member States are either officially free from bovine tuberculosis and bovine or caprine/ovine brucellosis, or reported no positive cases in 2005. However, in some of the non-free Member States prevalence at the levels of 3-4 % was still detected in bovine/sheep/goat populations.
The parasitic zoonoses, echinococcosis, and trichinellosis, accounted for 320 and 174 reported human disease cases respectively in 2005. *Trichinella* was rarely detected in slaughter animals. For both zoonoses, wildlife is an important reservoir of infections. There is a distinct geographical distribution of the findings of the parasites in the EU. The Toxoplasma parasite was reported from various animal species in 2005.
Four cases of human rabies were reported in 2005, but the infection originated from outside the EU. However, the increased reporting of cases in farm and wild animals in the eastern part of the EU is of concern.
The report also contains information about Bovine Spongiform Encephalopathy, Avian Influenza, Cysticerci and Sarcocystis parasites and Q fever in animal populations.

Discussion
Q: For how many countries are these cases notifiable?
A: The question is, what is notifiable? Not sure about the definitions for ‘probable case’, ‘most likely case’, ‘confirmed case’.
Q: Are figures available concerning economic costs due to these zoonoses?

A: EFSA does not have these figures. Presently there is no agreement on how to calculate the impact of an infection. ECDC is aware of this problem and is working on it.
Q: The increase in the use of fluoroquinolones may increase a human health problem. In general *Salmonella* or *Campylobacter* infections are not treated with fluoroquinolones. If humans are treated with these antibiotics, would that give a higher risk on infection with *Salmonella* or *Campylobacter*?
A: The data have not been interpreted in this detail. Presently a working group is looking at the data more closely.

2.3 Baseline studies

Frank Boelaert, EFSA, Parma, Italy

The European Union (EU) has agreed a programme for the reduction of *Salmonella* of public health significance in farm animals under Regulation EC No 2160/2003 on the control of *Salmonella* and other specified zoonotic agents. In order to provide the scientific basis for setting targets for reducing the prevalence of those *Salmonella* serovars, European Union-wide baseline surveys to determine the prevalence of *Salmonella* are conducted. These surveys are coordinated by the European Commission, whereas the European Food Safety Authority (EFSA) is responsible for analysing the collected data.
A first baseline survey was conducted in 2004-2005 in holdings of laying hen flocks. *Salmonella* was detected in 30.8 % of the laying hen holdings in the EU. In the specific Member States, the observed holding prevalence of *Salmonella* ranged from 0 % to 79.5 %. A total of 20.4 % of the laying hen holdings was positive for *S.* Enteritidis and/or *S.* Typhimurium. The Member State-specific observed holding prevalence of *S.* Enteritidis and/or *S.* Typhimurium varied greatly, from 0 % to 62.5 %. Based on the analysis results the Community target for the reduction of *Salmonella* Enteritidis and *Salmonella* Typhimurium in adult laying hens was set to be as follows: (a) an annual minimum percentage of reduction of positive flocks of adult laying hens equal to at least; i) 10 % if the prevalence in the preceding year was less than 10 %, ii) 20 % if the prevalence in the preceding year was between 10 and 19 %, iii) 30 % if the prevalence in the preceding year was between 20 and 39 %, iv) 40 % if the prevalence in the preceding year was 40 % or more, or (b) a reduction of the maximum percentage to 2 % or less; however, for Member States with less than 50 flocks of adult laying hens, not more than one adult flock may remain positive. The first target should be achieved in 2008 based on the monitoring starting in the beginning of that year. With regard to the target in 2008, the results of the baseline survey will be used as the reference.
A second baseline survey was conducted in 2005-2006 in broiler flocks. The Community observed prevalence of *Salmonella*-positive broiler flocks, in all flocks raised over the one year period of the baseline survey, was 23.7 %. The *Salmonella* prevalence varied widely amongst the Member States, from 0 % to 68.2 %. A total of 11.0 % of the broiler flocks was estimated to be positive for *Salmonella* Enteritidis and/or *Salmonella* Typhimurium. The Member State-specific observed flock prevalence of *S.* Enteritidis and/or *S.* Typhimurium varied also greatly, from 0 % to 39.3 %. The *Salmonella* serovar distribution varied amongst the Member States, many of them having a specific distribution pattern of their own. Based on the analysis results the Community target for the reduction of *Salmonella* Enteritidis and *Salmonella* Typhimurium in broiler flocks was set to be a reduction of the maximum percentage of flocks of broilers remaining positive of *Salmonella* Enteritidis and *Salmonella* Typhimurium to 1 %, or less, by 31 December 2011.
Two other *Salmonella* baseline surveys - in fattening pigs and in turkeys - are currently being conducted throughout the EU. Other baseline surveys that are currently being discussed at the

Community-level are; *Salmonella* in breeding pigs, *Campylobacter* in broiler flocks and *Salmonella* and *Campylobacter* in broiler meat.

Discussion
Q: When will the monitoring in broiler carcasses start?
A: This will be discussed on 8 May at DG-Sanco, but most probably it will start early 2008. A new aspect may be quantitative enumeration.
Q: The isolates from earlier studies were stored for phagetyping and antimicrobial resistance testing. Is there any development to perform these tests?
A: This will also be discussed at DG-Sanco on 8 May. First of all it is necessary to find out who will finance this.

2.4 WHO Global Salm-Surv programme

Jaap Wagenaar, Faculty of Veterinary Medicine, Utrecht University, Utrecht and Animal Sciences Group, Lelystad, the Netherlands.

WHO Global Salm-Surv (GSS) was initiated in 2000 as a global network of national and regional public health, veterinary and food laboratories involved in isolation, identification and antimicrobial resistance testing of *Salmonella* and surveillance of salmonellosis. From 2001, *Campylobacter* was included in GSS and more recently *E. coli* and *V. cholera* were introduced. The aim of GSS is to reduce the global burden of foodborne illness by strengthening laboratory-based surveillance. GSS is coordinated by the WHO, the Technical University of Denmark, the US Centres for Disease Control and Prevention, Institute Pasteur, the Public Health Agency of Canada, the US Food and Drug Administration, EnterNet, OzFoodNet-Australia, and the Dutch Animal Sciences Group.
GSS activities includes international training courses covering microbiology as well as epidemiology, an Electronic Discussion Group (EDG), an External Quality Assurance System (EQAS) for typing and determination of antimicrobial resistance, a Country Databank with the fifteen most frequently isolated *Salmonella* serotypes, free reference testing services, and focused regional and national projects.
As of May 2007, WHO Global Salm-Surv had 894 general members from 142 countries. Thirty-four international training courses have been conducted in six languages in nine regions with more than 300 participants from 91 countries. Regional Centres have been established in South America, Central America, Southeast Asia, Eastern Europe, and the Caribbean. In 2000-2007, at least 178 distinct laboratories from at least 91 countries participated in EQAS. Country-specific projects were launched in Fiji, the Philippines, Slovenia, the Caribbean, and Jordan.
Through GSS, interaction between epidemiologists and laboratories from public health and animals/food safety sectors is fostered. The program will continue to expand its activities by strengthening the capacity of Regional Centres to lead the development and implementation of regional food safety efforts. New Regional Centres will be designated and additional region- and country-specific training and research activities will be launched.

