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The eleventh CRL-*Salmonella* workshop
9 May 2006, Saint Malo, France

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Abstract

The eleventh CRL-*Salmonella* workshop

The eleventh workshop organised by the Community Reference Laboratory for *Salmonella* (CRL-*Salmonella*) was held in Saint Malo, France on 9 May 2006. Participants included representatives of the National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) of the Member States of the European Union and of the European Commission. Presentations were given by representatives of the European Commission and the CRL-*Salmonella*. Subjects discussed were European legislation on *Salmonella* criteria for food, results of the EU baseline study to determine the prevalence of *Salmonella* in laying hens, future baseline studies for determining the prevalence of *Salmonella* in turkeys and fattening pigs, standardisation of methods in ISO and CEN, research activities of CRL-*Salmonella*, intercomparison studies organised by CRL-*Salmonella* (2005 and 2006) and the CRL-*Salmonella* work programme for 2006 and 2007. In the presentations of the baseline studies (past and future) it was made clear that the EU Member States will have to make large efforts in measuring the prevalence of *Salmonella* and reducing the bacterium in poultry and in pigs. Representatives of CRL-*Salmonella* announced that two interlaboratory comparison studies on the detection of *Salmonella* spp. will be organised in the second half of 2006. One study will include a food matrix and the other study will include animal faeces. A discussion on the choice of the matrices and the methods to be used evolved from the presentation.

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

Rapport in het kort

De elfde CRL-*Salmonella* workshop

De elfde workshop georganiseerd door het Communautair Referentie Laboratorium voor *Salmonella* (CRL-*Salmonella*) werd gehouden op 9 mei 2006 in Saint Malo, Frankrijk. Deelnemers waren vertegenwoordigers van de Nationale Referentie Laboratoria voor *Salmonella* (NRLs-*Salmonella*) van de lidstaten van de Europese Unie alsmede van de Europese Commissie. Presentaties werden gegeven door vertegenwoordigers van de Europese Commissie en van CRL-*Salmonella*. Onderwerpen die bediscussieerd werden waren: Europese wetgeving op het gebied van *Salmonella* criteria voor levensmiddelen, resultaten van de basisstudies in de EU voor het vaststellen van de prevalentie van *Salmonella* bij leghennen, toekomstige basisstudies voor het vaststellen van de prevalentie van *Salmonella* bij kalkoenen en mestvarkens, standaardisatie van methoden in ISO en CEN, onderzoeksactiviteiten van CRL-*Salmonella*, ringonderzoeken georganiseerd door CRL-*Salmonella* (2005 en 2006) en het werkprogramma van CRL-*Salmonella* voor 2006 en 2007. De presentaties van de basisstudies (verleden en toekomst) maakten duidelijk dat de EU-lidstaten grote krachtinspanningen moeten leveren voor het vaststellen van de prevalentie van *Salmonella* en in het reduceren van de bacterie in pluimvee en varkens. Door het CRL-*Salmonella* werd uitgelegd dat er twee ringonderzoeken in de tweede helft van 2006 georganiseerd zullen worden, welke betrekking hebben op de detectie van *Salmonella.* Eén studie zal met levensmiddelen als matrix uitgevoerd worden en de andere studie zal betrekking hebben op dierlijke feces. De presentatie resulteerde in een discussie over de keuze van de matrices en over de te gebruiken methoden.

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

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Summary

On 9 May 2006, the Community Reference Laboratory for *Salmonella* (CRL-*Salmonella*) organised a workshop in Saint Malo, France. Representatives of the National Reference Laboratories (NRLs-*Salmonella*) were present, as well as representatives of the European Commission (DG-Sanco). A total of 37 participants were present at the workshop.

The programme of the workshop consisted of two parts.
During the morning session, presentations were given on the results of the EU baseline study to determine the prevalence of *Salmonella* in laying hens and on the future baseline studies for determining the prevalence of *Salmonella* in turkeys and fattening pigs. Also a presentation was given on the European legislation on *Salmonella* criteria for food.
During the afternoon session, a presentation was given on standardisation activities in ISO and CEN and results of the interlaboratory comparison studies of 2005 and 2006 were presented as well as the designs of future studies were discussed. The workshop was closed with a presentation on the work programme of CRL-*Salmonella* for 2006 and 2007.

In the presentations of the baseline studies (past and future) it was made clear that the EU Member States will have to make large efforts in measuring the prevalence of *Salmonella* and reducing the bacterium in poultry and in pigs.
The presentation on the interlaboratory comparison studies made clear that two interlaboratory comparison studies on the detection of *Salmonella* spp. will be organised in the second half of 2006. One study will include a food matrix and the second study will include animal faeces. A discussion on the choice of the matrices and the methods to be used evolved from the presentation. It was concluded that the ‘food’ study will be organised in September 2006 with minced meat as matrix of choice and ISO 6579 as prescribed method and Annex D of ISO 6579 as optional method. The second study will be organised in November 2006 with pig faeces as matrix of choice and Annex D of ISO 6579 as prescribed method.

The full presentations given at the workshop can be found at:
http://www.rivm.nl/crlsalmonella/workshop/index.html

List of abbreviations

A	Answer
BPW	Buffered Peptone Water
CEN	European Committee for Standardization
cfp	colony forming particles
CRL	Community Reference Laboratory
DG-Sanco	Directorate General on Health and Consumer Protection (Santé et Protection des Consommateurs)
EC	European Commission
EFSA	European Food Safety Authority
ENL	EnterNet Laboratory
EU	European Union
HPA	Health Protection Agency
ISO	International Organization for Standardization
MKTTn	Mueller Kauffmann Tetrathionate novobiocin broth
MS(s)	Member State(s)
MSRV	Modified Semi-solid Rappaport Vassiliadis
NRL	National Reference Laboratory
Q	Question
RIVM	National Institute for Public Health and the Environment
SC	Sub Committee
SE	*Salmonella* Enteritidis
SPan	*Salmonella* Panama
STM	*Salmonella* Typhimurium
TC	Technical Committee
UK	United Kingdom
XLD	Xylose Lysine Deoxycholate agar
WG	Working Group
WHO	World Health Organization

1. Introduction

In this report abstracts of the presentations given at the CRL-*Salmonella* workshop of 2006 are presented as well as a summary of the discussion that followed the presentation. The full presentations itself are not provided within this report, but can be found at the CRL-*Salmonella* website: http://www.rivm.nl/crlsalmonella/workshop/index.html

The lay-out of this report is according to the programme of the workshop.

