RIVM report 330300005/2004

The ninth workshop organised by CRL-*Salmonella*
Bilthoven (the Netherlands), 13-14 May 2004

H. Korver and K.A. Mooijman (editors)

Contact:
H.Korver
Microbiological Laboratory for Health Protection (MGB)
Hans.Korver@rivm.nl

This investigation has been performed by order and for the account of the European Commission , Législation Vétérinaire et Zootechnique within the framework of project V/330300/03/CS by the Community Reference Laboratory for Salmonella.

RIVM, P.O. Box 1, 3720 BA Bilthoven, telephone: 31 - 30 - 274 91 11; telefax: 31 - 30 - 274 29 71
European Commission, Legislation Vétérinaire et Zootechnique, Rue de la Loi 86, B-1049 Bruxelles, Belgique, telephone 32-2-2959 928; telefax: 32-2-2953 144

Abstract

The ninth workshop organised by CRL-*Salmonella*

The ninth workshop organised by the Community Reference Laboratory for *Salmonella* (CRL-*Salmonella*) was held on 13 and 14 May 2004 in Bilthoven, the Netherlands. The representatives of the National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) of the Member States of the European Union (including the 10 new Member States) were present. Also representatives of the European Commmission (DG-Sanco) participated in the workshop. Presentations were given by representatives of DG-Sanco, of the NRLs and of CRL-*Salmonella* and by some guest speakers. Subjects which were discussed were: the new Zoönoses Directive and Regulation, tasks and duties of CRLs and NRLs, the Zoönoses report of 2002, methods (PCR confirmation, validation), antibiotic resistance, intercomparison studies organised by NRLs and by CRL (2003, 2004 and 2005), and the work programme of CRL-*Salmonella* for the coming year.

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

Rapport-in-het-kort

De negende workshop georganiseerd door het CRL-*Salmonella*

De negende workshop georganiseerd door het Communautair Referentie Laboratorium voor *Salmonella* (CRL-*Salmonella*) werd gehouden op 13 en 14 mei 2004 in Bilthoven, Nederland. De vertegenwoordigers van de Nationale Referentie Laboratoria voor *Salmonella* (NRLs-*Salmonella*) van de lidstaten van de Europese Unie (inclusief de 10 nieuwe lidstaten) waren aanwezig. Ook vertegenwoordigers van de Europese Commissie (DG-Sanco) namen deel aan de workshop. Presentaties werden gehouden door vertegenwoordigers van DG-Sanco, van de NRLs en van CRL-*Salmonella* en door enkele gastsprekers. Onderwerpen die werden bediscussieerd waren: de nieuwe Zoönosen Richtlijn en Verordening, taken en plichten van CRLs en NRLs, het Zoönosen rapport van 2002, methoden (PCR bevestiging, validatie), anti-microbiële resistentie, ringonderzoeken georganiseerd door NRLs en door het CRL (2003, 2004 en 2005), en het werkprogramma van het CRL-*Salmonella* voor het komende jaar.

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

Contents

Summary **7**

1. Thursday 13 May 2004: day 1 of the workshop **9**

1.1 Opening and introduction *9*
1.2 The new EU Zoonoses Directive and Regulations *10*
1.3 Progress in ISO/TC34/SC9 *12*
1.4 Monitoring Salmonella spp. in laying hens *13*
1.5 Bacteriological sampling to detect Salmonella in poultry flocks *14*
1.6 Results bacteriological detection study VII – 2003 *16*
1.7 Discussion on design bacteriological detection study VIII – 2004 *17*
1.8 Results typing study IX – 2004 : phage typing *19*
1.9 Results typing study IX – 2004 : serotyping and design typing study X (2005) concerning serotyping and phage typing *20*
1.10 Results typing study IX – 2004: antibiotic resistance testing *21*
1.11 Draft monitoring scheme for antibiotic resistance testing *22*
1.12 Discussion on design typing study X (2005) concerning antibiotic resistance testing *23*

2. Friday 14 May 2004: day 2 of the workshop **25**

2.1 Overview on the zoonoses report 2002 *25*
2.2 Tasks and duties CRLs and NRLs *27*
2.3 Questionnaire comparative testing *31*
2.4 National comparative testing programme in Poland *33*
2.5 PCR confirmation directly from MSRV agar plates *34*
2.6 European validation and certification of methods *35*
2.7 Validation of methods at national or laboratory level *37*
2.8 Work programme CRL-Salmonella 2005 and closure *38*

Annex 1. Participants **39**

Annex 2. Programme of the workshop **41**

Annex 3. Slides of presentation 1.1 **45**

Annex 4. Slides of presentation 1.2 **48**

Annex 5. Slides of presentation 1.3 **56**

Annex 6. Slides of presentation 1.4 **62**

Annex 7. Slides of presentation 1.5 **68**

Annex 8. Slides of presentation 1.6 90

Annex 9. Slides of presentation 1.7 99

Annex 10. Slides of presentation 1.8 102

Annex 11. Slides of presentation 1.9 107

Annex 12. Slides of presentation 1.10 116

Annex 13. Slides of presentation 1.11 130

Annex 14. Slides of presentation 1.12 136

Annex 15. Slides of presentation 2.1 140

Annex 16. Slides of presentation 2.2 163

Annex 17. Slides of presentation 2.3 171

Annex 18. Slides of presentation 2.4 180

Annex 19. Slides of presentation 2.5 191

Annex 20. Slides of presentation 2.6 198

Annex 21. Slides of presentation 2.7 207

Annex 22. Slides of presentation 2.8 214

Summary

From 13 till 14 May 2004 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*) of the EU Member States (including the ten new member states) were present, as well as representatives of the European Commission (DG-Sanco). A total of 44 participants were present at the workshop.

The programme of the workshop consisted of several parts. At the first day presentations were given about the “New EU Zoonoses Directive and Regulations” and “Progress in ISO/TC34/SC9”. Furthermore papers were presented about “Monitoring *Salmonella* spp. in laying hens” and “Bacteriological sampling to detect *Salmonella* in poultry flocks”. The results of two interlaboratory comparison studies organised by CRL-*Salmonella* were also presented and were open for discussion.
On the second day of the workshop the following subjects were presented: “Zoonoses report 2002” en “Tasks and duties of CRLs and NRLs”.
The results of a questionnaire about national comparative studies, held in spring 2004 among the NRLs-*Salmonella*, were discussed. Furthermore papers were presented about the validation of methods on European, national and laboratory level.

The presentations which can be found in Annex 3 till 22 (pages 43 – 215) are printed in black and white. For colour rendering see the website of CRL-*Salmonella*:
http://www.rivm.nl/crlsalmonella/workshop

1. Thursday 13 May 2004: day 1 of the workshop

1.1 Opening and introduction

*Kirsten Mooijman, Head CRL-*Salmonella*, Bilthoven, the Netherlands (see Annex 3)*

After a warm welcome to all participants, some changes at the CRL-*Salmonella* when compared to the workshop of 2003 were explained. The main changes were:

- Since 1 May 2004, 10 countries have become new Member States of the EU and thus the number of NRLs also increased by the number of 10;
- In the past, two participants per NRL have been invited to the workshop. As the number of NRLs have increased significantly, the EC has decided to fund in principle only one participant per NRL;
- Personnel changes:
    - André Henken changed at 1 September 2003 from Head of the Microbiological Laboratory for Health Protection (MGB/RIVM) and from Head CRL-*Salmonella* to Director of the Division Public Health of the RIVM;
    - New Head of MGB has become: Anne Mensink;
    - New Head of CRL-*Salmonella* has become: Kirsten Mooijman;
    - New technician of CRL-*Salmonella* since 1 May 2004: Christiaan Veenman.
- The website (http://www.rivm.nl/crlsalmonella) will be used more frequently to inform the NRLs. For instance the Newsletter is no longer distributed on paper, but is since the first of January 2004 only available via the website. All presentations of the workshop will be placed on the website as soon as possible after the workshop.

Aims of the workshop:

- Discuss issues of relevance for CRL and NRLs:
    - EU level (new Directive and Regulation)
    - Tasks and duties CRL and NRLs
    - Exchange of information on methods (ISO, validation, PCR);
- Past (2003) and future intercomparison studies CRL;
- Exchange of information between NRLs (research activities);
- Exchange of information with representatives of the EC (DG-Sanco);
- Needs and expectations NRLs;
- Discuss future activities CRL.

Programme of the workshop:
13 May

- New Directive and Regulation: status, implementation, methods;

- Intercomparison studies: detection and typing (2003, 2004 and 2005);
- Antibiotic resistance.

14 May
- Zoonoses report 2002;
- Task and duties CRL and NRLs, including comparative testing;
- Methods: PCR confirmation, validation;
- Work programme second half 2004 and 2005.

A more detailed programme of the workshop is presented in Annex 3.

1.2 The new EU Zoonoses Directive and Regulations

Sarolta Idei, European Commission, Brussels, Belgium (see Annex 4)

Zoonoses are diseases and infections that can be transmitted between animals and humans, directly or through food in particular. Zoonoses may cause severe human suffering and economic losses to food business operators. Zoonoses present particularly at the level of primary production must be adequately controlled. The main principle is: safe food from healthy animals.
Council Directive 92/117/EEC concerns measures for protection against specified zoonoses and specified zoonotic agents in animals and products of animal origin provided for the establishment of a monitoring system for certain zoonoses and of controls on *Salmonella* in poultry. The Directive laid down compulsory monitoring for four pathogens and voluntary monitoring of other ones, in animals, food, feed and in humans, mostly based on national systems. Member States were required to implement minimum control measures to eradicate *Salmonella* Enteritidis and *Salmonella* Typhimurium in breeding flocks of poultry. These two serotypes of *Salmonella* account for over 70% of the total *Salmonella* serotypes isolated from humans.
Directive 92/117/EEC is repealed and replaced, with effect from 12 June 2004, by Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents and Regulation (EC) No 2160/2003 on the control of salmonella and other specified food-borne zoonotic agents (both adopted by co-decision on 11 November 2003 and applicable on 12 June 2004).
Directive 2003/99/EC on monitoring aims to obtain comparable data to evaluate related risks. The monitoring of eight zoonoses and zoonotic agents is mandatory and others should be monitored according to the epidemiological situation in each Member State.
From 2005 the European Food Safety Authority (EFSA) will be responsible for the production of the Community summary report, instead of CRL-Epidemiology, Berlin. EFSA is in the process of contracting a Zoonoses Collaborator Centre and is creating an internet reporting system. Nevertheless prioritised harmonisation of monitoring schemes will be decided by the Commission with Member States.

The member States should send their national report 2003 to CRL-Epidemiology, which is due to produce the summary report by the end of 2004. No change is intended for the collection of monitoring data of 2004. The Member States should send their national report 2004 to the Commission, and EFSA will produce the Community report in the course of 2005. Full data on human cases shall be part of the report covering the year 2004 throughout. New Member States are expected to produce a national report covering the full year 2004. Moreover discussions are now starting with Member States to prepare for the implementation of the new provisions in 2005.
The commission informed the Member states, that the scheme for Campylobacter is almost ready to be laid down whilst the scheme for antimicrobial resistance monitoring is under discussion. Both schemes are prepared by the CRL-Epidemiology.
The sampling frame for Campylobacter is based on sampling of broiler chickens at slaughterhouse at time for evisceration.
Regulation (EC) No 2160/2003 is a framework legislation, which prescribes that controls on zoonoses and zoonotic agents should cover the whole food chain, but principally at the level of primary production. The primary aim of the Regulation is to ensure that effective measures are taken to decrease the occurrence of *Salmonella* serotypes of significance for public health (and other zoonotic agents in the future where appropriate) progressively, in different categories of poultry (breeding flocks, laying hens, broilers, turkeys) and pigs.
Community salmonella reduction targets will be set and national programmes should be operational 18 months after setting the Community targets.
A scheme for a baseline study on salmonella in poultry laying flocks was drafted by a Commission expert group and discussed with Member States representatives in order to gain comparable data on prevalence in different Member States. This is a prerequisite for setting the target on reduction of *Salmonella* in laying flocks, which is second target (after breeding flocks) to be set under Regulation No 2160/2003. Not only faecal material, but also environment (dust) shall be sampled, aiming to maximise the sensitivity of sampling. One single analytical method (the one proposed by CRL-*Salmonella* for ISO standardisation) is retained for the scheme.
The European Commission has asked EFSA for a scientific opinion on the use of vaccines and antimicrobials for the control of *Salmonella.* These opinions are due by mid 2004.

Discussion

Q: What are the plans for Community Reference Laboratories other than CRL-*Salmonella* ?
A: This will be discussed by J.J.Cavitte at another time during this workshop.
Q: Why is the monitoring done at the end and not in the beginning of the laying hen period ?
A: Some countries do not have vaccination programmes. Consensus about the end of the laying period in hens.
Q: Should poultry meat be tested for *Salmonella* ?
A: Criteria not set up. Over the years these criteria will be discussed when meat will contain *Salmonella*.

1.3 Progress in ISO/TC34/SC9

*Kirsten Mooijman, CRL-*Salmonella*, Bilthoven, the Netherlands (see Annex 5)*

Since several years there is a need for a standard method for the detection of *Salmonella* spp. in poultry faeces. The existing standard, ISO 6579, is primarily intended for isolation of *Salmonella* spp. from food and feeding stuffs and is less suitable for analysing matrices like poultry faeces. In 2003 it was requested to Sub Committee 9 (SC9: Microbiology) of ISO Technical Committee 34 (TC34: Food products) to prepare a standard (or an annex to ISO 6579) for the detection of *Salmonella* spp. in poultry faeces. For this purpose the scope of SC9 needed to be extended, which was realised by the end of 2003. Next, CRL-*Salmonella* wrote a document, which summarised information from literature and from two CRL comparison studies on the use of semi-solid media for the detection of *Salmonella* spp. in poultry, faeces (and other matrices). This document was sent to SC9 in February 2004 and discussed at the plenary meeting of SC9 in Parma, Italy on 21 April 2004. At this meeting it was agreed that CRL-*Salmonella* would prepare a draft annex to ISO 6579:

- With the scope: 'Detection of *Salmonella* spp. from animal faeces and the primary production stage'. Whether this latter part of the scope can be remained would depend on the availability of data of the primary production stage (e.g. detection of *Salmonella* spp. in environmental samples like dust).
- The method of choice will be ISO 6579 in which both liquid selective enrichment media will be replaced by the semi-solid medium Modified Semi-solid Rappaport Vassiliadis (MSRV) to be incubated at (41.5 ±1) °C for 2 x 24 h (if negative after 24 h, incubated for an extra 24 h).
- A 'warning' will be added to the scope that MSRV is less appropriate for non-motile *Salmonellae* and if non-motiles would be expected it will be advised to pick off non-typical colonies and/or to use beside MSRV also a liquid selective enrichment.
- The diagram of the new annex will become the following:
  1. Pre-enrichment in BPW, (18 ± 2) h at (37 ± 1) °C;
  2. Selective enrichment on:
     - MSRV for 2 x (24 ± 3) h at (41.5 ± 1) °C;
     - if non-motile *Salmonellae* are expected, pick off non-typical colonies and/or also selective enrichment in either RVS or MKTTn (follow ISO 6579);
  3. Plating-out on:
     - XLD (follow ISO 6579);
     - second agar of choice
  4. Confirmation, on/in media mentioned in ISO 6579

The draft annex will be prepared and sent to SC9 as soon as possible.
The document in which the information of the semi-solid media was summarised will be worked out as a report, also including the conclusions from the meeting of ISO/TC34/SC9. This report will become available to the NRLs and SC9.

Discussion

Q: Incubation time and temperature of MSRV not too strict?
A: As much as possible ISO 6579 is followed.
Q: Can an indication be given for the amount of sample?
A: This should be given in international or national regulations. Perhaps an indication can be added to the method.
Q: What do you mean with non-motile bacteria ?
A: When bacteria are not swarming over the medium plate.
Remark: You do not have the possibility to agglutinate directly from the MSRV plates.
Remark: Validation studies have to be undertaken because of the introduction of MSRV.

1.4 Monitoring *Salmonella* spp. in laying hens

Arjen van de Giessen, NRL-the Netherlands, Bilthoven, the Netherlands
(see Annex 6)

A programme for a baseline study on the prevalence of *Salmonella* spp. in laying hen flocks was drafted by a working group of DG SANCO. The objective of this programme is to estimate the prevalence of *Salmonella spp*. in the population of commercial laying hens (*Gallus gallus*) at the end of the production period in the Member States of the European Union. The study should yield comparable results from the different MSs, which will be used to set Community targets pursuant to Regulation (EC) No 2160/2003. The study should cover a one year period commencing from 1 October 2004. The sampling scheme should cover holdings with at least 1000 hens. MSs with a large proportion of the population kept in holdings with less than 1000 hens should include also smaller holdings.
Countries shall sample the number of flocks as calculated based on the following criteria:
a target prevalence of 20%, a confidence level of 95% and an accuracy of 3%. For selection of flocks, the population of laying hens should be stratified according to holding size. Sampling should be conducted as close as possible to depopulation based on a notification system. Only one flock per holding should be sampled (flock definition according to the Regulation). The samplings should be equally distributed over the year and shall be performed by the competent authority or under its supervision. In order to maximise sensitivity of sampling, both faecal material and the environment shall be sampled. There should be 7 pooled samples taken in any selected holding, the type of samples depending on the type of production/type of facilities. Member States may collect additional samples, in which case they shall report the data separately. Samples shall be sent by fast mail or courier to the relevant laboratory and should be kept refrigerated until examination. For detection, a modification of ISO 6579 (2002) should be used, where one semi solid medium (MSRV) is used as the single selective enrichment medium. One isolate from each positive sample shall be serotyped at least and it is strongly recommended that at least one isolate of *S.* Enteritidis and *S.* Typhimurium from each positive sample should be phagetyped. For

epidemiological purposes, it is strongly recommended that, where possible, one isolate per serovar per flock is used for antimicrobial susceptibility testing. MSs shall collect information for each flock/holding sampled and for each sample examined in the laboratory. This information, results of the study as well as a description of the implementation of the programme should be reported to the Commission by the national authority.

Discussion

Q: Can you tell something more about the inclusion of environmental samples ?
A: Increase of sensitivity and from dust samples one can get positives at all stages of the hen's life.
Remark: In one study *S.* Typhimurium was isolated from eggs as well as dust samples.

1.5 Bacteriological sampling to detect Salmonella in poultry flocks

Robert Davies, NRL-United Kingdom, Addlestone, UK (see Annex 7)

The types of samples collected are often dictated by the type of test used, for example serology which requires serum or egg yolk. The detection of *S.* Gallinarum / Pullorum is ideally carried out on serum or post-mortem tissues or rapid methods, which may perform poorly with standard faecal culture without enhanced sample preparation. Sampling may be carried out for a variety of purposes but for control programmes the sensitivity should be maximised as far as practicable by increasing sample sizes and numbers and concentrating on focal points of contamination, eg. on droppings or egg belting or ventilation systems, which may be variable between farms. Where it is necessary to compare prevalences sampling should be carried out in a standardised way. This is not possible to do on a farm basis because of variables in farm design so ideally such monitoring should be based on individual animals. This normally involves a much larger number of samples, particularly if the prevalence is low, and in the case of poultry is best conducted on caecal contents from slaughtered birds. For statutory confirmation of infection a statistically derived number of samples is designated and this is usually 59 birds to detect a 5% prevalence of infection with 95% confidence, assuming that the test is 100% sensitive, which it is not. Individual samples may be pooled to reduce test costs but unless large sample volumes are used this reduces the contribution of the individual sample so if contamination is clustered infection may be more likely to be missed. In the case of faecal/caecal samples pooling may increase the proportion of competitive flora if the prevalence of *Salmonella* is low so that it may become overgrown during culture. On the other hand inclusion of a greater number of individual samples in the test increases the chance that a positive sample will be included in the pool. For faecal samples it appears that pooling up to 20 individual samples has little detrimental effect on detection of an individual positive component. In the case of tissues gathered aseptically at

post-mortem it is likely to be possible to pool a much greater number of individual samples but care must be taken where sample matrices contain inhibitory substances and numbers of organisms are low, as in egg contents. More work on optimisation of sample pooling is required.

Several studies have demonstrated the superiority of environmental samples for detection of *Salmonella* in farms, abattoirs, hatcheries and feedmills. It is often argued that identifying *Salmonella* in such situations does not always mean that infection is currently present. This may be true in some cases in hatcheries where isolation of *Salmonella* from incubator fluff may indicate endemic or recycling contamination of equipment rather than hatching of eggs from infected breeding flocks, but in the case of farms finding infection in key environmental sites is a sensitive indicator of the flock/herd as a source, even though the individual animal prevalence may be low. To identify suitable sampling sites it is necessary to consider the flow of outputs such as faeces, eggs and air from the building. Contamination tends to accumulate preferentially at the collection ends of eggs belts, scraper systems and droppings belts, where material is naturally pooled. *Salmonella* also survives preferentially in dust so accumulations of dust on air exhausts, egg elevators, beneath cages, on ledges, etc., and in sections of the house which are separated from the birds but where low level dust can settle, such as service corridors or storage areas close to pens, are good samples to use for screening.

In houses where there is no collection system for eggs or faeces it is necessary to collect representative samples from the floor area, which may be slatted or bedded with straw or shavings based litter. Traditionally pooled faeces or litter picks have been taken but it has proved to be difficult to encourage samplers to take these in a systematic way, thus compromising the sensitivity of the sample. Litter sampling can also be problematic since deep litter material is biologically active and inappropriate transport and storage conditions may accelerate the loss of *Salmonella* once the sample is removed from the source of continuous addition of faeces in the house. This is less of a problem with thin layers of litter from scratching areas or niches in wall cracks, etc., since thin layers tend to desiccate rather than developing an antagonistic flora.

To overcome the problems of litter or faeces sampling drag swabs were developed and used, primarily in the USA. In their original form these complex assemblies were cumbersome to use, especially in densely populated houses and attempts were made by commercial organisations to reduce the size and replace large moist gauze pads with small presoaked sponges. This adapted apparatus lacks sensitivity however and boot swabs, which comprise rolls of tubegauze bandage or fabric overshoes, are more commonly used and are as effective as the original drag swabs.