Discussion
Q: The quality of the antisera will largely influence the quality of the serotyping. It would be good to have a scientific evaluation of the quality of antisera.
A: Indeed, this may be an issue for collaboration.
Q: Are you able to harmonise methodologies in the framework of WHO Salm-Surv?
A: We use standardised protocols for detection, serotyping and antimicrobial susceptibility

testing. The protocols are based on ISO methods and are available through the website: www.who.int/salmsurv/en. Protocols for molecular typing are not yet available.
Q: What are the selection criteria to participate in the ring trials?
A: In principle all countries can participate. The parcels are sent from Denmark to approximately 87 countries in the world every year. Last year the mailing was performed in two steps in some countries. First the parcels were sent to a central point in a country. Next the parcels were distributed from this central point over the country.
Q: Do you audit laboratories to check whether they follow the methods?
A: We have audited some 'lower-level' laboratories. However, it is sometimes difficult to see whether they really do what they say they do, due to language problems.
Q: Will WHO Salm-Surv also organise ring trials for the detection of *Salmonella*?
A: The focus is presently on serotyping and antimicrobial susceptibility testing. There may indeed be a need for detection studies, but the funding is presently the limiting factor.

2.5 Results typing study XII – 2007: phagetyping

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

Salmonella strains for phage typing in the XII – 2007 interlaboratory comparison study on the typing of *Salmonella* were provided by the Laboratory of Enteric Pathogens (LEP), of the Health Protection Agency (HPA), London, England. Ten *Salmonella* Enteritidis and ten *Salmonella* Typhimurium strains were selected from the current LEP culture collection.

Eight National Reference Laboratories (NRLs) and 17 Enter-Net Laboratories (ENLs) took part in the phage typing study. Results from two of the ENLs are still outstanding. Overall the results of this year's study were good: twelve laboratories (6 NRLs and 6 ENLs) correctly identified the ten *S.* Enteritidis strains. Ten laboratories (2 NRLs and 8 ENLs) correctly identified nine of the ten *S.* Enteritidis strains and one ENL correctly identified six.

The ten *S.* Typhimurium strains were correctly identified by eleven of the laboratories (5 NRLs and 6 ENLs). Four laboratories (2 NRLs and 2 ENLs) correctly identified nine of the ten *S.* Typhimurium strains and seven ENLs correctly identified eight. Five of the ten *S.* Typhimurium strains were correctly identified by one NRL. One strain of *S.* Typhimurium – PT 208 caused the most problems in this years study and was only correctly identified by 13 laboratories (5 NRLs and 8 ENLs).

All 20 strains in the study were correctly identified by eight laboratories (4 NRLS and 4 ENLs). Seven laboratories (3 NRLs and 4 ENLs) correctly identified 19 of the 20 strains and six ENLs correctly identified 17. One NRL and one ENL correctly identified 14 of the 20 strains in this year's study.

Discussion
Q: Do you provide a follow-up or training for laboratories that face problems with phagetyping?
A: In the past the HPA has offered training. Presently we are making plans for trainings, to start early next year. The idea is to have trainings for 4 participants per occasion and to give these trainings twice a year.
Q: Does the strains show instability in their reactions with phages, e.g. after storage?
A: Yes, this can happen sometimes. *Salmonella* Typhimurium is more stable in its phagetypes than *Salmonella* Enteritidis.

2.6 Results typing study XII – 2007: serotyping; Proposal typing study 2008

Petra Berk, CRL-Salmonella, Bilthoven, the Netherlands

The twelfth interlaboratory comparison study on the typing of *Salmonella* was organised by the Community Reference Laboratory for *Salmonella* (CRL-*Salmonella*, Bilthoven, the Netherlands) in collaboration with the Health Protection Agency (HPA, London, United Kingdom) in March 2007. 26 National Reference Laboratories for *Salmonella* (NRLs-Salmonella), including Norway, and 29 Enter-Net Laboratories (ENLs), three of which are also NRLs, participated in the study. In total, 20 strains of the species *Salmonella enterica* subspecies *enterica* (from group B, C1, C2-C3, D, E1, E4, G and I) were selected for serotyping.

In general, no problems were encountered with the typing of the O-antigens. 98 % of the NRLs were able to correctly type the O-antigens. A few laboratories had problems typing the H-antigens. The H-antigens were typed correctly by 96 % of the NRLs. 95 % of the NRLs indicated correct serovar names for the 20 serotyping strains. Eight strains were correctly typed by all NRLs, five strains were correctly typed by 25 of the 26 NRLs and four strains were correctly typed by 24 of the 26 NRLs. The two strains causing most problems were *S.* Thompson and *S.* Paratyphi B var. Java, which were typed correctly by respectively 19 and 20 of the 26 NRLs.

A proposal was made to define good performance of the NRLs. In this proposal a distinction is made between the five most important serovars (*S.* Enteritidis, *S.* Typhimurium, *S.* Infantis, *S.* Hadar and *S.* Virchow) and all other strains. It was proposed to give four penalty points if a NRL incorrectly typed *S.* Enteritidis, *S.* Typhimurium, *S.* Infantis, *S.* Hadar or *S.* Virchow or if a NRL assigned one of the serovar names of these five serotypes to another strains. One penalty point is given for all other incorrect typings. NRLs with less than four penalty points (20 NRLs) reach the level of good performance. Six NRLs had four or more penalty points and therefore did not reach the level of good performance, for these NRLs there will be a follow-up.

The typing study of 2008 will be comparable to the typing study of 2007. Twenty strains will be selected for serotyping and ten *S.* Enteritidis and ten *S.* Typhimurium strains will be selected for phagetyping.

Discussion

Q: How many penalty points do you give per mistake? For instance, does it make difference if a wrong serovar name is caused by one mistake or by several mistakes?
A: We will have a closer look at this. A distinction may be made between only one mistake in the O-antigens and mistakes in both O- and H –antigens.
Q: Is it possible to obtain more information from the CRL to improve the serotyping at the NRL?
A: Next year we will propose to use another table in the test report, in which more details can be given on the antisera used. This information may help in finding reasons for problems with serotyping.
Remark: Germany isolates many rough strains which are hard to be serotyped further, or can not be serotyped. Often these rough strains are *Salmonella* Enteritidis strains from poultry. Germany uses therefore an additional PCR test to be able to identify these strains.

