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**The tenth CRL-*Salmonella* workshop**
28 and 29 April 2005, Bilthoven, the Netherlands

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# Abstract

**The tenth CRL-*Salmonella* workshop**

The tenth workshop organised by the Community Reference Laboratory for *Salmonella* (CRL-*Salmonella*) was held on 28 and 29 April 2005 in Bilthoven, the Netherlands. 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, the NRLs and CRL-*Salmonella*, as well as by several guest speakers. Subjects discussed were European legislation on zoonoses and feed and food, baseline studies in the EU to determine the prevalence of *Salmonella* in laying hens, broiler and breeding flocks, the zoonoses report of 2003, typing methods (PCR, phagetyping), *Salmonella* monitoring in pigs, research activities, intercomparison studies organised by CRL-*Salmonella* (2004 and 2005) and the work programme of CRL-*Salmonella* for the coming year. The presentations on European legislation made clear that the CRL and NRLs not only have responsibilities for veterinary samples, but also for food and feed samples. Presentations on the intercomparison studies resulted in discussion on the type of samples used in all studies up to now. Research results of CRL-*Salmonella* showed a negative effect of glycerol on the growth of *Salmonella*. As the faeces samples used in all interlaboratory comparison studies were mixed with glycerol, this might have affected the results of the studies. A special item at the workshop was the future on phagetyping. Continuity in the availability of materials and knowledge for this way of typing was guaranteed by the Health Protection Agency, London, United Kingdom.

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

# Rapport in het kort

**De tiende CRL-*Salmonella* workshop**

De tiende workshop georganiseerd door het Communautair Referentie Laboratorium voor *Salmonella* (CRL-*Salmonella*) werd gehouden op 28 en 29 April 2005 in Bilthoven, Nederland. Deelnemers betroffen 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, de NRLs en CRL-*Salmonella,* alsmede door enkele gastsprekers. Onderwerpen die bediscussieerd werden waren: Europese wetgeving op het gebied van zoonosen en op het gebied van levensmiddelen en diervoeders, basisstudies in de EU voor het vaststellen van de prevalentie van *Salmonella* bij leghennen, broedkuikens en vermeerderingskoppels, zoonosen rapport 2003, typeringsmethoden (PCR, faagtypering), *Salmonella* monitoring bij varkens, onderzoeksactiviteiten, ringonderzoeken georganiseerd door CRL-*Salmonella* (2004 en 2005) en het werkprogramma van CRL-*Salmonella* voor het komende jaar. De presentaties over de Europese wetgeving maakten duidelijk dat het CRL en de NRLs niet alleen verantwoordelijkheden hebben voor veterinaire monsters, maar ook voor monsters ten aanzien van levensmiddelen en diervoeder. De presentaties over de ringonderzoeken gaven aanleiding tot dicussie over de type monsters welke in de ringonderzoeken tot nu toe zijn gebruikt. Onderzoek uitgevoerd door het CRL-*Salmonella* toonde een negatief effect aan van glycerol op de groei van *Salmonella*. De fecesmonsters welke gebruikt zijn bij de ringonderzoeken waren tot nu toe altijd gemengd met glycerol. Dit kan effect hebben gehad op de resultaten van de studies. Een speciaal onderwerp tijdens de workshop betrof de toekomst van de faagtypering. Continuïteit in de beschikbaarheid van materialen en kennis op dit gebied werd gegarandeerd door de ‘Health Protection Agency’, Londen, Engeland.

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

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# Summary

From 28 till 29 April 2005 the Community Reference Laboratory for *Salmonella* (CRL-*Salmonella*) organised a workshop in Bilthoven, the Netherlands. On both days representatives of the National Reference Laboratories (NRLs-*Salmonella*) were present, as well as representatives of the European Commission (DG-Sanco). A total of 42 participants were present at the workshop.

The programme of the workshop consisted of several parts.
During the morning session of the first day, presentations were given on current EU legislation on zoonoses and related issues (zoonoses report 2003, baseline studies in the EU to determine the prevalence of *Salmonella* in laying hens, broiler and breeding flocks, activities of the European Food Safety Authority).
During the afternoon session of the first day, results of research activities of CRL-*Salmonella* were presented and results of the interlaboratory comparison studies of 2004 and 2005 as well as designs of future studies were discussed.
On the second day of the workshop, the implications of the new feed and food regulation were presented. Furthermore information was provided on phagetyping and Pulsed Field Gel Electrophoresis (PFGE) typing. Also monitoring of *Salmonella* in pigs was discussed and finally the work programme of the CRL-*Salmonella* for the next year was presented.

The presentations on European legislation made clear that the CRL and NRLs not only have responsibilities for veterinary samples, but also for food and feed samples. Presentations on the intercomparison studies resulted in discussion on the type of samples used in all studies up to now. Research results of CRL-*Salmonella* showed a negative effect of glycerol on the growth of *Salmonella*. As the faeces samples used in all interlaboratory comparison studies were mixed with glycerol, this might have affected the results of the studies. A special item at the workshop was the future on phagetyping. Continuity in the availability of materials and knowledge for this way of typing was guaranteed by the Health Protection Agency, London, United Kingdom.

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

An evaluation of the workshop revealed 12 responses, which were in general positive.

# List of abbreviations

| | |
|---|---|
| BPW | Buffered Peptone Water |
| 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 |
| IRMM | Institute for Reference Materials and Methods |
| ISO | International Organization for Standardization |
| MIC | Minimal Inhibition Concentration |
| MKTT | Mueller Kauffmann Tetrathionate broth |
| MS | Member State |
| MSRV | Modified Semi-solid Rappaport Vassiliadis |
| NRL | National Reference Laboratory |
| PCR | Polymerase Chain Reaction |
| PFGE | Pulsed Field Gel Electrophoresis |
| RIVM | National Institute for Public Health and the Environment |
| SE | *Salmonella* Enteritidis |
| SPan | *Salmonella* Panama |
| STM | *Salmonella* Typhimurium |
| UK | United Kingdom |
| XLD | Xylose Lysine Deoxycholate agar |

# 1. Introduction

In this report abstracts of the presentations given at the CRL-*Salmonella* workshop of 2005 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.
In chapter 2 all presentations of the first day are given.
In chapter 3 all presentations of the second day are summarised.

After the workshop an evaluation form was sent to all NRLs. Responses on this evaluation are summarised in chapter 4.

# 2. Thursday 28 April: day 1 of the workshop

## 2.1 Opening and introduction

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

After welcoming all participants to the 10th CRL-*Salmonella* workshop, Kirsten Mooijman of the CRL-*Salmonella* explained shortly the aims and the programme of the workshop.

**Aims of the workshop:**

- Discuss issues of relevance for CRL and NRLs:
    - EU legislation
    - Baseline studies (layers, broilers, breeders)
    - *Salmonella* monitoring in pigs
    - Typing methods
    - Research activities
- Past and future intercomparison studies organised by CRL-*Salmonella*;
- Exchange of information between NRLs;
- Exchange of information with representatives of the EC (DG-Sanco);
- Discuss future activities of the CRL-*Salmonella*.

**Programme of the workshop:**

28 April 2005

- Zoonoses legislation:
    - Zoonoses report
    - Baseline studies
    - EFSA activities
- Intercomparison studies on detection and typing of *Salmonella* spp. (2004, 2005 and 2006).

29 April 2005

- Feed and Food regulation;
- Matrices and Proficiency testing (results questionnaire);
- Typing: phagetyping, PFGE;
- Monitoring of *Salmonella* in pigs;
- Work programme second half 2005 and 2006;

A more detailed programme of the workshop is presented in Annex 2.

## 2.2 Zoonoses report *Salmonella* 2003

*Annemarie Kaesbohrer, CRL-Epidemiology, Berlin, Germany*

The basis of the report on trends and sources of zoonotic agents in EU and Norway on the year 2003 is Directive 92/117/EEC on zoonoses, which laid down rules on collection of information on zoonoses and zoonotic agents in humans, animals, food and feeding stuffs. This is the last report which has been prepared by the Community Reference Laboratory for the Epidemiology of Zoonoses.

Altogether, this data collection covered 11 zoonoses and antimicrobial resistance in two zoonotic agents and one indicator bacterium in all Member States of the EU and Norway. Four new Member States provided a report on the situation in 2003 voluntarily.

In 2003, again salmonellosis and campylobacteriosis were by far the most frequently reported zoonoses in **humans**, with approximately 135 000 cases each. For salmonellosis the decreasing tendency observed over several years continued and for campylobacteriosis, the downward trend observed since 2002 continued. In 2003, the overall reduction was 7 % for salmonellosis and 9 % for campylobacteriosis.

In **poultry breeding flocks** (*Gallus gallus*), the very favourable situation has continued in 2003 as regards *S.* Enteritidis and *S.* Typhimurium in Finland, Ireland, Sweden and Norway. In the other countries, infection rates reported in 2003 ranged between 0 % and 8.4 % for *S.* Enteritidis and *S.* Typhimurium infections. As in previous years, *S.* Enteritidis is the dominating serovar, sharing 38 % of all isolates reported compared to 42 % in 2002. *S.* Typhimurium was reported in 4.5 % of all isolates from breeding flocks, which is comparable to 2002. *S.* Virchow on the second position.

In 2003, the reported infection rates for *S.* Enteritidis and *S.* Typhimurium in **laying hens** ranged from 0 % to 11.2 %. The prevalence rate for all *Salmonella* serovars ranged up to 18 %. An increasing trend was observed in several countries (i.e. Germany, Spain and Great Britain). In layers, there was a slightly lower proportion of *S.* Enteritidis isolates, 53.7 % in 2003 compared with 57.7 % in 2002. Nine percent of all isolates were *S.* Typhimurium. On the second place is *S.* Infantis with a share of 10.5 %.

The infection rates reported in **broiler flocks** ranged from 0 % to 24.3 % which reflects an increase in the *Salmonella* infection rate observed in some countries. *S.* Enteritidis was the predominant type in broilers and poultry meat, representing 40 % and 13 % of all isolates respectively. In broilers, *S.* Paratyphi B var. Java moved to the second position in 2003, representing 15 % of all isolates compared with 20 % in 2002. Again, all isolates were reported in the Netherlands. *S.* Infantis, *S.* Livingstone and *S.* Virchow are on the next places and this is in line with the situation in 2002.

As seen in 2002, in the overall distribution the serovar pattern in **poultry meat** is more divers compared to the live birds. *S.* Enteritidis and *S.* Typhimurium are dominating in poultry meat, followed by serotypes *S.* Saintpaul, *S.* Heidelberg and *S.* Blockley.

The serovar pattern in **pigs** and **pork** remained unchanged. *S.* Typhimurium is clearly dominating in the overall figure, and this is true in most of the individual countries. The next frequent serovar is *S.* Derby. Interestingly, *S.* Infantis and *S.* Enteritidis isolates from pigs and pork were reported from several countries.

All countries included some information on **antimicrobial resistance in *Salmonella***. Nine Member States reported some resistance to ciprofloxacin or enrofloxacin. Most Member States reported resistance to nalidixic acid, which is an indicator of developing resistance to fluoroquinolones.

All details are given in the Commission document SANCO/339/2005.