In any situation where there is a low prevalence infection, especially where the flock is subdivided into several small pens, *Salmonella* contamination is likely to be clustered and it is possible for a limited number of swabs to become loaded before reaching the contaminated

areas. For this reason the author prefers to use large gauze swabs (Kleenex Readiwipes), which have been autoclaved in 225 ml Buffered Peptone Water. The jars containing the swabs are taken to the farm and representative areas swabbed vigorously so that a series of samples is taken to represent the whole house. These samples in BPW must be returned to the laboratory and culture begun on the day of collection or overgrowth may occur. Ideally swabs should be cultured individually but they can be pooled for economy. Alternatively incubated BPW broth from several samples can be pooled, or separate inocula dropped onto separate places on a semi-solid selective agar plate. The gauze swabs are also ideal for sampling houses after cleaning and disinfection. Another useful method is to place gauze or sponge swabs in drainage channels during the washing of the house and recover these for culture. These drainage channels also act as a concentrator of contamination.

In summary, there are a variety of sampling methods which may be used for detection of *Salmonella* in poultry houses and for basic sampling in non-cage houses a combination of boot swabs and dust is recommended. In cage houses, pooled faecal material from belts or scrapers, dust beneath cages and dust from egg belt elevators is most suitable. Whatever sample is taken the greatest detection sensitivity will be achieved with the maximum number of sample cultures rather than multiple enrichment methods for a restricted number of samples.

Discussion

Q: Will this presentation be placed on the website as soon as possible ?
A: Yes

1.6 Results bacteriological detection study VII – 2003

*Hans Korver, CRL-*Salmonella*, Bilthoven, the Netherlands (see Annex 8)*

A seventh bacteriological interlaboratory comparison study was organised by the Community Reference Laboratory for *Salmonella* (CRL-*Salmonella*, Bilthoven, the Netherlands), in Fall 2003. Twenty National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) participated in the study. This was the first time that NRLs from the candidate countries could participate. Reference materials in combination with or without the presence of chicken faeces, as well as naturally contaminated faecal samples (containing *Salmonella* Muenchen) were tested by all laboratories. The reference materials existed of gelatin capsules containing *Salmonella* Typhimurium (STM), *Salmonella* Enteritidis (SE) or *Salmonella* Panama (SPan) at different contamination levels.
In addition to the performance testing of the laboratories a comparison was made between the media described in the ISO 6579: 2002 [(being Rappaport Vassiliadis Soya Broth (RVS), Mueller Kauffmann Tetrathionate-novobiocin broth (MKTTn) and Xylose Lysine

Deoxycholate agar (XLD)] and the alternative media Modified Semi-solid Rappaport Vassiliadis (MSRV) and Brilliant Green Agar (BGA).
Significantly more positive isolations were obtained from capsules containing circa 500 cfp/capsule of SE than, in declining order, from capsules with circa 100 cfp/capsule of SE, from capsules containing circa 100 cfp/capsules of STM and from capsules with circa 10 cfp/capsule of STM, analysed in the presence of (*Salmonella* negative) chicken faeces. The use of MKTTn was significantly better in relation to RVS for the SE capsules. MSRV scored significantly better than RVS for all capsules.The results of the naturally contaminated samples revealed significantly better results for MKTTn and MSRV versus RVS and for XLD in relation to BGA.

Discussion

Q: Could the disappointing results of this study be due to the long transport times?
A: Long transport times did not always result in bad results.
Q: Could the backgroud flora of the faecal samples used have been inhibiting?
A: This will be analysed.
Q: Could fecal samples harbouring different types of *Salmonella* be used to better reflect the real situation? And could environmental samples be included in the study?
A: CRL will try to take these aspects into account.

1.7 Discussion on design bacteriological detection study VIII – 2004

Kirsten Mooijman, CRL-Salmonella*, Bilthoven, the Netherlands (see Annex 9)*

Three issues were discussed concerning the bacteriological detecion study VIII of 2004:
1. Temperature recording during transport of the samples;
2. Transport of the samples as diagnostic specimens instead as dangerous goods;
3. Discussion on the design of the study.

1. During the last detection study (VII) of 2003, for the first time, small electronic temperature recorders were included with the samples. These recorders give important information concerning temperature and time during transport. This information can be of use in trying to explain ‘deviating results’. It was stressed again that the recorders are only useful if they are immediately returned to the CRL-*Salmonella* after receipt of the parcel. The recorders will also be included in the parcels of the next detection study. For this purpose the CRL will order extra recorders, because of the increase in the number of NRLs since the new Members States have joined the EU.
2. Discussion with experts and courier have revealed that the materials for the detection studies can be transported as diagnostic specimens and transport as dangerous goods would not be necessary. For the next detection study it will be tried to transport the

samples in this way. The advantages will be: door-to-door transport, faster delivery and probably less expensive.

3. The 2004 detection study will probably be organised in November 2004. The following samples were proposed:
   - 10 capsules without poultry faeces (controls), including STM10, SE100, SPan5, blank;
   - 25 capsules + 10 g *Salmonella* negative poultry faeces, including STM10, STM100, SE100, SE500, blank;
   - naturally contaminated (with *Salmonella*) poultry faeces (20 x 10 g) and/or environmental samples (e.g. dust) naturally or artificially (with capsules) contaminated.

   Different from the study of 2003 will be that the laboratories will not be informed in advance on the number per type of capsules. This may be better for the randomisation of the study. The use of environmental samples, like dust, during the study is not yet sure. This will depend on the availability of the materials and on the experiences of the CRL.
   For the methods to be used the following was proposed:

- 'New draft annex to ISO 6579' (MSRV); also see the presentation on the progress in ISO/TC34/SC9;
- Own method(s).

Discussion

Q: Dust may be interesting in a next study, but we prefer to have no increase in the amount of work.
A: We prefer to keep the faeces as before. If we add dust to the next study we can consider to lower the amount of samples of naturally polluted faeces to analyse. Furthermore, the amount of work will already decrease as the 'new draft annex to ISO 6579' (MSRV) will be the only prescribed method.
Q: Will other materials like pigs faeces be included in later studies ?
A: This will be an option for future studies.
Q: Many samples had to be tested in the last few studies ? Can we change this ?
A: For the next study of 2004 lesser media have to be tested.
Q: How many samples will be tested in the next study when environmental samples will be included ?
A: We do not know yet, but this could be e.g. instead of 20 naturally contaminated samples ten dust samples and ten naturally contaminated samples.
Q: For accreditation purposes these interlaboratory comparison studies are being used. Study criteria are needed in the future.
A: Minimum criteria will be set in the near future. Also the homogeneity of faecal samples have to be tested in the future.
Q: Would it be possible to include more Salmonella serovars in one batch of faeces in the next study ?

A: This will be an option.

1.8 Results typing study IX – 2004 : phage typing

Linda Ward, Health Protection Agency, London, UK (see Annex 10)

Twenty strains of *Salmonella* were supplied for the study. Ten were *Salmonella* Enteritidis phage types (PT) 1b, 1, 14b, 12, 2, 3, 21, 9b, 24 and 4 and ten were *S.* Typhimurium PT41, 1, 104, 22, 9, 120, 208, 18, 136 and 193. These strains were selected from the collection of the *Salmonella* Reference Unit of the Health Protection Agency, Laboratory of Enteric Pathogens. The majority of the *S.* Enteritidis types had been implicated in outbreaks in England and Wales during 2003. A number for example *S.* Enteritidis PT14b had been linked with imported Spanish eggs.
Final reports have been received and assessed from 14 participating laboratories; 7 NRL's and 7 ENL's. Thirteen laboratories typed both *S.* Enteritidis and *S.* Typhimurium and one laboratory only typed the *S.* Enteritidis strains. All of the laboratories typed *S.* Enteritidis PT1b, 1, 14b, 2 and 9b correctly. The *S.* Enteritidis type giving most problems was PT12 where five NRL's and four ENL's obtained incorrect identifications. This type is rather unstable and does require plating and selecting single colonies. The second problem strain was PT24. The laboratories with an incorrect identification had a low reading with phage 9 where an SCL reading is expected. It is possible that with these laboratories the phage was not at the required titre.
S. Typhimurium phage types 41, 1, 104, 22, 9, 208 and 136 were identified correctly by 13 laboratories. All six NRL's and six of the seven ENL's identified all ten *S.* Typhimurium strains correctly. One ENL had problems with PT18 and PT193. In summary one ENL identified all 20 strains correctly. Four NRL's and four ENL's identified nine and three NRL's and two ENL's identified eight of the *S.* Enteritidis strains correctly. All the participating laboratories apart from one ENL had correct identifications for all ten of the *S.* Typhimurium strains. At least 85% correct results were obtained by all participating laboratories.

Discussion

Q: What can be done when accreditation for phage typing is wanted ?
A: This will have our attention.

1.9 Results typing study IX – 2004 : serotyping and design typing study X (2005) concerning serotyping and phage typing

Hans Korver, CRL-Salmonella*, Bilthoven, the Netherlands (see Annex 11)*

In spring 2004 the ninth proficiency test on serotyping 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). This was the first study in which also the reference laboratories of the new Menber State countries could participate. Twenty-three NRLs-*Salmonella* of the EU Member States and NRL-Norway and NRL-Romania participated in this study.
A total of 20 strains of the species *Salmonella enterica* subspecies *enterica* were selected by the CRL-*Salmonella*. The strains had to be typed with the method routinely used in their own laboratory, this could also include sending of the strains for serotyping to another specialised laboratory in their country. Most problems were encountered when typing the H-antigens. More than half of the NRLs typed strain S-1 (*S.* Banana) as *S.* California. In almost each of the former studies laboratories faced problems when typing *Salmonella* strains with the H-antigens g, m, t in relation to m, t strains. Some NRLs interpreted on the basis of multi factor sera which may lead to incorrect results. By analysing the data of antisera from different manufacturers certain sera showed positive reactions with strain S-1, whereas the same sera from other manufacturers tested negative with this particular strain. Some kind of quality control of the antisera would be necessary and/or detailed information from the manufacturer should be asked.

Discussion

Q: From time to time we have problems with typing of *Salmonella* Paratyphi B var. Java.
A: Indeed sometimes difficult. Some sera may contain rough antibodies. Best to check with supplier of the sera.
Q: Is it possible to include the biochemical results in the report?
A: This will be added.
Q: How to perform quality control of sera?
A: Using a panel of strains is possible, but the amount of strains to be used may be very large. Results of control strains are also not always the same. We will think on some guidelines for quality control.

1.10 Results typing study IX – 2004: antibiotic resistance testing

Dik Mevius, CIDC, Lelystad, the Netherlands (see Annex 12)

The conclusions from the CRL-workshop held in 2003 were:
CRL-*Salmonella* should develop an External Quality Assurance System (EQAS); EU-should encourage quantitative testing; Panel of strains for the trial of 2004 should be based on resistance phenotype, preferably incl.: *S.* Java; NCCLS is reference method used and the participating laboratories use their routine method.
Moreover a panel of antibiotics to be included in the reference panel was agreed upon.

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

CRL-1	*S.* Dublin	Cattle	AST-2
CRL-2	*S.* Enteritidis Pt 6a	Human	AST-8
CRL-3	*S.* Blockley	Human	AST-3
CRL-4	*S.* Typhimurium : Ft 508	Human	AST-9
CRL-5	*S.* Enteritidis Pt 4	Human	AST-4
CRL-6	*S.* Livingstone	Pig	AST-6
CRL-7	*S.* Hadar	Poultry	AST-1
CRL-8	*S.* Muenchen	Poultry	AST-10
CRL-9	*S.* Paratyphi B. var.Java	Poultry	AST-7
CRL-10	*S.* Kentucky	Human	AST-5

All MICs were confirmed by retesting with broth micro dilution or E-test for amoxicillin-clavulanic acid and streptomycin. At the time of the CRL-meeting 25 laboratories had supplied their results: 19 provided zone diameters and 7 MICs (one participant provided both zone diameters and MICs).
For all either highly susceptible or very resistant bacteria antibiotic combinations 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 caused a lot of confusion and many errors were made, specifically on amoxicillin-resistant but clavunalate susceptible strains numbers CRL 2, 5, 6 and 10.
Primarily those laboratories determining MICs, compared to disk diffusion resulting only in a few errors made, made major errors on these strains. The reason is that clavulanic acid in the agar or broth used is in competition with the betalactamase enzyme produced by the salmonella. In the tests used the amount of clavulanic acid is not unlimited and once it is all

used, the strains will grow slightly resulting in elevated MICs. However this is artificial and needs to be taken into account when testing this antibiotic combination.
Another combination causing confusion was trimethoprim sulphamethoxazole. A strain is by definition only resistant to this combination if it is resistant to both individual drugs. This was only the case for strain CRL-6. In general when testing sulphonamides or trimethoprim antagonists present in the growth medium used will affect determinations of the endpoints. Specifically strains that were resistant to one of the two drug causing problems (strains 2, 4 and 9), interestingly for this combination disk diffusion was the source of most errors.

It was concluded that the EQAS provided very valuable information, both for the participants and for the reference laboratory, providing that the reference values are 100% reliable. During the meeting it was presented how complex this matter is and the suggestion of a confirmation by another lab was suggested.

Discussion

Q: How should we see the results and presented information in relation to other actions in this field?
A: There are many different actions in the field of standardisation of antibiotic resistance testing. Results from the CRL-*Salmonella* intercomparison studies can be of use to improve further testing and monitoring.
Remark: MIC plates with a certain lay out can be ordered especially for trials.

1.11 Draft monitoring scheme for antibiotic resistance testing

Kirsten Heckenbach, CRL-Epidemiology, Berlin, Germany (see Annex 13)

In Article 7 of the zoonoses Directive 2003/99/EEC the implementation of a monitoring scheme on the occurence of antimicrobial resistance is laid down. A first draft for a common baseline approach, for minimal rules to achieve comparable data, was already presented and circulated to the Member States. The presented draft included a revision on the items surveillance and methods.

The overall objective is to provide the essential criteria for monitoring the prevalence and potentially trends of antimicrobial resistance in *Salmonella* spp., *Campylobacter (C.) jejuni, C. coli* and *Escherichia (E.) coli* from cattle, pigs, poultry and food of animal origin derived from those species in the Member States.

Several monitoring approaches for zoonotic bacteria will be implemented according to the Directive 2003/99/EEC and the Regulation 2160/2003/EC. Isolates from these sampling could be used to draw a sample for the antimicrobial resistance testing, or to get more

isolates. Also the sampling scheme could be used for further testing, one sample could be tested for *Salmonella* , *Campylobacter* and *Escherichia coli*.

The primary aim of this protocol is the estimation of the prevalence of resistance to a defined set of antimicrobials in zoonotic agents and commensal bacteria.

The critical point for the comparability of data are the methods for the isolation of bacteria and the antimicrobial susceptibility testing by itself. In the Member States a diversity of methods is established. In addition different scales to discriminate resistance from susceptible are used. The first draft fixed the methods to ISO Norms for the isolation and the NCCLS standards for the antimicrobial susceptibility testing. This proposal was revised, the Member States demanded for more flexibility. The new proposal took this into account. The method for the monitoring should provide quantitative data in the unit of measurement µg/ml. The comparability of the results in the laboratories should only be achieved by an external control, ring trials conducted by Community reference laboratories. The result of the ring trials must be submitted in addition to the test results.

The panel of antimicrobials is based on the recommendation of ARBAO (Antibiotic Resistance in Bacteria of Animal Origin), the Workshops of the CRL-*Salmonella* and the Workshops of the CRL-Epidemiology. Some options were added since the first draft. The addition of antimicrobials testing extended spectrum lactamases is of special interest in the treatment of salmonellosis in children. If an agreement in principle on the methods took place, a detailed form could be developed.

Discussion

Q/A: Antibiotic resistance testing may only be of interest for special cases and serotypes, else it may give very little extra information. It may also be relevant when performing monitoring. In case of outbreaks a resistance marker on a strain may be very valuable as epidemiological marker.
Q: How many strains should be tested? All strains coming into the laboratory, or a selection of e.g. 100 strains?
A: For the nordic countries it may be a problem to obtain 100 strains. If the prevalence is low it may be enough to analyse less strains.

1.12 Discussion on design typing study X (2005) concerning antibiotic resistance testing

Dik Mevius, CIDC, Lelystad, the Netherlands (see Annex 14)

Based on the results the following suggestions were made:
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 from MEDVETNET.

2. For monitoring purposes
   d. Exclude AMCL because it is not reliable
      i. In stead: Ampi/amox, Cefotaxime and ESBL confirmation with Etest
   e. Streptomycin's value is disputed
   f. Trim/Sulpha is disputed, preferable the individual components.
      i. Include:
      ii. Sulphamethoxazole
      iii. Nal and ciprofloxacin (not enro)
      iv. Neomycin (not kanamycin)

Discussion

Q: Is it required in the Directive to use quantitative methods for antibiotic resistance testing?
A: Need to check the Directive, not yet sure what is written.

2. Friday 14 May 2004: day 2 of the workshop

2.1 Overview on the zoonoses report 2002

Kirsten Heckenbach, CRL-Epidemioplogy, Berlin, Germany (see Annex 15)

The Directive 92/117/EEC covers a single harmonised monitoring programme, the monitoring of breeding flocks of *Gallus gallus*. This scheme was approved for 7 Member States by the Commission. At least *S*. Enteritidis and *S.* Typhimurium are notifiable, if not all *Salmonella* serovars are targeted. The comparability of the data from other production lines and animals under the current directive is constricted. The member states differ in their monitoring and sampling schemes. The notification system, the kind of samples, the location samples are taken and the allocation herd or animal is restricted to the individual countries. A general limitation is the availability of data from single countries, therefore given trends reflect only a part of the European Union.

The very favourable situation in breeding flocks has continued in 2002 as regards *S.* Enteritidis and *S.* Typhimurium in Denmark, Finland, Ireland, Sweden and Norway. The *Salmonella* control programmes run in Finland, Sweden and Norway have documented that also the prevalence of other *Salmonella* serovars in poultry is low.

In the other countries, infection rates reported in 2002 range between 0% and 6% for *S.* Enteritidis and *S.* Typhimurium infections. *S.* Enteritidis is the dominating serovar, sharing 45% of all isolates reported in breeding flocks (*Gallus gallus*) in the reporting countries.

In layer breeders in all five countries (Denmark, Finland, Ireland, Sweden, Norway) which are running an approved control programme for several years no *Salmonella* findings were reported. In Finland and Norway all flocks of laying hens were *Salmonella* negative. In Ireland, no *S.* Enteritidis and *S.* Typhimurium were detected but a few other serovars were found. In Sweden, three positive layer flocks were detected. The serovars detected were *S.* Livingstone, *S.* Typhimurium and *S.* Subspecies II. In Denmark the prevalence of *Salmonella* has been reduced compared to the previous year. In 2002, 2.7 % of 330 rearing flocks and 2.6 % of 619 flocks producing table-eggs for authorised egg-packing centres were infected with *Salmonella*, and most of them were infected with *S.* Enteritidis. In the other countries, as far as a trend can be assessed, situation remained at a low level or improved slightly. The reported infection rates for *S.* Enteritidis and *S.* Typhimurium in laying hens ranged from 0.06 % to 7.2 %.

In layers, *S.* Enteritidis is dominating in all countries except in Ireland and Sweden, where this serovar was not isolated.

A *Salmonella* prevalence above 1 % in table eggs was reported in four out of eight reporting countries. As the sampling schemes are not described in detail, it is not clear whether the

differences observed reflect differences in sampling and pooling of samples or true differences in the prevalence rate. In contrast, in raw material for egg products, no *Salmonella* at all were reported. In final egg products, which are usually heat treated, in three countries the *Salmonella* prevalence was above 1 %. Usually, *S.* Enteritidis is the dominating serovar.

In the meat production sector situation is not as favourable as reported in the egg production sector. In Sweden and Norway, all broiler breeder flocks were *Salmonella* negative. In Finland and Ireland, no *S.* Enteritidis and *S.* Typhimurium were detected. In Great Britain, no findings of *S.* Enteritidis and *S.* Typhimurium were reported whereas in the other countries the prevalence for these two serovars ranged between 0.06 % and 6 % infected flocks.

Altogether, infection rates in broiler flocks are higher compared to the breeder level. No *Salmonella* infection was detected in Norway. In Sweden, one broiler flock infected with *S.* St Paul was detected. In Finland, the share of *Salmonella* positive flocks was 0.35 %. In Denmark, 1.5 % of the broiler flocks were *Salmonella* positive on the average. The monthly prevalence rate ranged from 0.5 % to 2.7 %. The most frequently occurring serovar was *S.* Indiana. The infection rates reported in broiler flocks in the other countries ranged from 2.0 % to 16.9 %.

In cattle, results of the surveillance programme at slaughterhouses and cutting plants run in Finland, Sweden and Norway showed that the *Salmonella* situation continued to be at low level. In lymph node samples and carcass swabs *Salmonella* were rarely detected.

Salmonella was detected in beef at lower rates compared to poultry meat and pork in several countries. In beef, contamination level ranged from 0 % to 3.2 %. At retail level, slightly higher contamination levels were reported compared to the data at slaughterhouse and processing plants.

Altogether, 145,231 cases of human salmonellosis have been reported by the 15 Member States of the European Union and Norway in 2002. This means an overall decrease by 10% compared to 2001,

As in previous years, *S.* Enteritidis was dominating in human salmonellosis, causing 67.1 % of all notified cases in the European Union and Norway. Rates in the individual countries ranged between 88.6 % in Austria and 30.9 % in France. *S.* Typhimurium was on the second place, causing 17.0 % of all cases. Next to *S.* Enteritidis and *S.* Typhimurium, most cases are caused by *S.* Infantis, *S.* Virchow and *S.* Hadar.

In humans, the main phagetypes of *Salmonella* remained phagetypes PT 4, PT 8, PT 21, PT 1 and PT 6. These phagetypes are also among the most frequent ones in poultry. The pattern in the individual countries is different. In Austria, Italy and the Netherlands, PT 4 is by far the most frequent phagetype in man and poultry. In contrast, in Denmark the main causative agent in humans and frequently isolated in layers is *S.* Enteritidis PT 8. The pattern of *S.* Typhimurium is different in the individual countries, but *S.* Typhimurium DT 104 was isolated in all Member States.

The comparability of the data on antimicrobial susceptibility testing is also limited for several reasons. Not all countries provided the source of the isolates, if the isolates come from an active or passive monitoring programme. The main constraint of the data, are the different test methods used in the individual countries. Therefore the summary of the results must be done very carefully.

For all species Tetracyclin, Streptomycin and Ampicillin is common. Pigs have a higher resistance rate of Chloramphenicol in comparison to cattle. Also resistance to Trimethoprim alone and in combination with Sulfonamide is common in isolates from pigs. In addition, in isolates from poultry resistance to nalidixic acid is common. Resistance to Cephalosporins occurs in single countries.