2.7 Results interlaboratory comparison study on bacteriological detection of *Salmonella* – FOOD I – 2006

Angelina Kuijpers, CRL-Salmonella, Bilthoven, the Netherlands

In September 2006 the first (pilot) interlaboratory comparison study on the bacteriological detection of *Salmonella* spp. in a food matrix (minced beef) was organised. In total 25 NRLs-*Salmonella* participated, including Norway. Some problems arose when organising this food study, as seven NRLs indicated not to be the right laboratory for performing food analyses. These NRLs handed over the samples to another laboratory in their institute or country which is more acquainted to the microbiological analyses of food samples.

The set up of the study was comparable to the earlier 'faeces studies'. *Salmonella* negative minced beef was artificially contaminated with reference materials. The reference materials were the same as used in former studies, consisting of capsules containing artificially contaminated milk powder with *Salmonella* Typhimurium at two levels (circa 10 and 100 colony forming particles (cfp) per capsule) or *Salmonella* Enteritidis at two levels (circa 100 cfp and 500 cfp per capsule). As control samples also reference materials containing *Salmonella* Panama (circa 5 cfp per capsule) were used.

The prescribed method was ISO 6579 of 2002 (Anonymous, 2002). In this ISO procedure two selective enrichment media are prescribed, being Rappaport Vassiliadis broth with Soya (RVS) and Mueller Kauffmann Tetrathionate broth with novobiocin (MKTTn). Furthermore the laboratories were requested to use, additionally, draft Annex D of ISO 6579 of September 2006 (Anonymous, 2006b). The selective enrichment medium in Annex D is Modified semi-solid Rappaport Vassiliadis agar (MSRV). Additional to the prescribed and requested methods the participants could use another (own) method.

The results per laboratory were judged following the criteria for 'good performance' as agreed at the CRL-*Salmonella* workshop in 2005 (Mooijman, 2005). One laboratory did not sent any results. All laboratories, except one, used all three methods (ISO 6579 and Annex D of ISO 6579). Although ISO 6579 is especially intended for the analyses of food samples, the best results were found when using the method as described in Annex D of ISO 6579. The scope of Annex D is detection of *Salmonella* spp. in samples from the primary production. However, the selective enrichment medium MSRV has shown to be well applicable for the detection of *Salmonella* spp. in other matrices as well. The results of all laboratories using MSRV fulfilled the criteria for good performance. With the selective enrichment medium MKTTn (ISO 6579) the lowest number of positive samples were found.

The study showed to have been successful for the number of NRLs-*Salmonella* participating in this study, as well as for the good results.

Discussion

Q: Some quality differences may exist between different brands of MSRV. Do you have information whether this may have influenced the results of the study?
A: We have not seen this influence in this study.
Q: MSRV is not prescribed for the analyses of food samples. However, in this study good results are found. Will MSRV be included in the future version of ISO 6579 for the analyses of *Salmonella* in food?
A: This may depend on the voting on ISO 6579 when it is send around for five years review (by the end of 2007). If many countries vote for revision of the ISO, MSRV may perhaps be included.

2.8 Results interlaboratory comparison study on bacteriological detection of *Salmonella* – FAECES X – 2006

Angelina Kuijpers, CRL-Salmonella, Bilthoven, the Netherlands

In November 2006 the tenth interlaboratory comparison study on the bacteriological detection of *Salmonella* spp. in animal faeces (from pigs) was organised. In total 27 NRLs-*Salmonella* participated, including Norway and Candidate Country Romania.

The set up of the study was comparable to the study of 2005, although this time pig faeces were used instead of chicken faeces. The pig faeces were negative for *Salmonella* and artificially contaminated with reference materials. The reference materials were the same as used in former studies, consisting of capsules containing artificially contaminated milk powder with *Salmonella* Typhimurium at two levels (circa 10 cfp and 100 cfp per capsule) or *Salmonella* Enteritidis at two levels (circa 100 cfp and 500 cfp per capsule). As control samples also reference materials containing *Salmonella* Panama (circa 5 cfp per capsule) were used. The prescribed method was draft Annex D of ISO 6579 of September 2006 (Anonymous, 2006b).
Additional to the prescribed method the participants could use another (own) method.

The results per laboratory were judged following the criteria for 'good performance' as agreed at the CRL-*Salmonella* workshop in 2005 (Mooijman, 2005). One laboratory did not send any results. Three NRLs did not meet these criteria of good performance. However, the same laboratories scored 'good performance' with the same method (Annex D of ISO 6579) when analysing artificially contaminated meat samples in the pilot 'food study' two months earlier. Still the three laboratories were contacted by the CRL-*Salmonella* in January 2007 to ask for any explanation for the deviating results. Furthermore, the possibility was offered to perform some extra analyses. In the follow-up of this study the three laboratories scored 'good performance'.

Discussion
Q: Is it correct that the performance with pig faeces is better than the performances in earlier studies with poultry faeces?
A: Since we do not longer mix the faeces with glycerol, the performances are improved, independent of the the origin of the faeces.
Q: Is it possible to use Diasalm as alternative medium for MSRV?
A: Diasalm is not the prescribed medium as indicated in Annex D of ISO 6579. Alternative methods are allowed if they are validated against the reference method. Here the reference method is MSRV.

2.9 Proposal on design of the interlaboratory comparison study on detection of *Salmonella* – 2007

Kirsten Mooijman, CRL-Salmonella, Bilthoven, the Netherlands

In the fall of 2007, two interlaboratory comparison studies are planned. One study (planned in September 2007) concerns the serological detection of *Salmonella* spp. (see section 3.6). The other study will deal with the bacteriological detection of *Salmonella* spp. After consultation of DG-Sanco, preference is given to a study on the detection of *Salmonella* spp. in a food matrix. This study is

proposed to be organised in November 2007, with a similar set-up as the pilot study organised in fall 2006 (see section 2.7). However, it is proposed to use reference materials with a lower contamination level for this 2007 study. The reason for this proposal is the fact that the last 2-3 years almost all NRLs score 100 % positives with all samples. Since the matrix is not longer mixed with glycerol the results of the interlaboratory comparison studies were very much improved. To obtain more information on the performance of the laboratories and of the method it is more interesting to test low level samples close to the detection limit, beside high level samples which are approximately ten times above the detection limit. It is expected that when low level samples at the detection limit are tested, circa 50 % of the tested samples will be negative. The CRL-*Salmonella* has performed some experiments (and will do some more in the coming months) to find out what the contamination level of the reference materials should become to be close to the detection limit. From the results of these experiments and from the results of the pilot 'food-study' of 2006, the following draft set-up for the study of 2007 is proposed:

- Matrix: minced beef or minced pork (negative for *Salmonella*);
- Reference materials (consisting of capsules) will be added to the matrix: 25 capsules (positives and blank) each added to 10 g matrix;
- Ten control reference materials (no matrix added);
- Contamination level of the serovars in the reference materials:
    - *Salmonella* Typhimurium, circa 5 cfu/capsule (STM5);
    - *Salmonella* Typhimurium, circa 50 cfu/capsule (STM50);
    - *Salmonella* Enteritidis, circa 10 cfu/capsule (SE10);
    - *Salmonella* Enteritidis, circa 100 cfu/capsule (SE100);
- Methods:
    - Prescribed method: ISO 6579: 2002. 'Microbiology of food and animal feeding stuffs – Horizontal method for the detection of Salmonella spp.' The selective enrichment media of this method are: Rappaport-Vassiliadis medium with Soya (RVS) and Muller-Kauffmann tertathionate-novobiocin broth (MKTTn). The selective plating medium is Xylose lysine deoxycholate (XLD) agar.
    - Requested method: (draft) Amendment 1 to ISO 6579: 2007. 'Annex D: Detection of Salmonella spp. in animal faeces and in environmental samples from the primary production stage.' The selective enrichment medium of this method is: Modified semi-solid Rappaport-Vassiliadis medium (MSRV). The selective plating medium is Xylose lysine deoxycholate (XLD) agar.
    - Optional method: Any laboratory (routine) method (for example a PCR method).

For the follow-up of the study in principle the same 'rules' as used for the former detection studies will be followed. This will concern sending of extra samples in case of 'poor performance', soon after the study. For the definition of good performance the agreements made at the workshop of 2005 (Mooijman, 2005) will be followed, unless the results lead to a different approach. Good performance will be defined as follows:

- Positive control capsules (no matrix added):
    - High level: all samples positive;
    - Low level: 1 out of 2 samples may be negative;
- Blank control capsules (no matrix added): all samples negative;
- Blank capsules + matrix: at least 80 % of the samples negative;
- Low level capsules (STM5 and SE10) + matrix: at least 50 % of the samples positive;
- High level capsules (STM50 and SE100) + matrix: at least 80 % of the samples positive.

Discussion
Q: Is it possible to dissolve the capsules at lower temperature to prevent growth during the dissolution step?
A: A temperature of about 37 °C is needed to dissolve the gelatine. Experiments with *Escherichia coli* (a fast growing bacterium) in capsules showed that growth was not visible up to approximately 45 minutes of dissolving time of the capsules. The present protocol describes a reconstitution time of the capsules of 45 minutes, after which the matrix has to be added. Very little to no growth is expected in these first 45 minutes.
Q: Is it possible to organise a ring trial with animal feed?
A: This is not anymore feasible for this year, but could perhaps be organised next year, or later. The CRL-*Salmonella* has contact with researchers in Sweden who are testing several methods for the detection of *Salmonella* in animal feed. The results will be published as soon as all information is available. It would be good to wait for the outcome of this study to have more information on optimal methods for the detection of *Salmonella* in animal feed.

Conclusions from the presentation and the discussion:
All NRLs for *Salmonella* agree with the proposed design of the interlaboratory comparison study on the detection of *Salmonella* in minced meat, to be organised in fall 2007.

2.10 *Salmonella* Dublin in cattle

Dorte Lau Baggesen, Department of Microbiology and Risk Assessment, National Food Institute, Technical University of Denmark

Difficulties in the isolation of *Salmonella* Dublin promote research in relation to improve sensitivity of bacteriological methods. Evaluation of different conventional strategies for isolation of *S.* Dublin from cattle herds showed that the performance of strategies differed significantly among different bacterial strains and sources of faecal material. Detailed investigation of the faecal flora (pathogens and other flora) and the interaction with chemical factors may result in developing an improved method for detection of *S.* Dublin. Overall, the Modified Semi-solid Rappaport Vassiliadis (MSRV)-culture medium had the most reliable detection capability, whereas detection with Selenite Cysteine-broth and Müller-Kauffmann Tetrathionate broth with Novobiocin in combination with both indicative media varied more and rarely reached the same level of detection as MSRV in this experiment. Xylose Lysine Deoxycholate agar was the most reliable indicative media compared to Brilliant Green Agar, especially in combination with MSRV.
Use of an automatic method based on immunomagnetic separation (PATHATRIXTM) identified the same positive samples as the conventional standard method. As the automatic methods were both more expensive and time consuming, the new method would not be a cost-effective alternative to replace the current ISO standard method for *S.* Dublin detection.
A real-time PCR technique, identifying the presence of a *Salmonella* specific gene (the ttr-gene encoding the tetrathionate respiration) showed improved sensitivity compared to the standard culturing method. In a study including 10 animals, four samples, representing three animals, were positive with the traditional bacteriological culture method, while 18 samples, representing 4 animals, were positive with the PCR technique, including those that were positive by traditional culture. In another study, the culturing method found 3 herds and 9 manure tanks positive whereas the PCR method found 7 herds and 15 manure tanks positive in 9 confirmed *S.* Dublin outbreak herds. Diagnosis by real time PCR opens, however, the discussion on the applicability and reliability of diagnostic methods where the

positive results are not verified by isolation of the causable organism. Therefore, further investigations will be performed to optimise the PCR-test. Furthermore, the specificity and usefulness in relation to diagnosis and control of *S.* Dublin in the cattle production will be evaluated.

Following the Danish tradition for control and eradication of infection in animal production, the Danish Cattle Federation (DCF) and the Food and Veterinary Administration have agreed to the following plan for eradication of *S.* Dublin from the Danish cattle population before end of 2014:
Phase 1: Voluntary intervention in cattle herds with technical support from DCF and local advisors (2007-2009). Farmers will be encouraged to use the principles developed in this project.
Phase 2: Sanctions may be imposed on infected herds by differential milk/meat pricing (2010-2012).
Phase 3: Regulatory restrictions may be imposed to control infection (2013-2014).

Several research projects have been established in order to improve knowledge on *S.* Dublin in cattle and support the eradication campaign.

Discussion
Q: Does *Salmonella* Dublin cause problems in milk or other food products?
A: In milk it seems to cause little problems. Presently a surveillance is running on the presence of *Salmonella* Dublin in meat and it seems that the numbers are slowly increasing.