2. CRL-*Salmonella* workshop, 9 May 2006

2.1 Opening and introduction

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

Kirsten Mooijman, head of CRL-*Salmonella*, opened the 11th workshop of CRL-*Salmonella* welcoming all participants in Saint-Malo, France on 9 May 2006. She memorised the turbulent year of the CRL, especially because of the sudden death of CRL staff member Hans Korver. A moment of silence was shared in his memory. Due to this tragic situation, a temporary replacement was sought and found in Petra Berk. Since 8 May 2006 a more permanent replacement was found in the new technician Angelina Kuijpers.
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 Baseline study in laying hens

2.2.1 *Salmonella* observed prevalence in large-scale laying hen holdings in the EU

Frank Boelaert and Pia Mäkelä, EFSA, Parma, Italy

Pursuant to Regulation (EC) No 2160/2003 on the control of *Salmonella* and other specified zoonotic agents, a Community target must be established for reducing the prevalence of *Salmonella* in populations of laying hens. In order to set that target, comparable data on the prevalence of *Salmonella* spp. in populations of laying hens in Member States (MS) should be available. Such information was not available and therefore a baseline study was carried out in order to estimate that prevalence. The Commission coordinated this EU-wide study and EFSA analysed the collected data.
The objectives, the sampling frame, the diagnostic testing methods as well as the collection of data, evaluation and reporting, and the timelines of this study were specified in Commission Decision 2004/665/EC. The primary objective was to estimate the *Salmonella* holding observed prevalence at the global EU-level as well as for each Member State

specifically. The study was conducted on commercial large-scale laying hen holdings with at least 1000 laying hens in the flock. Samples were taken from flocks of laying hens during the last nine weeks of their production. One flock per each holding was sampled by taking five faeces samples and two dust samples. The study was carried out in all Member States, and the sampling of the holdings took place during the period of 1 October 2004 to 30 September 2005. Norway participated in the study on a voluntary basis.
On 7 April 2006 EFSA submitted a preliminary report on the analyses to the Commission. In total 5317 laying hen holdings in the EU were included in this study. A clean dataset, comprising 4797 holdings, was mainly used to analyse the results. The results show that at the global EU-level 20.3 % of the large-scale laying hen holdings are bacteriological positive for *Salmonella* Enteritidis and/or *Salmonella* Typhimurium. The Member States'-specific *Salmonella* Enteritidis – *Salmonella* Typhimurium holding observed prevalence estimates varied largely, from a minimum of 0 % to a maximum of 62.5 %. The holding observed prevalence for any *Salmonella* was, in general, higher. At the global EU-level the presence of any *Salmonella* spp. was detected in 30.7 % of the large-scale laying hen holdings. The range of the Member States'-specific *Salmonella* spp. holding observed prevalence was also wide, from a minimum of 0 % to a maximum of 79.5 %. The number of positive samples in a holding varied between 1 and 7, and an important proportion of the holdings was found positive on the basis of only one or two positive samples. The five most frequently isolated *Salmonella* serovars in the EU were, in descending order: *Salmonella* Enteritidis, *Salmonella* Infantis, *Salmonella* Typhimurium, *Salmonella* Mbandaka and *Salmonella* Livingstone.

Discussion

Q: When will the report be published?
A: The final report is delayed to circa October 2006. EFSA is presently performing a new data revision. However, no big changes are expected. EFSA is considering to publish the data on their website in June/July 2006.
Q: Why does it take so long before having the data complete?
A: The laying hens study was the first baseline study and did not go complete smoothly. Several problems existed with the reporting system. For instance, data were not reported correctly, which needed to be checked and if necessary cleaned-up afterwards.
Q: Will the results of the phagetyping and of the antimicrobial resistance testing also be reported?
A: These data were not yet evaluated. A discussion is going on whether these variables give useful information or not when they are analysed on a voluntary basis.
Q: Have the results of this baseline study been compared with the zoonoses reports?
A: A preliminary comparison has been made. However, the sampling scheme of the baseline study has been very sensitive and may differ of the schemes as used for gathering data for the zoonoses reports. Comparing prevalences in different studies is difficult.

2.2.2 Results QA serotyping

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

From 1 October 2004 to 1 October 2005 the baseline study on the prevalence of *Salmonella* in laying flocks of *Gallus gallus* was performed. According to the technical specifications of the baseline study, at least one isolate from each positive sample had to be serotyped by the NRL-*Salmonella* following the Kaufmann-White scheme. For quality assurance of the serotyping, each NRL had to send at maximum of 16 typable strains and 16 non-typable isolates to the CRL-*Salmonella*. In accordance with this, the CRL received a total of 302 typable strains and 67 non-typable strains. Fourteen strains of the 302 typable strains were serotyped differently by the CRL. Forty-four of the 67 non-typable strains were further identified to serovar names by the CRL. Seventeen of the latter 'non-typables' concerned *Salmonella* Enteritidis PT7. The results of the quality assurance were regularly reported to the NRLs. At the end of the baseline study the NRLs were asked whether they used the CRL results to correct reported results. The majority of the NRLs replied either yes or indicated that they had reported the results to the National authority. However, the NRLs did not always know whether this authority had communicated the results to the European Commission or not. All results on the quality assurance were recently reported to DG-Sanco and to the EFSA. A report in which country names were replaced by labcodes was sent to the NRLs a week before the workshop.