The whole report is available at a webpage of the Commission.

Discussion

Q: Are there differences in most occurring serovars in relation to animal species ?

A: Yes. Pigs for instance, include *S.* Brandenburg, *S*. Derby and *S*. Bovismorbificans. For cattle include *S*. Anatum.

2.2 Tasks and duties CRLs and NRLs

Jean-Charles Cavitte, European Commission, Brussels, Belgium (see Annex 16)

There is a network of laboratories involved in official control of food at Community level. Community Reference Laboratories (CRLs) are appointed at EC level; as tasks and organisation are defined in EC legislation. National Reference Laboratories (NRLs) are appointed by Member States authorities pursuant to EC legislation; Usually there is only one NRL per field of activity per country. Routine laboratories (national/regional) are operational on national level.

The current situation of CRLs in the area of biological risks

Twelve CRLs have been designated in the area of food safety:

- 6 CRLs for biological risks: Paris; Berlin (zoonoses report); Bilthoven; Vigo; Weymouth; Weybridge;
- 4 CRLs for residues: Bilthoven; Fougères; Berlin; Rome;
- 1 CRL for additives for use in animal nutrition: Joint Research Center-JRC (will probably be operational in mid-October);
- 1 CRL for GMO’s: JRC (April 2004).

(1) Tasks of CRLs for biological risks (they may differ slightly according to the specific legislation through which CRL are appointed):

- provide NRLs with details of analytical methods;
- coordinate application of methods by NRLs, by organising comparative testing in particular;
- co-ordinate research on new methods;
- conduct training for NRLs;
- assistance to E Commission;
- cooperate with laboratories in third countries;
- help NRLs implement QA.

(2) Tasks of NRLs for biological risks (they may differ according to the specific legislation through which CRL are appointed):

- co-ordination activities of NLs;
- assisting the national competent authorities;
- organising on regular basis comparative tests between NLs;
- disseminating information supplied by the CRLs to authorities and NLs;
- collaboration with CRLs.

Revision of legislation on Official Feed/Food Control (OFFC): Com(2003)52 final

This new legislation, applicable from January 2006, will recast the general missions/requirements for CRLs/N(R)Ls. Further detailed requirements can be laid down. It also clarifies that accreditation ISO 17025 is required for laboratories in official control (including CRLs/NRLs).
Member States shall arrange for the designation of 1 or more NRL for each field , where a CRL has been designated. They may choose 1 in another Member State or EFTA country, if no NRL is appointed in the particular field of activity in a country in question. There should be close cooperation if more than 1 NRL is designated for the same domain within a country. Member States shall communicate the name and address of each NRL to the Commission, the relevant CRL and other Member States.

(3) General tasks of CRLs resulting from OFFC Regulation:

- Providing NRLs with details of analytical methods, including reference methods;
- Coordinating application by the NRLs of the methods referred to in (a), in particular by organising comparative testing and by ensuring an appropriate follow-up of such comparative testing in accordance with internationally accepted protocols, when available;

- Coordinating, within their area of competence, practical arrangements needed to apply new analytical methods and informing NRLs of advances in this field;
- Conducting initial and further training courses for the benefit of staff from national reference laboratories and of experts from developing countries;
- Providing scientific and technical assistance to the Commission, especially in cases where Member States contest the results of analyses;
- Collaborating with laboratories responsible for analysing feed and food in third countries.

(4) General tasks of NRLs resulting from OFFC Regulation:

- Collaborate with the CRL;
- Coordinate the activities of official laboratories;
- Where appropriate, organise comparative tests between the official national laboratories and ensure an appropriate follow-up;
- Ensure dissemination of CRL information to the competent authority and official national laboratories;
- Provide scientific and technical assistance to the CA for the implementation of coordinated control plans.

Zoonoses Legislation

Directive 92/117/EEC repealed from 12/6/2004 and replaced by Dir 2003/99/EC and Regulation 2160/2003:

Need to reappoint CRL epidemiology zoonoses - Berlin until end 2004 (EFSA taking over the preparation of Community report in order to provide technical assistance to the COM from January 2005)

Need to reappoint CRL-*Salmonella* - Bilthoven (at least until end 2005 –OFFC has reappointed CRL-*Salmonella* with effect from January 2006) and clarify area of competence and responsibilities (and subsequently those of NRLs)

Likely need to appoint additional CRLs in the future (probably on the basis of OFFC Regulation)

(5) CRL salmonella: EC proposal

General missions: those in OFFC

Additional specific missions:

- Technical assistance to EC in the organisation of monitoring schemes for salmonella and related anti-microbial resistance;
- Technical assistance to ECommission in the setting of Community targets pursuant to Regulation (EC) No 2160/2003;

- Advice to EC on aspects related to salmonella vaccine strains and other specific control methods;
- Participation, as appropriate, in international fora relating to the areas of competence identified in point 1 above, and concerning in particular the standardisation of analytical methods and their implementation;
- Gathering of data and information on the activities developed and methods used in national reference laboratories;
- Keeping abreast of developments in salmonella epidemiology;
- Cooperate, as appropriate, with other relevant Community structures involved in salmonella surveillance, in particular the structures appointed pursuant to Decision No 2119/98/EC setting up a network for the epidemiological surveillance and control of communicable diseases in the Community;
- (Possibly, building up and maintenance of an up-to-date data bank of salmonella strains, as appropriate).

Areas of competence:

- identification and development of bacteriological methods for the detection and as appropriate quantification of zoonotic salmonella in livestock, feed and food, as well as in environmental samples;
- subtyping of zoonotic *Salmonella*, in particular serotyping, and other subtyping, including phenotypic and genetic methods;
- antimicrobial susceptibility testing on isolates of zoonotic *Salmonella*;
- identification and development of immunological methods for zoonotic *Salmonella*;
- identification and development of sampling methods.

(6) NRLs-*Salmonella*: EC proposal

General missions: those in OFFC
Additional specific missions: mirroring CRL:

- Co-ordination in the Member State of, and, as appropriate, participation in monitoring schemes for salmonella and related anti-microbial resistance pursuant to Directive 2003/99/EC;
- Co-ordination in the Member State of the analysis and testing of salmonella pursuant to Regulation (EC) No 2160/2003;
- Inform as appropriate CRL on aspects related to salmonella vaccine strains and other specific control methods;
- Gathering of data and information on the activities developed and methods used in laboratories (*and feed back to CRL*);
- Keeping abreast of developments in salmonella epidemiology;

- (conducting as appropriate training courses for the benefit of staff from relevant laboratories);
- (possibly, building up and maintenance of an up-to-date data bank of salmonella strains, a appropriate).

The Commission intends to present a proposal in the Standing Committee in June 2004, so that the 2 CRLs would be reappointed with effect from 12 June 2004. The responsibilities for live poultry would apply from that this date and, for other areas, from January 2005.

Conclusions

EC is finalising the revision of its food safety legislation, including consolidation of provisions on laboratory analysis. CRLs/NRLs are important components for official control, by coordinating laboratory activities up to field laboratories. A reflection was initiated by the Directorate General on the need for additional CRLs, and subsequently for need to appoint NRLs by Member States.

Discussion

Q: Is there a formal link between CRL/NRLs with Pulsenet and/or MedVetNet?
A: For epidemiological purposes it may be good to have a link.
Q: Tasks will increase. How do the national authorities become informed?
A: It will be discussed in the Standing Committee, meaning that the Member States should know what is decided. The list of tasks does not mean that the year programmes of the CRL or the NRLs should contain everything every year.
Q: Would it not be better to have separate CRLs for techniques like genotyping, antibiotic resistance?
A: Nothing is decided yet. Genotyping in a separate CRL is perhaps not the most optimal situation. For antibiotic resistance it is not yet sure what would be best. The EC should adopt the most efficient system.

2.3 Questionnaire comparative testing

*Kirsten Mooijman, CRL-*Salmonella*, Bilthoven, the Netherlands (see Annex 17)*

Based on the EU zoonoses legislation, one of the tasks of a NRL is to organise comparative tests between the official national laboratories. Comparative or proficiency testing is used to compare the performance of laboratories undertaking testing. This in order to help ensure comparability of test results and to identify, and improve, the performance of poorly performing laboratories. It is also an essential aspect of laboratory accreditation.

Discussion between the Commission and EU CRLs has focussed on the status of comparative testing among NRLs in Europe. The picture seems quite variable. It has been agreed that CRLs should seek to establish the current status of comparative testing among NRLs in their various fields. To make an inventory on this subject the CRL-*Salmonella* has sent a questionnaire to the 27 NRLs-*Salmonella.* At the beginning of April 2004 a total of 21 completed questionnaires was received by the CRL-*Salmonella*. The results of the questionnaires were summarised in a draft report and presented at the workshop per question. The aim of the questionnaire was not to appoint the NRLs who did not (yet) organise comparative tests (proficiency tests), but to identify difficulties NRLs are experiencing with their duty of national proficiency testing organisation.

The following conclusions were drawn from the questionnaires:

- Majority of NRLs-*Salmonella* organise proficiency tests (either alone or in partnership);
- In most MS it is compulsory to participate;
- In some of the MS laboratories have to pay for participation;
- The number of participants vary per country;
- Majority of NRLs organise 1-2 studies per year (typing and/or detection);
- Methods are mostly prescribed (official methods);
- Test materials are mainly spiked animal faeces and/or (pure) strains;
- *Ca* half of the NRLs have a scoring system and a follow-up system for laboratory performance;
- The majority of the organising NRLs still have problems in carrying out the proficiency testing programme. Most mentioned problems are:
  - Resources
  - Test materials
- Main problems mentioned by not organising NRLs:
  - Resources
  - Experiences
  - Test materials (including distribution)

The following possible support to the NRLs could be given:

- In October 2004 (28 & 29) a workshop on proficiency testing for microbiology in food and veterinary laboratories will be organised at the Institute of Reference Materials and Methods (IRMM) of the EC Joint Research Centre (JRC) in Geel, Belgium. This workshop is especially intended for NRLs who (still) have to start with the organisation of proficiency testing and/or have major problems with the organisation of proficiency testing.
- Capsule reference materials, as used by CRL-*Salmonella* in the intercomparison studies, will not become available on short term. Alternatively to the capsules, NRLs can order ‘lenticules’ at the Health Protection Agency (HPA) in Newcastle, UK. HPA would need to have the following information of an NRL:
  - Number of lenticules required per distribution per year;

- The strains involved and the target levels;
- Need of single strains or mixtures per lenticule.

The contact at HPA Newcastle will be: Danka Tharagonnet: danka.tharagonnet@hpa.org.uk

Discussion

Q: It would be helpful to have information on preparation of samples, number of samples, mailing of samples, analyses of results, etc.
A: It is plannend to summarise the results of the questionnaire in a report. In annexes in this report some relevant information can be added.

2.4 National comparative testing programme in Poland

Andrzej Hoszowski, NRL-Poland, Pulawy, Poland (see Annex 18)

National Reference Laboratory for *Salmonella* (NRL-*Salmonella*) was established by the Regulation of 12 February 2003 of Ministry of Agriculture and Rural Development. It was located in National Veterinary Research Institute in Pulawy. One of the main NRL-*Salmonella* tasks is proficiency testing of regional veterinary laboratories.
During the year 2003 NRL-*Salmonella* carried out 2 proficiency tests regarding *Salmonella* isolation from samples of animal origin and one study on *Salmonella* serotyping.
The first proficiency test on *Salmonella* isolation was organised in April, 2003. Five lyophilized samples of bovine faeces were sent to each of 16 participating regional veterinary laboratories. The samples were spiked with *S.* Typhimurium on 3 levels: 3 x 10^2 cfu (2 samples per laboratory), 3 x 10^3 cfu and 5 x 10^6 cfu. The fifth sample was not contaminated ("blank"). In general, the percentage of correct results reached 85%, however it differed between spiked and blank samples. *Salmonella* was found in all contaminated samples but 12 out of 16 laboratories reported false-positive results in the case of "blank" samples. It was concluded that efficacy of laboratories regarding *Salmonella* isolation is not satisfactory.
The next proficiency test on *Salmonella* isolation was organised in September 2003 and comprised 37 laboratories (16 regional laboratories and their branches). The objective was to check the improvement of diagnostic efficacy in *Salmonella* isolation in comparison with the previous trial. Each of 37 laboratories received 4 lyophilized "blank" samples of bovine faeces and 2 *Salmonella* positive samples spiked with circa 14 cfu of *S.* Typhimurium. All spiked samples were found positive. The correctness of the results reported for "blank" samples reached 95%.
The proficiency test for *Salmonella* serotyping was also organised in September 2003. Each of the participating laboratories received 5 strains: *S.* Enteritidis, *S.* Typhimurium,

S. Gallinarum and 2 out of *S.* Agona, *S.* Derby, *S.* Dublin or *S.* Schwarzengrund. Most of the laboratories were able to define serovars of epidemiological importance such as *S*. Enteritidis, *S*. Typhimurium and *S*. Gallinarum. However, regional laboratories encountered some problems with serotyping of less prevalent *Salmonella* serovars.

Discussion

Q: What are the plans for studies in the future?
A: Plans to increase the number of samples, containing *Salmonella* at different levels, to increase the competitive flora, poultry faeces instead of bovine faeces.

2.5 PCR confirmation directly from MSRV agar plates

Erik Eriksson and Anna Aspán, NRL-Sweden, Uppsala, Sweden (see Annex 19)

Since two years, PCR confirmation on suspected *Salmonella* colonies from BG & XLD agar plates, is routinely used on feed samples in our laboratory, by using the real-time PCR assay of the *invA* gene according to Hoorfar J, Ahrens P, Radstrom P. Automated 5' nuclease PCR assay for identification of *Salmonella enterica. J Clin Microbiol*. 2000 Sep;38(9):3429-35. The method is simple and straight forward, including the following steps:

- A suspected colony is picked by a touch of a loop on the agar plate, and transferred directly to the PCR-master mix. Lysis is performed in the PCR-machine before cycling starts;
- The master-mix is prepared in advance, and kept in freezer before use. Confirmation is completed within two hours.

The method has been "in-house validated", and accreditated by Swedac.

***Salmonella* samples from the VII collaborative study**

We were interested in applying this real-time PCR-method, to shorten analysis time on faecal samples from the collaborative study. Four different ways to prepare and detect *Salmonella*, after pre-enrichment, were compared:

1. 100 µl BPW was transferred to 900 µl BHI-medium, incubated at 37º C, 3h, and 10 µl BHI- medium was used as template for real-time PCR.
2. A touch of a loop on the MSRV agar plates after 2 days incubation was transferred directly to the PCR-master mix, followed by real-time PCR.
3. A touch of a loop on the MSRV agar plates after 2 days incubation, was transferred to 500 µl BHI-medium, that was subsequently incubated at 37º C, 3h. 10 µl BHI- medium was used as template for real-time PCR
4. Gel-BAX-salmonella was used according to the manufacturers instructions. (Lot:3143, Exp date 9/30/05)

BPW from Oxoid was used for the pre-enrichments 16-20 h.

Results and Conclusions

- For the control samples, not containing faeces, all methods used to prepare and detect salmonella worked excellently.
- For the faecal samples spiked with salmonella, only method 2 & 3 gave reliable results.
- For the 20 samples of naturally contaminated faeces, again method 2 & 3 gave the best results.
- Faecal samples from poultry are highly inhibitory to PCR
- A second enrichment step is not sufficient to overcome inhibition
- Gel-BAX salmonella is not suitable for analysis of these samples

Discussion

Remark: One NRL also performed PCR via Diassalm. Similar results as culture. Promising method.

2.6 European validation and certification of methods

Pauline Kalkman, Microval, Delft, the Netherlands (see Annex 20)

Numerous and diverse alternative methods for microbiological research are being offered to the market as a result of recent developments. These often more rapid and or convenient methods are of great interest to the food industry since they can provide better and/or faster means to monitor raw materials, processes and products. Microbiological tests are also very important in the governmental food inspection, in international trade, in commercial relationships between trade partners and in product liability matters. The results of these tests should be reliable and it is therefore very important that all parties involved agree with and accept the methodology employed.
However, before a new microbiological method can be widely accepted not only must its intrinsic technical quality be established objectively, but, as mentioned, there must also be a guarantee that interested parties will accept the results obtained using such a method.

MicroVal (MV)
The MV project started with the aim of setting up a European validation procedure and of creating such conditions that the results of the procedure would be accepted as far as possible by all interested parties in Europe. The MV Rules and Certification Scheme were developed, describing the methodology and the organisation to be used for the European certification by an independent organisation. The validations will be performed using EN ISO 16140, Protocol for the validation of alternative methods.

EN ISO 16140

This standard covers the validation the procedures and requirements for the validation of both quantitative and qualitative alternative methods. Both of these require a method comparison study as well as an interlaboratory study to be performed by MV expert laboratories. For the purpose of validation, EN ISO 16140 is also required by the EU (SANCO/4198/2001/rev9).

MicroVal Certification organisation

MicroVal has a balanced representation and is constituted of a group of independent MicroVal Certification Bodies (MCB's), with a common MV General Committee (MGC), a common Secretariat and a European network of sub-contractors: laboratories, reviewers and auditors. The MCB's are headed by an impartial MGC which has a European composition and consists of public authorities, manufacturers, users and MicroVal third parties, as well as a secretariat for which the Netherlands Standardization Institute (NEN) is responsible.

Why MicroVal

MicroVal, as an organisation, aims to provide a single accepted method validation and certification system in Europe. One of its primary goals in doing so is to lower the entry barrier for manufacturers wishing to enter the European market. It will remove the need for multiple national certifications which are expensive in both time and money. In addition to this, it fulfils the requirements of European Legislation for rapid methods as stated in the Draft EU Microbiological Criteria Document (SANCO/4198/2001, rev. 9), art. 5.

Recent developments

Recently Lloyds and TNO Certification entered MV. The MV secretariat performed a market survey, which showed an obvious need for MV certification. Furthermore progress is being made on all fronts. For example, interested parties, e.g. food industry and FLEP (Food Law enforcement Practitioners) are supportive and recognized by EC. On going discussions with AOAC and NordVal are taken place to establish mutual recognition agreements.

Conclusion

There is still a lack of uniformity in Europe as to which validation system should be used. A number of national or regional validation systems exist which tend not to be accepted outside their particular region. MicroVal is aiming to address these issues. For more information, visit www.microval.org.

Discussion

Q: What will be the approximate costs?
A: Depends on the participating laboratories etc, but a certification may cost circa € 30 000.
Q: What will be the time frame for a full validation?
A: circa 9 months

2.7 Validation of methods at national or laboratory level

Henk Stegeman, Rikilt, Wageningen, the Netherlands (see Annex 21)

The standard EN-1SO 16140:2003 gives the general principle and a technical protocol for the validation of alternative methods in the field of microbiological analysis of food, animal feeding stuff and environmental and veterinary samples. The technical protocol has two parts: validation of alternative qualitative and quantitative methods. The validation protocol is based on comparison study of the alternative method against a reference method with regard to the performance characteristics such as detection limit, robustness, sensitivity, specificity, accuracy, repeatability and reproducibility.
The Dutch Board for Livestock, Meat and Eggs (PVE) has applied ISO 16140 at a national level for the validation of a PCR and other rapid methods against the Dutch MSRV standard method for the detection of *Salmonella* in poultry matrices (see the 2003 CRL-*Salmonella* workshop).

The Probellia^{TM} PCR method was already validated for food, but not for the poultry matrices fluff, faeces and skin. Therefore the data on the detection limit and on inclusivity and exclusivity were known. The relative accuracy, the relative specificity and relative sensitivity were determined in a comparative study between the alternative and reference method for the matrices fluff, faeces and skin, using for each matrix approximately 30 positive and negative naturally contaminated samples. For fluff it was necessary to use some artificially contaminated samples. Instead of one expert laboratory the study was done by two routine laboratories which were accreditated by ISO 17025 for the MSRV method. Each laboratory has investigated 60 samples for each matrix. For laboratory 1 there was no statistical difference between the two methods; laboratory 2 found more positive samples for faeces and fluff with the PCR method. It was not possible to organize an interlaboratory study with 10 laboratories according ISO 16140 at a national level; at that moment only 3 laboratories were equipped. However, these laboratories participated with good results in the national proficiency testing programme of RIVM.

On the results of this validation study the Dutch Board allows now the PCR as an alternative analysis method for the control of Salmonella in the poultry chain. The same protocol has been used for the validation of the real-time PCR and an immunological method for these poultry matrices.

Discussion

Q: Is it possible to use the PCR method directly after pre-enrichment in BPW ?
A: Yes, but sometimes inhibition will occur. Dilute your sample 1:10 to overcome inhibition.

2.8 Work programme CRL-Salmonella 2005 and closure

*Kirsten Mooijman, CRL-*Salmonella*, Bilthoven, the Netherlands (see Annex 22)*

Programme coming year

Concerning the proficiency testing the following activities are planned:

- Performance analyses over at least four intercomparison studies (detection and typing);
- Start with developing a score system for laboratory performance;
- Start with developing a follow-up system for addressing poor laboratory performance;
- Organisation of the 9th bacteriological detection study (fall 2004) with poultry faeces and if possible also with environmental samples;
- Organisation of the 10th typing study (spring 2005).

Concerning research the following activities are planned:

- Continuation of the stability studies of the reference materials;
- Stability studies of poultry faeces (*Salmonella* and background flora), stored at different temperatures;
- Detection of *Salmonella* spp. in other matrices than poultry faeces, e.g. environmental samples (like dust) of the primary production stage of poultry, faecal samples of e.g. pigs, animal feed;
- Molecular biological and immunological methods;
- Working out the draft annex for ISO 6579.

Concerning communication and other activities the following is planned:

- Newsletter 4x/year through website;
- Update website;
- Accreditation according to ISO 17025;
- Ad hoc activities on own initiative or on request;
- Workshop in circa May 2005

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, contributing to the success of this workshop.