2.11 The importance of environmental monitoring during outbreak investigations

Susanne Surman-Lee, Director Health Protection Agency London, Regional Food, Water & Environmental Microbiology Services Laboratory, Centre for Infections, London, United Kingdom

Investigations into foodborne outbreaks of gastrointestinal disease often fail to reveal the source because there is either insufficient or no food left over for microbiological examination from the batch consumed.
The detection of pathogens in suspected food sources by itself may not indicate the extent of public health risk in a food production / kitchen environment.
In several outbreak investigations we have found that taking additional targeted environmental samples provided substantive evidence of poor personal and kitchen hygiene practices. Such information is essential to ensure appropriate remedial measures are taken e.g. HACCP plans updated; deep cleaning; changing hygiene practices and ensuring adequate training is given. It can also be used to support any enforcement action to be taken.
Several outbreaks were discussed where this supporting evidence indicated poor personal hygiene, cross contamination and poor kitchen hygiene which had a significant impact on the outcome of the investigation.
For example: in one investigation of an outbreak, linked to a bakery, *Salmonella* Enteritidis PT5C (the patient strain) was found in egg mayonnaise and a bagel with egg mayonnaise filling. Other samples revealed other strains of Salmonella, i.e. *Salmonella* Enteritidis PT1 present in salami; *Salmonella* Enteritidis PT 12 in an egg and water glaze and a swab from a toilet door handle which was also contaminated with a *Salmonella* Enteritidis PT 1. The latter showed that there was not only a problem with the product preparation (contaminated egg) but also possible transfer from food handler to salami (or vice versa). Without this supporting environmental evidence appropriate remedial action may not have been taken to ensure there were no further cases from this premises.

Discussion
Q: How to prevent problems in e.g. restaurants?
A: Some countries have national programmes. In the United Kingdom a 'safe food training' exists. However, very often it is also a language problem (e.g. in Chinese restaurants).

2.12 A *Salmonella* Typhimurium outbreak from cheese

Rob de Jonge. National Institute for Public Health, Laboratory for Zoonoses and Environmental Microbiology, Bilthoven, the Netherlands

A regional cluster of an unusual high number of cases with *Salmonella* Typhimurium phagetype 560 (ST560: Dutch phagetyping system; DT7 in the Colindale system) was detected early February 2006. Over 2006 more than 200 laboratory-confirmed cases of ST560 were found. This corresponds to an estimated 3400 cases of ST560 gastroenteritis in the community. In the first six 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 kilos of raw milk per day for the local production of cheese, sold at the farm, as well as regionally and nationally. Cheese production area, manure and dairy cattle of this farm were positive for ST560. However, no *Salmonella* could be found in any dairy product of the farm up to the end of October 2006. In August-September 2006 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. Growth of *Salmonella* in Gouda-type cheeses is unlikely, but survival is very well possible. Closer scrutiny of the cheeses indeed demonstrated ST560, although in very low concentrations (4 cfp/ kg), showing the limited chances of detection in the legally required quantity of 25 grams to be tested. The number of cases dropped considerably after all new, recent and old batches that could be traced from the farm were screened and destroyed if found positive.
Salmonella is well able to survive in hard cheeses. Due to long shelf life, contaminated hard cheeses may therefore cause such prolonged outbreak. Very low numbers of *Salmonella* were seemingly responsible. These low numbers must survive the human stomach in order to cause an infection. Inclusion in fat, adaptation to the low pH cheese environment and attachment to cheese may have contributed to this low infectious dose.

Discussion
Q: Is it allowed to use milk for cheese production in case the herd is positive for *Salmonella*?
A: Not sure about the exact rules, but the problem was that the milk was negative!
Q: Would it not be better to set rules to always pasteurise the milk in case of positive findings in the herd?
A: Indeed this would be better. However, in this case the milk was pasteurised (after a while) and still the cheese was positive for *Salmonella*. This could have been caused by contamination through dirty boots. Hygiene measures are therefore also very important.

3 Tuesday 8 May 2007: day 2 of the workshop

3.1 Introduction NRL from 'new Member State' Romania

Monica Vanghele, Institute for Diagnosis and Animal Health (IDAH), Romania

In Romania there are three NRLs: NRL-*Salmonella* for human strains, NRL-*Salmonella* for food strains and NRL-salmonellosis for animal origin strains. The last one is a main part of the 'Institute for Diagnosis and Animal Health' (IDAH) from Bucharest, the only national state institute for veterinary diagnosis. This institute has 11 departments and 36 NRLs and it is accredited according to EN ISO 17025:2005. Specialists from two departments of IDAH ('Bacteriology Department' and 'Molecular Biology and Genetic Modified Organisms') work for NRL-salmonellosis. The main tasks of the 'Bacteriology Department' are:

- the general bacteriology for investigation of pathological materials from different animal species;
- the technical coordination, the training and the control of the diagnosis activities in the county state of veterinary laboratories for bacteriology;
- the organisation of the interlaboratory comparison tests at national level for bacteriology.

The second department, 'Molecular Biology and Genetic Modified Organisms', executes molecular tests for the current diagnosis of wild and domestic animal species and implements the methods and methodologies recommended by Reference Laboratories of EU in veterinary diagnosis and for the identification of Genetic Modified Organisms (GMOs). The activity of NRL-salmonellosis is composed by the isolation and identification of *Salmonella* spp. in pathological materials from different animal species using the method recommended by CRL-*Salmonella*, the confirmation of *Salmonella* strains isolated in county laboratories, monitoring of the antimicrobial resistance in *Salmonella* strains. Furthermore, the NRL participates in the CRL-*Salmonella* interlaboratory comparison studies on the detection of *Salmonella* spp. with very good results, including Real Time PCR test. The NRL-salmonellosis organises the national intercomparison tests for the detection of *Salmonella* spp. at the national level. In the next future, NRL-salmonellosis from Romania will implement the typing methods for *Salmonella* (serotyping, phagetyping and genotyping) and it will participate in the ring-trials for serotyping and phagetyping organised by CRL-*Salmonella*.
Finally, this year for the first time, the NRL is involved in surveillance on the slaughter pigs sampled in slaughter houses in Romania, a baseline study with Community financial contribution.

3.2 Introduction NRL from 'new Member State' Bulgaria

Aleksander Maramsky, National Diagnostic and Research Veterinary Institute, Sofia, Bulgaria

The presentation included a brief introduction concerning the history and structure of the National Diagnostic & Research Veterinary Medical Institute 'Prof. George Pavlov'- Sofia. The basic part of the presentation was devoted to the National Reference Laboratory "Salmonella" which is a part of the food safety section 'Veterinary and sanitary expertise of products of animal origin for human consumption and raw materials'. Attention was paid to its organisation, tasks and activity. Information was given on the technical basis and methods of investigation and detection of *Salmonella* spp.