***Discussion***
Q: What progress has been made after 10 years of zoonoses reports?
A: A lot of progress! Ten years ago there was little knowledge on how to collect the data. Nowadays all Member States collect information. Still there are difficulties, but Member States learn from each other. Also the communication between the veterinary field and the human field has made good progress in the past 10 years.

## 2.3 Zoonoses legislation

*Jean-Charles Cavitte, European Commission, Brussels, Belgium*

In 2003 revision took place of the ‘old’ EU zoonoses legislation. Directive 92/117/EEC was replaced by Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents and by Regulation 2160/2003 on the control of *Salmonella* and other specified food-borne zoonotic agents.
Important items of Directive 2003/99/EC:

- Surveillance throughout the food chain;
- Monitoring is based on existing systems in the Member States;
- Eight zoonoses are listed for mandatory monitoring, some others according to the epidemiological situation;
- Antimicrobial resistance monitoring on *Salmonella* and *Campylobacter* is also included;
- Co-operation at national level between authorities in animal/feed/food/human health sectors;
- Interpretation of monitoring results at national level for yearly reporting.

The yearly (synthesis) report on zoonoses (based on the national reports) will be prepared by the European Food Safety Authority (EFSA).
In May 2005 the European Centre for Disease Prevention and Control (ECDC) will become operational.
Good co-operation between EFSA and ECDC is important to combine data from the animal, feed and food sector with the data from the human health sector.

Important items of Regulation 2160/2003:

- Creates a framework for zoonoses control by setting targets for the reduction in prevalence of pathogens (*Salmonella*) in different animal populations. The setting of targets is for breeding flocks under process, for laying hens this should be set by the end of 2005, for broiler flocks by the end of 2006 and for turkeys and pigs by the end of 2007.
- When targets are established:
    - Member States should prepare a national control programme;
    - This programme need to be approved by the Commission;
    - Programmes should become operational 18 months after setting the target.
- Set rules on trade in live animals and hatching eggs;
- Predefined specific measures:
    - Fowl breeding flocks infected with *Salmonella* Enteritidis or *Salmonella* Typhimurium should be slaughtered/ heat treated/ destructed;
    - Table eggs have to originate from negative flocks by the end of 2009;
    - (relevant) Poultry meat should be free from *Salmonella* in 25 g by the end of 2010.

## 2.4 Baseline study in laying hens

### 2.4.1 Interim results collected at EC

*Sarolta Idei, European Commission, Brussels, Belgium*

The objective of the study is to estimate the prevalence of *Salmonella* serotypes in the population of laying hens (*Gallus gallus*) for the production of table eggs in the Member States of the European Union, in adult laying hens at the end period of production (9 weeks before depopulation). The results should be used to prepare setting of a Community target pursuant to Regulation (EC) No 2160/2003. The study covers a one year period commencing from 1 October 2004.

Sampling shall take place in a proportion of holdings randomly selected. Member States shall ensure randomisation of the sampling on the basis and geography and season. The sampling should take place in one selected flock at each selected holding. Holdings should be selected at random, but in such a way that the holdings sampled represent 80 % of the laying hen population in the country. Sampling shall be performed /supervised by the competent authority. In order to maximise sensitivity of sampling, both faecal and environmental material shall be sampled. Samples shall be placed in transport media as appropriate and sent by fast mail or courier to the national reference laboratory. The NRL shall coordinate/supervise the testing by involved laboratories; laboratories shall be involved in official control and be accredited (ISO 17025); Laboratories shall be trained in the method; NRLs shall do the serotyping. **Examination** should be carried out **within 48 hours** after receipt of the sample. Strains isolated shall be stored, using the normal method for NRL culture collection, as long as it ensures integrity of the strains for a minimum of **2 years**.

The detection method recommended by the CRL shall be used. NRL shall follow the Kaufmann-White scheme in serotyping.

A holding is considered positive for the purpose of this study if the presence of S*almonella* is confirmed in at least one of the sample. However, all serotypes will be reported separately, including untypable serotypes. NRLs were asked to send typable /non-typable strains to the CRL-*Salmonella* on a quarterly basis for quality assurance purposes.

According to the technical specification of the baseline study SANCO/34/2004 Rev3 Article 7(b) a progress report on the first 3 months of the implementation of the study should be submitted to Commission. Member States were asked to submit information on timing /selection of holdings/outcome of sampling/testing/difficulties if encountered. All countries started to implement the study and no serious difficulties were reported. The rates of flocks positive for *Salmonella* varied a lot (0-84.4 %) The study started with delay in some countries, because of the fact that no flocks were scheduled for slaughter within the time period.
Due to the low capacity of the NRL-*Salmonella*, in certain countries, the delegation of tasks to other laboratories was required. In some countries, farms terminated egg production, resulting in the fact that randomly selected farms had to be replaced by others.
Member States agreed on providing raw results on the study electronically to the EC.

### 2.4.2 Interim results QA serotyping

*Anjo Verbruggen, CRL-Salmonella, Bilthoven, the Netherlands*

The interim results of the quality assurance of the serotyping of *Salmonella* strains isolated from laying hens in the context of the baseline study on the prevalence of *Salmonella* in laying flocks of *Gallus gallus* were presented.
From the 25 EU countries seventeen National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) have sent 79 typable and 16 non-typable strains to the Community Reference Laboratory for *Salmonella* (CRL-*Salmonella*). At present a total of 51 typable and 16 non-typable *Salmonella* strains were investigated at CRL-*Salmonella*. Strains belonging to serovars (one strain each) *S.* Altoni, *S.* Bovismorbificans, *S.* Bredeney, *S.* Cerro, *S.* Corvallis, *S.* Lexington, *S.* Livingstone, *S.* Saintpaul and *S.* Tennessee were typed accurately by the NRLs.
The serotyping of strains belonging to serovars *S.* Hadar (n=3), *S.* Heidelberg (n=2), *S.* Infantis (n=5), *S.* Mbandaka (n=3), *S.* Senftenberg (n=3), *S.* Typhimurium (n=2) and *S.* Virchow (n=2) was in accordance with the results obtained by CRL-*Salmonella*. One NRL was not able to designate a serovar name to a strain that belonged to serovar *S.* Carno and another NRL to a strain that belonged to *S.* Veneziana.
Two strains, sent by two NRLs, were identified by CRL-*Salmonella* as *S.* Montevideo. One of these strains was also identified by the NRLs as *S.* Montevideo, but the other one could not be identified. The same was found for two strains belonging to serovar *S.* Derby. Two strains of *S.* Oranienburg (sent by two NRLs) could not be identified due to the absence of biochemical reactions.
Twenty-four strains from 12 NRLs were identified by CRL-*Salmonella* as belonging to *S.* Enteritidis. Twenty-one of these strains were also typed by the NRLs as *S.* Enteritidis. One of these strains was identified as group D1 and the remaining two strains could not be identified due to their roughness. The latter two strains were identified by CRL as *S.* Enteritidis (phagetype 7).

### 2.4.3 Discussion/ questions/ evaluation

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

**Sampling**

- It was remarked that the collection of dust samples in Germany is difficult as a German law prescribes that stables should be clean;
- Some difficulties were mentioned when working with dust. When an equal volume of Buffered Peptone Water (BPW) is added to the dust sample, clumps will be formed which are hard to handle;

- It was requested to send all comments to CRL-*Salmonella.* The information can be used when new studies have to be drafted.

**Detection method**

It was asked whether it was allowed to store samples longer than 48 h before starting the analyses. Long storage of the samples may have a negative effect on the detection of *Salmonella.* It is important to start the analyses as soon as possible after receiving the samples. The maximum of 48 h of storage should be respected.

A possibility which was suggested was storage of the BPW culture or MSRV plates at 5 °C (e.g. during the weekend), which would not affect the results. However, another NRL mentioned to find no positives if the BPW culture is stored at 5 °C, but good results were found when the BPW culture was stored at room temperature. As the influences of storage do not seem to be very clear, it would be better not to store samples and cultures. If this is not possible, the storage time should be kept as short as possible.

**Typing**

Some remarks/questions were addressed to the quality assurance of serotyping of isolates from the baseline study:

- Up to now only 17 NRLs have sent in isolates. As it is a legal requirement, the NRLs were urgently stressed to send strains to the CRL-*Salmonella*, using the official CRL forms;
- It was asked whether there is a rule for selection of the strains which should be sent to the CRL. It was indicated that it would be preferable to send in as much as possible different serovars and preferably the ‘unusual’ strains;
- If an NRL is able to type all strains, still only the maximum number of typable strains should be sent and not some extra for replacing non-typable strains;
- Occasionally problems arise with typing rough strains. It was advised to try, if possible, to use ‘special’ culture methods to return the strain to the smooth stage, so that it can be fully typed.

**Reporting**

For the detection of *Salmonella*, the prescribed method is MSRV. However, some laboratories use other media in parallel to MSRV. It was asked how the results should be reported in case the other media would give positive results whereas MSRV does not. CRL-*Salmonella* and representatives of EC DG-Sanco will look further at this item and will soon inform the NRLs.

## 2.5 Proposed baseline study in broilers

*Antonia Ricci, NRL-Italy, Legnaro, Italy*

**Introduction**

The control of food borne diseases must be based on a 'farm to fork' approach, in which primary production represents a critical point for contamination spreading, and is therefore a key point for any control activity. At the European level, such a strategy is clearly identified by the new zoonoses legislation (Directive 2003/99/CE and Regulation (CE) 2160/2003), which provides for the monitoring and the control of food borne zoonoses at primary production.

Regulation 2160/2003, 'on the control of *Salmonella* and other specified food-borne zoonotic agents', points out the necessity, for some zoonoses, to establish specific control measures, which should be based on targets for prevalence reduction. Such targets will be set for the agents and following the timetable described in Annex I of the Regulation.

In order to set targets following the deadlines reported in table 1, the Commission shall obtain comparable data on infection prevalence in different species and categories of animals, in all Member States. Since these data do not arise from routine surveillance programmes, annual baseline studies will be defined, in order to assess *Salmonella* spp. prevalence in different animal populations, and therefore to get the necessary data every year to set the targets. The first of these baseline studies concerns laying hens, and will be performed in EU countries from October 2004 to September 2005 (Decision C (2004) 3512 of 22/09/04). After the establishment of targets for laying hens, a similar study will be performed in broilers, in order to get also in this case comparable data on which, at the end of 2006, targets will be set.

This monitoring scheme has been drafted by a working group, which has initially defined a proposal consisting in two different options: sampling at slaughterhouses or sampling at holdings. After a first discussion with Member States, the second option has been chosen.

**Sampling scheme**

In this scheme, the sampling frame should cover primarily holdings representing at least 80 % of the total population. This can usually be achieved by including all holdings with at least 5000 birds. Preferably, only one flock per holding should be sampled. If the calculated number of flocks to be sampled is higher than the number of holdings available with at least 5000 birds, up to four flocks may be sampled from the same holding to achieve the calculated number of flocks. In this case, only one flock per season from the same holding may be included in sampling and different houses should be selected.

Primarily, the number of flocks to be tested (sample size) has to be calculated. It is calculated on the basis of the following criteria:

Target prevalence: 50 %
Desired confidence level: 95 %
Accuracy: 5 %
The population size (total number of flocks in a MS), will be estimated, considering that on average it is expected to have 2 houses per holding and 6 broiler flocks raised per house per year.
Consequently, to establish the population size, the number of holdings in a MS should be multiplied by the factor 12.