Discussion

Remark: *Salmonella* Enteritidis PT7 is generally known as rough.
Q: Is the method used for typing rough strains the same as used at the WHO reference laboratory in Paris?
A: Not sure, there has been no contact with Paris about the procedure used.
Q: Would there come a standard procedure for dealing with rough strains?
A: Some experiments have been performed by the CRL. More information will be given in the next presentation.

2.2.3 Dealing with rough strains

Anjo Verbruggen and Henny Maas, CRL-Salmonella, Bilthoven, the Netherlands

During the baseline study, the CRL received 67 non-typable strains. Non-typable strains include (among others) 'rough' strains, mucoid strains (which make a mucus layer), strains which can not be (fully) typed due to lack of a complete set of sera. Of the 67 non-typable

strains received, 18 were marked ‘Rough’. These latter strains were studied further at the CRL-*Salmonella*.
The strains were inoculated from the original tubes in diluted Nutrient Broth (1 part Nutrient broth mixed with 3 parts aquadest; NBaqua) and incubated at 37 °C for 2-3 days. From the upper part of the NBaqua tubes, all strains were again inoculated in NBaqua (2nd inoculation) and on a Columbia-agar plate (CAS). The latter NBaqua tubes were incubated overnight in a shaking waterbath at 37 °C. The CAS plate was incubated overnight in a 37 °C incubator. The CAS plate showed rough and smooth colonies. An H-suspension was created by treating the NBaqua tubes overnight with formaldehyde (end concentration 0.5%). By heating a part of the formaldehyde-treated NBaqua suspension for 45 minutes in a 100 °C water bath, the O-suspension was created.
The H-suspensions were tested with H-agglutination mixed antisera. This test is performed in small glass tubes with antisera originally used for slide agglutination: HMA, HMB, HMC and HMD. The tubes, each containing an antiserum, were inoculated with the H-suspension, incubated at 50 °C for 2 hours and read for agglutination.
The antisera used for the O-agglutination test also originated from the slide agglutination test: OMA, OMB and OMC. The tubes were inoculated with the O-suspension, incubated at 50 °C overnight and read for agglutination.
From 8 of the 18 rough strains, the O-antigen could be determined and of all strains at least 1 H-phase. Disadvantages of this method are:

- The test is very time-consuming;
- Many antisera are needed.

Further research for typing of the rough strains is recommended (for example O-antigen, U-tube passage, testing second phase etcetera).

Discussion

Q: What will be the future procedure for dealing with rough strains isolated from, for instance, a baseline study?
A: There is no standard procedure yet. At the CRL some further experiments will be done to try to develop a ‘standard’ procedure.
Q: How should the NRLs deal with results reported to the EC or to EFSA when from the quality assurance a different result is found?
A: The Commission has been consulted and it has indicated that reports should be updated in order to contain the most correct/specific information if such information becomes available before the deadline set for reporting.
Q: What is the meaning of *Salmonella* rough strains? Is it still infectious to humans?
A: A *Salmonella* strain will become rough to protect itself against adverse conditions. Rough strains are therefore often found in the environment (e.g. dust and spices). However, rough and smooth isolates have also been isolated from a human patient. At optimal conditions a

rough strain may become smooth again. Rough strains can cause infections in humans and are therefore equally important as smooth strains.

2.2.4 Discussion/ questions/ evaluation

Arjen van de Giessen, CRL-Salmonella, Bilthoven, the Netherlands

A general mentioned problem for the baseline studies was the time constraint. The time frame for the baseline studies is laid down in EU legislation and is very strict. Several Member States need more time to organise everything and would prefer more flexibility with the deadlines. However, the European Commission does not seem to allow more flexible deadlines.
Another problem which was mentioned was the sampling of dust. Samplers are not always able to collect the right amount of dust for analyses. In the UK this was initially also a problem. However, after further explanation about the possible places where dust generally will accumulate, the samplers were in most cases able to collect more dust. In some cases where not sufficient dust was available, extra boot swabs were taken. Some more guidance on this aspect from the European Commission would be welcome.

2.3 Future baseline studies: Sampling and analytical aspects

Frank Boelaert and Pia Mäkelä , EFSA, Parma, Italy

Regulation (EC) No 2160/2003 on the control of *Salmonella* and other specified zoonotic agents provides for the setting of Community targets for reducing the prevalence of *Salmonella* serovars with public health significance in several animal populations. The animal populations specified in Annex I of the regulation are: breeding flocks of *Gallus gallus*, laying hens, broilers, turkeys, herds of slaughter pigs, and breeding herds of pigs. The establishment of reduction targets requires first comparable data on the prevalence of *Salmonella* to be available in those animal populations. To this end, European Union (EU)-wide baseline studies are carried out or planned.
The primary objective of these baseline studies is to estimate the prevalence of *Salmonella* and other specified zoonotic agents, at the global EU-level as well as for each Member State (MS) specifically. A first pilot baseline study was carried out in 2004-2005 in laying hens on the basis of which results a target is currently discussed at the Community level. A second baseline study is currently ongoing in broilers. Two other baseline studies - in fattening pigs and in turkeys - are to be conducted from 1 October 2006 to 30 September 2007.
The fattening pigs baseline study that is currently proposed and discussed at the Community-level, is based on Annex III ‘Baseline study on the prevalence of *Salmonella* in fattening pigs