Annex 1. Participants

European Commission	Jean-Charles Cavitte Sarolta Idei Maija Hatakka
CRL – *Salmonella*	Kirsten Mooijman Hans Korver Christiaan Veenman Henny Maas
CRL – Epidemiology of Zoonoses	Kirsten Heckenbach
Guest speakers (the Netherlands)	Dik Mevius (CIDC, Lelystad) Pauline Kalkman (Microval, Delft) Henk Stegeman (RIKILT, Wageningen)
Guest speaker (United Kingdom)	Linda Ward (HPA, London)
Invited persons (the Netherlands)	Anne Mensink **(**Head of Microbiological Laboratory for Health Protection, RIVM) Wilfrid van Pelt (CIE, RIVM)

National Reference Laboratories for *Salmonella*

AUSTRIA	Heimo Lassnig
BELGIUM	Hein Imberechts
CYPRUS	Economides Constantinos
CZECH REPUBLIC	Iva Bernardyova Marketa Tomsickova
DENMARK	Dorte Lau Baggesen Steen Nordentoft
ESTONIA	Toomas Kramarenko

FINLAND	Tuula Johansson Henry Kuronen
FRANCE	Marylène Bohnert
GERMANY	Christina Dorn
GREECE	Eleni Valkanou
HUNGARY	Erzsebet Andrian
IRELAND	John Egan John Ward
ITALY	Antonia Ricci
LATVIA	Andra Utinane
LITHUANIA	Ceslova Butrimaite-Ambrozeviciene
LUXEMBOURG	Joseph Schon
NETHERLANDS	Arjen van de Giessen Anjo Verbruggen
NORWAY	Bjarne Bergsjo
POLAND	Andrzej Hoszowski
PORTUGAL	Alice Amado
SLOVAK REPUBLIC	Milan Sasik
SLOVENIA	Vojislava Bole-Hribovsek
SPAIN	Christina de Frutos Escobar
SWEDEN	Erik Eriksson
UNITED KINGDOM	Robert Davies

Annex 2. Programme of the workshop

Programme of the CRL-*Salmonella* workshop IX, 13-14 May 2004, Bilthoven

General information

Hotel: Hotel Biltsche Hoek; De Holle Bilt 1; De Bilt; The Netherlands;
tel.: +31 30 2205811
http://www.valk.com/nl/vestigingen/body/show.phtml?nummer=5
http://www.rivm.nl/en/route (pdf file)

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 10 May 2004.
In the meeting room the following is available for the presentations: overhead projector, beamer+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 12 May 2004

Arrival of representatives of the NRLs at Hotel De Biltsche Hoek.

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

Thursday 13 May 2003 (T007)

Morning chair: Kirsten Mooijman

8.30	Departure from hotel De Biltsche Hoek to RIVM
9.00 - 9.15	Opening and introduction (Kirsten Mooijman)
9.15 - 9.45	The new EU Zoonoses Directive and Regulations (Sarolta Idei)
9.45 - 10.15	Progress in ISO/TC34/SC9 (Kirsten Mooijman)
10.15 – 10.45	Monitoring *Salmonella* spp. in laying hens (Arjen van de Giessen)

10.45 - 11.15 Coffee/tea

11.15 – 11.45	Bacteriological sampling to detect *Salmonella* in poultry flocks (Robert Davies)
11.45 – 12.15	Results bacteriological detection study VII – 2003 (Hans Korver)
12.15 – 12.45	Discussion on design bacteriological detection study VIII - 2004 (Kirsten Mooijman)

12.45 - 13.30 Lunch

Afternoon chair: Arjen van de Giessen

13.30 - 13.50	Results typing study IX - 2004 : phagetyping (Linda Ward)
13.50 - 14.20	Results typing study IX - 2004: serotyping and design typing study X (2005) concerning serotyping and phagetyping (Hans Korver)
14.20 – 14.40	Results typing study IX - 2004: antibiotic resistance testing (Dik Mevius)

14.40 - 15.15 Coffee/tea

15.15 – 15.45	Draft monitoring scheme for antibiotic resistance testing (Kirsten Heckenbach)
15.45 - 16.15	Discussion on design typing study X (2005) concerning antibiotic resistance testing (Dik Mevius)
16.30	Transport to hotel de Biltsche Hoek
18.00 – onwards	Evening programme and dinner

Friday 14 May (T007)

Chair: Arjen van de Giessen

8.45	Departure from hotel Biltsche Hoek to RIVM
9.00 - 9.30	Overview on the zoonoses report 2002 (Kirsten Heckenbach)
9.30 - 10.00	Tasks and duties CRLs and NRLs (Jean-Charles Cavitte)
10.00 - 10.30	Questionnaire comparative testing (Kirsten Mooijman)
10.30 – 10.45	National comparative testing program in Poland (Andrzej Hoszowski)

10.45 - 11.15 Coffee/tea

Chair: Kirsten Mooijman

11.15 – 11.30	PCR confirmation directly from MSRV agar plates (Erik Eriksson)
11.30 - 12.00	European validation and certification of methods (Microval; Pauline Kalkman)
12.00 – 12.30	Validation of methods at national or laboratory level (Henk Stegeman)
12.30 – 13.00	Work programme CRL-*Salmonella* 2005 and closure (Kirsten Mooijman)

13.00 - 14.00 Lunch

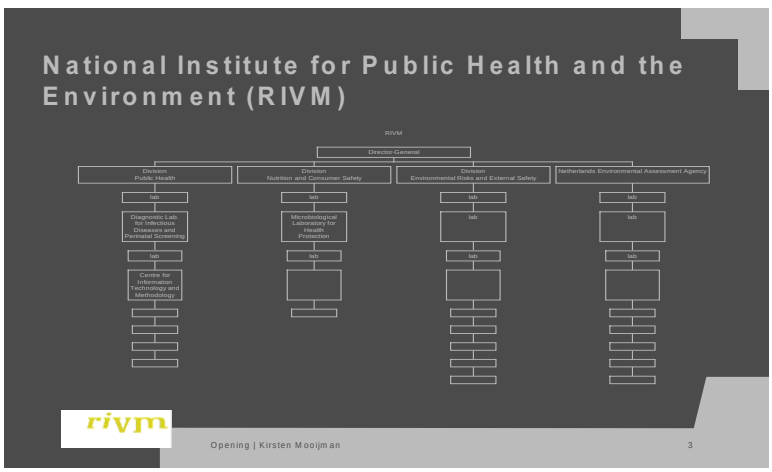
14.00 Departure to train station Bilthoven

Annex 3. Slides of presentation 1.1

Slide 1

Slide 2

Slide 3



Slide 4

Changes

- 10 new Member States
- 1 participant per NRL
- Personnel:
  - André Henken
  - new Head MGB: Anne Mensink
  - new Head CRL-*Salmonella*: Kirsten Mooijman
  - new technician CRL-*Salmonella*: Christiaan Veenman
- Website: http://www.rivm.nl/crlsalmonella

rivm

Opening | Kirsten Mooijman 4

Slide 5

Introduction participants

rivm

Opening | Kirsten Mooijman 5

Slide 6

Aims of workshop

- Discuss issues of relevance for CRL and NRLs:
  - EU level (new Directive and Regulation)
  - Tasks and duties CRL and NRLs
  - Exchange info on methods (ISO, validation, PCR,….)
- Past (2003) and future intercomparison studies CRL
- Exchange info between NRLs (research activities)
- Exchange info with representatives EC (DG-Sanco)
- Needs and expectations NRLs
- Discuss future activities CRL

rivm

Opening | Kirsten Mooijman 6

Slide 7

Programme 9th CRL-S workshop (I)

13 May:

- New Directive and regulation: status, implementation, methods
- Intercomparison studies: detection and typing (2003 and 2004, 2005)
- Antibiotic resistance

rivm

Opening | Kirsten Mooijman 7

Slide 8

Programme 9th CRL-S workshop (II)

14 May:

- Zoonoses report
- Task and duties CRL and NRLs, including comparative testing
- Methods: PCR confirmation, validation
- Work programme second half 2004 and 2005

rivm

Opening | Kirsten Mooijman 8

Slide 9

General information

- Language: English
- Speakers: hand-over pp-presentations in time
- Use badges and table cards
- coffee, tea, lunches
- copies of tickets
- transport to and from the hotel
- 13 May: 10.45 h group picture
- 13 May social evening programme: 18.00 h lobby hotel; followed by dinner (info to Loes/Jeanette)
- 14 May, closure ca 14.00 h (after lunch); transport to Bilthoven train station

rivm

Opening | Kirsten Mooijman 9

Annex 4. Slides of presentation 1.2

Slide 1

Zoonoses Legislation

CRL Salmonella workshop Bilthowen
12-14/05/2004
Sarolta Idei - European Commission DG SANCO

1

Slide 2

Zoonoses legislation

- Dir.No 92/117/EC-(will be repealed on the 12 of June 2004 by):
- Directive No 2003/99 on monitoring
- Regulation No 2160/2003 on control
- Other relevant legislations-Hygiene package,OFFC,
- Decision No 2119/98/EC setting up a network for epid.surveillance and control of communicable diseases,reinforcement of data collection

2

Slide 3

References to Dir.No 92/117/EC

- Establishment of monitoring systems certain zoonoses-salmonella in poultry flocks,draw up plans for monitoring salmonella in poultry,report to COM

3

Slide 4

Connected to food safety legislation

OFFC

Hygiene package

4

Slide 5

II.

- White paper on food safety-new framework on food safety legislation-regulations,co-decision(EP,Council)
- General Food law-Reg.178/2002/EC

  High level of consumer protection-coordinated and integrated approach-RA(science based approach)traceability,precaution

5

Slide 6

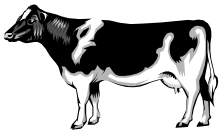
1.Hygiene package-Hygiene of foodstuffs,hygiene rules for food of animal origin,official controls for food of animal origin

2.OFFC-by MS, Community controls

6

Slide 7





Directive on monitoring I.

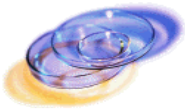
•Establishment of a monitoring system for certain(8 agents compulsory) zoonoses on national and Community level,COM

collects and compiles the results of monitoring from MS's yearly

Aim-harmonisation,based on national sytems

7

Slide 8



Directive on monitoring II.

•Surveillance throughout the food chain-all food
•Co-operation between competent authorities(food/feed/animal/human health sector)
•ECDN-data in humans collected

8

Slide 9

III.

- Monitoring on a harmonised basis(harmonised schemes)evaluate trends and sources,basis for RA on zoonotic organisms
- Monitoring antimicrobial resistance
- Foodborne outbreaks provide the opportunity to identify the pathogen(the food vehicle,factors in the handling contributed to the outbreak)

9

Slide 10

IV.

- Monitoring-at stages of the food chain-influenced by occurence in animal and human,food and feed-the gravity and effects for humans
- Monitoring antimicrobial resistance:

  Animal species,bacterial strains,sampling strategy,laboratory methodology used for the detection,methods used for the data collection

10

Slide 11

MS's role on reporting trends and sources

- MS's(responsibility for establishing and maintaining monitoring systems lie with MS's) report to COM-forwarded to EFSA,to examine and publish by the end of November,and made available to the public
- Obligations of MS's(ensure that data are collected,analysed,published,designate a competent authority act as contact point with COM,investigation of food-borne outbreaks,designate NRL's where CRL has been established,inform COM)

11

Slide 12

Monitoring programme on antimicrobial resistance

- Comparable data on the occurrence of antimicrobial resistance in zoonotic agents
- Salmonella spp.;Campylobacter jejuni.C.coli;E.coli from cattle,pigs,poultry and food derived from those species-implement a monitoring scheme(initial approach restricted to S.;C;E coli
- Slaughterhouses to be sampled-selected on a statistical basis(geographical distribution)
- Monitoring of food-retail level to reflect the risk for the consumer

12

Slide 13

Monitoring scheme for Campylobacter

- MS's-collection of comparable data-to identify hazards,characterise risks related to zoonozes
- Harmonized schemes for Campylobacter-age related colonisation,rapid spread within the flock-low infectious dose-seasonality

13

Slide 14

Food business operators duties

Arrange for preservation of isolates,keep results,communicate

14

Slide 15

Data collection system-current and future

MS's-COM-EFSA

15

Slide 16

EFSA's role in the preparation of Community report

- Take over the production of the Community report on zoonoses from 2005 based on data 2004
- Appointment of a Zoonoses Collaboration Center(EZCC)
- EFSA gives technical assistance to develop harmonised schemes on monitoring,guidelines for harmonised data collection and reporting system –but prioritisation given by MS's and COM
- Setting up WG's on the report and on the review of the reporting tables/manual for the reporting of data on zoonoses-creation of a new zoonoses database and internet reporting system
- MS's feedback on priorities:Salmonella throughout the food chain;Campylobacter in broiler/retail/slaughter batch;Listeria monocytogenes in foodstuffs

16

Slide 17



Regulation on control covers the whole foodchain

Target based approach,reduction of the prevalence of pathogens

17

Slide 18

Community targets

- Reduction of the prevalence(salm.-public health significance)
- Time limit,within which the target must be achieved
- Definition of the testing schemes
- Breeders,laying hens,broilers,turkeys
- Possibility to extension

18

Slide 19

Community targets established-MS's prepare a national control programme-approved by COM

Progressive approach

Salmonella with public health significance-transitional period for poultry

Successive years for poltry breeders,layers,broilers,turkeys,pigs

19

Slide 20

Baseline study

- Prevalence of salmonella in laying flocks of Gallus gallus
- Prepare setting of a Community targets pursuant to Reg.on control
- Random selection of holdings(geography, season)
- Hens shall be sampled at the end of the laying period

20

Slide 21

Timetable

Community targets set,National plans operational 18 months later

Specific measures

Breeding flocks infected with S.e/S.t.-slaughter/heat treatment/destruction

Table eggs-have to originate from salmonella negative focks

Poultry meat-criterion of absence of salmonella in 25 g/industrial heat treatment

21

Slide 22

Control programmes

- Continous,cover a period of at least 3 years
- Detection of zoonozes,minimum sampling schemes

22

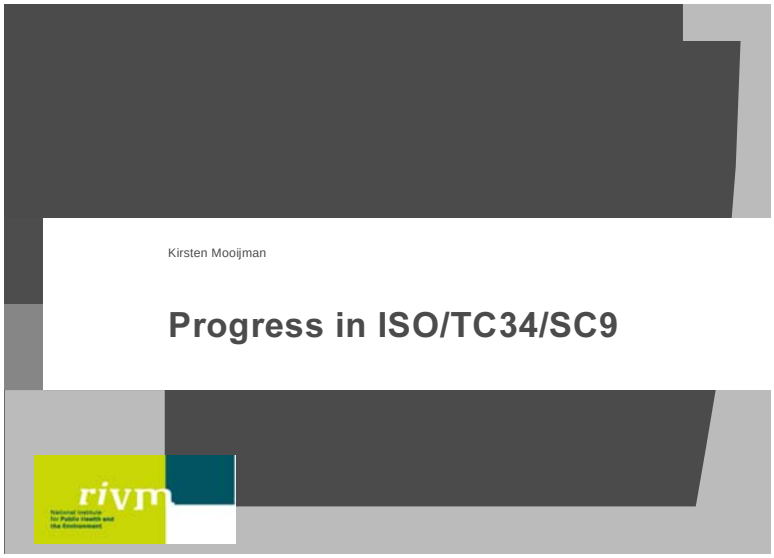
Slide 23

Thank you for the attention!

23

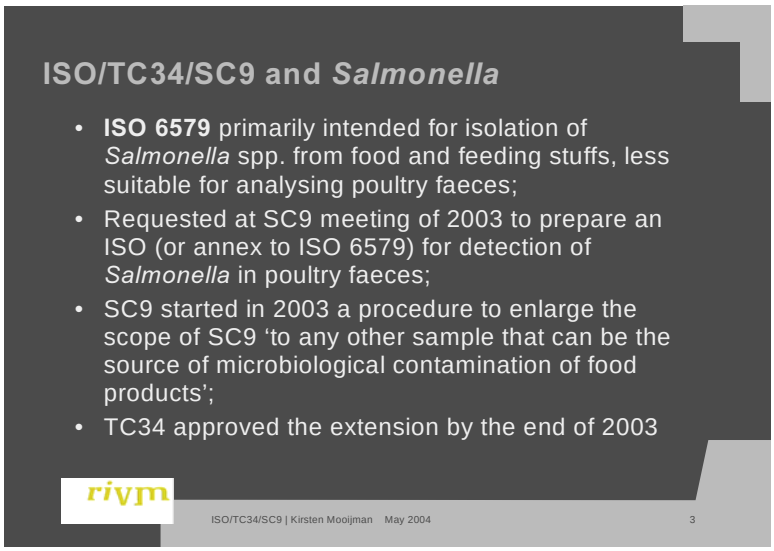
Annex 5. Slides of presentation 1.3

Slide 1

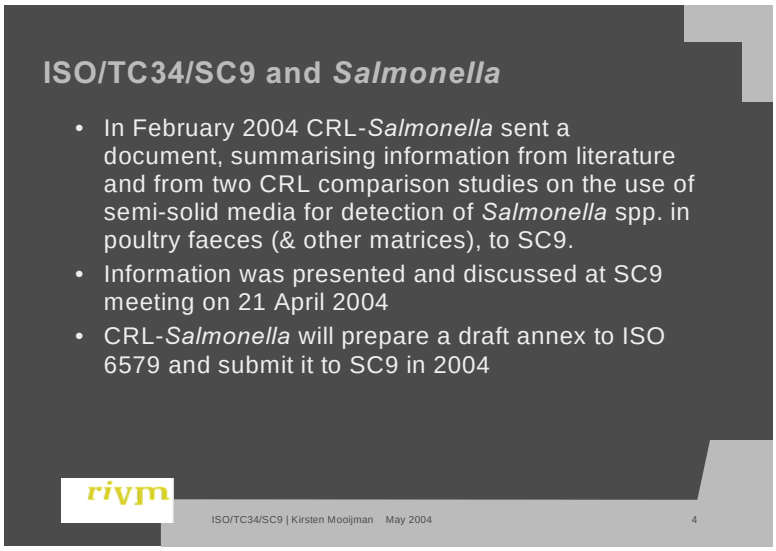


Slide 2

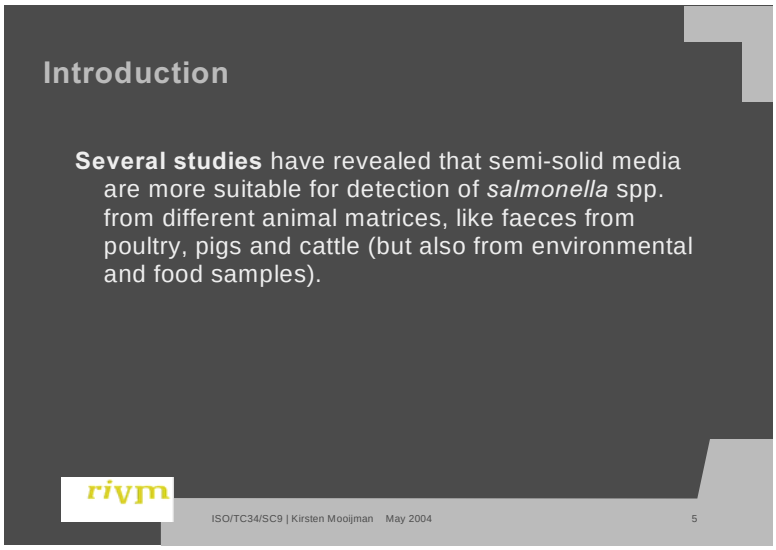
Slide 3



Slide 4



Slide 5



Slide 6

First study of Hartman (1999)

Matrix: Poultry faeces, 2249 samples

Selective enrichment medium	% *Salmonella* confirmed positive after 24 h of total *Salmonella* positives after 48 h	% *Salmonella* confirmed positive after 48 h of total *Salmonella* positives after 48 h
RV	47	62
RVS	62	69
MSRV	79	93
Diasalm	49	90
MSRV + RV	90	94
MSRV + RVS	95	94
Diasalm + RV	66	96
Diasalm + RVS	82	94

rivm

ISO/TC34/SC9 | Kirsten Mooijman May 2004 6

Slide 7

Salmonella isolates (Hartman, 1999)

Salmonella	Diasalm		MSRV		RV		RVS	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
SB (%)	62	97	82	94	58	63	74	79
SC (%)	23	77	65	83	63	77	44	60
SE (%)	20	85	95	100	40	55	55	50
Total (%)	44	89	79	91	50	66	62	69

SB: Serogroup B; SC: Serogroup C; SE: *Salmonella* Enteritidis

rivm

ISO/TC34/SC9 | Kirsten Mooijman May 2004 7

Slide 8

Study of Voogt *et al* (2001)

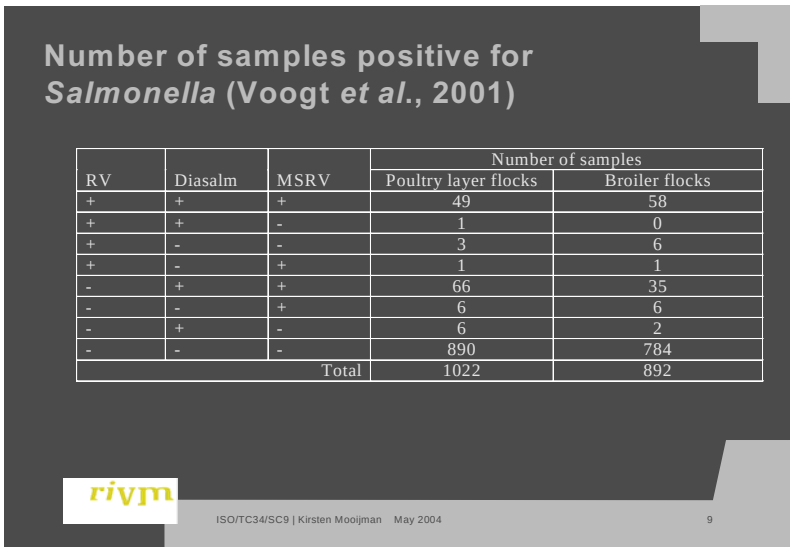
Matrix: Faecal samples from layer flocks (1022) and broiler flocks (892)