If the calculated population size turns out to be less than 10 000 flocks, the sample size should be adjusted, considering anyway that a minimum of 154 flocks should be sampled in each country. This is necessary to enable detection of a reduction in prevalence from 50 % to 40 % with a power of 80 %, and will allow to keep the same sampling scheme also with the aim of evaluating the efficacy of control programs, once the Commission will set the reduction targets.

**Sampling protocol**
There are three different production types:
Free range (animals have access to outside)
Free range, organic
Conventional (animals are kept inside houses)

In order to maximise sensitivity of sampling, faecal material equivalent to about 300 individual samples shall be collected, throughout five pairs of ‘boot’ or ‘sock’ swabs, taken at farm. This is considered to give a detection sensitivity equivalent to 300 faeces samples (Skov *et al*., 1999), providing 95 % confidence of detection of 1 % within flock prevalence assuming the test is 100 % sensitive.

**Laboratory methods**
National Reference Laboratories (NRLs) for *Salmonella* are the laboratories where detection and serotyping shall take place. In case the National Reference Laboratory does not have the capacity to perform all analyses or if it is not the laboratory that performs detection routinely, the competent authorities may decide to designate a limited number of other laboratories involved in official control of *Salmonella* to perform the analyses, These laboratories should have proven experience of using the required detection method and have a quality assurance system complying with ISO standard 17025 and be submitted to the supervision of the National Reference Laboratory.

***Detection method***

The method recommended by the Community Reference Laboratory for *Salmonella* in Bilthoven, the Netherlands, shall be used: the method is a modification of ISO 6579 (2002), where a semi solid medium (MSRV) is used as the single selective enrichment medium. The semi-solid medium should be incubated at (41.5 ± 1) °C for 2x (24 ± 3) h.

***Serotyping***

At least one isolate from each positive sample shall be typed in the NRL for *Salmonella*.
The NRL shall follow the Kaufmann-White scheme.
For quality assurance, a proportion of the typable strains and of the non-typable isolates shall be sent to the CRL, with a maximum of 16 typable strains and 16 non-typable isolates.
A proportion of these isolates should be sent to the CRL on a quarterly basis.

***Storage of strains***

Strains isolated shall be stored, using the normal method for NRL culture collection, as long as it ensures integrity of the strains for a minimum of 2 years.

***Phagetyping***

It is strongly recommended that at least one isolate of *S.* Enteritidis and *S.* Typhimurium from each positive sample should be phagetyped, using the Protocol defined by the Health Protection Agency, Colindale, London.

***Testing of antimicrobial susceptibility***

For epidemiological purposes, it is recommended that, where possible, one isolate per serotype per flock is used for antimicrobial susceptibility testing. As far as possible, quantitative methods should be implemented and NCCLS standards should be used.

**Reporting**

A flock is considered positive for the purpose of this study if the presence of *Salmonella* spp. is detected in at least one of the samples. However, all serotypes shall be reported separately, including untypable serotypes.

**Acknowledgments**

The baseline study has been drafted by a working group, composed by Annemarie Kaesbohrer, Arjen van de Giessen, Rob Davies, Christina Dorn, Antonia Ricci.

## 2.6 Monitoring in breeder flocks

*Jean-Charles Cavitte, European Commission, Brussels, Belgium*

According to Regulation 2160/2003 on the control of *Salmonella* and other specified food-borne zoonotic agents, targets should be set for the reduction in prevalence of pathogens (*Salmonella*) in different animal populations. The setting of targets is done by using monitoring schemes to verify achievement of the targets. The targets for breeding flocks should already have been set by the end of 2004 and is presently under process. The targets for layer flocks should be set by the end of 2005, for broiler flocks by the end of 2006 and for turkeys and pigs by the end of 2007. Attention is paid to *Salmonella* serovars with public health significance. The 5 most frequently found serovars in humans are presently: *Salmonella* Enteritidis (SE), *Salmonella* Typhimurium (STM), *Salmonella* Hadar (SH), *Salmonella* Infantis (SI) and *Salmonella* Virchow (SV).
The collection of prevalence data for breeding flocks was still based on the sampling regime pursuant to the 'old' zoonoses Directive 92/117. For this monitoring, the method of analyses was still flexible, although it was recommended to use the CRL-*Salmonella* recommended method.
The monitoring took place in 2004 and the results were submitted in February 2005. All Member States (including Norway) reported results. Using these results a draft Regulation for setting the Community target on breeding flocks was set-up:

- Maximum 1 % of adult breeding flocks may remain positive (for SE, STM, SH, SI, SV) within 3 years of implementation of national control programmes (i.e. by the end of 2009);
- Enter into force: 1 July 2005;
- Testing scheme would be very detailed for number of samples, type of samples, frequency of sampling, method for analyses.

## 2.7 EFSA activities in relation with *Salmonella*

*Pia Makela, EFSA, Parma, Italy*

The European Food Safety Authority (EFSA) is a new Community agency established in 2002. Its mission is to provide scientific advice as well as scientific and technical support for the Community's legislation and policies in all fields, which have a direct or indirect impact on food and feed safety. This remit covers also zoonoses, including both the food-borne and the non-food-borne ones.

EFSA's main task is to issue scientific opinions. Questions related to zoonoses, including *Salmonella*, are dealt with by two on the Scientific Panels: the Panel on Biological Hazards (BIOHAZ) and the Panel on Animal Health and Welfare (AHAW), depending on the scope of the question. One of the main objectives of EFSA is to improve the support provided to the Scientific Panels. To this end EFSA is strengthening the secretariats of the panels by recruiting qualified personnel to these units. These persons would be able to assist the panels in drafting the opinions and collecting information needed. In addition a new structure, Scientific Expert Services, has been created in EFSA, with the aim to provide further support to the panels. The persons in these services have each their specific field of expertise where they can offer in-depth assistance to the panels. The Scientific Expert Services will employ, among other things, veterinary and human epidemiologists, who will be capable of assisting the panels with questions related to zoonoses.

So far the opinions on zoonoses issued by the panels represent qualitative risk assessments based on the best available information on the subject in question. EFSA is now taking steps to introduce methods of (semi-)quantitative risk assessments into the preparation of certain opinions related to zoonoses. Two scientific opinions regarding *Salmonella* have so far been issued by the BIOHAZ panel. These are the opinion on the use of antimicrobials to control *Salmonella* in poultry flocks, and the opinion on the use of vaccines to control *Salmonella* in poultry flocks. Both the opinions were delivered in 2004. EFSA is currently working with a new request for an opinion from the Commission, which deals with monitoring and control of *Salmonella* in pig production.

EFSA has also the task to collect, analyse and report data on zoonoses, and it is responsible for publishing of the annual Community Summary report on zoonoses. EFSA is currently developing and improving this data collection system. A new web-based reporting system and database have been established, and the reporting and the monitoring will be further harmonised in order to provide more comparable and high quality data. In this exercise EFSA is assisted by a Task Force, where the Member States, the Commission, WHO and OIE are represented. The zoonoses monitoring team will also assist the Scientific Panels, when requested. Concerning *Salmonella*, the zoonoses monitoring team has got requests from the Commission to prepare draft monitoring schemes for *Salmonella* in poultry meat and for antimicrobial resistance in general. The zoonoses monitoring team will also analyse the results from the *Salmonella* baseline studies carried out in the Community.

***Discussion***

Q: It would be helpful to have a website at EFSA where results of a Member State can be placed and where also results from other countries can be found (e.g. on certain phagetypes).

A: In principle all data received from the Member States will be published on the website. This information is then available for all Member States. It is not yet clear who may have access to the data in the database (may be sensitive data). This is still under discussion.

## 2.8 Results research activities CRL-*Salmonella*

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

An overview was given on

1. The progress with standardisation of the method for detection of *Salmonella* spp. in the primary production stage (draft Annex D);
2. Research activities with poultry faeces and pigs' faeces.

1. Draft Annex D of ISO 6579:
   The first proposal for standardisation of the detection of *Salmonella* spp. in poultry faeces was made in December 2002 at a meeting of ISO/TC34/SC9 in Bangkok. Many steps have been made since then to become at the present stage of voting on the New Work Item Proposal (April 2005). In June 2005 the results of voting and the comments on the draft Annex D will be further discussed at a meeting of ISO/TC34/SC9 in Warsaw.

2. Several research activities were performed at CRL-*Salmonella* in relation with draft Annex D and/or in relation with the samples as used in the interlaboratory comparison studies. The following was investigated:
   - *Novobiocin concentration in MSRV*. A higher percentage of positive *Salmonella* samples were found when pig faeces was analysed with MSRV containing 0.01 g/L novobiocin, when compared to MSRV containing 0.02 g/L novobiocin. The results with poultry faeces were less pronounced, although for poultry faeces as well as for pigs faeces the 'spreading' of *Salmonella* was larger on MSRV containing 0.01 g/L novobiocin than on MSRV containing 0.02 g/L novobiocin. It was therefore decided to prescribe 0.01 g/L novobiocin in MSRV in draft Annex D.
   - *Stability of Salmonella and aerobic total count in chicken faeces*. *Salmonella* positive chicken faeces mixed with peptone/glycerol (30 %) showed stable results for *Salmonella* Enteritidis ($10^3$-$10^4$cfp/g) when stored at -20 °C, for at least 14 days. Storage at +5 °C showed almost 2 $\log_{10}$ decreases in the number of cfp after 7 days of storage. At +20 °C the number of *Salmonella* came below the detection limit after (already) 2 days of storage. The number of aerobic total count (*ca* $10^9$ cfp/g) remained stable for at least 14 days at -20 °C, +5 °C and +20 °C, independent whether the faeces was mixed or not with peptone/glycerol.

- *Incubation of chicken faeces and pigs faeces in dilution steps of 1/10 or 1/100 in BPW*. For faeces not mixed with peptone/glycerol and originated from chicken or from pigs, no differences were found in the two dilutions. For chicken faeces mixed with peptone/glycerol (30 % v/v glycerol) the results were variable. More positives were found in a 1/10 dilution if the BPW was incubated for only 4 h. However, if the same BPW was incubated for 18 h the 1/100 dilution gave (much) more positive results.
- *Incubation of chicken faeces and pigs faeces in BPW for 4 h and for 18 h. Salmonella* negative faeces mixed with peptone/glycerol (30 % v/v glycerol) and artificially contaminated with *Salmonella* reference materials gave in general more positive results after 18 h of incubation of BPW than after 4 h of incubation, but not all samples were 100 % positive. *Salmonella* positive (naturally contaminated) chicken faeces, mixed with peptone/glycerol (30 % v/v glycerol) showed opposite results (much more positives after 4 h of incubation). If the faeces was not mixed with peptone/glycerol, originated from chicken and from pigs, 100 % positive results were found after 18 h of incubation of BPW. After 4 h of incubation only a few sample were found positive.
- *Influence of glycerol on the detection of Salmonella.* Chicken faeces (negative for *Salmonella*) was mixed (1:1) with Tryptone Soya Broth (TSB)/glycerol 30 %, TSB/glycerol 15 %, peptone/glycerol 30 %, peptone/glycerol 15 % and not mixed at all. All faeces samples were artificially contaminated with *Salmonella* reference materials. Hundred percent positives were found with the non-mixed faeces. Lower numbers of positives were found with the mixed faeces with 15 % glycerol. Very low numbers of positives were found with the mixed faeces with 30 % glycerol.