in the EU' of the Opinion 'Risk assessment and mitigation options of Salmonella in pig production' of EFSA's Scientific Panel on Biological Hazards. The objective is to estimate the prevalence of *Salmonella* infection in fattening pigs slaughtered in MSs of the EU and in pork carcasses produced at slaughterhouses in the MSs. Every MS would sample about 2400 carcasses. From each selected carcase at least 5 lymph nodes in the ileocaecal regions and swab samples from the carcass would be taken. The detection method recommended by the Community Reference Laboratory for *Salmonella* would be used (a modification of ISO 6579 (2002)). All strains isolated and confirmed as *Salmonella* spp. are proposed to be serotyped according to the Kaufmann-White scheme. For quality assurance, 16 typable strains and 16 non-typable isolates would be sent to the CRL. All isolates of *Salmonella* Typhimurium and *Salmonella* Enteritidis should be phagetyped by methods described by WHO reference centre for phagetyping of *Salmonella* of the Health Protection Agency, Colindale, UK. For epidemiological purposes, at least one isolate per serovar per month should be tested for anti-microbial susceptibility.
The proposed turkeys baseline study aims at estimating the prevalence of *Salmonella* spp. in flocks/holdings of turkeys in the MSs of the EU. The sampling frame includes fattening turkeys as well as breeding turkeys. In total some 4,000 flocks or more could be sampled at the Community-level. Five pooled faeces samples in each flock would be taken. The detection method, the serotyping, phage typing and antimicrobial susceptibility testing would be covered by an analogous protocol of the other baseline studies.
Another future baseline study that is currently discussed at the Community-level is *Campylobacter* in broiler flocks. Moreover, proposals for *Salmonella* and *Campylobacter* in broiler meat as well as *Salmonella* in breeding pigs are currently drafted.

Discussion
Q: When are the protocols for the baseline studies of fattening pigs and of turkeys expected to be finished?
A: Hopefully they will be finished before the summer break.
Q: Why are immunological methods not involved in the pigs study?
A: This was chosen as it is important to obtain the isolate, which is possible with bacteriological detection methods but not with immunological methods. Furthermore, the prescribed bacteriological method has been evaluated in all MSs by using it in earlier studies. For immunological methods no standard procedure is available yet.
Q: Will it still be possible to use immunological methods in the future monitoring programmes?
A: It is proposed to the European Commission to apply both methods (bacteriological and immunological) during the baseline study on a voluntary basis and to give some resources for the laboratories willing to do so. In this way a comparison of both types of methods will be

possible. After this it will be evaluated whether a further validation of one or more immunological methods (organised through CRL-*Salmonella*) is possible.
Q: What is the public health relevance of *Salmonella* in pigs?
A: This may be less than in case of *Salmonella* in laying hens, but still there may be a risk. Some other types than *Salmonella* Typhimurium can cause from time to time some problems in humans.
Q: In the (draft) technical specifications of the baseline study on turkeys it is prescribed to collect faecal samples. Are boot swabs not allowed?
A: Here boot swabs are intended. Using boot swabs is a tool to gather faeces.
Q: Will the new CRL on antibiotic resistance be involved in the studies?
A: Not yet, but may be in the future.

2.4 *Salmonella* criteria for foodstuffs

Maija Hatakka, European Commission, Brussels, Belgium

Commission Regulation (EC) No 2073/2005 of 22 December 2005 on microbiological criteria for foodstuffs, which is an implementing measure of the Hygiene Package, entered into force from 11 January 2006.
The main objectives of the Regulation are to ensure a high level of consumer protection with regard to food safety and to harmonise the microbiological criteria in the EU. Food business operators have to ensure that their products are in compliance with the criteria set down in the Regulation. Therefore, food business operators must be aware of the microbiological risks associated with their products and on case-by-case basis evaluate the need of sampling and testing.

Two types of criteria have been set down in the new Regulation:

- ***A food safety criterion*** defining the safety of a product or a batch. This criterion applies to products placed on the market. If the criterion is not met the product/batch must be withdrawn from the market.
- ***A process hygiene criterion*** indicating the correct functioning of the manufacturing process. This criterion applies during manufacturing process, but not to products placed on the market. If the criterion is not met, improvement of production hygiene and possibly the selection of raw material is required.

Salmonella process hygiene criteria have been laid down for carcases. These provisions permit a certain tolerance for *Salmonella* depending on animal species. Results of 50 samples taken during 10 consecutive weeks are evaluated. The results are still considered satisfactory

when *Salmonella* is detected in a maximum of 2 out of 50 samples taken from carcasses of cattle, sheep, goat and horses, 5 out of 50 samples taken from carcases of pigs and 7 out of 50 samples taken from carcases of broilers and turkeys. The use of lower c-values than 2, 5 or 7 for carcasses is possible in Member States, where the prevalence of *Salmonella* is low. These criteria shall be revised in the light of changes observed in *Salmonella* prevalence.

Salmonella food safety criteria have been laid down for the following food categories including certain ready-to-eat products and for other products considered to pose a risk in relation to *Salmonella*: minced meat and meat preparations, mechanically separated meat, certain meat products, certain dairy products, egg products, ready-to-eat foods containing raw eggs, cooked crustaceans and molluscan shellfish, live bivalve molluscs, sprouted seeds, pre-cut fruit and vegetables, unpasteurised fruit vegetable juices, dried infant formulae. The result is unsatisfactory if *Salmonella* is detected in any of the 5 samples tested.

Analytical reference methods have been set down in Regulation (EC) No 2073/2005, which means that the limits for micro-organisms are fixed with the reference method. Internationally recognised horizontal methods, such as CEN and ISO methods when available, have been selected as reference methods. The Regulation provides flexibility for the food business operator to choose methods for in-house control purposes. The use of alternative proprietary methods (rapid methods) is acceptable when the methods are validated against the reference method and certified by a third party in accordance with the protocol set out in EN/ISO standard 16140 or other internationally accepted similar protocol.