Selective enrichment medium	Poultry layer flocks		Poultry broiler flocks	
	% *Salmonella* confirmed pos. after 24 h of total *Salmonella* pos. after 48 h	% *Salmonella* confirmed pos. after 48h of total *Salmonella* pos. after 48 h	% *Salmonella* confirmed pos. after 24 h of total *Salmonella* pos. after 48 h	% *Salmonella* confirmed pos. after 48 h of total *Salmonella* pos. after 48 h
RV	35 %	41 %	57 %	60 %
MSRV	89 %	92 %	92 %	93 %
Diasalm	87 %	92 %	82 %	88 %
MSRV + RV	91 %	95 %	96 %	98 %
Diasalm + RV	90 %	95 %	89 %	94 %

rivm

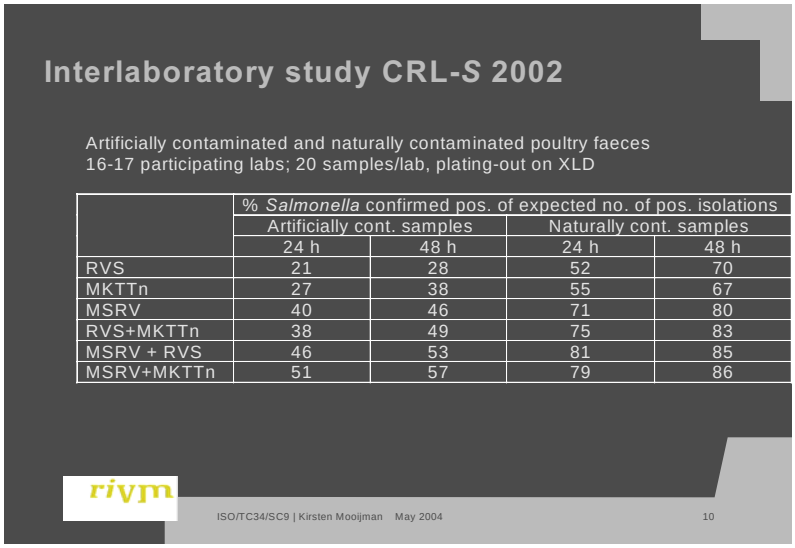
ISO/TC34/SC9 | Kirsten Mooijman May 2004 8

Slide 9



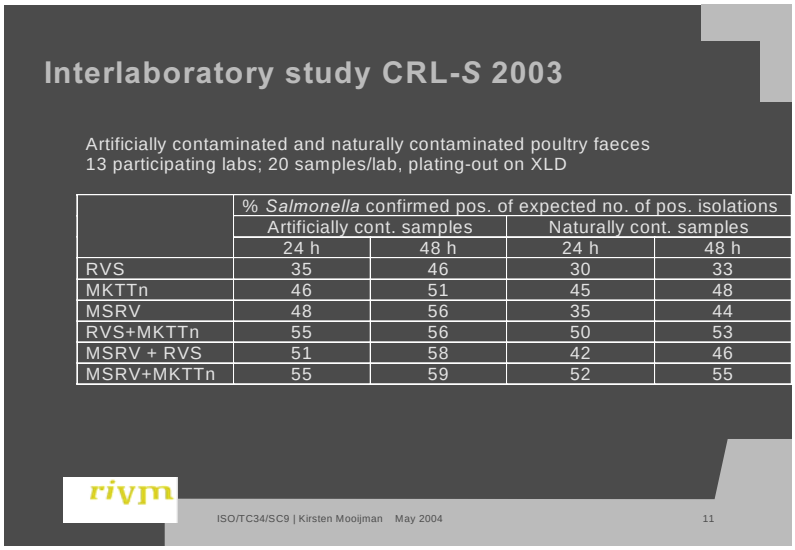
RV	Diasalm	MSRV	Number of samples	
			Poultry layer flocks	Broiler flocks
+	+	+	49	58
+	+	-	1	0
+	-	-	3	6
+	-	+	1	1
-	+	+	66	35
-	-	+	6	6
-	+	-	6	2
-	-	-	890	784
		Total	1022	892

Slide 10



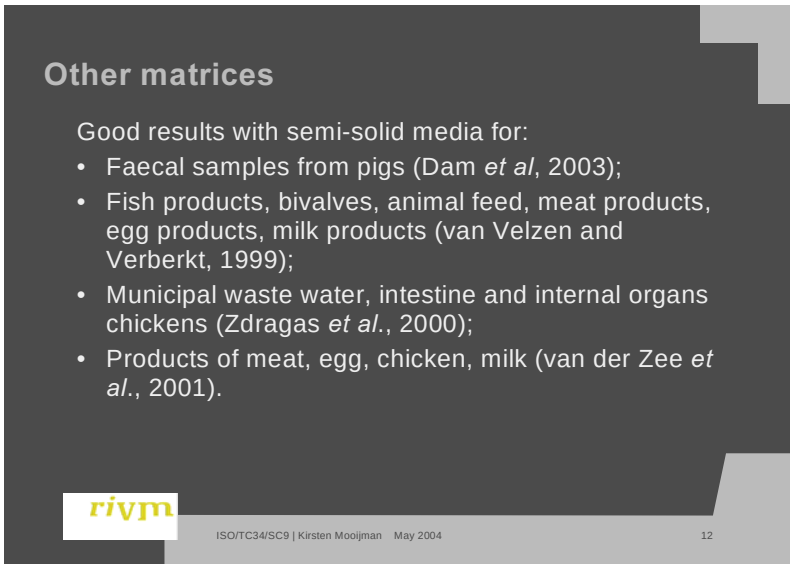
	% *Salmonella* confirmed pos. of expected no. of pos. isolations			
	Artificially cont. samples		Naturally cont. samples	
	24 h	48 h	24 h	48 h
RVS	21	28	52	70
MKTTn	27	38	55	67
MSRV	40	46	71	80
RVS+MKTTn	38	49	75	83
MSRV + RVS	46	53	81	85
MSRV+MKTTn	51	57	79	86

Slide 11

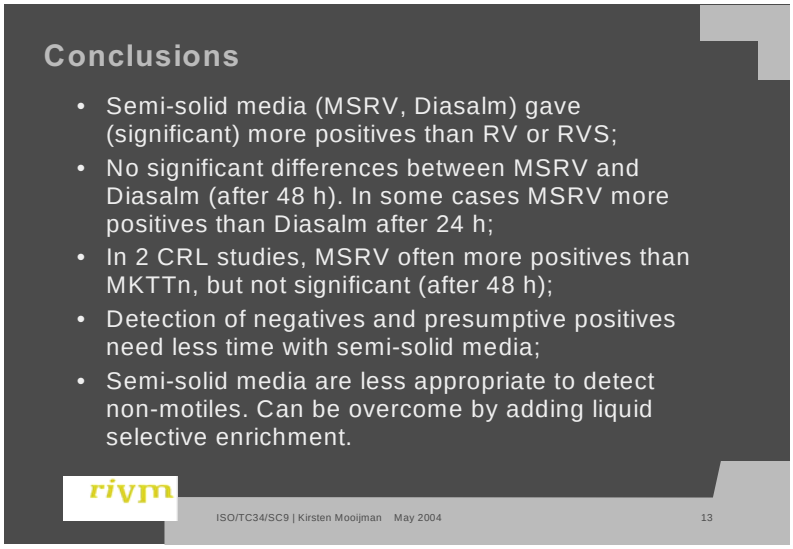


	% *Salmonella* confirmed pos. of expected no. of pos. isolations			
	Artificially cont. samples		Naturally cont. samples	
	24 h	48 h	24 h	48 h
RVS	35	46	30	33
MKTTn	46	51	45	48
MSRV	48	56	35	44
RVS+MKTTn	55	56	50	53
MSRV + RVS	51	58	42	46
MSRV+MKTTn	55	59	52	55

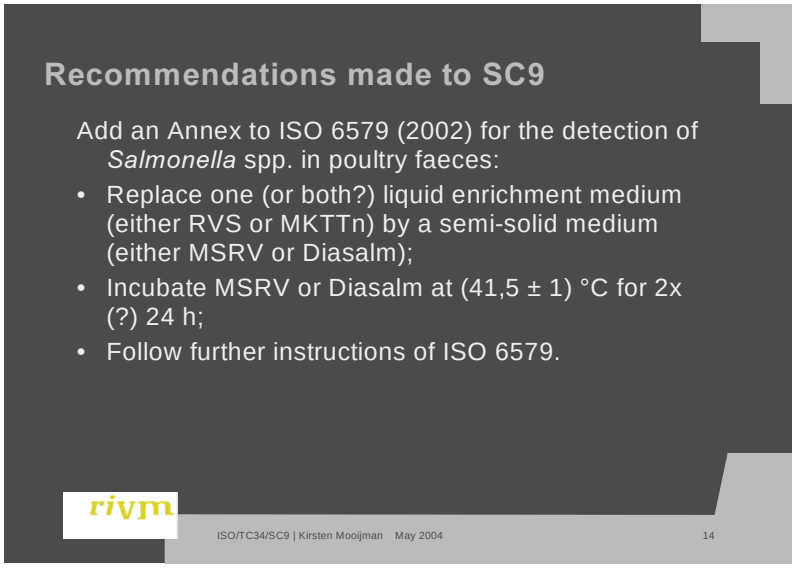
Slide 12



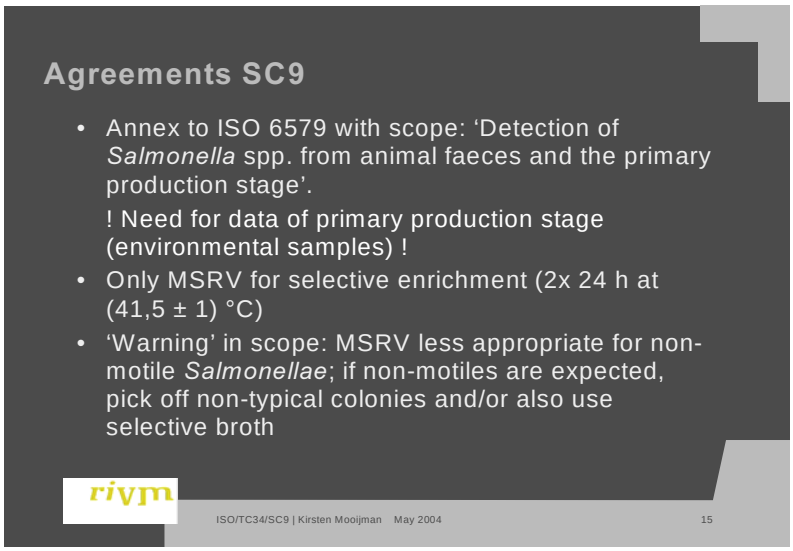
Slide 13



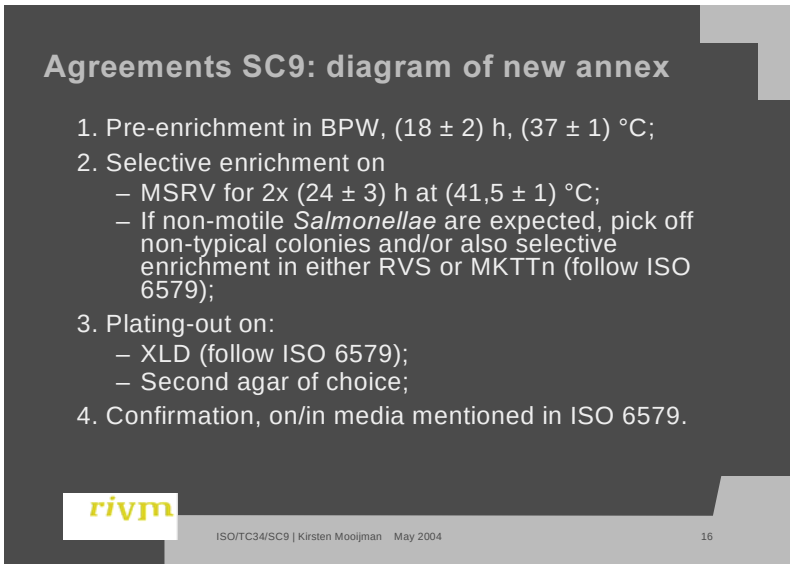
Slide 14



Slide 15



Slide 16



Annex 6. Slides of presentation 1.4

Slide 1

Draft scheme for a baseline study on the prevalence of *Salmonella* spp. in laying flocks of Galllus *gallus* in the EU

EC (DG SANCO) working group:

- Jean-Charles Cavitte (SANCO)
- Sarolta Idei (SANCO)
- Anne Käsbohrer (CRL-E)
- Antonia Ricci (I)
- Ivar Vagsholm (S)
- Rob Davies (UK)
- Arjen van de Giessen (CRL-S)

Slide 2

Contents

- objectives of the study
- sampling frame
- sample size
- stratification of the population
- samples
- testing methods
- reporting
- time schedule

Slide 3

Objectives of the study

- to estimate the prevalence of *Salmonella* spp. in the population of commercial laying hens at the end of the production period in the EU MSs
- to obtain comparable results in different MSs
- to set Community targets pursuant to Regulation 2160/2003 on the control of salmonella and other foodborne zoonotic agents
- study should cover a one year period

Slide 4

Sampling frame

- scheme should cover holdings with at least 1000 hens
- MSs with a large proportion of the population kept in holdings <1000 should include also smaller holdings
- the population of laying hens should be stratified according to holding size
- sampling should be conducted as close as possible to depopulation based on a notification system
- only one flock per holding should be sampled (flock definition according to the Regulation)
- samplings should be equally distributed over the year
- sampling shall be performed by the competent authority

Slide 5

Sample size

- primary sample size provides the number of flocks to be tested
- calculation should be based on the following criteria:
  - target prevalence: 20%
  - confidence level: 95%
  - accuracy: 3%
- or based on prevalence known from pre-existing measures

Slide 6

Stratification to holding size (data 2000)

	NUMBER OF HOLDINGS BY NUMBER OF HEADS PRESENT					
	1.000-2.999	3.000-4.999	5.000-9.999	10.000-29.999	>=30.000	**total 2**
COUNTRY						
BE Belgique/België	50	70	150	240	180	**690**
DK Danmark	50	50	90	80	40	**310**
DE Deutschland	1.280	420	410	440	360	**2.910**
GR Elláda	150	110	120	80	30	**490**
ES España	230	160	330	580	430	**1.730**
FR France	400	370	810	1.000	670	**3.250**
IE Ireland	60	30	50	30	10	**180**
IT Italia	190	120	190	330	340	**1.170**
LU Luxembourg						
NL Nederland	230	220	430	780	480	**2.140**
AT Österreich	420	140	130	90	20	**800**
PT Portugal	20	20	30	60	90	**220**
FI Suomi/Finland	450	160	130	80	10	**830**
SE Sverige	90	60	120	130	60	**460**
UK United Kingdom	380	220	470	620	300	**1.990**
EUR15	**4.000**	**2.150**	**3.460**	**4.540**	**3.020**	**17.170**

Slide 7

Nr. of holdings to be selected per country / size categorie

Size categorie Country	Total number	1,000-2,999	3,000-4,999	5,000-9,999	10,000-29,999	≥ 30,000
BE Belgique/België	***343***	25	35	75	119	90
DK Danmark	***213***	34	34	62	55	28
DE Deutschland	***553***	243	80	78	84	68
GR Elláda	***285***	87	64	70	47	17
ES España	***490***	65	45	93	164	122
FR France	***564***	69	64	141	174	116
IE Ireland	***142***	47	24	40	24	8
IT Italia	***431***	70	44	70	122	125
LU Luxembourg						
NL Nederland	***518***	56	53	104	189	116
AT Österreich	***368***	193	64	60	41	9
PT Portugal	***166***	15	15	23	45	68
FI Suomi/Finland	***375***	203	72	59	36	5
SE Sverige	***275***	54	36	72	78	36
UK United Kingdom	***508***	97	56	120	158	77
Total EUR15	5233					

Slide 8

Samples (1)

- Both faecal material and environmental samples
- 7 pooled samples per flock
- Cage flocks
  - 5 samples of faeces from dropping belts, scrapers or deep pits, depending on type of cage houses
    (total: 300 g; tested as 5 pools of 60g)
  - 2 samples of dusty material beneath cages (2x250ml)

Slide 9

Samples (2)

- Barn or free-range houses
  - 5 pairs of boot swabs (1 pair =1 pool);
    or 2 pairs of boot swabs (1 pair=1 pool) and
    3 samples of litter from scratching areas
    (total: 200 g pooled in 3);
  - 1 sample of dust from egg belts (250 ml)
  - 1 sample of dust collected in different places of the house (250 ml)
- Additional samples may be taken and reported separately

Slide 10

Testing method (1)

- Analyses of the samples to be performed by NRLs!?
- Samples should be kept refrigerated until examination
- Examination within 48 hours after receipt
- Detection method:
  - the method recommended by CRL Salmonella
  - amendment to ISO 6579 (2002) for detection in animal faecal and environmental samples in preparation
  - MSRV the single selective enrichment medium
  - if non-motiles are expected: additional liquid medium or non-typical colonies from MSRV

Slide 11

Testing method (2)

- Serotyping
  - at least one isolate from each positive sample
  - Kaufmann-White scheme
  - 2% of typable strains and non-typable isolates shall be sent to the CRL, for quality assurance
- Phagetyping
  - at least one isolate of SE and STM from each positive sample
  - protocol defined by PHLS Colindale
- Antimicrobial susceptibility testing
  - one isolate per serovar per flock is recommended
- Isolates shall be stored for a minimum of 2 years

Slide 12

Reporting (1)

Information to be collected for each flock (holding):

- Number of hens in the holding, number of flocks in the holding
- Number of hens in the flock tested
- Age of hens at sampling
- Expected depopulation date
- Date and place of sampling
- Flock (Holding) production type
- Type of samples taken

Slide 13

Reporting (2)

Information to be collected for each sample:

- ID of the laboratory (in case several laboratories are involved)
- Date of testing
- Type of specimen
- Weight / volume of the specimen
- Means of transport of samples
- Detection media used
- Result for the individual sample tested (negative or salmonella serovar or untypable)
- Results of antimicrobial susceptibility testing and/or phagetyping

Slide 14

Reporting (3)

Information on the programme to be reported to the EC:

- Description of the population under study stratified according to holding size
- Description of randomisation procedure (including notification system),
- Sample size calculated and realized
- Sampling method used
- Testing method used
- Details of authorities and laboratories involved in sampling/testing/typing

Slide 15

Reporting (4)

Results of the programme should include:

- Number of flocks tested
- Number/proportion of positive samples; number/proportion of each serotype divided per
  - obligatory and voluntary samples
  - quarter
  - type of production system
  - size category of holdings
  - type of specimen tested
- Details on additional voluntary sampling
- Details on specimen tested and number of samples positive per flock
- Age of hens at sampling

Slide 16

Time schedule

- First draft presented to MSs on 22 April 2004
- Second meeting with MSs scheduled for 24 May 2004
- Monitoring programme from autumn 2004-autumn 2005
- Establishment of Community targets in December 2005
- Implementation of control programme 18 months after establishment of targets

Annex 7. Slides of presentation 1.5

Slide 1

Sampling of Poultry Flocks for Bacteriological Detection of *Salmonella*

Rob Davies

Veterinary Laboratories Agency - Weybridge

[Data derived from Defra funded research and surveillance]

Sampling Slide 1

Slide 2

Types of *Salmonella* tests available

- Conventional culture: Reference method v. optimum method for sample type / purpose
- Rapid Methods: IMS, ELISA, PCR, Conductance Impedance - not fully optimised for faeces and environmental samples in all labs
- Serology: Eg. ELISA for SE, STM, Mix ELISA
  - most sensitive method for invasive serovars included in test
  - not (so) susceptible to suppression by antimicrobials

Sampling Slide 2

Slide 3

Objectives of Sampling

1. To detect *Salmonella* with maximum sensitivity for control purposes
   - large sample sizes and numbers
   - variable according to farm design
   - environment / focal points
2. To compare prevalences over time or between locations
   - standardised sampling
   - sensitivity not main issue - comparability
   - individual animal based - droppings (faecal / caecal)
     - cloacal swabs
     - post-mortem tissues
     - eggs
3. Confirmation of infection for slaughter/compensation - post-mortem - statistically derived number of birds (eg. 59 birds to detect 5% prevalence with 95% if 100% sensitive test)

Sampling Slide 3

Slide 4

Types of Samples

Intestinal / Faecal

[gives prevalence but large nos. for sensitive detection of positive flock]

[can be pooled - ? Pool size]

[Vaccination - ↓ shedding]

Cloacal swabs
- low volume/poor sensitivity/external contamination
- 'Home Office' Procedure

Floor faeces
- caecal > faecal droppings
- laborious
- attached litter / dust / other faeces

Intestinal contents
- whole caeca / ileocaecal junction best
- cross-contamination at slaughter
- best for prevalence estimates

Sampling Slide 4

Slide 5

Naturally Pooled Environmental Samples
Non-Cage Houses

Faeces	Boot Swabs / 'Socks' - moist / absorptive
	Gauze Swabs / Sponges - focal points - weigher, ramps, perches, platforms, nest boxes
	Drag Swabs
	Pooled Litter Picks
Dust	Enhanced survival of *Salmonella* ⟶ historical record of infection
	Delay 1-2 w after first infection
	Naturally mixed at exhaust vents
	Variable quantities and access - beams, ledges, pipes
	Salmonella concentrated in egg belt related dust - elevators, conveyors, grinding equipment
Post cleaning	Floor sweepings
	Washings (inc. splashed water)
	Mice - pooled intestine liver, spleen

Boot Swabs and Dust ideal - ? Separate or pooled sample

Sampling Slide 5

Slide 6

Naturally Pooled Environmental Samples
Cage Systems

Pooled Faeces	Droppings belts - scrapers at end of belt
	Scraper system - scrapers after use
	Step cage - gauze swabs / picks from manure rows
Dust	Dust beneath cages
	Egg belt spillage
	Fan exhausts
	Beams, ledges, pipes, belting pulleys, egg belt brushes

Sampling Slide 6

Slide 7

Optimising Test Sensitivity for Salmonella from faecal and environmental samples

Maximise number of sampling points	+++	
Maximise number of individual tests	+++	
Maximise sample volumes	++	
Ensure best sample handling	+	
Ensure best test method	++	[? Also culture 1:10 dilution]
24 and 48 hour enrichment	++	
Multiple enrichment methods	+	[Non motile *Salmonella* will grow in Diasalm !]
Multiple agar plates	+	
Multiple colony confirmation / methods	+	
Best confirmatory tests	+	

Sampling Slide 7

Slide 8

Drag Swabs

Assembly of Drag Swabs:

3 x 3 inch sterile gauze pads

Complex large Y shaped assembly

Each sample takes 20-30 minutes - traverse 1/2 house at least twice

Repeat with second drag swab

BUT - swab saturated after 35 m ∴ poor detection of clustered contamination

Comparison of % houses with drag swabs positive when 1-4 assemblies used (Caldwell et al 1994)

No. Swab positive	Vacant	Occupied
1 of 4	73.3	39.3
2 of 4	20.0	25.0
3 of 4	6.7	21.4
4 of 4	0	14.3

Sampling Slide 8

Slide 9

Drag Swabs (Cont'd)

Holding Media - 2x skim milk if kept moist (Opara et al 1992)

- no need if not held in liquid - unless prolonged storage
- best to incubate and store incubated broth

Drag Swab v. 5g litter

	No. Positive House		
	Drag Swab	**5g litter**	(Kingston 1980)
Breeders	8/96 (8.3)	8/96 (8.3)	
Broilers	9/16 (56.3)	2/16 (12.5)	

Litter culture	2/13 (15.4)
Caecal culture (50 → 10g)	7/13 (53.8)
Drag Swab	8/13 (61.5)

Sampling Slide 9

Slide 10

Drag Swabs v. Boot Swabs

4 drag swab assemblies [= 12 gauze pads / house]

v.