The main conclusion from the experiments was that glycerol has a negative effect on the growth of *Salmonella* (*S.* Typhimurium as well as *S.* Enteritidis).

***Discussion***

Q: Is there a way to solve the ‘glycerol problem’?

A: For the moment we see as only solution to use fresh, not mixed faeces in the next interlaboratory study.

During the discussion it was suggested to test the influence of glycerol on the growth of *Salmonella* and background flora in BPW by taking samples every hour. Furthermore another possible preservation medium for mixing the faeces was suggested, being double strength skim milk. Both suggestions will be studied by the CRL.

## 2.9 Results bacteriological detection study VIII - 2004

*Kirsten Mooijman/Hans Korver, CRL-Salmonella, Bilthoven, the Netherlands*

The eighth interlaboratory comparison study on the detection of *Salmonella* was organised by the Community Reference Laboratory for *Salmonella* (CRL-*Salmonella*, Bilthoven, the Netherlands), in fall 2004. Twenty-eight National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) participated in the study.
Reference materials in combination with or without the presence of chicken faeces, as well as naturally contaminated faecal samples (containing *Salmonella* Enteritidis) were tested by all laboratories. The reference materials existed of gelatine capsules containing *Salmonella* Typhimurium (STM), *Salmonella* Enteritidis (SE) or *Salmonella* Panama (SPan) at different contamination levels. The contamination levels for *S.* Typhimurium were *ca* 10 cfp/capsule and *ca* 100 cfp/capsule, for *S.* Enteritidis *ca* 100 cfp/capsule and *ca* 500 cfp/capsule and for *S.* Panama *ca* 5 cfp/capsule. Furthermore a number of blank capsules (containing sterile milk-powder) had to be tested.
The prescribed procedure for analyses was:

- Pre-enrichment in Buffered Peptone Water (BPW) with two incubation times (4 h and 18 h);
- Selective enrichment on Modified Semi-solid Rappaport Vassiliadis agar (MSRV);
- Plating-out on Xylose Lysine Deoxycholate agar (XLD).

Each laboratory was also permitted to test the materials with their own medium combination(s).

The specificity for the blank capsules (without faeces) for both incubation times of BPW was 100 %. The sensitivity for the SPan 5, STM 10, SE 100 and SE 500 capsules (without faeces) with an incubation time in BPW of 4 h was 40 %, 40 %, 30 % and 80 %, respectively. The sensitivity for the same capsules (without faeces) after 18 h of incubation was respectively 96 %, 100 %, 98 % and 100 %.The accuracy rate for all capsules (without faeces) was 52 % after 4 h and 99 % after 18 h of incubation in BPW.

The specificity, sensitivity and accuracy of capsules with the addition of 10 g negative chicken faeces were as follows. The specificity for the blank capsules was 97 % after 4 h and 93 % after 18 h of incubation in BPW. The sensitivity for the STM 10, STM 100, SE 100 and SE 500 capsules with an incubation time in BPW of 4 h was 37 %, 70 %, 22 % and 59 %, respectively. The sensitivity for the same capsules after 18 h of incubation was respectively 81 %, 81 %, 62 % and 79 %. The accuracy for all capsules was 49 % after 4 h and 77 % after 18 h of incubation in BPW.

The sensitivity of the naturally contaminated samples (containing *Salmonella* Enteritidis) was 81 % after 4 h and 55 % after 18 h of incubation in BPW.

***Discussion***

Q: Are the differences between the results of the artificially contaminated samples and the naturally contaminated samples for the different incubation times of BPW only due to the effect of glycerol?

A: This is not sure, but it looks like it, especially if you look at the research results when non-mixed faeces was tested.

## 2.10 Discussion on design bacteriological detection study IX – 2005

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

For the interlaboratory comparison study on detection of *Salmonella* it was agreed that:

1. Transport of the samples would again be performed as diagnostic specimens. Good results (fast transport) were obtained with this way of mailing in the 2004 study;
2. Again temperature recorders will be included with the samples to record the temperature during transport;
3. The study will be organised in (early) November 2005.

Discussion items were:

4. Choice of samples and methods;
5. Follow-up in case of poor performance.

4. The following samples were proposed:
   Use of poultry faeces **not** mixed with glycerol and stored at 5 °C. Resulting in the fact that naturally polluted poultry faeces can not be used, as *Salmonella* is not stable in the faeces.
   For the artificially contaminated poultry faeces it was proposed to use a similar set-up as earlier studies, to check the effect of absence of glycerol:
   - 10 control capsules without poultry faeces (controls), including STM, SE, SPan, blank;
   - 25 capsules + 10 g *Salmonella* negative poultry faeces, including STM10, STM100, SE100, SE500, blank;

For naturally contaminated (with *Salmonella*) samples it was suggested to use *Salmonella* positive dust (10x 10g), as it was shown that *Salmonella* remains present in dust for at least half a year when stored at 5 °C.

For the methods the following was proposed:

- Draft annex D of ISO 6579: MSRV (+0.01 g/L novobiocin) 2x 24 h, plating on XLD. A problem might arise when the new working item proposal on Annex D would not be accepted in ISO.
- For comparison with study 2004 and for further check on the effect of glycerol in earlier studies it was proposed to incubate the BPW again for two incubation times (4 h and 18 h);
- Optional: Own method(s), following own procedures.

5. A proposal was made for the follow-up in case of 'poor performance':
    - Sending extra materials soon after the study;
    - How to define 'poor performance'? The following was suggested to define '**good**' performance:
        - Positive control capsules should all be positive, except for Span5 of which 1 out of 2 may be negative;
        - Blank control capsules should all be negative;
        - Of blank capsules + faeces, 80 % should be negative, meaning that 1 out of 3 samples may be positive;
        - Of STM100, SE500 + faeces, 80 % should be positive, meaning that 3 out of 4 samples should be positive;
        - Of STM10, SE100 + faeces, *ca* 50 % should be positive, meaning that 3-4 out of 7 samples should be positive.

***Discussion***

*Dust*

Q: How to exclude cross contamination when working with dust?

A: Work in a safety cabinet, work carefully and clean immediately after working with dust. In the United Kingdom already many dust samples have been analysed, with very little problems with cross contamination.

The majority of the NRLs were positive about the idea of including dust in the next study. The CRL will therefore try to collect *Salmonella* positive dust samples.

*Methods*

Q: In France also MKTT is used beside MSRV and more positives are found. Can MKTT be added to the method?

A: In principle draft Annex D will be the method for use, and this will include MSRV only (2x 24 h). It will depend on the discussion in ISO whether draft Annex D will change a lot.

Furthermore, it was agreed to test again the 4 h and 18 h incubation of BPW, not only to have a better comparison with the 2004 study where mixed faeces was used, but also to have an indication whether the total procedure could be shortened by shortening the pre-enrichment step.

*Follow-up*
The NRLs agreed with the suggestions for follow-up in case of poor performance and with the suggestions for definition of poor or good performance.

## 2.11 Results typing study X – 2005: phagetyping

*Linda Ward, Health Protection Agency, London, United Kingdom*

The Laboratory of Enteric Pathogens (LEP), of the Health Protection Agency in England, provided ten *Salmonella* Enteritidis strains and ten *Salmonella* Typhimurium strains for phagetyping in the X – 2005 interlaboratory comparison study on typing. The strains were selected from the LEP current culture collection. Results have been obtained from ten laboratories of which seven are NRLs and three EnterNet laboratories (ENLs). Results from the remaining ENLs are awaited. On the whole the results are good with seven laboratories (5 NRLs and 2 ENLs) correctly identifying all ten of the *S.* Enteritidis strains and the remaining three laboratories, identifying nine of the strains. Six laboratories (4 NRLs and 2 ENLs) identified the ten *S.* Typhimurium strains correctly, with two NRLs having correct results for nine strains and one ENL correctly identified eight *S.* Typhimurium strains. Four of the ten laboratories (3 NRLs and 1 ENL) identified all 20 strains correctly. Five laboratories (4 NRLs and 1 ENL) had one misidentification and the remaining ENL had two incorrect results.

## 2.12 Results typing study X – 2005: serotyping

*Arjen van de Giessen/Hans Korver, CRL-Salmonella, Bilthoven, the Netherlands*

In spring 2005 the tenth interlaboratory comparison study on 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) in London and the Central Institute for Animal Disease Control – Section Infectious Diseases

(CIDC, Lelystad, the Netherlands). The main goal of this study was to compare the results among the National Reference Laboratories (NRLs-*Salmonella*) and among the EnterNet Laboratories (ENLs). Twenty-five NRLs-*Salmonella* of the EU Member States and NRL-Norway participated in the serotyping part of this study.
A total of 20 strains of the species *Salmonella enterica* subspecies *enterica* were selected by the CRL-*Salmonella*. Among these twenty strains, twelve strains were included which belong to the most frequently occurring serotypes within the European Union. Furthermore, some strains were included that caused problems in serotyping in earlier interlaboratory comparison studies. Six strains of group B, six strains of group C1, two strains of group C2-C3, two strains of group D1, two strains of group G and one strain of group J were included in the panel of *Salmonella* strains which had to be investigated.
As in earlier studies, most problems were encountered when typing the H-antigens. In the study of 2002 strain *S*. Oranienburg was accurately serotyped by 59 % of the NRLs, while the same strain scored 65 % of accurate serotyping in the 2005 study. Strain *S*. Banana of the 2004 study scored 44 % of accurate typing results and 81 % in the study of 2005. This improvement may be caused by the fact that the strain of the 2005 study was the reference strain for serovar *S*. Banana and the strain of the 2004 study was a field isolate. However, also a better understanding of the interpretation of typing results, especially for the H-antigens g, m, t in relation to m, t strains may have lead to this improvement.
Hundred percent of accurate serotyping was obtained by 13 laboratories while eight *Salmonella* strains were typed correctly by all participating NRLs.
The serotyping results of the five most important serovars within the European Union over a ten year period were as follows: 100 % of accurate serotyping for *S*. Typhimurium, 99 % for *S*. Infantis, 98 % for *S*. Enteritidis and *S*. Virchow, and 92 % for *S*. Hadar.

***Discussion***
Q: Is it possible that the quality of the sera has influenced the results?
A: This can not be excluded. It is difficult to distinguish between the influence of the quality of the sera and a problem with analyses.
Q: Will there also come a ‘definition’ for good/poor performance for the next typing study and a follow-up?
A: This still need to be worked out. A suggestion would be that for strains with human significance a score of 100 % correct typing should be found. For other strains this may be e.g. 95 %.