Discussion

Q: Slaughter animals are subject to process criteria. In the mean time future targets are set. Will both been brought in line with each other?
A: It is the intention to bring them in line in the future.
Q: At what level should producers evaluate the microbiological criteria? What should be the sampling frequency?
A: Food has to be in compliance with criteria set down in Regulation (EC) No. 2073/2005. The point of the food chain where the criterion applies is set down in the Regulation. A fixed sampling frequency, once a week, has been set down at the EU level for carcasses, minced meat, meat preparations and mechanically separated meat. For other products the sampling frequency will be up to the food business operator on risk basis.
Q: What to do if a positive result is found?
A: The food business operator should check the process, e.g. by more frequent sampling. For this no details are given in the Regulation. It is important to follow the trends in a process.
Q: What methods should be used for the microbiological analyses?

A: In the Regulation only reference methods are laid down (mostly ISO or CEN methods). Other methods are allowed if it is shown that they give equivalent results.
Q: How should a validation be organised?
A: The protocol of EN/ISO 16140 is intended for validation of alternative methods (rapid methods). There are international validation/certification organisations. Currently, EN/ISO 16140, which is the basis of validations/certifications carried out by these organisations, is under revision. Experience has been gained during the validation work and as a result shortcomings in the EN/ISO standard have been revealed. For example, no acceptance criteria for alternative methods have been set down. The experiences of the validation organisations would be very valuable for the revision of the standard. The target of the revision should be to set down harmonised rules for the assessment and the acceptance of alternative microbiological methods.

2.5 State of play in ISO and CEN

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

Activities in ISO/TC34/SC9 (Food products/microbiology) and in CEN/TC275/WG6 (Food analysis –Horizontal methods-Microbial contaminants) in which CRL-*Salmonella* plays a role and/or which may be of interest for the NRLs-*Salmonella* are:

- Draft amendment ISO 6579 Annex D: 'Detection of *Salmonella* spp. in animal faeces and in samples from the primary production stage' (CRL-*Salmonella* projectleader).
  The voting on the document terminated on 21-02-2006. Twenty-three members voted in favour. Although 100 % of the members voted positive, still many comments were received (20 pages!). Kirsten Mooijman prepared reactions on these comments and amended the Annex in accordance to the comments. The table with comments and reactions and the amended Annex D were sent to the secretariat of Sub Committee (SC) 9 in April 2006. Next these documents were discussed in the new ISO working group on 'Primary Production'. Reactions of this working group will be sent to Kirsten Mooijman, after which she will further amend the documents (if necessary) for the discussion in the plenary meeting of ISO/TC34/SC9 in Prague (Czech Republic) by the end of June 2006.
  An aspect which seems to be a discussion issue is the concentration of novobiocin in the MSRV medium. The participants of the workshop were requested to send any information about comparison of the two novobiocin concentrations (10 mg/L or 20 mg/L) to Kirsten Mooijman.
- New Working Item: Enumeration of *Salmonella* (CRL-*Salmonella* projectleader).
  At the ISO/TC34/SC9 meeting in Warsaw in June 2005, the following resolution was adopted: 'New working item: Enumeration of *Salmonella*. SC9 members invited Kirsten

Mooijman (CRL-*Salmonella*) to collect methods for this topic to include semi-quantitative enumeration and to present an overview at the next SC9 meeting'. Kirsten Mooijman explained that she was presently collecting information from the literature. Any information on enumeration techniques from the NRLs would still be welcome.

- New ISO Working Group: 'Primary Production'.
  At the ISO/TC34/SC9 meeting in Warsaw in June 2005, the following resolution (no. 252) was adopted: 'The secretariat will launch an enquiry to SC9 members to nominate members for a working group to deal with the primary production stage'. 'The first priority of this working group will be to consider the preparation of samples from the primary production stage'. The first meeting of this working group took recently place on 3 and 4 May 2006. Results of this meeting will be reported on the plenary meeting of ISO/TC34/SC9 in Prague by the end of June 2006.
- ISO Working Group on Proficiency Testing in microbiology (CRL-*Salmonella* active member).
  This working group started in January 2005 with the aim to prepare a guidance document for organising Proficiency Tests for microbiological analyses. By the end of 2005 the working group had prepared a rough outline and first parts of the document. One week before the workshop a first draft document was sent to the members of the working group, which will be discussed in Prague by the end of June 2006.
- New ISO Working Group on Validation of microbiological methods (CRL-*Salmonella* active member).
  This working group was raised as many comments existed on ISO 16140. This latter ISO document is applicable for validation of e.g. test kits, but is 'too heavy' for validation of e.g. culture methods. In January 2006 this new working group met for the first time. At this meeting it was decided to split ISO 16140 into 5 parts on which 5 subgroups will work upon:
    - Terminology (definitions of the relevant items);
    - Validation of proprietary methods (revision of the present ISO 16140);
    - Intermediate validation (e.g. of culture methods);
    - Method verification (e.g. setting precision data for accreditation);
    - In-house method validation (with/without a reference method).

  The next meeting of the working group will be held in conjunction with the meeting of ISO/TC34/SC9 by the end of June 2006.
- CEN mandate on validation of microbiological methods (CRL-*Salmonella* proposed as projectleader concerning Annex D of ISO 6579).
  Early 2006 an EC mandate was addressed to CEN/TC275/WG6 for the validation of 15 microbiological methods. The validation should be performed in accordance to ISO 16140 or to the new project in ISO. Candidate projectleaders had to react before

15 March 2006. One of the projects concerned validation of Annex D of ISO 6579. CRL-*Salmonella* has proposed projectleadership for the validation of this latter method, by using the NRL network. For this the available data in the literature will be checked as well as whether the results of the ring trials as organised by the CRL fulfil the criteria of ISO 16140. It might be necessary to organise a ring trial with another matrix than chicken faeces or dust.

Discussion

Q: Is there any harmonisation between ISO and CEN?