4 individual boots per house (spun olefin fibres) (Caldwell et al 1998)

	Positive Sampling Occasions (3/flock)	**Positive Houses**
Drag Swab	17/27 (62.9)	9/9
Boot Swab	14/27 (51.8) N.S.	9/9

Sampling Slide 10

Slide 11

‘Socks’ and Sampling Times

(Gradel et al 2002)

5 pairs ‘socks’ as 5 pools

2 pairs ‘socks’ as 1 pool

60 faeces as 1 pool (60g)

2 pairs ‘socks’ (41%) ≡ 60 faeces (32%)

Best sample type agreements between 2 pairs and 5 pairs ‘socks’ (45%)

3 weeks > 33-40 days for sampling broilers

Sampling Slide 11

Slide 12

‘Socks’ (Cont’d)

(Skov et al 1999)

Broiler Flocks

5 pairs ‘socks’ ≡ 300 (60 x 5 faeces) [? sample weight]

[15/23 (65.2) flocks] [15/23 (65.2) flocks]

Paper sheets and 1 pair ‘socks’ inferior

[9/23 (39.1)] [8/23 (34.8)]

Sampling Slide 12

Slide 13

Detection of *S.enteritidis* contamination of poultry houses after antibiotic treatment - *Salmonella* isolation from various sample sites

Poultry unit code	Litter		Nest box		Chain feeders		Drinkers		Beams		Slave feed hopper	
	No. of samples	No. positive for *Salmonella* (%)	No. of samples	No. positive for *Salmonella* (%)	No. of samples	No. positive for *Salmonella* (%)	No. of samples	No. positive for *Salmonella* (%)	No. of samples	No. positive for *Salmonella* (%)	No. of samples	No. positive for *Salmonella* (%)
A	16	0	16	2 (12.5)	16	0	16	0	16	0	4	0
B	16	4 (25.0)	16	5 (31.2)	16	2 (12.5)	16	4 (25.0)	16	0	4	0
C	16	0	16	3 (18.7)	16	0	16	0	16	0	4	0
D	16	4 (25.0)	16	6 (37.5)	16	0	16	1 (6.3)	16	2 (12.5)	2	1 (50.0)
E	16	3 (18.7)	16	8 (50.0)	16	0	16	0	16	5 (31.2)	2	0
Totals	80	11 (13.7)	80	24 (30.0)	80	2 (2.5)	80	5 (6.2)	80	7 (8.7)	16	1 (6.2)

Sampling Slide 13

Slide 14

Sample Comparison over a 33-month period in Eight Broiler Houses persistently infected with *S.*Montevideo

Crop No.	Houses positive Company sampling	Samples positive End crop litter	Houses positive End crop litter	Samples positive Dust	Houses positive Dust	Samples positive Boot swabs	Houses positive Boot swab
1.	0/8 [L]	31/64 (48.4)	8/8 (100.0)	57/64 (89.1)	8/8 (100.0)	NS	NS
2.	4/8 (50.0) [L]	20/64 (31.3)	7/8[I] (87.5)	52/64 (81.3)	8/8[T] (100.0)	NS	NS
3.	2/8[O] (25.0) [L]	1/64 (1.6)	1/8 (12.5)	14/64 (21.8)	5/8 (62.5)	NS	NS
4.	2/8 (25.0) [L]	6/64 (9.3)	2/8 (25.0)	22/64 (34.4)	5/8 (62.5)	NS	NS
5.	1/8 (12.5) [L]	3/64 (4.7)	2/8 (25.0)	18/64 (28.1)	7/8 (87.5)	NS	NS
6.	1/8 (12.5) [L]	3/64 (4.7)	3/8 (37.5)	11/64 (17.2)	5/8 (62.5)	NS	NS
7.	1/8 (12.5) [L]	8/64 (12.5)	3/8 (37.5)	19/64 (29.7)	6/8 (75.0)	NS	NS
8.	3/8 (37.5) [B]	9/64 (14.1)	4/8 (50.0)	36/64 (56.3)	8/8 (100.0)	6/16 (37.5)	6/8 (75.0)
9.	4/8 (50.0) [B]	4/64 (6.3)	4/8 (50.0)	41/64 (64.1)	7/8 (87.5)	4/16 (25.0)	3/8 (37.5)
10.	2/8 (25.0)[I] [B]	2/64 (3.1)	2/8 (25.0)	21/64 (32.8)	4/8 (50.0)	1/16 (6.3)	1/8 (12.5)
11.	0/8 [B]	0/64	0/8	5/64 (7.8)	2/8 (25.0)	0/16	0/8
12.	0/8 [B]	0/64	0/8	1/64 (15.6)	1/8 (12.5)	0/16	0/8
14.*	0/8 [B]	0/64	0/8	2/64 (3.1)	1/8 (12.5)	1/16 (6.3)	1/8 (12.5)
16.*	1/8 (12.5) [B]	0/64	0/8	2/64 (3.1)	1/8 (12.5)	1/16 (6.3)	1/8 (12.5)
18.*	0/8 [B]	0/64	0/8	8/64 (12.5)	1/8 (12.5)	1/16 (6.3)[I]	1/8 (12.5)
21.*[CE]	0/8 [B]	0/64	0/8	3/64 (4.6)	2/8 (25.0)	0/16	0/8
16 crops	21/128 (16.4)	87/1024 (8.5)	36/128 (28.1)	312/1024 (30.5)	71/128 (55.5)	14/144 (9.7)	13/72 (18.1)

Key: [L] litter; [B] boot swab; [I] including *S.*Typhimurium in one house; [T] including *S.*Typhimurium in two houses; [O] including *S.*Ohio in one house; [I] *S.*Indiana (in house -ve by other sampling); * missed flock sampling due to disease precautions or ownership changes; [CE] after 2 x flock CE treatment at hatchery

Sampling Slide 14

Slide 15

Comparative Results During Equivalent Sampling Periods

Company litter	11/56 (19.6)	Houses pos. during litter sampling period	44/56 (78.6)	% litter/total = 25.0%
Company boot swabs	10/72 (13.9)	Houses pos. during boot sampling period	27/72 (37.5)	% boot swab/total = 37.0%

Study Boot Swab Period: End Crop

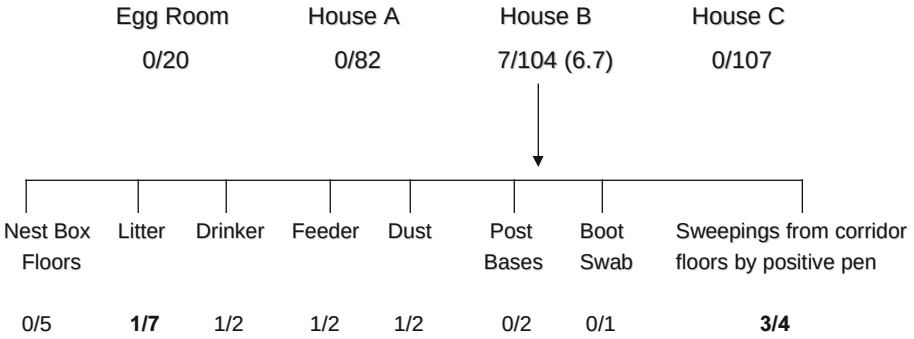
	Samples	Houses
litter:	15/576 (2.6)	10/72 (13.9)
boot swab:	14/144 (9.7)	13/72 (18.1)
dust:	119/576 (20.7)	27/72 (37.5)

Sampling Slide 15

Slide 16

Distribution of *Salmonella Mbandaka* on occupied turkey breeder site

(% Samples positive for *Salmonella*)



Sampling Slide 16

Slide 17

Distribution of *Salmonella* contamination on commercial pullet rearing farm

No. samples positive for *Salmonella* / No. samples taken (%)

Age of Flock	Sample Type	House 1	House 2	House 3	House 4	House 5	House 6
'Day old'	bulked (x10) delivery box liners	0/4	0/4	2[a]/3	2[a]/3	2[a]/2	2[a]/2
1 week	bulked litter	0/4	0/4	0/4	4[a]/4	1[a]/6	1[a]/3
3-4 weeks*	"	0/3	0/3	1[a]/3	1[a]/3	0/3	1[a]/3
7 weeks*	bulked cloacal swabs (x60)	0/1	0/1	0/1	0/1	0/1	0/1
12 weeks	"	0/1	0/1	0/1	0/1	0/1	0/1
	boot swabs	0/1	0/1	0/1	0/1	0/1	0/1
16 weeks	"	0/1	0/1	0/1	0/1	0/1	0/1
	litter/faeces/boot swab/drinkers	0/48	0/48	0/48	0/48	0/48	0/48
	dust	0/12	0/12	0/12	0/12	0/12	8[a]/12 (75.0)
Post C&D	floor surfaces	-	-	-	-	-	2[a]/53 (3.7)
	ventilation ducts	-	-	-	-	-	4[a]/34 (11.8)
	equipment	-	-	-	-	-	1[a]/63 (1.6)
	ante-room	-	-	-	-	-	3[a2,b1]/14 (21.4)
	outside houses	-	-	-	-	-	10[a4,b5,c1]/40 (25.0)
	cleaning contractors' vehicles	-	-	-	-	-	4[c2,d2]/6 (66.7)
Next Flocks - 16 weeks	litter/dust	0/60	0/60	0/60	0/60	0/60	0/60

* after fluoroquinolone/competitive exclusion treatment C&D cleaning and disinfection - not sampled

[a] *S.* Enteritidis PT6 [b] *S.* Montevideo [c] *S.* Thomasville [d] *S.* Havana
number after superscript = no. of isolates of each serovar

Sampling Slide 17

Slide 18

Distribution of *Salmonella* Contamination in Cage Layer Flocks

No. samples positive for *S*.Enteritidis [other serovars]/ No. samples taken (% *S*E) [% total *Salmonellas*]

Flock	Droppings Belts/ Bulked Faeces	Egg Belt Spillage	Dust	Spillage Under Cages	Egg Belts
L/1(v)	2/34 (5.9)	6/16 (37.5)	6/34 (17.6)	10/30 (33.3)	0/10
L/2	7/32 (21.8)	0/16	2/32 (6.2)	4/24 (16.7)	0/8
SGL/1	NS	4[1][a]/7 (57.1)[71.4]	3/4 (75.0)	NS	NS
CK/I	0/4	1/5 (20.0)	2/21 (9.5)	0/15	0/5
CK/2(v)	1/5 (20.0)	0 [1][b]/5 [20.0]	1/20 (5.0)	0/15	0/5
CK/3	0/5	0/5	0/20	4/15 (26.7)	0/5
F/1	NS	5/6 (83.3)	5/6 (83.3)	3/4 (75.0)	NS
F/2	NS	5/6 (83.3)	4/6 (66.7)	2/4 (50.0)	NS
G/1	14/16 (87.5)	6/8 (75.0)	13/26 (50.0)	19/20 (95.0)	1/8 (12.5)
G/2	7/8 (87.5)	9/16 (56.2)	17/20 (85.0)	12/16 (75.0)	3/8 (37.5)
SUT/1	20/23 (86.9)	7/8 (87.5)	1[13][ae]/21(4.8) [66.7]	8[2][ae]/14 (57.1) [71.4]	2/8 (25.0)
SUT/2	11[10][ae]/26 (42.3) [80.8]	6[3][a]/12 (50.0) (75.0]	6[12][ae]/24 (25.0) [75.0]	2[3][a]/6 (33.3) [83.3]	7[1][a]/12 (58.3) [66.6]
SUT/3	0[23][e]/24 [95.8]	0[11][e]/12 [91.7]	0[6][e]/6 [100.0]	0[6][a]/6 [100.0]	1[9][a]/12 (8.3) [83.3]
ST(v)	1/98 (1.0)	2/28 (7.1)	1/14 (7.1)	1/14 (7.1)	0/14
Total	**63[33]/275 (22.9) [34.9]**	**51[16]/150 (34.0) [44.7]**	**61[31]/254 (24.0) [36.2]**	**65[11]/183 (35.5) [41.5]**	**14[10]/95 (14.7) [25.3]**

Key: NS - not sampled; (v) vaccinated; [a] *S*.Livingstone; [b] *S*.Ohio; [c] *S*.Agama; [d] *S*.Braenderup; [e] *S*.Infantis

Sampling Slide 18

Slide 19

Sampling Slide 19

Slide 20

Sampling Slide 20

Slide 21

Sampling Slide 21

Slide 22

Sampling Slide 22

Slide 23

Sampling Slide 23

Slide 24

Sampling Slide 24

Slide 25

Sampling Slide 25

Slide 26

Sampling Slide 26

Slide 27

Sampling Slide 27

Slide 28

Sampling Slide 28

Slide 29

Sampling Slide 29

Slide 30

Sampling Slide 30

Slide 31

Sampling Slide 31

Slide 32

Sampling Slide 32

Slide 33

Sampling Slide 33

Slide 34

Sampling Slide 34

Slide 35

Sampling Slide 35

Slide 36

Sampling Slide 36

Slide 37

Sampling Slide 37

Slide 38

Sampling Slide 38

Slide 39

Sampling Slide 39

Slide 40

Sampling Slide 40

Slide 41

Sampling Slide 41

Slide 42

Sampling Slide 42

Slide 43

Sampling Slide 43

Slide 44

Sampling Slide 44

Slide 45

Sampling Slide 45

Slide 46

Sampling Slide 46

Slide 47

Sampling Slide 47

Slide 48

Sampling Slide 48

Slide 49

Sampling Slide 49

Slide 50

Sampling Slide 50

Slide 51

Sampling Slide 51

Slide 52

Sampling Slide 52

Slide 53

Sampling Slide 53

Slide 54

Sampling Slide 55

Slide 55

Sampling Slide 56

Slide 56

Sampling Slide 57

Slide 57

Conclusions

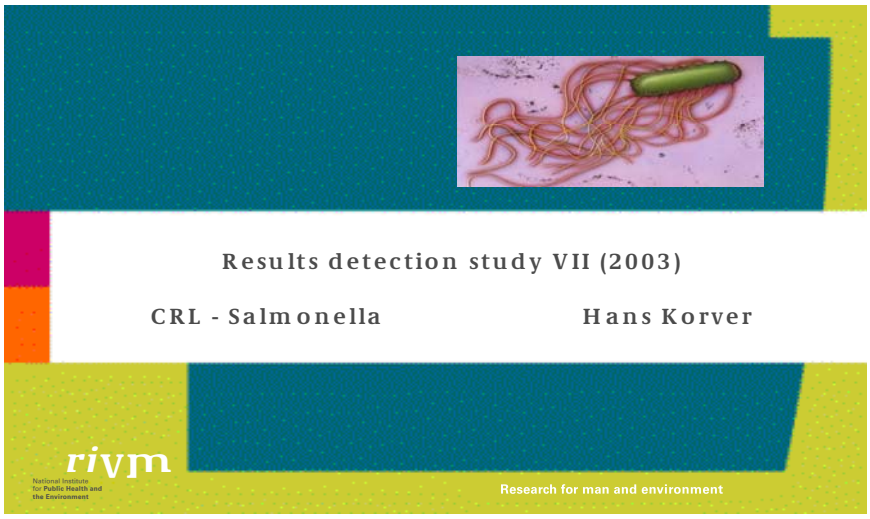
- Dust best sample type for sensitivity
  - proportional to abundance
  - focal concentrations - best sites
- Boot swabs and dust best for deep litter
- Boot swabs / scratching area litter and dust best for barn / free-range
- Droppings belts / scrapers, egg belt dust, dust under cages best for cage houses
- Caecal samples best for prevalence / comparability
- Large representative samples effectively mixed and subsampled improve sensitivity
- More samples cultured by BPW / MSRV method

  → increased sensitivity compared with dual enrichment / plating for serovars of zoonotic significance

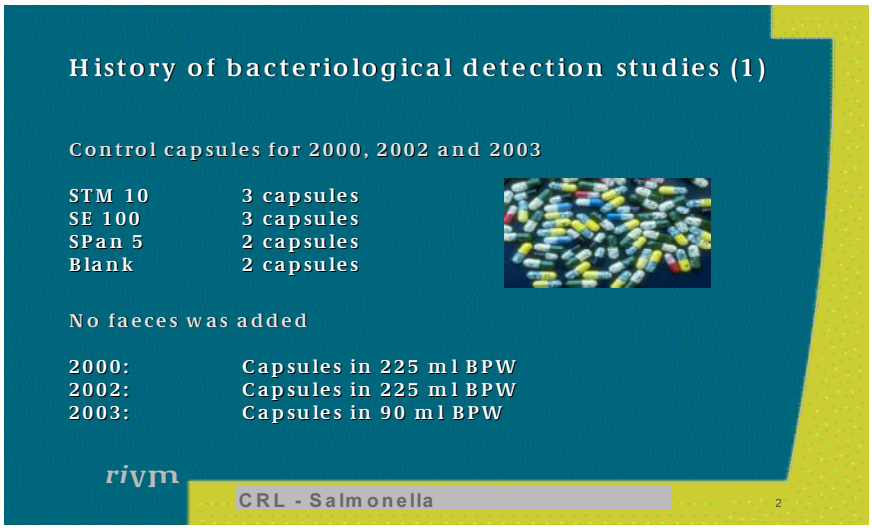
Sampling Slide 58

Annex 8. Slides of presentation 1.6

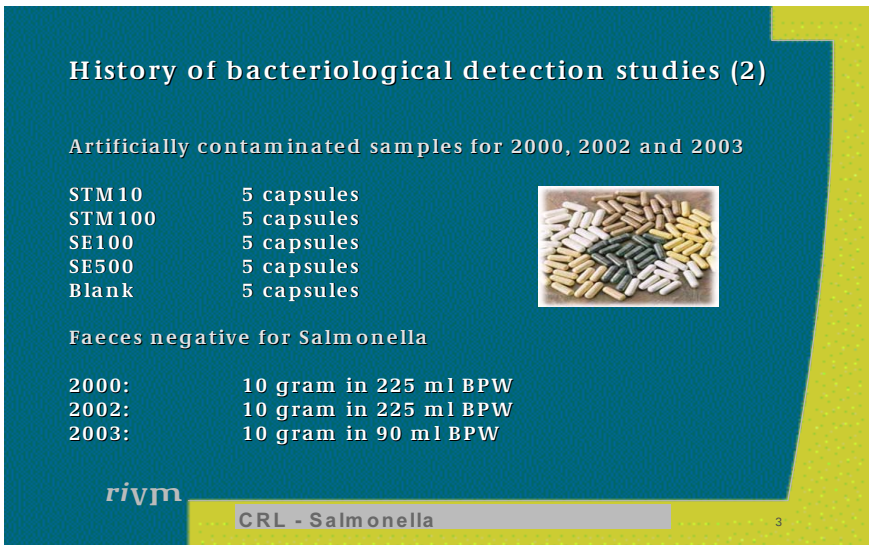
Slide 1



Slide 2



Slide 3



Slide 4

History of bacteriological detection studies (3)

Naturally contaminated samples for 2000, 2002 and 2003

Number of samples = 20

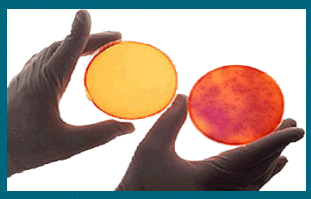
Faeces positive for Salmonella

2000:	25 gram in 225 ml BPW
2002:	25 gram in 225 ml BPW
2003:	10 gram in 90 ml BPW

rivm CRL - Salmonella 4

Slide 5

Media for study 2003



rivm CRL - Salmonella 5

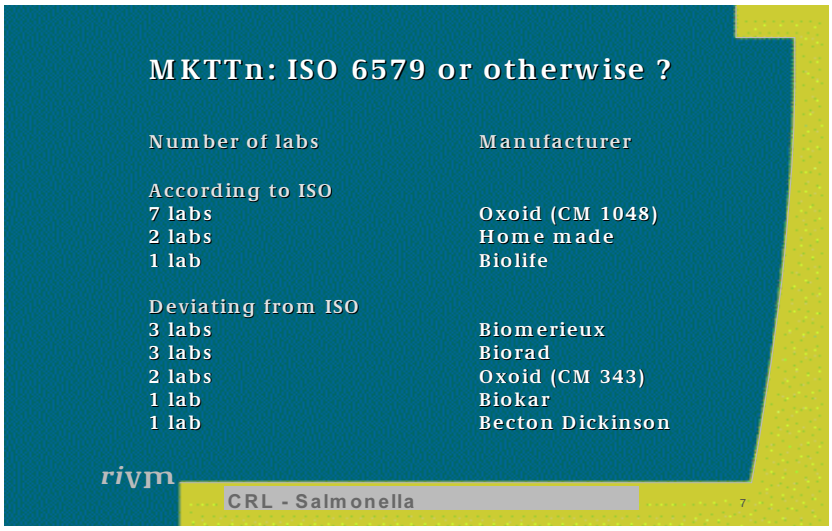
Slide 6

History of bacteriological detection studies (media)