## 2.13 Results typing study X – 2005: antibiotic resistance testing

*Dik Mevius, CIDC, Lelystad, the Netherlands*

The conclusions from the CRL-workshop held in 2004 were:

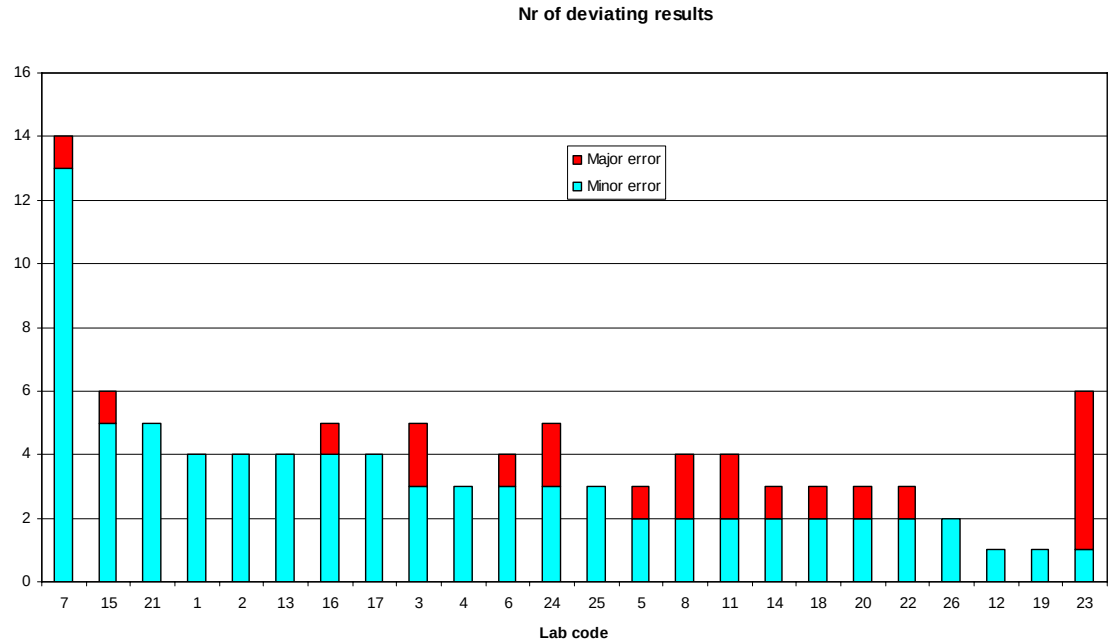
1. **For EQAS-reference MICs:**
    a. No more e.g. neomycin or kanamycin tested, because cross resistance is not 100 %.
    b. Genetic profiles of ß-lactam resistance may assist in the understanding of the MIC results.
    c. Or wait for EU-project results starting as part of MEDVETNET network project.
2. **For monitoring purposes**
    a. Exclude AMCL because it is not reliable.
        Instead: Ampi/amox, Cefotaxime and ESBL confirmation with E-test.
    b. Streptomycin's value is disputed.
    c. Trim/Sulpha is disputed, preferable the individual components;
        Including:
        - Sulphamethoxazole
        - Nal and ciprofloxacin (not enro)
        - Neomycin (not kanamycin)

Based on their MIC-profile, the following strains were selected:

| **2005** | **Source** | **Serotype** |
|---|---|---|
| **AST-1** | Patient | *S.* Enteritidis |
| **AST-2** | Minced meat | *S.* Typhimurium |
| **AST-3** | Patient | *S.* Typhimurium |
| **AST-4** | Patient | *S.* Corvallis |
| **AST-5** | Broiler | *S.* Infantis |
| **AST-6** | Patient | *S.* Enteritidis |
| **AST-7** | Chicken | *S.* Hadar |
| **AST-8** | Patient | *S.* Typhimurium |
| **AST-9** | Patient | *S.* Kentucky |
| **AST-10** | Patient | *S.* Typhimurium |

All MICs were confirmed by retesting with broth micro dilution or E-test for amoxicillin-clavulanic acid and streptomycin. At the time of the workshop 25 laboratories had supplied their results: 18 provided zone diameters and 8 MICs (one participant provided both zone diameters and MICs).

For all either highly susceptible or very resistant bacteria, the level of agreement was very high and only a small number of errors were made. For those bacteria that were intermediate or borderline susceptible (close to the breakpoint), the numbers of inconsistencies were, as expected, higher.
Streptomycin gave fewer errors than in 2003 because most isolates were resistant.
The combination amoxicillin-clavulanic acid again caused a lot of confusion and many deviating results were produced, specifically on amoxicillin-resistant but clavunalate susceptible strains numbers AST 1, 3, 7, 8, 9 and 10. Because in monitoring programmes it is the specific purpose to detect the emergence of and trends in resistance, the inclusion of amoxicillin clavanulate will result in an over reportage of AmpC-type strains.
In study IX (2004), trimethoprim/sulphamethoxazole caused a lot of variety in the results. However in the 2005 ring trial for this antibiotic combination no deviating results were produced, which indicates a positive effect of the inter laboratory comparison studies. Because when testing this combination a strain will only be designated R if it is resistant to both trimethoprim and sulphamethoxazole, for monitoring purposes it is advisable to include the individual compounds in the antibiotic panel tested.




The numbers of deviating results made in 2005 are presented in the figure.

Based on the results the following suggestions were made:

- Amoxicillin/clavulanic acid is not a reliable indicator for inhibitor resistant ß-lactamases, test instead:
  - Ampi/Amox, Cefotaxime and Ceftazidime (breakpoint 1 µg/ml);
  - Additionally test a limited number of ESBL suspected strains:

- Ceftazidime/Cefotaxime with and without clavulanic acid (Etest or double disk test)
  - For ESBL determination
- Cefoxitin
  - For AmpC phenotype

■ Use Trim and Sulpha in monitoring; not the combination.

***Discussion***

Q: Will the antibiotic resistance activities continue in CRL-*Salmonella*?
A: It is intended to appoint a CRL for antibiotic resistance. If such a CRL will start, the activities on antibiotic resistance of the CRL-*Salmonella* will be handed over to this new CRL. This may already be the case in 2006, else in 2007.

## 2.14 Discussion on design typing study XI - 2006

*Arjen van de Giessen/ Hans Korver, CRL-Salmonella, Bilthoven, the Netherlands*

As in previous years the organisation of the eleventh typing study will be done by CRL-*Salmonella* in collaboration with the Health Protection Agency (HPA, London, UK) and (if necessary) with the Central Institute for Animal Disease Control (CIDC, Lelystad, the Netherlands). The eleventh typing study will take place in spring 2006.
Again twenty *Salmonella* strains have to be investigated by the National Reference Laboratories for *Salmonella* for serotyping. The five most important serovars within the European Union will be an important part in this eleventh interlaboratory comparison study on the typing of *Salmonella*. Furthermore, strains that caused problems in the past could be included in the panel of twenty strains.
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.
It is not sure whether the antimicrobial susceptibility testing will still be a part of the eleventh typing study of CRL-*Salmonella*. This will depend on whether new CRLs will be established by the end of 2005/ early 2006. One of the new CRLs will be a CRL for antimicrobial susceptibility testing. In fall 2005 it will be decided whether the typing study of 2006 (to be organised by CRL-*Salmonella*) will still include susceptibility testing or not. If so, this will be done on 10 strains with a panel of approximately 14 antibiotics.

***Discussion***

The NRLs agreed with the suggested lay-out for the next study.

# 3. Friday 29 April 2005: day 2 of the workshop

## 3.1 Implications of the new feed and food regulation

*Sarolta Idei/Jean-Charles Cavitte, European Commission, Brussels, Belgium*

*Community legislation in force* includes some microbiological criteria for foodstuffs of animal origin. These criteria are laid down in different pieces of legislation. These criteria are applicable at the stage of production, in intra community trade and in controls of imports from third countries.
In 1999, the *Scientific Committee on Veterinary Measure Relating to Public Health* gave its opinion on current microbiological criteria and concluded that the criteria were varied and not based on risk assessment. The Committee recommended that microbiological criteria should be relevant and effective in relation to public health protection and harmonised.
The principle of the EU food safety policy is to ensure a *high level* of *human health* and *consumer protection*. It underlines the importance of greater involvement by *food business operators* (implementation of *good hygiene practice* and the application of *HACCP-based principles)*.
In the framework of the *recast of Community food hygiene legislation* it was considered necessary to set up a strategy to create microbiological criteria for foodstuffs. The legal basis to set microbiological criteria is *Regulation EC 852/2004*.
Food business operators must comply with the microbiological criteria set in the context of their *HACCP-procedures*. This should include testing against the values set for certain criteria through sampling, the conduct of analyses and the implementation of corrective actions.
*Official controls* are to verify that the criteria set for food business operators are fulfilled. Member States shall ensure that official controls are carried out regularly, on a risk basis, as it is laid down in *Regulation EC 882/2004.*
According to the *Codex standard* a microbiological criterion includes: the foodstuff, the microorganism, the analytical method, the sampling plan, the limits, stage of application, actions to be taken in case of unsatisfactory results.
The draft Regulation lays down *two types of criteria (food safety* and *process hygiene criteria*). For each criterion a *reference method* is included. Test results are dependent on the analytical method used. There is a possibility for food business operators to use other (*alternative*) analytical methods other than the reference methods if they provide *equivalent results*.

***Discussion***

Q: Is it possible to say more about the official food and feed activities in relation with microbiological activities?
A: Presently draft guidelines are being prepared for the official regulators.
Q: What is the present situation for appointing new CRLs?
A: Hopefully within a few weeks a call will be placed for new CRLs for Listeria, *E. coli* (VTEC), Campylobacter, Staphylococci, Antibiotic resistance, parasites, Brucellosis. If a new CRL is appointed, automatically Member States need to appoint NRLs.
Q: What is the length of the working period for nominated CRLs?
A: In principle a 5 year framework contract need to be signed by each CRL. It may be possible to prolongate the contract with another 5 year. The designation is not forever!
Q: Is it obliged that laboratories performing the official analyses are accredited?
A: The legislation requires that all official laboratories are accredited from 010106.

## 3.2 Questionnaire matrices and follow-up Proficiency testing

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

According to the EU legislation on Zoonoses and on Feed and Food, the activities of CRL-*Salmonella* as well as of the NRLs-*Salmonella* should not only focus on *Salmonella* in livestock, but also on *Salmonella* in feed and food as well as in environmental samples. To make an inventory on the experiences of the NRLs-*Salmonella* with the detection of *Salmonella* in different matrices a questionnaire was sent to all NRLs-*Salmonella* in February 2005. Beside this inventory, this questionnaire was also intended as a follow-up of the questionnaire on proficiency testing as was sent to the NRLs in 2004. In April 2005 completed questionnaires of all 26 NRLs were received by the CRL-*Salmonella*.
The results of the questionnaires were summarised in a draft report and presented at the workshop per question.

The following conclusions were drawn from the questionnaires:

**Matrices**

- All NRLs perform bacteriological detection of *Salmonella* spp.;
- All, except one, NRLs perform serotyping of *Salmonella* spp.;
- Seven NRLs ‘subcontract’ another laboratory in their Member State for specific activities;
- The NRLs are able to analyse a wide variety of matrices. Fourteen or more NRLs analyse:

- Faeces of poultry, pigs, cattle, wild animals and pets;
- Meat of poultry, pigs and cattle;
- Feed for poultry, pigs and cattle;
- Environmental samples (like dust);
- Meconium (poultry);
- Down/fluff;
- Chicks dead in shells of eggs;
- Eggs/egg products.