Discussion
Q: Is Bulgaria participating in the baseline studies?
A: Probably, but not sure about it.

3.3 Introduction NRL from 'Candidate Country' Former Yugoslav Republic of Macedonia (FYROM)

Pavle Sekulovski, Food Institute at Faculty of Veterinary Medicine, Skopje, Macedonia

The Food Institute is one of the two reference laboratories for food control in the Republic of Macedonia. It was established in 1927 as part of the National Veterinary Institute which was merged with the Faculty of Veterinary Medicine in 2004. At the moment the Faculty of Veterinary Medicine consists of 4 different Institutes, one of them is the Food Institute. The major mission of the Faculty of Veterinary Medicine, besides education, is applied research in veterinary medicine, as well as diagnostic laboratory services. This latter is particularly concentrating on prophylaxis and diagnosis of infectious diseases, including zoonoses, and for safety of food of animal origin. At the same time the Faculty of Veterinary Medicine acts as the Reference Laboratory responsible for the diagnosis of animal infectious diseases and control of food of animal origin and feedstuffs, evaluates the quality of veterinary biology, and provides the expertise service for the Veterinary Administration. In 2006, the Central Veterinary Office designated the Food Institute as National Reference Laboratory for *Salmonella*.
In May 2005, the Faculty of Veterinary medicine has been certified in accordance with ISO 9001 (Anonymous, 2000) Quality Assurance System, by the British Standardisation Institute (BSI). At the moment the Food Institute is implementing ISO 17025 (Anonymous, 2005) and seeking international accreditation by the end of 2007. In March this year the Food Institute successfully participated in a Proficiency testing scheme for *Salmonella* organised by FEPAS.
Presently, the Food Institute consists of four departments: Food Chemistry, Food Microbiology, Contaminants and Residues, and Animal feed. The Scientific staff has a multidisciplinary background: 6 doctors of veterinary medicine, 4 chemical engineers, 1 biochemist, 1 biologist and 1 pharmacologist. Four of them have reached PhD, and another four Master of Science (MSc) level. They have received extensive training in different laboratories in Europe and USA, and participated in many international workshops and scientific conferences. The testing in the laboratories of the Food Institute is carried out by using the methods and protocols recommended by international standardisation bodies like ISO, AOAC as well as some USDA and FDA methods. Equipment used for the testing and analyses is GC, HPLC, GC-MS, ELISA, Automated Microbial Identification System etc.

3.4 Use of serology for monitoring and control of *Salmonella* in pigs

Thomas Blaha, University of Veterinary Medicine Hannover, Field Station for Epidemiology, Bakum, Germany

The argument about 'serology vs. bacteriology'
Several EU countries (Denmark, Germany, United Kingdom, Ireland, the Netherlands and Belgium), have implemented national (or regional) *Salmonella* monitoring and control programmes, either on a

voluntary or a mandatory basis. All of these programmes are, in contrast to the long-standing control programmes in Sweden, Finland and Norway, built on a serological monitoring scheme that semi-quantitatively estimates the intra-herd prevalence of *Salmonella* antibody positive animals. However, there is no unanimous agreement on the usefulness of the serological approach, since two issues are often raised: a) since *Salmonella* antibodies are a human health risk, but the *Salmonella* organisms, measuring *Salmonella* antibodies has nothing to do with food safety; and b) there is no exact correlation between the occurrence of antibodies and the occurrence of *Salmonella* organisms.
The two major reasons for this opinion are: a) the traditional concept of the indirect detection of an infection via serology, where the detection of antibodies in an animal leads directly to a decision over the animal in question (treatment, separation or culling); and b) the idea that the *Salmonella* infected animal is the direct threat to human health so that treating infected animals differently (e.g. use for cooked or otherwise processed products) would be a solution against the introduction of *Salmonella* into the food chain (the real problem, however, is the contamination of the slaughter environment at slaughter, not the meat of the infected animal!).
In the following, the major arguments for an approach that is both population-oriented (estimating the intra-herd prevalence of *Salmonella* antibody carrying animals) and risk-oriented (classifying herds according to their relative probability to carry more or less *Salmonella* into the slaughterhouse environment) are explained in detail.

Using serology as 'classification tool'
The EFSA report 'Opinion on risk assessment and mitigation options of Salmonella in Pigs' (Anonymous, 2006c) differentiates between EU member states with a low *Salmonella* prevalence (Sweden and Finland, and Norway as well), with a medium *Salmonella* prevalence (Denmark) and with a relatively high *Salmonella* prevalence (all other EU Member States). It has been shown by Denmark that it is the most cost-efficient approach to monitor and control *Salmonella* in pigs in high prevalence countries, to first determine the pig herds with the relatively highest intra-herd prevalence (Category III herds). Once these herds are identified, measures to reduce the cross-contamination at slaughter (logistic slaughter), and the intra-herd prevalence of the Category III herds (implementing herd-specific *Salmonella* reduction plans in the Category III herds) can be implemented. In Denmark, this strategy together with increased food hygiene measures at slaughter and meat processing has led to a reduction of the national *Salmonella* load in pork by approximately 50 %. Once such a reduction is achieved, the strategy has to be shifted to address the lower, but existing *Salmonella* load of all Category II and Category I herds, which then will justify to also switch to a bacteriological approach of monitoring the pig herds (comparable to the current monitoring in Sweden, Finland and Norway.

In so far, the serological monitoring of pig herds to estimate the intra-herd prevalence in a national pig population is not in general the approach of choice, but in countries with a higher *Salmonella* load in the pork chain, it is a cost-effective tool to identify the herds that pose the highest risk of introducing *Salmonella* into the pork production chain, which, in turn, is the basis for targeted *Salmonella* reduction measures both at farm level and at slaughter.

Discussion
Q: The number of pigs in Category III (high prevalence) is only a relatively small proportion. The penalties should be quite severe to lower the numbers in Category III.
A: Not all Member Sates have already started a national programme. In most countries the farmers participate on a voluntary basis. It is important that all farmers should be part of the programme and take samples.
Q: Is this strategy of using different categories also possible for other animals?