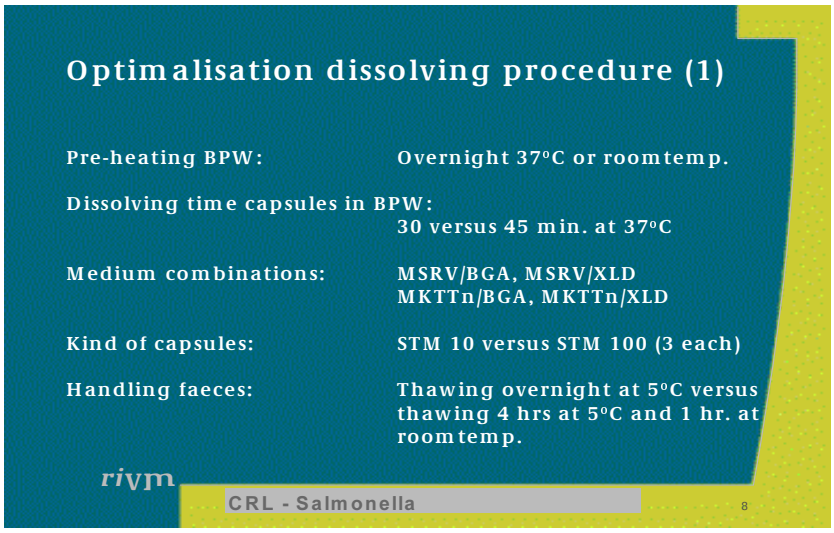
Year	Sel.enrichm.	Plating-out
2000	RV or RVS MSRV Own	XLD BGA
2002	RVS MKTTn MSRV Own	XLD BGA Own
2003	Same as 2002	

rivm CRL - Salmonella 6

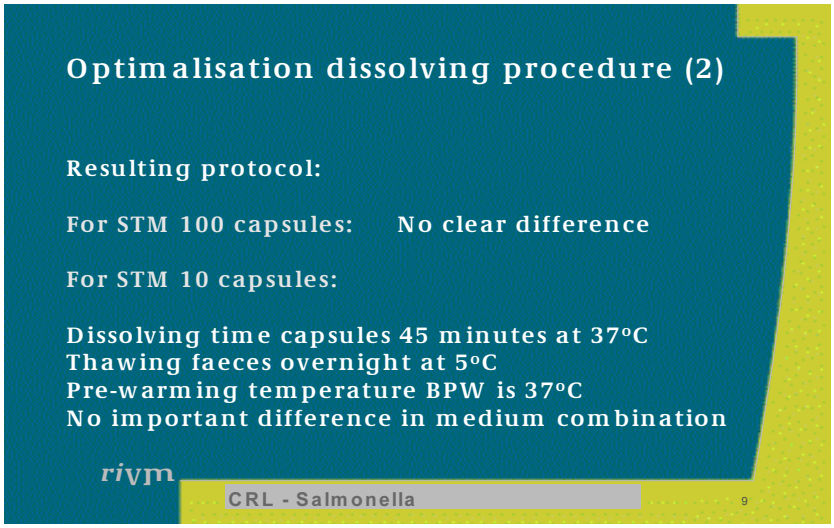
Slide 7



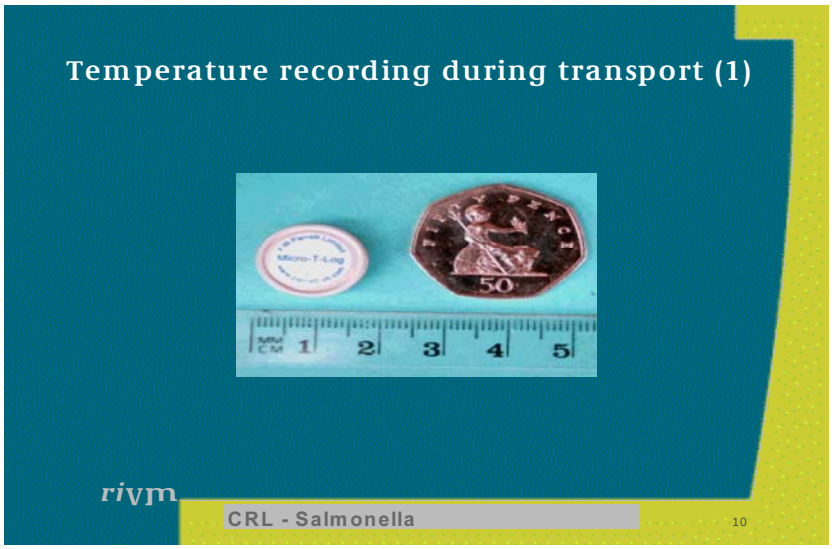
Slide 8



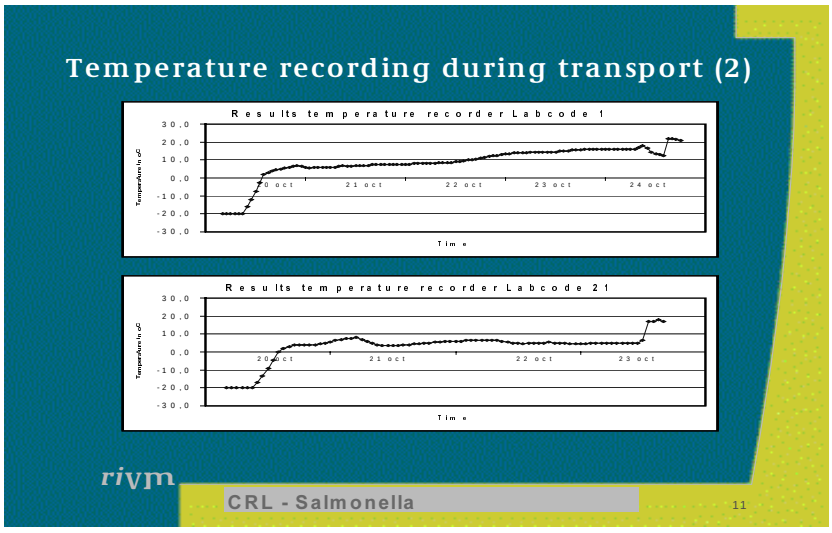
Slide 9



Slide 10



Slide 11



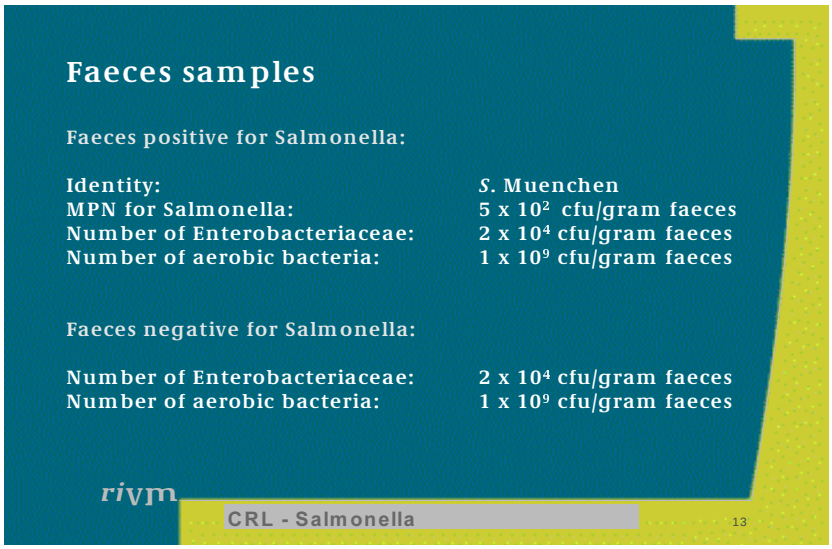
Slide 12

Level of contamination and homogeneity of SE and STM capsules

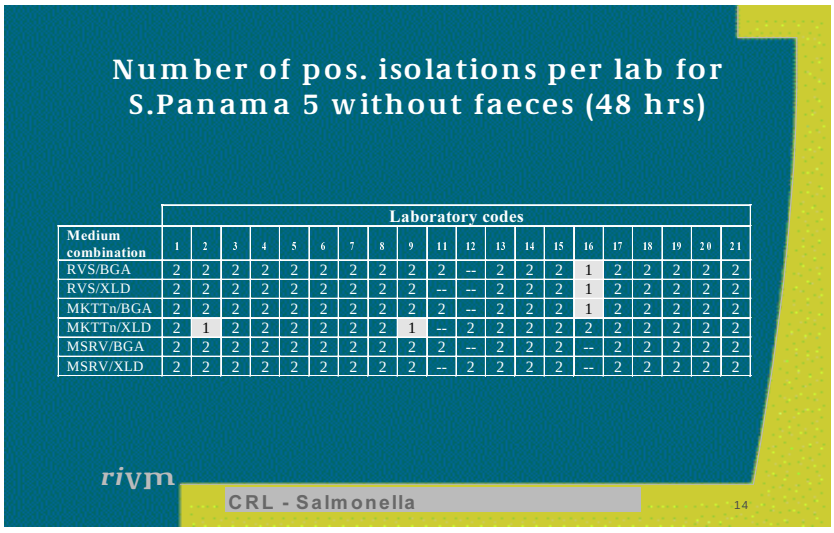
	Test batch (n=25)		Final batch (n=25)	
	Mean cfp per capsule	Homogeneity (T_2 / (I-1)	Mean cfp per capsule	Homogeneity (T_2 / (I-1)
SE 100	117	1.14	127	1.28
SE 500	585	0.80	595	1.21
SPan 5	8	0.53	9	0.87
STM 10	11	0.75	12	1.06
STM 100	101	1.11	96	0.84

rivm
CRL - Salmonella
12

Slide 13

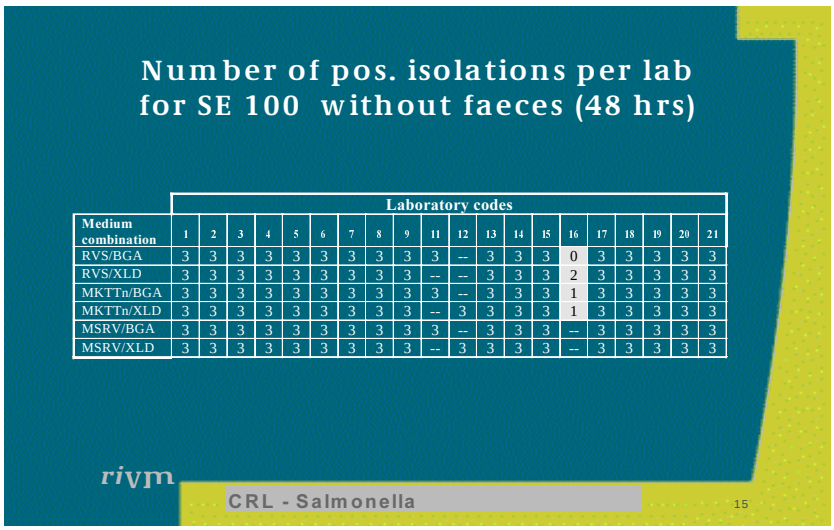


Slide 14



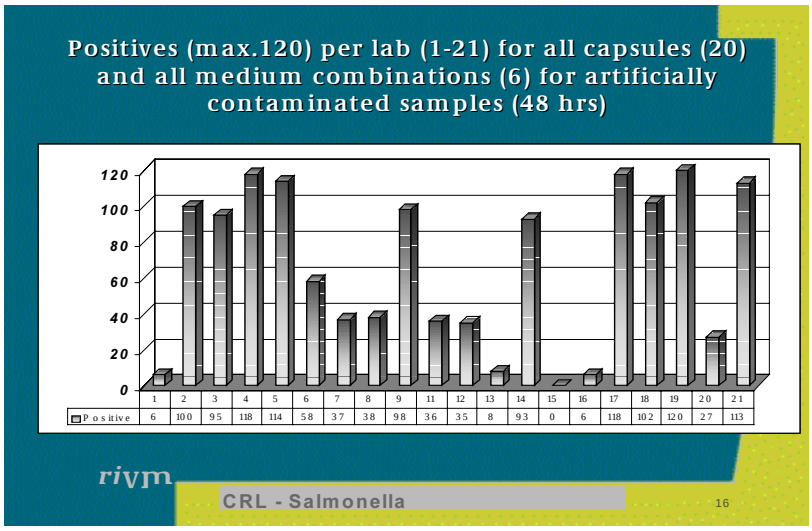
	Laboratory codes																			
Medium combination	1	2	3	4	5	6	7	8	9	11	12	13	14	15	16	17	18	19	20	21
RVS/BGA	2	2	2	2	2	2	2	2	2	2	--	2	2	2	1	2	2	2	2	2
RVS/XLD	2	2	2	2	2	2	2	2	2	--	--	2	2	2	1	2	2	2	2	2
MKTTn/BGA	2	2	2	2	2	2	2	2	2	2	--	2	2	2	1	2	2	2	2	2
MKTTn/XLD	2	1	2	2	2	2	2	2	1	--	2	2	2	2	2	2	2	2	2	2
MSRV/BGA	2	2	2	2	2	2	2	2	2	2	--	2	2	2	--	2	2	2	2	2
MSRV/XLD	2	2	2	2	2	2	2	2	2	--	2	2	2	2	--	2	2	2	2	2

Slide 15

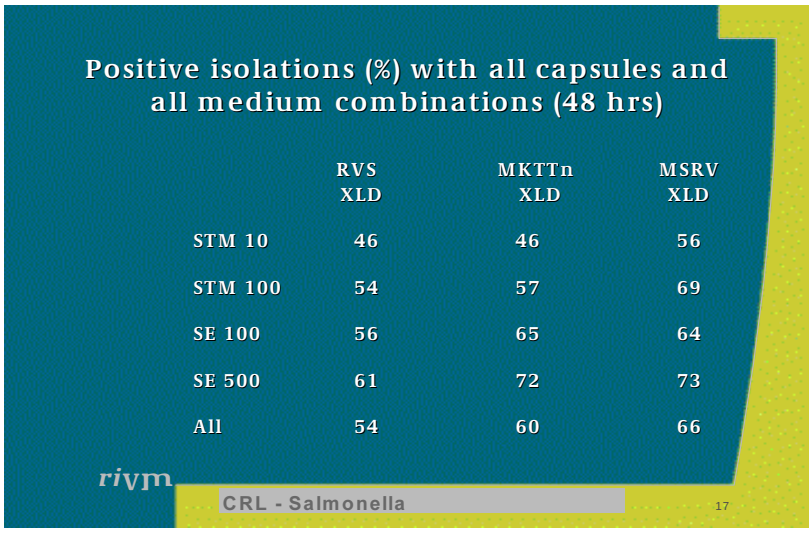


	Laboratory codes																			
Medium combination	1	2	3	4	5	6	7	8	9	11	12	13	14	15	16	17	18	19	20	21
RVS/BGA	3	3	3	3	3	3	3	3	3	3	--	3	3	3	0	3	3	3	3	3
RVS/XLD	3	3	3	3	3	3	3	3	3	--	--	3	3	3	2	3	3	3	3	3
MKTTn/BGA	3	3	3	3	3	3	3	3	3	3	--	3	3	3	1	3	3	3	3	3
MKTTn/XLD	3	3	3	3	3	3	3	3	3	--	3	3	3	3	1	3	3	3	3	3
MSRV/BGA	3	3	3	3	3	3	3	3	3	3	--	3	3	3	--	3	3	3	3	3
MSRV/XLD	3	3	3	3	3	3	3	3	3	--	3	3	3	3	--	3	3	3	3	3

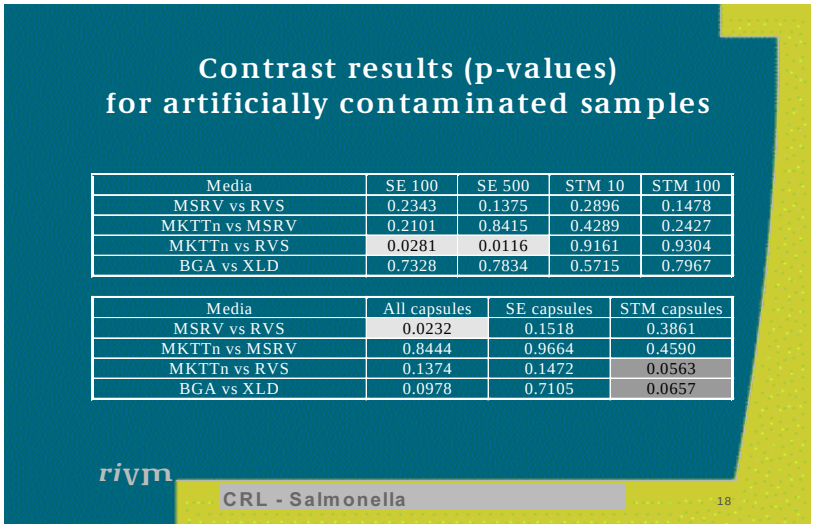
Slide 16



Slide 17



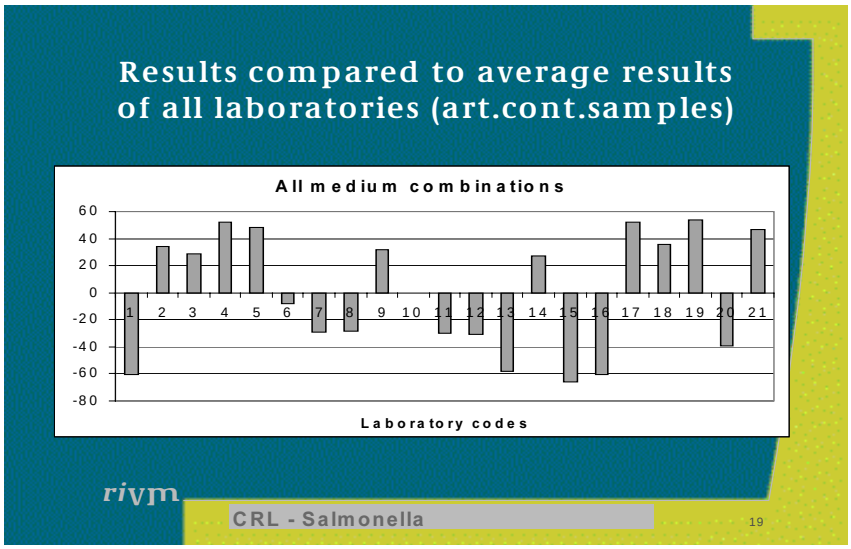
Slide 18



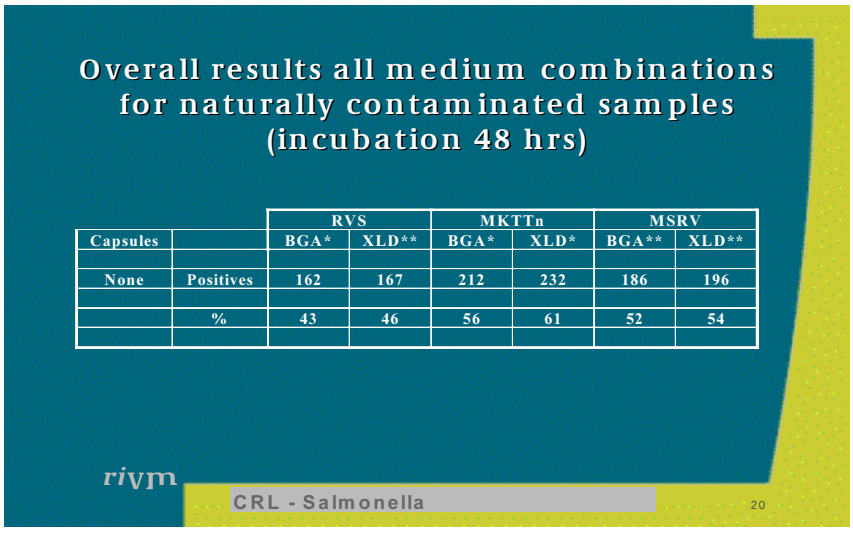
Media	SE 100	SE 500	STM 10	STM 100
MSRV vs RVS	0.2343	0.1375	0.2896	0.1478
MKTTn vs MSRV	0.2101	0.8415	0.4289	0.2427
MKTTn vs RVS	0.0281	0.0116	0.9161	0.9304
BGA vs XLD	0.7328	0.7834	0.5715	0.7967

Media	All capsules	SE capsules	STM capsules
MSRV vs RVS	0.0232	0.1518	0.3861
MKTTn vs MSRV	0.8444	0.9664	0.4590
MKTTn vs RVS	0.1374	0.1472	0.0563
BGA vs XLD	0.0978	0.7105	0.0657

Slide 19

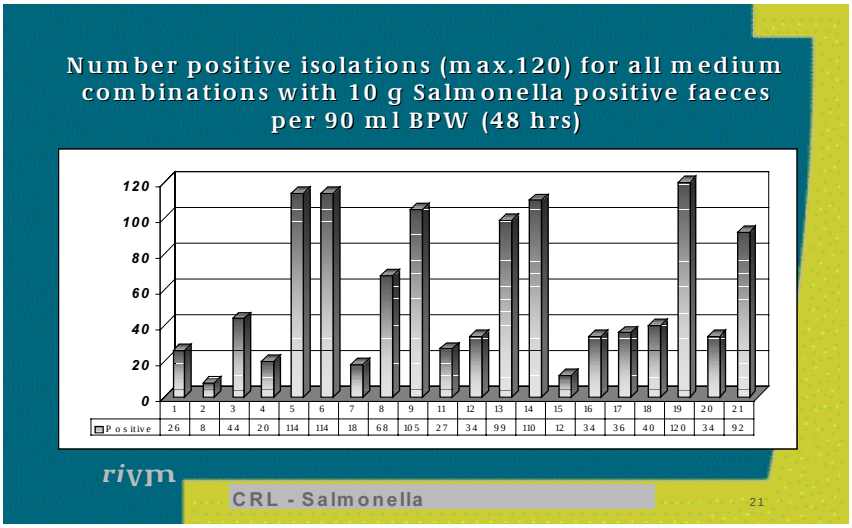


Slide 20



		RVS		MKTTn		MSRV	
Capsules		BGA*	XLD**	BGA*	XLD*	BGA**	XLD**
None	Positives	162	167	212	232	186	196
	%	43	46	56	61	52	54

Slide 21



Slide 22

Contrast results (p-values) for naturally contaminated samples

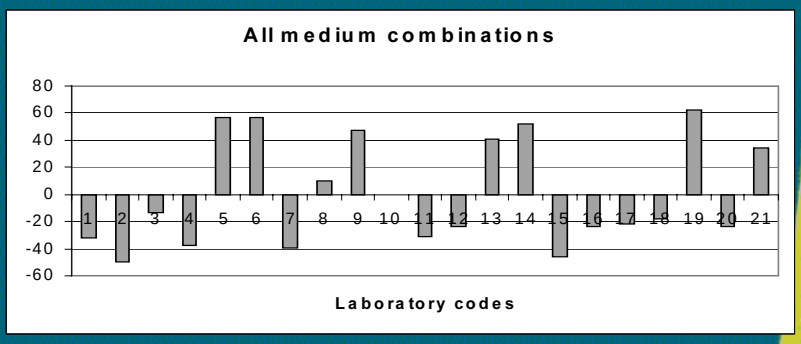
Media	p - values
MSRV vs RVS	0,0454
MKTTn vs MSRV	0,2991
MKTTn vs RVS	0,0230
BGA vs XLD	0,0049

rivm

CRL - Salmonella 22

Slide 23

Results compared to average results of all laboratories (naturally contaminated samples)