**Follow-up Proficiency Testing**

- From the questionnaire of 2004 the information was available that 6 NRLs did not yet organise national proficiency tests;
- Sixteen (out of 26) NRLs-*Salmonella* participated in the workshop on Proficiency Testing held at the IRMM in Geel, Belgium in October 2004. Five of the non-organising NRLs also participated in this workshop;
- All NRLs, except one, found the information of the workshop useful;
- Eleven (out of 18) NRLs which already organised Proficiency Tests indicated some changes in their studies (number of participants, number of studies, materials, methods);
- Six (out of 8) NRLs which did not yet organise Proficiency Tests indicated to have started or will soon start with the organisation of Proficiency Tests;
- The NRLs gave many suggestions to the CRL, how the CRL can facilitate the NRLs with organisation of Proficiency Tests. Items were:
  - Need for guidelines on the organisation of Proficiency Tests (like no. of strains, contamination levels, no. of samples, how to analyse the results). For this item it was mentioned that recently a working group in ISO/TC34/SC9 has started to work on the subject, especially for the microbiological field. CRL-*Salmonella* is a participant in this working group.
  - Need for reference materials. Up to now, only a few NRLs have tried the lenticules of Health Protection Agency (HPA) in Newcastle, UK. CRL-*Salmonella* has recently made an order at HPA for reference materials containing similar serovars and levels as of the reference materials as used in the CRL interlaboratory comparison studies. Information on the usefulness of this type of reference materials will be sent to the NRLs as soon as it will become available.

***Discussion***

Q: In Denmark lenticules were tested, but it was not possible to obtain lenticules with the more ‘usual’ serovars. Also the contamination levels of the lenticules were low.

A: The CRL has recently contacted HPA to order lenticules with the usual serovars (*Salmonella* Typhimurium and *Salmonella* Enteritidis) with the usual contamination levels (as used in the capsules for the ring tests). When the lenticules are received and tested the results will be reported to the NRLs.
Q: Is it possible that CRL will make a list with types and numbers of lenticules needed by the NRLs and communicate this with HPA?
A: This is possible. The CRL can start doing so after the lenticules as mentioned above have been tested.

## 3.3 Phagetyping, now and in future

*Linda Ward/ Elizabeth de Pinna, Health Protection Agency, London, UK*

*Salmonella* phagetyping has been carried out in the Laboratory of Enteric Pathogens (LEP), formerly the Enteric Reference Laboratory, since the nineteen forties, starting with *Salmonella* Typhi Vi-phagetyping.

Today there are ten phagetyping schemes used routinely in the LEP, namely *Salmonella* Typhi, *S.* Paratyphi A, *S.* Paratyphi B, *S.* Enteritidis, *S.* Typhimurium, *S.* Virchow, *S.* Hadar, *S.* Thompson, *S.* Agona and *S.* Pullorum. In addition the LEP has phagetyping schemes for *S.* Newport, *S.* Bredeney and *S.* Kedougou that can be used in outbreak situations. The schemes, particularly for *S.* Typhi, *S.* Paratyphi A, *S.* Paratyphi B, *S.* Enteritidis and *S.* Typhimurium are in use worldwide and are continually being developed and improved. All the typing phages are propagated in the LEP and all laboratories are provided with identical preparations.

In recent years there has been an increasing demand for *Salmonella* phagetyping and this has added to the work load of the LEP, resulting in some delays in the distribution of the phage-typing reagents. However with some reorganisation we hope that our service will improve and that the LEP will continue to provide typing phages and training in *Salmonella* phage-typing. It is possible that the LEP will have to charge for the phagetyping reagents in the near future.

***Discussion***
During the discussion it was made clear that continuity in the availability of materials and knowledge for the phagetyping is very important. Up to now the Health Protection Agency (HPA) did not charge the materials. This may change in the future. For most of the laboratories it is very acceptable to pay for it. Also training and exchange of information was

considered very important. HPA is willing to continue in giving trainings for small groups. Besides training, also a special meeting/workshop related to phagetyping would be of interest. It would be most efficient if such a meeting/workshop could be organised in relation with another (relevant) meeting.
Furthermore, it was mentioned that it would be very useful to have a central database in which phagetyping data of veterinary samples could be collected. New and special types could then also be added to this database. Such a database already exists for human isolates and is managed by HPA in London. It was discussed whether a veterinary database should be linked to this human database or if it should be separate (e.g. managed by CRL-*Salmonella*, or EFSA, or another institute). No final decisions were made. Finally it was mentioned by EC DG-Sanco that as phagetyping is a stable, recognised and valid method to investigate e.g. food born outbreaks, it would be important that more laboratories perform phagetyping (by now only 7 NRLs perform phagetyping).

## 3.4 Pulsed-Field Gel Electrophoresis (PFGE) typing of *Salmonella* spp.; Salm-gene

*Max Heck, Diagnostic Laboratory for Infectious Diseases and Perinatal Screening, RIVM, Bilthoven, the Netherlands*

A major part of the prevention and control of salmonellosis is reliant on early outbreak recognition combined with prompt epidemiological investigation. With increasing travel and global trade, outbreaks involving widely scattered cases are occurring more frequently. In order to recognise any link between such cases, it is necessary to use rapid subtyping methods and pool the resulting data promptly.
Rapid sub-typing of all *Salmonella* isolates nationally, through serotyping, phagetyping and the use of antimicrobial resistance patterns, has been the basis of successful international surveillance. However, across many countries certain *Salmonella* serotypes and phagetypes predominate, hindering our ability to recognise geographically or temporally scattered increases in cases involving these strains. The introduction of more discriminatory subtyping methods needs consideration as this may considerably improve the outbreak detection power of current laboratory typing.
The use of molecular subtyping methods as an adjunct to phenotypic methods in the investigation of outbreaks has been valuable. However its value as a tool for food borne disease surveillance in Europe remains to be established and was investigated in the Salm-gene project.
The overall aims of the Salm-gene study were: 1) Study whether molecular sub-typing will enhance the timely recognition of international outbreaks recognised through the current

international laboratory-based surveillance network for human enteric pathogens – Enter-net. 2) Establish the feasibility of capturing molecular information on *Salmonella* strains internationally in real-time for surveillance purposes.
The aims were achieved through: 1) The development of a standard laboratory operating procedure for Pulsed-Field Gel Electrophoresis (PFGE) including an external quality assurance scheme. PFGE is currently the gold standard for subtyping of *Salmonella*.
2) A specification of the range and incidence of gene-print subtypes of the major *Salmonella* serotypes and phagetypes in participating countries. 3) The creation a searchable database of gene-print information. The Salm-gene database was created in the latest version of BioNumerics (v.3.1, beta), a software package specifically designed for the capture and analysis of microbiological data such as gel images. 4) A large sample of *Salmonella* strains in each participating country was Gene-printed in real-time and data were analysed continuously. By the end of 2004, approximately 23 000 strains of *Salmonella* (excluding control strains) had been entered into the Salm-gene database since the start of the project, of which 51 % were *S.* Enteritidis and 26 % were *S.* Typhimurium. 5) The cost effectiveness was studied and recommendations were made for incorporating gene-printing into national laboratory based surveillance of *Salmonella.*
The usefulness of Salm-gene was demonstrated by the several 'possible outbreaks' that were identified by different incidences of strain profiles from January to June 2004: 4 (Jan-Feb); 5 (Mar-Apr); 3 (May-Jun). The number of strains involved in each ranged from 6 to 100 strains and involved mostly commonly reported patterns.
The Salm-gene project has finished 31st December 2004 and a final report will be published shortly. The Salm-gene data will be migrated to PulseNet EU by the end of 2005. It is proposed that PulseNet participants have access in the first instance and then the database be handed over to the PulseNet EU Steering Committee. PulseNet EU is part of MedVetNet, whether this means that MedVetNet will also have access to the Salm-gene database needs to be clarified formally.
Further detailed information can be found on the Salm-gene website: www.salmgene.net

***Discussion***

Q: Is the continuity of the database guaranteed?
A: Project Salm-gene is now finished and it was decided to hand-over the database to PulseNet Europe. However, PulseNet Europe is still waiting for funding.
Q: Is phagetyping included in the database?
A: Various information per strain is available, like serotype, phagetype, antibiotic resistance.

## 3.5 Bacteriological monitoring of *Salmonella* in pigs

*Arjen van de Giessen, NRL-Netherlands, Bilthoven, the Netherlands*

In the Netherlands, from 1998 till 2004, a surveillance programme for zoonotic bacteria in finishing pigs was conducted at herd level. In 2000-2004, the prevalence of *Salmonella* spp. approximated 30 % and no significant trend was observed. Serotype discrimination showed the predominance of *S.* Typhimurium. By using the samples from this study, a comparison study was conducted in which three different selective enrichment media, i.e. RV, MSRV and DIASALM, were compared for the isolation of *Salmonella* spp. from pig faeces. Both MSRV and DIASALM scored significantly better compared to RV. By using logistic regression analysis of farm and herd specific data, potential risk factors for *Salmonella* spp. in finishing pig herds were identified and quantified including the seasons spring (Odds Ratio (OR) = 3.51; *P* value = 0.0164), summer (OR = 2.74; *P* = 0.0358) and autumn (OR = 3.76; *P* = 0.0077) all compared to winter, a moderate farm size (501-1000 animals) (OR = 4.22; *P* = 0.0032) compared to a small farm size (201-500 animals), one specific feed supplier (OR = 12.3; *P* = 0.0027) compared to the reference supplier, the use of fermented liquid feed (OR = 0.37; *P* = 0.0280) compared to its non-use, the use of natural ventilation (OR = 6.18; *P* = 0.0231) compared to mechanical ventilation, presence of cats on the farm (OR = 0.25; *P* = 0.0222) compared to the absence of animals and an age of 2-3 months (OR = 0.17; *P* = 0.0446) compared to an age of 3-4 months.

***Discussion***

Q: When you find a phagetype which is commonly found in different animal species, how do you decide which percentage is related to pigs?

A: This is based on relative prevalence of certain phagetypes in the reservoirs. The percentages are therefore approximations.

Q: Which phagetyping system is followed in the Netherlands?

A: For *Salmonella* Enteritidis a change was made from the NL-system to the UK-system. For *Salmonella* Typhimurium the NL-system as well as the UK-system are performed. The NL-system is more sensitive and therefore no change was made here to the UK-system.

Q: Is there any information on drugs given to the herds and the relevant resistance to *Salmonella*?

A: We do not fully know the influence of antimicrobials given to the herds on the detection of *Salmonella*.

## 3.6 UK *Salmonella* monitoring and control programme in pigs

*Rob Davies, NRL-UK, Addlestone, United Kingdom*

A national survey of pathogens carried by red-meat animals at slaughter carried out between 1999 and 2000 identified 25 % of pigs carrying *Salmonella* at slaughter. High proportions of these strains were DT104 and related multiple antimicrobial resistant strains such as U302. Following the introduction of national control programmes in Denmark and Ireland a similar scheme was set up in UK – The *Salmonella* Zoonoses Action Plan (ZAP) Scheme. This was incorporated into national quality assurance schemes operated by British pig industry organisations. The target for the scheme is a 25 % reduction in the prevalence of *Salmonella* in three years.