A: The basic principles can be the same for other animals. It may depend on the prevalence of *Salmonella*. The production cycles in poultry are in general shorter than for pigs and therefore it may be easier to reduce the prevalence of *Salmonella* in poultry than in pigs.
Q: This step-by-step approach in the reduction of the prevalence of *Salmonella* sounds realistic and it has proven to work. However, the European Commission seems to think different about this aspect and follow the prevalence by bacteriological analyses of lymph nodes. Is there a correlation between the two approaches?
A: It does correlates, but it is not a 1:1 correlation.
Q: Is there a correlation between bacteriology and serology?
A: There is a correlation, but you can only find it when you analyse large numbers. Serology can be useful for identification of single herds. If pigs are infected during transport, the infection can reach the lymph nodes in only two hours time. Resulting in positive lymph nodes when tested with bacteriology, but negative when tested with serology as antibodies are not yet present. For the baseline study it was advised to perform bacteriological analyses of lymph nodes, as this can give an identification at national level.
Q: If you find antibodies in a herd, should you check it with bacteriology to identify the serovar?
A: Yes and no. When the monitoring is started it is good to do so to obtain more information about the *Salmonella* serovars circulating at national level. However, it is not absolutely necessary. The primary goal is to reduce *Salmonella* in the herds. For this it is important to identify the farm specific infection, to find out where the infection came from (e.g. environment, feed, water, rodents, etc) and to increase the hygiene. The approach for the reduction of *Salmonella* may be different per farm. It may be too expensive to always perform bacteriology.

3.5 International ring trials for poultry serology organised by the Animal Health Service

Sjaak de Wit, Animal Health Service, Deventer, the Netherlands

The Animal Health Service started to organise ring trials for poultry antibody detection in 2000. At present 7 ring trials (*Salmonella*, Avian Influenza, Newcastle Disease, infectious bronchitis virus, *Mycoplasma gallisepticum* and *synoviae* and Gumboro virus) are organised annually, in which between 40 till 125 laboratories from Africa, Asia, Europe, Central and South America are participating. The sera that are used are mostly raised in SPF birds that are vaccinated or infected with reference virus or bacteria strains. Field sera can also be included. Each ring trial has negative, weak positive and strong positive lyophilised sera. The sera are tested twice in different test runs. The participating laboratories can use any test system they want, such as the virus neutralization tests (VNT), hemagglutination inhibition test (HI-test), enzyme-linked immunosorbant assays (ELISA), rapid plate agglutination test (RPA) and agar gel precipitation tests (AGPT). These global ring trials provide a large amount of data on variation within- and between laboratories and test systems used worldwide.

Discussion
Q: When comparing results between laboratories, are the differences in incubation times (long and short incubation) also taken into account?
A: In case of poultry the incubation time is in general the same (circa 1 hour). This may be different for pigs.

3.6 An attempt to come to harmonised serological methods

Petra Berk, CRL-Salmonella, Bilthoven, the Netherlands

3.6.1 Duplicate analysis of meat juice samples collected during the baseline study on slaughter pigs

The baseline study on slaughter pigs runs from October 2006 to October 2007. New in this study, in comparison with previous baseline studies, is the possibility to use a serological method in addition to the bacteriological method for the detection of *Salmonella* in pigs.
Ten NRLs (Cyprus, Denmark, France, Germany, Ireland, Lithuania, the Netherlands, Slovenia, Sweden and the United Kingdom) participate in this serology part of the baseline study. The NRLs are allowed to use their own serological methods. Sixty meat juice samples have to be sent to the CRL-*Salmonella* together with the serological results and the bacteriological results. The CRL-*Salmonella* will analyse the samples with one single serological method. At this point, 94 samples were received from 5 NRLs. Three NRLs used the Salmotype PigScreen (Labor Diagnostik), 1 NRL used the HerdCheck Swine Salmonella (Idexx Laboratories) and 1 NRL used Vet Sign Salmonella Elisa test (Guildhay). In general the CRL-*Salmonella* found less positive results than the NRLs. When comparing different methods the HerdCheck SS found less positive results than the Salmotype PigScreen. The results from the bacteriology and serology were compared to each other. Most samples gave the same results for both bacteriology and serology, however there were also some samples negative with bacteriology and positive with serology and vice versa.

3.6.2 Interlaboratory comparison study on serological methods

In the fall of 2007 an interlaboratory comparison study will be organised on serological methods. The participants will be the same (10) NRLs who participated in the serology part of the baseline study. Forty sera will be sent to the NRLs by the CRL-*Salmonella*. The NRLs will have to test these sera with their own serological method. The sera will have different levels and will include negative controls. At this point the CRL-*Salmonella* has sera available from infection studies with *S.* Tyhpimurium, *S.* Brandenburg, *S.* Livingstone, *S.* Goldcoast and *S.* Panama. If the participating NRLs can supply the NRLs with sera they can also be included in the study.

Discussion
Q: How to compare results (baseline study) if different cut-off values are used?
A: It is indeed difficult to compare results if the information given is only ‘positive or negative for *Salmonella*’. It is therefore important that all NRLs should also indicate the OD% results. The most interesting samples will be the ones around the cut-off value. All serological tests are able to detect real low and high levels of *Salmonella*. More difficult are the samples around the cut-off value.

3.7 Work programme CRL-*Salmonella* second half 2007, first half 2008 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 2007 and for early 2008.

Protocol of DG-Sanco for participation of NRLs
In February 2007, DG-Sanco introduced a draft protocol in which 'stringent rules' are indicated for the management, by a CRL, in case of underperformance/lack of collaboration of NRLs. 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 summary, the CRL has to take the following actions:

- In case of underperformance (like failure in proficiency test):
  - The CRL should contact the NRL and provide assistance;
  - Repetition of the comparative test (if possible). If the results of this extra test show good performance, no further actions need to be taken. If the results show again poor performance, the Commission (DG-Sanco) shall be informed. The Commission will subsequently inform the competent authority of the Member State.
- In case of lack of collaboration (like not participating in a proficiency test or a workshop):
  - The CRL should contact the NRL for the justification of the lack of collaboration. This information shall be reported to the Commission;
  - At repetitive lack of response of the NRL, the CRL shall inform the Commission. Next the Commission will contact the competent authority of the Member State.

Interlaboratory comparison studies
As indicated in earlier presentations, two interlaboratory comparison studies are planned in fall 2007. The first study will concern the detection of *Salmonella* spp. with serological methods. This study is limited to the ten NRLs performing serological analyses of meat juice samples of the baseline study on slaughter pigs.
The second study will concern the detection of *Salmonella* spp. in a food matrix.
Another interlaboratory comparison study on typing of *Salmonella* spp. is planned in 2008.