A: Yes, there is a Vienna agreement by which CEN can easily take over ISO methods and vice versa. Furthermore the meetings of ISO/TC34/SC9 and CEN/TC275/WG6 are generally organised in conjunction with each other. Many participants of the ISO meetings also follow the CEN meetings and vice versa.

2.6 Results bacteriological detection study IX - 2005

Petra Berk, CRL-Salmonella, Bilthoven, the Netherlands

In 2005 the ninth interlaboratory comparison study on bacteriological detection of *Salmonella* spp. was organised by the Community Reference Laboratory for *Salmonella* (CRL-*Salmonella*, Bilthoven, the Netherlands). National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) of the EU Member States (26) and the NRLs of Norway and of Romania participated in the study. Reference materials in combination with or without the presence of chicken faeces, as well as naturally contaminated dust (containing *Salmonella* Virchow and *Salmonella* Livingstone) were tested. The reference materials existed of gelatine capsules containing *Salmonella* Typhimurium (STM), *Salmonella* Enteritidis (SE) or *Salmonella* Panama (SPan) at different contamination levels. In addition to the performance testing of the laboratories, a comparison was made between 4 h and 18 h incubation of the samples in the pre-enrichment broth Buffered Peptone Water (BPW), followed by selective enrichment on Modified Semi-solid Rappaport Vassiliadis (MSRV) and plating-out on Xylose Lysine Deoxycholate agar (XLD) and a second selective medium chosen by the laboratory.

Significant more positive isolations were obtained from the artificially contaminated samples (negative chicken faeces, artificially contaminated with reference materials) after 18 h of incubation in BPW when compared to 4 h incubation of BPW. The accuracy rates for the artificially contaminated samples were 57 % and 98 % after respectively 4 and 18 h of incubation in BPW. The results for the naturally contaminated dust samples revealed also

significant more positive results after 18 h of incubation. The accuracy rates for these samples were respectively 81 % and 99 % after 4 and 18 h of incubation in BPW.

Discussion

Q: If the specificity is 97 % does that mean that cross contamination has occurred in some cases?

A: That is possible. However, it could also have been the case that the faeces was not fully negative.

2.7 Discussion on design and timing of two bacteriological detection studies in 2006

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

According to Regulation (EC) No 882/2004 on official controls, the CRL-*Salmonella* as well as the NRLs-*Salmonella* should also work on food samples. Therefore, the CRL-*Salmonella* has planned a pilot interlaboratory comparison study on a food matrix, beside a study on animal faeces in fall 2006.

The study on the food matrix is proposed to be organised in week 39 (25 – 29 September) 2006, with as matrix minced pork or minced beef. The proposed set-up is similar to earlier studies: analyses of *Salmonella* negative minced meat artificially contaminated with *Salmonella* reference materials. These reference materials will consist of capsules filled with milk powder artificially contaminated with *Salmonella* Typhimurium (at a level of 10 or 100 cfp/capsule) or with *Salmonella* Enteritidis (at a level of 100 or 500 cfp/capsule). The proposed methods are: ISO 6579 and draft Annex D of ISO 6579 and optional an own method.

The study on animal faeces is proposed to be organised in week 48 (27 November – 1 December) 2006. The suggested matrix is pig faeces, because:

- Problems may be expected with mailing of chicken faeces due to Aviaire influenza;
- Relation to the baseline study on pigs;
- ‘New’ matrix for the validation of Annex D.

The proposed set-up is the same as described above for the ‘food study’. The proposed methods are (draft) Annex D of ISO 6579 and optional an own method.

Discussion

Q: Would it be possible to include animal feed instead of a food matrix?

A: It is important to start some activities on a food matrix. According to the Feed and Food regulation we have to do so. We prefer to start now with a food matrix in this pilot study and perhaps next year (or later) organise a study with animal feed.
Q: Could both studies (food and faeces) be organised in one week?
A: This would be difficult to organise. Also the work load would become high for the participants. CRL would prefer to organise the two studies in two different periods.
Q: Is it possible to organise the food study in another month than September, because of the timing of the baseline studies?
A: The time constraints because of the baseline studies will remain as they will run the whole year. As both studies need to be organised in 2006, it is almost impossible to avoid September.
Q: Will it be possible for the coming years to spread the studies more over the year?
A: CRL-*Salmonella* will certainly try to do so.
Q: In our laboratory we do not analyse food, therefore it will be difficult to perform the analyses of food.
A: The NRLs are allowed to forward the intercomparison samples to a 'food laboratory' in their country to perform the food analyses. In this way, the NRL will have the opportunity to cooperate with other laboratories. The contact persons of the NRLs will remain the same for the CRL-*Salmonella*.
Q: When we have to ask another laboratory to perform the food analyses it would be difficult to ask to use the MSRV method as they are not familiar to the method.
A: Perhaps better to prescribe in the protocol for the food study only the ISO 6579 methods (RVS and MKTTn) and optional the Annex D method (MSRV).

Conclusions from the presentation and the discussion:

- The 'food study' will be organised by the end of September 2006 (week 39). The matrix of choice will be minced meat (pork or beef). The prescribed method will be ISO 6579 and optional Annex D of ISO 6579 (MSRV).
- The study on animal faeces will be organised by the end of November 2006 (week 48). The matrix of choice will be pig faeces. The prescribed method will be Annex D of ISO 6579 (MSRV).