The scheme was initially set up aiming to test one meat juice sample per 50 pigs per slaughter batch but this led to insufficient samples from small farms so this was changed to three samples per batch, to provide a minimum of 15 samples per quarter year to determine the herd seroprevalence. The test used is a commercial ELISA kit (Guildhay Vetsign) operated by a commercial laboratory under contract to the British Pig Executive (BPEX) of the Meat and Livestock Commission (MLC). Sample/positive ratios of >0.25 are interpreted as positive results.

Herds are categorised from 1 (<65 % samples positive) to 3 (>85 % positive). ZAP 2 and 3 herds are obliged to take action to reduce the seroprevalence to ZAP 1 levels or after a series of stages their assurance scheme membership is suspended which means exclusion of pigs from major abattoirs.

In the first year of the scheme there was little change in the overall seroprevalence which remained around 21 %, but it was clear that there were significantly greater seroprevalences in England than in Scotland or Northern Ireland. The overall breakdown of farms in the quarter April to June 2004 was 92.6 % in ZAP 1, 5.7 % in ZAP 2 and 1.7 % in ZAP 3. ZAP 2 and 3 farms are being offered advisory sampling visits from the Veterinary Laboratories Agency as part of a Defra funded project.

Research work comparing bacteriology and serology showed a poor correlation, with one particular ELISA kit used at the 20 % Optical Density level giving the best correlation with the prevalence of pooled pen faeces samples. It was also possible to pool samples and use mean ODs or S/Ps as indicators of herd infection and this also gave a reasonable correlation with pen faeces. Pen faeces showed a better correlation with positive caeca at slaughter than

serology but there was a high proportion of outlying results. Analysis of the accuracy of designation of ZAP scores determined a sensitivity of 92 % and specificity of 85 %, with errors being made at the interfaces of the different scores.

It is concluded that serological monitoring via meat juice is a practical means of monitoring progress in overall *Salmonella* control programmes but cannot replace bacteriology for international monitoring and early warning schemes for emerging serovars.

## 3.7 Serological detection of *Salmonella* in swine

*Peter van der Wolf, Animal Health Service, Deventer, the Netherlands*

**Development**

The developed Elisa was tested for its efficacy using monospecific sera against *S*. Typhimurium, *S*. Infantis and SPF sera. A Lipopolysaccharide (LPS) coating (individual vs. mixed) was used as antigen in the Elisa. Reactions to specific sera were clearly distinguishable from SPF-sera. Correlation between the results from the individual coatings and the mixed coating with serogroup B- and C1-LPS was excellent. Several sample matrix (serum, colostrum, meat drip) were tested for their possibilities. All three can be used to test for *Salmonella* antibodies. For each an optimal sample dilution was determined. Also pooling of samples was studied. Pooling is possible and useful up to 10 sera given a low (herd) prevalence. Repeatability and reproducibility of the test were evaluated.
Evaluation of field sera from a longitudinal study in sows and their offspring showed that pigs with a B and D1 serogroup infection reacted in the test and that C2-infected pigs did not react, which was expected.

The original publication by Nielsen *et al*. from 1995 in Veterinary Microbiology also showed a good sero-response from infected animals. In the EU-funded research program SALINPORK (contract FAIR1-CT95-0400) the *Salmonella*-Elisa was used in five countries within the EU. Herds that were infected with *Salmonella* Typhimurium showed a high herd prevalence. ROC-curve analysis showed a reasonable combination of sensitivity and specificity (80 % both). Danilo Lo Fo Wong and Tine Hald, the editors of the final report, conclude ‘Thus, without further investigation, no distinction can be made between a ‘false positive’ serological result because of poor sensitivity of bacteriological testing or poor specificity of the serological test (i.e. cross-reactivity). However, under practical circumstances in endemic countries, poor sensitivity of bacteriological testing is generally believed to be the most likely cause of a ‘false positive’ serological result...’

From field studies a theoretical sensitivity could be calculated for the *Salmonella*-Elisa, for example in Denmark of 94.5 % (Baggesen *et al*., 1996) and in the Netherlands of 89 % (Wolf *et al*., 1999).

**Quality control**

Quality control is done at several levels. Initial test validation (new/modified assay) is done as described above. Further, every lot is validated separately against a standard serum panel. Internal kit controls are used in every ELISA-plate and plate results that deviate more than 10 % are discarded and samples are tested again. 'Blind' controls are infrequently tested additionally. Finally, ring trials are used, if available, to test across labs and different tests.

**Ring trial**

The first international ring trial was carried out by Harold van der Heijden, the Netherlands (2001). Participants came from Europe: 9 countries with 10 labs/tests, from the USA (2 labs) and from Australia (1 lab). The sample panel consisted of 47 lyophilized serum samples originating from Danish calibration sera (n = 5), negative sera (n = 5), 'specificity panel' (n = 6) including other entero-pathogens, two groups of post-inoculation samples I & II (n = 18, n = 5), serial dilution (n = 8) samples and detailed instructions. Overall the tests performed well with the exception of one in-house test from a university. Conclusions from the ring trial were that cross-reactivity with Y.enterocolitica O3 could not be totally excluded but otherwise the specificity was good. However, there were considerable differences in sensitivity. Finally, the need for international reference samples was stressed!

***Salmonella* herd and population prevalences in the Netherlands**

The *Salmonella*-Elisa developed by the AHS was used to test the population and herd prevalence in the Netherlands. At least 40 samples were collected from 406 finishing herds which showed that in about 10 % of the herds no antibodies could be demonstrated. In the other 90 % many herds had only a few positive samples but a few percent of the herds had 80 % or more positive samples. The population prevalence was estimated separately for sows and finishers using a two step sampling design. First, a sample out of all herds was randomly selected and secondly from these herds two random blood samples were tested.

| Parameter: | Value: | 95% C.I. |
|---|---|---|
| Apparent Prev.: | 24.5% | 22.6 – 26.4% |
| True Prevalence: | 28.2% | 26.2 – 30.2% |
| Sensitivity: | 85% | 82 – 88% |
| Specificity: | 99% | 99 – 100% |
| Likely hood + | 122,5 | |
| Likely hood - | 0.15 | |
| Pos. Predictive value | 0.98 | |
| Neg. Predictive value | 0.95 | |

In sows the prevalence was 60.4 % (95 % CI 58.2 – 62.6) and in finishers 24.5 % (95 % CI 22.5 – 26.5). Accordingly, the test characteristics as given in the table could be calculated.

**National monitoring programmes.**
In several countries national monitoring programs are operational, such as Denmark (Alban *et al*., 2002), Germany (Blaha, 2003), Ireland, United Kingdom and the Netherlands (Wolf *et al*., 2001). Private monitoring programs are operational or in the process of being set up in France, Belgium and Italy (see also the presentation of Arjen van de Giessen). Monitoring results are used to classify herds in low, medium and highly infected with the aim to identify problem herds and targeting intervention efforts at those problem herds.

**Conclusions**

1. *Salmonella* Elisa's are reliable tests (high specificity and reasonable sensitivity);
2. *Salmonella* Elisa's are useful for determining herd status as part of monitoring programs with the aim to target intervention efforts at highly infected herds;
3. High volume testing of blood or meat drip samples is possible through lab automation (video available of the lab Elisa robot at the Animal Health Service);
4. *Salmonella* Elisa's are cheaper than bacteriological investigation;
5. There is a need for internationally available reference sera and ring trials for test evaluation and quality control of *Salmonella*-Elisa's.

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## 3.8 Work programme CRL-*Salmonella* second half 2005, first half 2006, closure

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

**Programme coming year**
Concerning the interlaboratory comparison studies the following activities are planned:

- Performance analyses over at least four intercomparison studies (detection and typing), keeping in mind the influence of glycerol on the detection of *Salmonella* in these studies;
- Start with a follow-up system for addressing poor laboratory performance (of detection study);
- Organisation of the 9$^{th}$ bacteriological detection study (fall 2005) with non-mixed poultry faeces and dust;
- Organisation of the 11$^{th}$ typing study (spring 2005).

Concerning research the following activities are planned:

- Continuation of the stability studies of the reference materials;
- Continuation ISO activities (Annex D and working group on Proficiency Testing);
- Explore quantitative analyses of *Salmonella* (MPN/plate technique);
- Test other reference materials (lenticules);
- Explore other matrices beside poultry faeces (e.g. test stability of *Salmonella* and of the background flora), like dust, pigs' faeces, other e.g. poultry feed, eggs/products;
- Explore other methods for detection and typing of *Salmonella* spp.;
- Transfer activities of CRL-milk to CRL-*Salmonella*.

Reports need to be written about:

- Research activities;
- Questionnaires 2004 & 2005;
- QA serotyping baseline study laying hens;
- Two interlaboratory comparison studies of 2005;
- Workshop.

Concerning communication and other activities the following is planned:

- Newsletter 4x/year through website;
- Update website;
- Maintain accreditation according to ISO 17025; explore accreditation for organisation of Proficiency Testing;
- Ad hoc activities on own initiative or on request;
- Workshop in April 2006, taking into account I3S symposium on 10-12 May in France.

**Closure**

All participants were thanked for their presence and contributions to the discussions. The guest speakers were thanked for their interesting presentations. The European Commission was acknowledged for their support, also in financial terms, to make the workshop possible. The CRL-*Salmonella* team, including the secretariat, was thanked for their work of the previous year, including the presentations and for all the organisational work, which made the workshop a success.

# 4. Evaluation of the workshop

## 4.1 Introduction

A few weeks after the workshop an evaluation form was sent to the NRLs. The evaluation was intended to obtain the views of the participants on the workshop. This information can be used for the improvement of future workshops.
The evaluation form contained four questions, relating to the general organisation, a general view on the programme, a detailed view on the programme and on future workshops. The questions were ‘open’ so that participants could also add additional information and/or remarks which they felt were important. Twelve participants sent in their views.

## 4.2 What did participants think about the general organisation?

All twelve participants were enthusiastic about the general organisation. Everything concerning tickets, transport, hotel, location of meeting, lunch, dinner and the social program were well organised according to them.

Concerning the social program reactions were given as:
‘Good opportunity for interaction’;
‘Clever and entertaining’;
‘Unforgettable wonderful evening and physical relax too’;
‘Very good – though clogs were a bit challenging for my dodgy feet’.

## 4.3 What were the participants’ general views of the programme?

Eight of the participants were satisfied with the programme. They found the information and presentations useful and easy to follow and considered that well balanced aspects were being discussed. Also the number of presentations were considered to be good, but sometimes too tight (time).

One participant questioned whether the way the EU legislation news was presented might have caused some problems to the participants as, although the information was important, many may have found it difficult to keep their interest alive.

One participant mentioned that one of the difficulties they experience is linking the laboratory (NRL) role into the regulatory debates (monitoring/control) within the EU. This laboratory also mentioned that the presentations on sampling methods for monitoring/control activities for *Salmonella* in poultry are given for information purposes only. It would have been useful if the NRLs had collectively debated issues through the CRL over the last year.

One participant questioned if there would be any way to incorporate scientific programs/conferences with the workshop.