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):
  - Finalising Annex D of ISO 6579, which will most probably result in a final ISO/EN publication by the end of 2007;
  - Preparation of a protocol for enumeration of *Salmonella* spp. based on a microwell MPN technique called mini-MSRV (Fravalo et al., 2003). This protocol will be sent to the members of ISO/TC34/SC9 (microbiology of food and feeding stuffs) and to the NRLs for *Salmonella* for testing. The results will be summarised by the CRL-*Salmonella* and presented at the meeting of ISO/TC34/SC9 and at the CRL-*Salmonella* workshop in 2008;

  - Participation in the working group on proficiency testing. This working group is preparing a protocol for the organisation of microbiological proficiency tests;
  - Participation in the working group on validation of microbiological methods. This working group is revising ISO 16140 (Anonymous, 2003);
  - Working out the activities for the organisation of a validation study of Annex D of ISO 6579 in relation to the CEN mandate. In 2006 the EC has sent a mandate to CEN/TC275/WG6 (microbiology of food and feeding stuffs) for the validation of 15 microbiological methods. CRL-*Salmonella* has sent a proposal to CEN to become projectleader for the validation of Annex D. The project proposals for the validation of all 15 methods are presently under discussion in CEN and still need to be sent to the EC.
- Use of serological methods and molecular methods (continuation of the work started by the end of 2006, early 2007);
- Quality assurance activities in relation with the baseline studies:
  - Duplicate serotyping of typable and non-typable isolates;
  - Duplicate serological analyses of meat juice samples.

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.
The CRL-*Salmonella* website is amended and will soon be on line. The address of the site will remain the same (www.rivm.nl/crlsalmonella).
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.

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

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Annex 1 Participants

European Food Safety Authority (EFSA)	Frank Boelaert
CRL – *Salmonella*	Kirsten Mooijman Petra Berk Angelina Kuijpers Christiaan Veenman Henny Maas
Guest speaker (United Kingdom)	Elizabeth de Pinna (HPA, London) Susanne Surman-Lee (HPA, London)
Guest speaker (Germany)	Thomas Blaha (University of Veterinary Medicine, Hannover, Field Station for Epidemiology, Bakum)
Guest speaker (the Netherlands)	Rob de Jonge (RIVM, Bilthoven) Sjaak de Wit (GD, Deventer) Jaap Wagenaar (University of Utrecht)

National Reference Laboratories for *Salmonella*

AUSTRIA	Heimo Lassnig
BELGIUM	Hein Imberechts Katelijne Dierick
BULGARIA	Aleksander Maramsky
CYPRUS	Economides Constantinos
CZECH REPUBLIC	Iva Bernardyova
DENMARK	Dorte Lau Baggesen Steen Nordentoft
ESTONIA	Age Kärssin
FINLAND	Henry Kuronen
FYROM (Macedonia Republic)	Pavle Sekulovski
FRANCE	Marylene Bohnert
GERMANY	Reiner Helmuth
GREECE	Maria Passioutou-Gavala
HUNGARY	Erzsebet Andrian
IRELAND	Montserrat Gutierrez
ITALY	Lisa Barco
LATVIA	Andra Utinane
LITHUANIA	Tatjana Kutyriova
LUXEMBOURG	Joseph Schon
MALTA	Gertrude Gat-Lanzon
NORTHERN IRELAND	Sam Strain

NETHERLANDS	Arjen van de Giessen
	Wilfrid van Pelt
NORWAY	Bjarne Bergsjo
POLAND	Andrzej Hoszowski
	Remigiusz Pomykala
PORTUGAL	Alice Amado
ROMANIA	Luminitra Monica Vanghele
	Maria Ionescu
SLOVAK REPUBLIC	Milan Sasik
SLOVENIA	Vojka Bole-Hribovsek
SPAIN	Christina de Frutos Escobar
SWEDEN	Erik Eriksson
UNITED KINGDOM	Robert Davies

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Annex 2 Programme of the workshop

Programme of the CRL-*Salmonella* workshop XII
7 and 8 May 2007, 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 3 May 2007.

Sunday 6 May 2007

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 payed 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 7 May 2007

Morning chair: Arjen van de Giessen

9.00 - 9.15	Opening and introduction (Kirsten Mooijman)
9.15 - 9.45	EFSA report on trends and sources of Zoonoses in Europe (Frank Boelaert)
9.45 - 10.15	Baseline studies (Frank Boelaert)
10.15 - 10.45	WHO Global Salm-Surv programme (Jaap Wagenaar)
10.45 - 11.15	*Coffee/tea*
11.15 - 11.45	Results typing study XII - 2007 : phagetyping (Elizabeth de Pinna)
11.45 - 12.15	Results typing study XII - 2007: serotyping. Proposal typing study 2008 (Petra Berk)
12.15 - 13.45	*Lunch*

Afternoon chair: Kirsten Mooijman

13.45 - 14.15	Results interlaboratory comparison study on bacteriological detection of *Salmonella* – FOOD I – 2006 (Angelina Kuijpers)
14.15 - 14.45	Results interlaboratory comparison study on bacteriological detection of *Salmonella* – FAECES X – 2006 (Angelina Kuijpers)
14.45 – 15.00	Proposal on design interlaboratory comparison study on detection of *Salmonella* – 2007 (Kirsten Mooijman)
15.00 - 15.30	*Coffee/tea*
15.30 - 16.00	*Salmonella* Dublin in cattle (Dorte Lau Baggesen)
16.00 - 16.30	The importance of environmental monitoring during outbreak investigations (Susanne Surman-Lee)
16.30 - 17.00	A *Salmonella* Typhimurium outbreak from cheese (Rob de Jonge)
17.30 and onwards	Social programme and dinner

Tuesday 8 May

Chair: Kirsten Mooijman

9.00 - 9.15	Introduction NRL from 'New Member State' Romania (Monica Vanghele)
9.15 - 9.30	Introduction NRL from 'New Member State' Bulgaria (Aleksander Maramsky)
9.30 – 9.45	Introduction NRL from 'Candidate Country' Former Yugoslav Republic of Macedonia (FYROM) (Pavle Sekulovski)
9.45 - 10.15	Use of serology for monitoring and control of *Salmonella* in pigs (Thomas Blaha)
10.15 - 10.45	International ring trials for poultry serology organised by the Animal Health Service (Sjaak de Wit)
10.45 - 11.15	*Coffee/tea*
11.15 – 11.45	An attempt to come to harmonised serological methods: - 'Duplicate analyses' of meat juice samples collected during the baseline study on fattening pigs (Petra Berk) - Interlaboratory comparison study on serological methods, fall 2007 (Petra Berk)
11.45 – 12.15	Work programme CRL-*Salmonella* second half 2007, first half 2008 and closure (Kirsten Mooijman)
12.15 - 13.45	*Lunch*
14.00	Departure to train station Bilthoven