2.8 Results typing study XI – 2006: phagetyping

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

The Laboratory of Enteric Pathogens (LEP), of the Health Protection Agency (HPA), London, England, provided ten *Salmonella* Enteritidis and ten *Salmonella* Typhimurium

strains for phage typing in the XI - 2006 interlaboratory comparison study on typing. The strains were selected from the current LEP culture collection. Presently results have been obtained from 20 laboratories - seven NRLs (National Reference Laboratories) and 13 ENLs (Enter-Net Laboratories). The results from the remaining five ENLs are still outstanding. Overall the results are good with six laboratories (3 NRLs and 3 ENLs) correctly identifying all ten of the *S.* Enteritidis strains. Nine laboratories (3 NRLs and 6 ENLs) identified nine of the ten *S.* Enteritidis strains correctly. One NRL and two ENLs correctly identified eight of the *S.* Enteritidis strains and one ENL only correctly identified four of the ten strains.

Fifteen of the laboratories (6 NRLs and 9 ENLs) identified the ten *S.* Typhimurium strains correctly. One NRL and three ENLs correctly identified nine of the ten *S.* Typhimurium strains. One ENL correctly identified eight of the *S.* Typhimurium strains and one ENL had seven of the *S.* Typhimurium strains correctly identified.

Six laboratories (3 NRLs and 3 ENLs) correctly identified all 20 strains. Six of the laboratories (2 NRLs and 4 ENLs) had one incorrect result. Two NRLs and three ENLs misidentified two of the strains. Two ENLs had three incorrect results and one ENL correctly identified 11 of the 20 strains.

Discussion

Q: How many participating laboratories possess accreditation for phagetyping?
A: Do not know by the moment, but we can check on the test reports of the study.

2.9 Results typing study XI – 2006: serotyping

Petra Berk, CRL-Salmonella, Bilthoven, the Netherlands

The eleventh 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 2006. Twenty-six National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*), including Norway and 28 Enter-Net Laboratories (ENLs), participated in the study. In total, 20 strains of the species *Salmonella enterica* subspecies *enterica* were selected for serotyping. Ten strains of *Salmonella* Enteritidis (SE) and 10 strains of *Salmonella* Typhimurium (STM) were selected for phage typing. In general, no problems were encountered with the typing of the O antigens. Ninety-nine per cent 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 94 % of the NRLs. Ninety-three per cent of the

NRLs indicated correct serovar names for the 20 strains. Most problems were encountered with *S.* Yoruba and *S.* Oranienburg. Some problems were encountered with *S.* Montevideo, *S.* Senftenberg and *S.* Brandenburg. A total correct identification by all participants was obtained for three strains *S.* Stanleyville, *S.* Typhimurium and *S.* Tennessee.

Discussion

Q: The ring trials on serotyping and phagetyping are very important. How are the serovars selected?

A: The selection is made by the CRL-*Salmonella* using the following criteria: Serovars presently most frequently found; Serovars from different groups; Serovars which have caused problems in past studies, Emerging serovars.

Q: Will it be possible to include other subspecies than *enterica* (e.g. 'reptile strains')?

A: In some past studies this has been done, but these subspecies caused many problems. However, it may be worthwhile to include one or more of these strains again in the future studies, as in rare cases these kind of strains are also found in food.

Q: In our laboratory we have many problems with the quality of sera. Is there a good (standard) way to do quality control on the sera?

A: We use sera of different manufacturers and the quality is tested with some 'standard' strains. Furthermore, in a book of the WHO centre in Paris, information is given on the choice of strains and the method to perform the quality control of sera.

2.10 Discussion on design typing study XII - 2007

Arjen van de Giessen, CRL-Salmonella, Bilthoven, the Netherlands

As in previous years the organisation of the twelfth typing study will be done by CRL-*Salmonella* in collaboration with the Health Protection Agency (HPA, London, UK).
For serotyping, 20 strains will be selected by CRL-*Salmonella.* The following selection criteria will be followed:

- Include public health significant serovars, associated with pigs (in relation with the baseline study on pigs);
- Include uncommon serovars with antigens similar to those of public health significant serovars;
- Include serovars that have caused typing problems in previous studies;
- Include serovars from subspecies *enterica* from as many different groups as possible;
- Include 1 or more strains from another subspecies than *enterica* ('reptile strain').

The phagetyping will be carried out on ten *S.* Typhimurium and ten *S.* Enteritidis strains. The transportation of the strains for phagetyping will be organised by the HPA in London.

Discussion
Q: It would be helpful to add the evaluation criteria of the study in the protocol.
A: CRL-*Salmonella* will try to do so.

2.11 Work programme CRL-*Salmonella* second half 2006, first half 2007, 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 2006 and for early 2007.

Interlaboratory comparison studies
As indicated in earlier presentations, two interlaboratory comparison studies on the detection of *Salmonella* in a food matrix and in faecal material are planned in fall 2006. Another interlaboratory comparison study on typing of *Salmonella* is planned in 2007.

Research
CRL-*Salmonella* will finalise the stability studies on reference materials in 2006. Presently information on the stability of the materials for a storage period of 5 years is available. Furthermore, the CRL-*Salmonella* will perform the following activities:

- Continue the activities for ISO and CEN;
- Test other food matrices for their use in ring trials (e.g. minced beef);
- Explore activities concerning the detection of *Salmonella* in milk(products). In 2005, the activities concerning *Salmonella* in milk and milk products were moved from the CRL - 'milk' to the CRL-*Salmonella.* It needs to be explored whether the present NRLs-*Salmonella* will also perform the NRL activities for *Salmonella* in milk and milk products;
- Explore immunological and molecular methods. It is proposed that CRL-*Salmonella* may play a role in validating one or more immunological methods which have shown to be useful in the baseline study for fattening pigs.

In 2005 the CRL-*Salmonella* tested lenticule reference materials containing the same *Salmonella* strains and contamination levels as the capsules. The contamination levels as well as the homogeneity of the lenticules were very similar to the capsules. Also the use of the

lenticules in a ‘ring trial set-up’ gave similar results as the capsules leading to the conclusion that lenticules can well be used as standard samples for interlaboratory comparison studies.