One participant indicated that he found the programme 'about right, but maybe coverage of ring trials could be slightly more brief to allow review presentations of important aspects of *Salmonella*'.

## 4.4 What were the participants' detailed views of the programme?

In general the reactions were positive about the different subjects which were presented during the workshop. Some remarks were made as follows:

- Zoonoses report: 'I hope the new zoonoses collaborator will not diminish the scope and experiences of the present report and will only improve the report'.
- EU-legislation (zoonoses, feed and food): 'Difficult to follow and too much information in this short time'; 'Personally, as someone comparatively new to this specific area, I would like to have had more information about current legislation and the implications for control over the next number of years'; 'Really important matters, with far-reaching consequences. Perhaps the bacteriologists are knocked off by the bureaucratic way of presenting'; 'Very interesting'; 'Essential'.
- Monitoring/control activities for *Salmonella* in poultry: 'Of extreme importance'.
- EFSA Activities: 'Good presentation, but too long compared to the time that was reserved for it'; 'Helpful'.
- Results research CRL-*Salmonella*: 'Important theme and interesting to follow'; 'Interesting'.

- Results proficiency tests: ‘Important and interesting to follow’; ‘In the absence of an NRL for antibiotic resistance, it would have been useful to have more input on this topic’; ‘Clearly arranged’; ‘Interesting, but could be reduced’.
- Design future proficiency tests: ‘Good to discuss’; ‘Necessary for estimation a level’; ‘We very much value the annual ring trials. As a small laboratory there is considerable work involved for us. We do not wish for this reason to process additional samples. Hence, participating in studies to evaluate methods cause us difficulties at the present time’.
- General remark: ‘All subjects presented in the workshop were interesting and beneficial. Especially subjects presented by the CRL-*Salmonella*’.

## 4.5 What suggestions do participants have for future workshops?

Overall the length of the workshop and presentations were just right according to the participants.

Two participants wrote that smaller groups might be easier for discussions (i.e. people working on the same problem) followed by a general discussion with a larger group.

One participant would like to ‘see reviews/discussions on alternative (i.e. non-culture) means of diagnosis if for no other reason than to identify those areas where they are deficient and need improving’.

One participant mentioned that the major profit was to gather people working on the same topic and dealing with the same problems. This participant would therefore not favour to split the audience in smaller groups. Only if more persons per country would participate it may be possible to discuss different problems in smaller working groups (followed by general discussion).

One participant indicated that the information on the ring trials could be shortened so that more time would be available to have (review) presentations involving guest speakers. Possible subjects: Electronic surveillance networks, Epidemiological methods and risk assessments applied to *Salmonella*, Rapid test methods, *Salmonella* post-slaughter, Post-genomics and pathogenesis. Furthermore, this participant indicated that more discussion on best sampling methods and programmes for future EU initiatives would be helpful. Finally, he preferred the presentations with the large group.

## 4.6 Conclusions

From the reactions of the evaluation it may be concluded that there is no need to change much on the workshop. Care should be taken that the programme will not become overloaded and that sufficient time is reserved for all presentations. On the other hand, speakers should take care of their reserved time as well. It would be helpful if complicated subjects (e.g. EU legislation) are presented in a ‘lively’ and ‘simple’ way, without losing the important information. This is not easy, but may be a challenge for the next time such presentations will be given. Splitting the audience in small groups may be beneficial for the discussion, but on the other hand some NRLs may then miss some important information, or do not have the opportunity to discuss a subject in more detail. Splitting into smaller groups may work best if two persons per NRL participate in the workshop.

# Acknowledgements

The organisation of a workshop is always a complicated matter. For a workshop to be organised in April/May, the first steps for the organisation are already taken in November the year before. Such a workshop can not be organised without the help of many people. The author would therefore like to thank the important players in this field. Many of them worked only at the background, without being seen. However, without them the workshop would not have been a success.

Jeanette van Essen is thanked for arranging all the tickets and other organisational details, working together with Loes van Dijk.
Loes van Dijk is thanked for doing the major organisation, like arranging the meeting room, lunches, hotel, transport from and to the hotel, social programme and dinner. Without Loes and Jeanette it would have been a very quiet (no tickets available) and boring workshop.
Christiaan Veenman is thanked for his valuable help during the workshop for smoothly putting the presentations ready.
Arjen van de Giessen and Anne Mensink are thanked for helping with the programme, chairing sessions of the workshop and for critical reading of this report.
Hans Korver is thanked for carefully preparing several presentations, which he unfortunately could not present himself due to illness.
Noël Peters is thanked for summarising the responses to the evaluation of the workshop.

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# Annex 1. Participants

| | |
|---|---|
| **European Commission** | Jean-Charles Cavitte<br>Sarolta Idei |
| **European Food Safety Authority (EFSA)** | Pia Makela |
| **CRL – *Salmonella*** | Kirsten Mooijman<br>Christiaan Veenman<br>Anne Mensink<br>Henny Maas<br>Anjo Verbruggen |
| **CRL – Epidemiology of Zoonoses** | Annemarie Kaesbohrer |
| **Guest speakers (the Netherlands)** | Dik Mevius (CIDC, Lelystad)<br>Max Heck (RIVM, Bilthoven) |
| **Guest speaker (United Kingdom)** | Linda Ward (HPA, London)<br>Elizabeth dePinna (HPA, London) |

**National Reference Laboratories for *Salmonella***

| | |
|---|---|
| AUSTRIA | Heimo Lassnig |
| BELGIUM | Hein Imberechts |
| CYPRUS | Economides Constantinos |
| CZECH REPUBLIC | Iva Bernardyova |
| DENMARK | Dorte Lau Baggesen<br>Steen Nordentoft |
| ESTONIA | Lea Rander |
| FINLAND | Henry Kuronen |
| FRANCE | Marylène Bohnert |
| GERMANY | Andreas Schroeter |
| GREECE | Maria Gavala |
| HUNGARY | Erzsebet Andrian |

| | |
|---|---|
| IRELAND | John Egan |
| ITALY | Antonia Ricci |
| | Veronica Cibin |
| LATVIA | Andra Utinane |
| LITHUANIA | Ceslova Butrimaite-Ambrozeviciene |
| LUXEMBOURG | Joseph Schon |
| NORTHERN IRELAND | Sam Strain |
| NETHERLANDS | Arjen van de Giessen |
| NORWAY | Bjarne Bergsjo |
| POLAND | Andrzej Hoszowski |
| | Dariusz Wasyl |
| PORTUGAL | Alice Amado |
| SLOVAK REPUBLIC | Milan Sasik |
| SLOVENIA | Vojislava Bole-Hribovsek |
| SPAIN | Maria Christina de Frutos Escobar |
| SWEDEN | Erik Eriksson |
| UNITED KINGDOM | Robert Davies |

# Annex 2. Programme of the workshop

## Programme of the CRL-*Salmonella* workshop X, 28 & 29 April 2005, Bilthoven

**General information**

**Hotel:** Hotel Mitland Utrecht; Ariënslaan 1, 3573 PT Utrecht; tel: +31 30 2715824; fax: +31 30 2719003; www.mitland.nl

**Transport:** All transport indicated in the programme will be organised by CRL-*Salmonella*. Please make sure you will be present at the indicated time. For departures from the Hotel, please wait in the lobby of the Hotel at the indicated time

**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 20 April 2005.

In the meeting room the following is available for the presentations: overhead projector, beamer and pc, flip-over/white board

**Place of the workshop:** National Institute for Public Health and the Environment: RIVM
A. van Leeuwenhoeklaan 9; Bilthoven
tel. CRL-*Salmonella* (general): +31 30 274 2171/2661
Meeting room: T007

**Important: If you want to enter the RIVM buildings you have to identify yourself at the main entrance. Please do not forget to bring an identity paper when you are coming to the RIVM**

## Wednesday 27 April 2005

Arrival of representatives of the NRLs at Hotel Mitland.

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

# Thursday 28 April 2005 (T007)

***Morning session: Current EU legislation and related issues***
***Chair: Arjen van de Giessen***

8.30 Departure from hotel Mitland to RIVM
9.00 - 9.15 Opening and introduction (Kirsten Mooijman)
9.15 - 9.45 Zoonoses report *Salmonella* 2003 trends and sources, including the new MSs reporting on a voluntary basis (Annemarie Kaesbohrer)
9.45 - 10.15 Zoonoses legislation (Jean-Charles Cavitte)

*10.15 - 10.45 Coffee/tea*

*Monitoring/control activities for Salmonella in poultry*
10.45 - 11.30 Baseline study in laying hens:
- Interim results collected at EC (Sarolta Idei)
- Interim results QA serotyping (Anjo Verbruggen)
- Discussion/questions/evaluation (Arjen v.d. Giessen)

11.30 - 11.50 Proposed baseline study in broilers (Antonia Ricci)
11.50 - 12.10 Monitoring in breeder flocks (Jean-Charles Cavitte)
12.10 – 12.40 EFSA-activities in relation with *Salmonella* (Pia Makela)

*12.40 - 13.45 Lunch*

***Afternoon session: CRL/NRL activities***
***Chair: Kirsten Mooijman***

13.45 - 14.15 Results research activities CRL-*Salmonella* (including Draft Annex D of ISO 6579) (Kirsten Mooijman)
14.15 - 14.45 Results bacteriological detection study VIII – 2004 (Hans Korver)
14.45 - 15.15 Discussion on design bacteriological detection study IX – 2005 (Kirsten Mooijman)

*15.15 - 15.45 Coffee/tea*

15.45 - 16.05 Results typing study X - 2005 : phagetyping (Linda Ward)
15.05 - 16.25 Results typing study X - 2005: serotyping (Hans Korver)
16.25 - 16.45 Results typing study X - 2005: antibiotic resistance testing (Dik Mevius)
16.45 - 17.15 Discussion on design typing study XI-2006 (Hans Korver)

*17.30 – onwards Evening programme and dinner; return to the hotel*

# Friday 29 April (T007)

***Morning session: Future & other items***
***Chair: Anne Mensink***

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|---|---|
| 8.30 | Departure from hotel Mitland to RIVM |
| 9.00 - 9.30 | Implications of the new feed and food regulation; Official Feed and Food Control, future activities of CRLs (Jean-Charles Cavitte/ Sarolta Idei) |
| 9.30 - 9.50 | Questionnaire matrices and follow-up Proficiency testing (Kirsten Mooijman) |
| 9.50 - 10.30 | Phagetyping, now and in future (Linda Ward & Elizabeth DePinna) |
| *10.30 - 11.00* | *Coffee/tea* |
| 11.00 - 11.20 | Pulsed-Field Gel Electrophoresis (PFGE) typing of *Salmonella* spp.; Salmgene (Max Heck) |
| 11.20 - 11.40 | Bacteriological monitoring of *Salmonella* in pigs (Arjen v.d. Giessen) |
| 11.40 - 12.00 | UK *Salmonella* monitoring and control programme in pigs (Rob Davies) |
| 12.00 - 12.20 | Serological detection of *Salmonella* in swine (Peter v.d. Wolf) |
| 12.20 - 12.45 | Work programme CRL second half 2005, first half 2006, closure (Kirsten Mooijman) |
| *12.45 - 14.00* | *Lunch* |
| 14.00 | Departure to train station Bilthoven |