Communication and other activities
As before, the newsletter will be published 4 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.
The CRL-*Salmonella* website will soon be updated. Comments and suggestions are welcome.
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.
Together with DG-Sanco, the possible role of CRL-*Salmonella* in advising the European Commission with requests for the use of alternative methods will be discussed.

Closure
Kirsten Mooijman closed the workshop, thanking all participants, and guest speakers for their presence and contributions. Special thanks were given to Genevieve Clement (ISPAIA) for her help in organising the workshop and to Petra Berk (CRL-*Salmonella*) for her great help with the CRL activities since September 2005.

Discussion
Q: Would it be possible to add a discussion forum on the CRL-*Salmonella* website, to discuss problems with methods, quality control of sera, etcetera?
A: CRL-*Salmonella* will explore the possibilities.

Acknowledgements

This workshop could not have been organised in Saint Malo, France without the help of a local person. Genevieve Clement of ISPAIA (Ploufragan, France) has made a great effort to make the workshop a success. She took care of all local organisational aspects (hotel, meeting room and facilities, coffee, tea, food, etcetera). We would like to thank her very much for her very important and valuable help.

Petra Berk of the CRL-*Salmonella* is thanked for taking over very quickly and efficiently many activities for the CRL-*Salmonella* since September 2005.

Angelina Kuijpers of the CRL-*Salmonella* is thanked for making minutes of the workshop.

Arjen van de Giessen is thanked for helping with the programme, and for leading discussions during the workshop.

Loes van Dijk is thanked for all the administrative work before and after the workshop.

References

ISO 6579, 2002 (E). Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp. International Organisation for Standardisation, Geneve, Switzerland.

Draft Amendment ISO 6579:2002/DAmd 1, 2005. Amendment 1 Annex D: Detection of *Salmonella* spp. in animal faeces and in samples from the primary production stage.

Annex 1. Participants

European Commission	Maija Hatakka
European Food Safety Authority (EFSA)	Frank Boelaert
CRL – *Salmonella*	Kirsten Mooijman Petra Berk Angelina Kuijpers Anjo Verbruggen
Guest speaker (United Kingdom)	Elizabeth de Pinna (HPA, London)

National Reference Laboratories for *Salmonella*

AUSTRIA	Christian Kornschober
BELGIUM	Hein Imberechts
CYPRUS	Economides Constantinos
CZECH REPUBLIC	Iva Bernardyova
DENMARK	Dorte Lau Baggesen
ESTONIA	Age Kärssin
FINLAND	Henry Kuronen
FRANCE	Marianne Chemaly Francoise Lalande
GERMANY	Andreas Schroeter
GREECE	Maria Gavala
HUNGARY	Erzsebet Andrian
IRELAND	Montserrat Gutierrez
ITALY	Veronica Cibin Lisa Barco
LATVIA	Andra Utinane
LITHUANIA	Ceslova Butrimaite-Ambrozeviciene
LUXEMBOURG	Joseph Schon
NORTHERN IRELAND	Sam Strain

NETHERLANDS	Arjen van de Giessen
	Wilfrid van Pelt
NORWAY	Bjarne Bergsjo
POLAND	Andrzej Hoszowski
	Dariusz Wasyl
PORTUGAL	Alice Amado
SLOVAK REPUBLIC	Milan Sasik
SLOVENIA	Vojislava Bole-Hribovsek
SWEDEN	Karin Bergström
UNITED KINGDOM	Robert Davies
	Linda Ward

Annex 2. Programme of the workshop

Programme of the CRL-*Salmonella* workshop XI, 9 May 2006, St. Malo, France

General information

Hotel and place of the workshop:

Hotel l'Univers; 16 Place Chateaubriand; 35400 Saint-Malo, France
tel: +33 2 99 40 89 52
fax: +33 2 99 40 07 27
www.hotel-univers-saintmalo.com

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 4 May 2006.

Monday 8 May 2006

Arrival of representatives of the NRLs at Hotel l'Univers.

19.00 – 20.00 Registration and get-together in hotel l'Univers

- Final information concerning the programme
- Late handing over of presentations
- Declaration forms
- Handing over of copies of tickets and boarding passes

Tuesday 9 May 2006

Morning session:
Chair: Kirsten Mooijman

9.00 - 9.30 Opening and introduction (Kirsten Mooijman)
9.30 – 10.45 Baseline study in laying hens:

- *Salmonella* observed prevalence in large-scale laying hen holdings in the EU (Frank Boelaert)
- Results QA serotyping (Kirsten Mooijman)
- Dealing with rough strains (Anjo Verbruggen)
- Discussion/questions/evaluation (Arjen v.d. Giessen)

10.45 - 11.15 Coffee/tea

11.15 - 11.45 Future baseline studies: Sampling and analytical aspects (Frank Boelaert)
11.45 - 12.15 *Salmonella* criteria for foodstuffs (Maija Hatakka)

12.15- 13.45 Lunch

Afternoon session:
Chair: Kirsten Mooijman

13.45 - 14.15 State of play in ISO and CEN (Kirsten Mooijman)
14.15 - 14.45 Results bacteriological detection study IX – 2005 (Petra Berk)
14.45 - 15.15 Discussion on design and timing of two bacteriological detection studies in 2006 (Kirsten Mooijman)

15.15 - 15.45 Coffee/tea

15.45 - 16.05 Results typing study XI - 2006 : phagetyping (Elizabeth de Pinna)
15.05 - 16.25 Results typing study XI - 2006: serotyping (Petra Berk)
16.25 - 16.45 Discussion on design typing study XII-2007 (Arjen van de Giessen)
16.45 – 17.15 Work programme CRL second half 2006, first half 2007, closure (Kirsten Mooijman)

19.00 Dinner at Restaurant le Franklin; 4, Chaussée du Sillon; Saint Malo
tel: 02 99 40 50 93. *www.lefranklin.com*