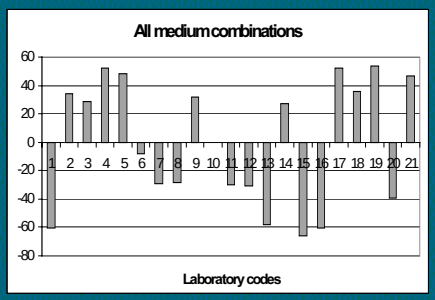
rivm

CRL - Salmonella 23

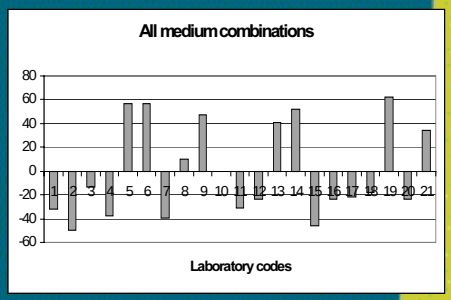
Slide 24

Comparison artificially and naturally contaminated samples per laboratory

Artificially contaminated



Naturally contaminated



rivm

CRL - Salmonella 24

Slide 25

Conclusions

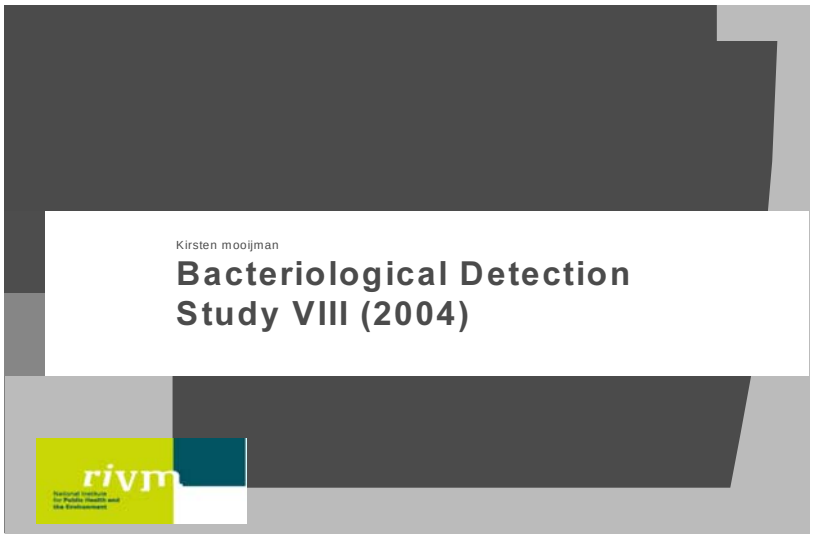
1. Optimalisation dissolving procedure
2. Temperature recording during transport
3. Isolation from STM 100 > SE 500 > STM 10 > SE 100
4. Significantly more positive isolations with MSRV in relation to RVS (art. contaminated samples)
5. MSRV showed more positive isolations than MKTTn but not significant (art. contaminated samples)
6. Differences between MSRV and MKTTn vs RVS for naturally contaminated samples significantly
7. XLD significantly better than BGA for naturally contaminated samples
8. Further analysis needed for combination : days of transport / results temperature and time / use of media / handling capsules and faeces samples

rivm

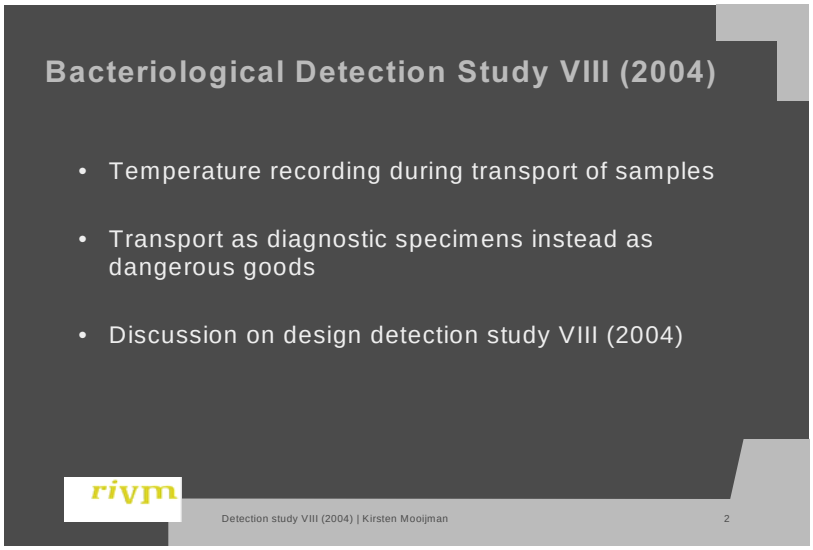
CRL - Salmonella 25

Annex 9. Slides of presentation 1.7

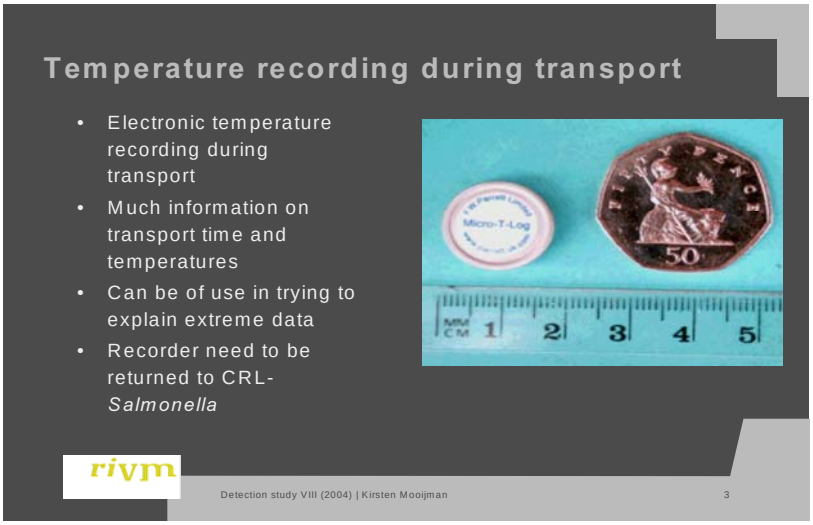
Slide 1



Slide 2



Slide 3



Slide 4

Transport as diagnostic specimens (I)

- Discussion with experts and courier revealed that materials for detection studies can be transported as diagnostic specimens
- Document International Civil Aviation Organisation:
  - '…specimens known or suspected of containing pathogens meeting the criteria for risk groups 2 or 3 may be transported as diagnostic specimens when they are transported for diagnostic or investigational purposes.'
  - 'Diagnostic specimens are any human or animal material including, but not limited to, excreta, secreta, blood and its componenets, tissue and tissue fluids being transported for diagnostic or investigational purposes, but excluding live infected animals.'
  - Excluded: '..cultures prepared for the intentional generation of pathogens (but not when intended for diagnostic purposes)'

rivm

Detection study VIII (2004) | Kirsten Mooijman 4

Slide 5

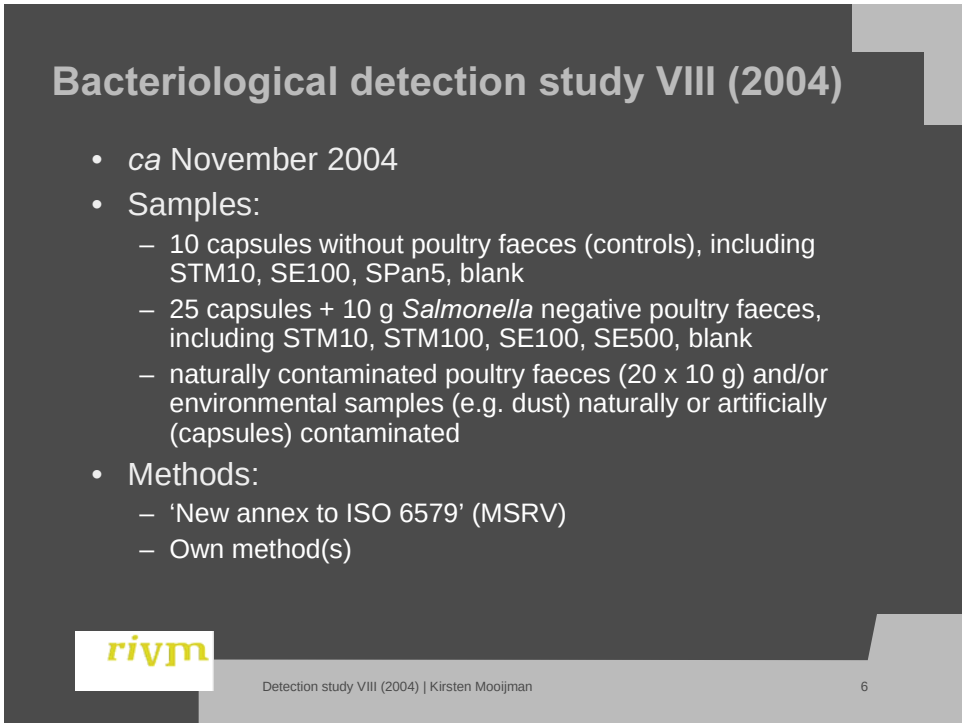
Transport as diagnostic specimens (II)

- Package similar to dangerous goods;
- Labeling and papers for dangerous goods not needed;
- package marked 'diagnostic specimen'
- door-to-door transport (DHL)
- faster (?) and less expensive (?)

rivm

Detection study VIII (2004) | Kirsten Mooijman 5

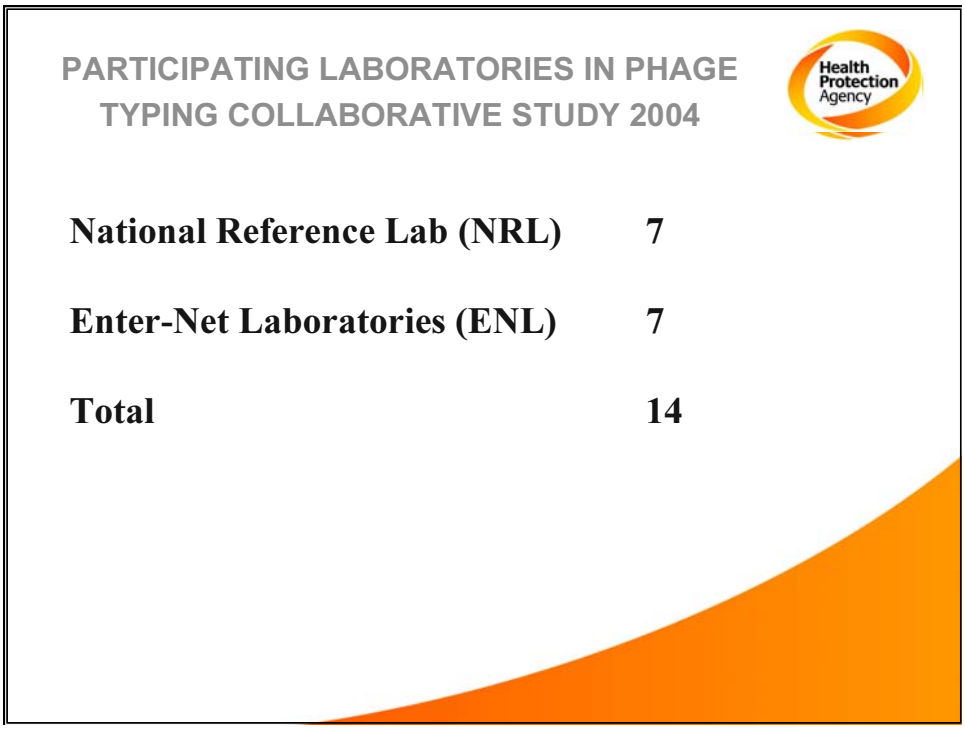
Slide 6


Bacteriological detection study VIII (2004)
• *ca* November 2004
• Samples:
– 10 capsules without poultry faeces (controls), including STM10, SE100, SPan5, blank
– 25 capsules + 10 g *Salmonella* negative poultry faeces, including STM10, STM100, SE100, SE500, blank
– naturally contaminated poultry faeces (20 x 10 g) and/or environmental samples (e.g. dust) naturally or artificially (capsules) contaminated
• Methods:
– 'New annex to ISO 6579' (MSRV)
– Own method(s)
rivm
Detection study VIII (2004) | Kirsten Mooijman
6

Annex 10. Slides of presentation 1.8

Slide 1

Slide 2



Slide 3

Results of *Salmonella* Enteritidis phage typing by the NRLs

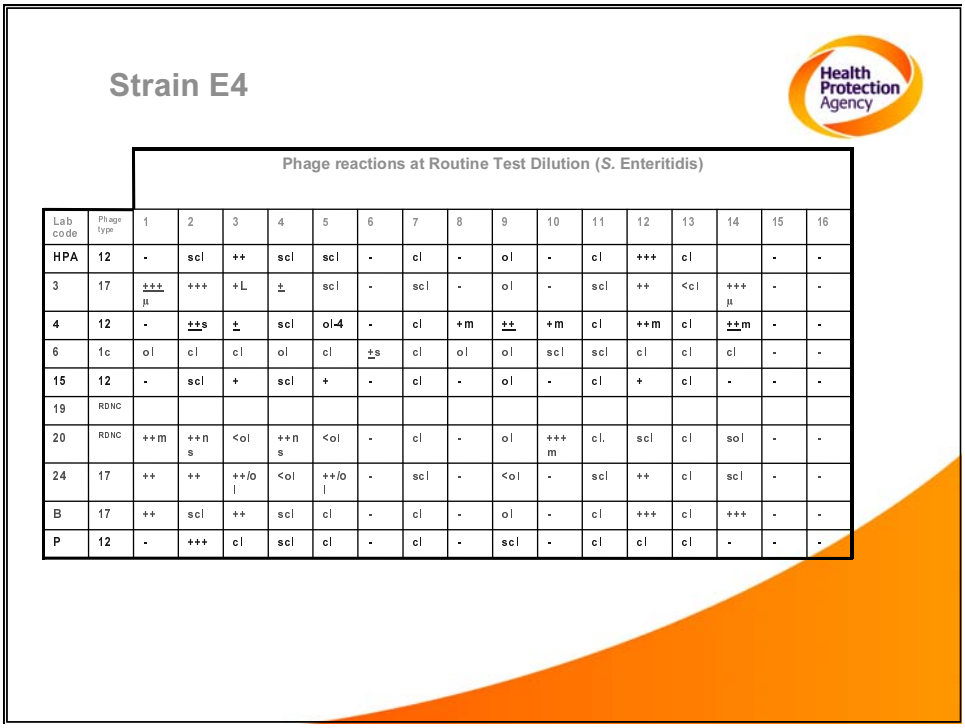
		Phage type of each laboratory						
Strain	PT	3	4	6	15	19	20	24
E1	1b	1b	1b	1b	1b	1b	1b	1b
E2	1	1	1	1	1	1	1	1
E3	14b	14b	14b	14b	14b	14b	14b	14b
E4	12	17	12	1c	12	RDNC	RDNC	17
E5	2	2	2	2	2	2	2	2
E6	3	22	3	3	22	3	3	3
E7	21	21	21	21	21	21	21	21
E8	9b	9b	9b	9b	9b	9b	9b	9b
E9	24	24	29	24	29a	29	24	24
E10	4	4	4	4	4	4	4	4

Slide 4

Results of Salmonella Enteritidis phage typing by the ENLs

		Phage type of each laboratory											
Strain	PT	A	B	C	E	F	H	L	M	N	P	S	W
E1	1b	1b	1b		1b			1b	1b/30		1b		1b
E2	1	1	1		1			1	1		1		1
E3	14b	14b	14b		14b			14b	14b		14b		14b
E4	12	17	17		12			RDNC	RDNC		12		12
E5	2	2	2		2			2	2		2		2
E6	3	3	3		3			3	3		3		3
E7	21	32	21		21			21	21/1		21		21
E8	9b	9b	9b		9b			9b	9b		9b		9b
E9	24	24	24		24			24	29		29a		24
E10	4	4	4		4			4	4		4		4a

Slide 5

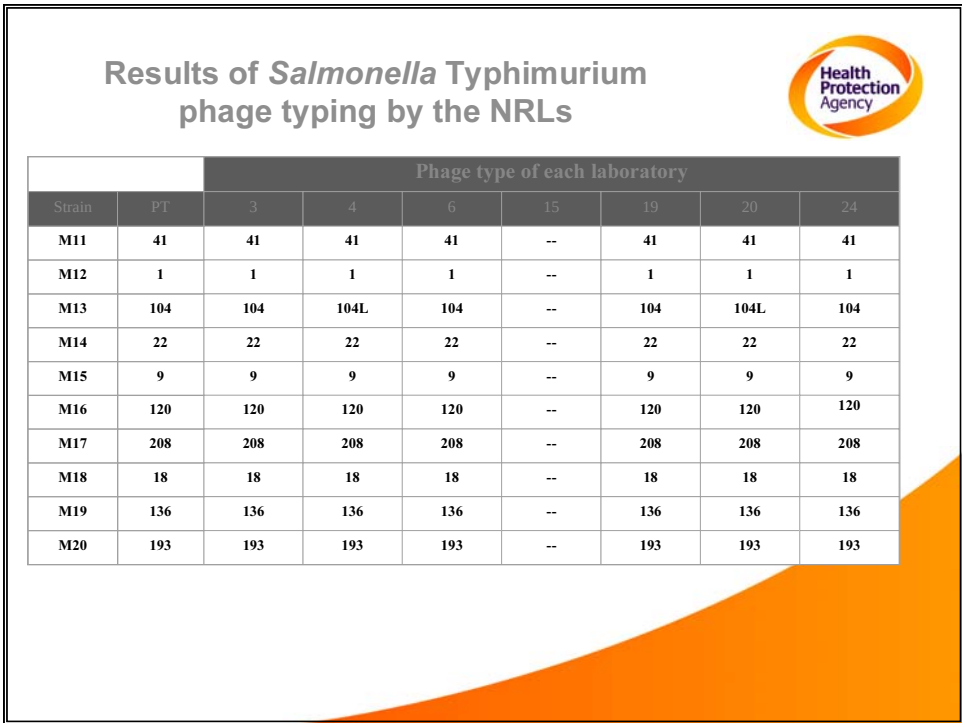


Strain E4

Phage reactions at Routine Test Dilution (*S.* Enteritidis)

Lab code	Phage type	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
HPA	12	-	scl	++	scl	scl	-	cl	-	ol	-	cl	+++	cl		-	-
3	17	+++ µ	+++	+L	±	scl	-	scl	-	ol	-	scl	++	<cl	+++ µ	-	-
4	12	-	++s	±	scl	ol-4	-	cl	+m	++	+m	cl	++m	cl	++m	-	-
6	1c	ol	cl	cl	ol	cl	±s	cl	ol	ol	scl	scl	cl	cl	cl	-	-
15	12	-	scl	+	scl	+	-	cl	-	ol	-	cl	+	cl	-	-	-
19	RDNC																
20	RDNC	++m	++ns	<ol	++ns	<ol	-	cl	-	ol	+++m	cl.	scl	cl	sol	-	-
24	17	++	++	++/ol	<ol	++/ol	-	scl	-	<ol	-	scl	++	cl	scl	-	-
B	17	++	scl	++	scl	cl	-	cl	-	ol	-	cl	+++	cl	+++	-	-
P	12	-	+++	cl	scl	cl	-	cl	-	scl	-	cl	cl	cl	-	-	-

Slide 6



Results of *Salmonella* Typhimurium phage typing by the NRLs

		Phage type of each laboratory						
Strain	PT	3	4	6	15	19	20	24
M11	41	41	41	41	--	41	41	41
M12	1	1	1	1	--	1	1	1
M13	104	104	104L	104	--	104	104L	104
M14	22	22	22	22	--	22	22	22
M15	9	9	9	9	--	9	9	9
M16	120	120	120	120	--	120	120	120
M17	208	208	208	208	--	208	208	208
M18	18	18	18	18	--	18	18	18
M19	136	136	136	136	--	136	136	136
M20	193	193	193	193	--	193	193	193

Slide 7

Results of Salmonella Typhimurium phage typing by the ENLs

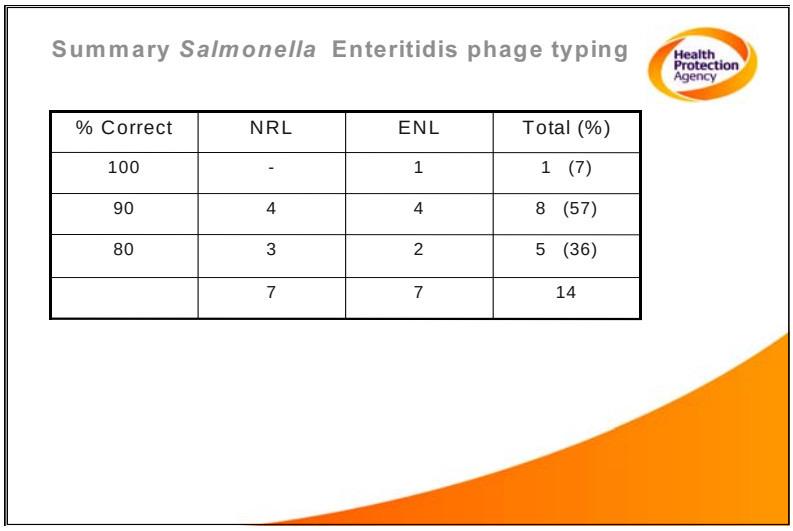
		Phage type of each laboratory											
Strain	PT	A	B	C	E	F	H	L	M	N	P	S	W
M11	41	41	41		41			41	41		41		41
M12	1	1	1		1			1	1		1		1
M13	104	104	104		104			104	104		104		104
M14	22	22	22		22			22	22		22		22
M15	9	9	9		9			9	9		9		9
M16	120	120	120		120			120	120		120		120
M17	208	208	208		208			208	208		208		208
M18	18	18	116		18			18	18		18		18
M19	136	136	136		136			136	136		136		136
M20	193	193	193a		193			193	193		193		193

Slide 8

Types identified correctly by all laboratories

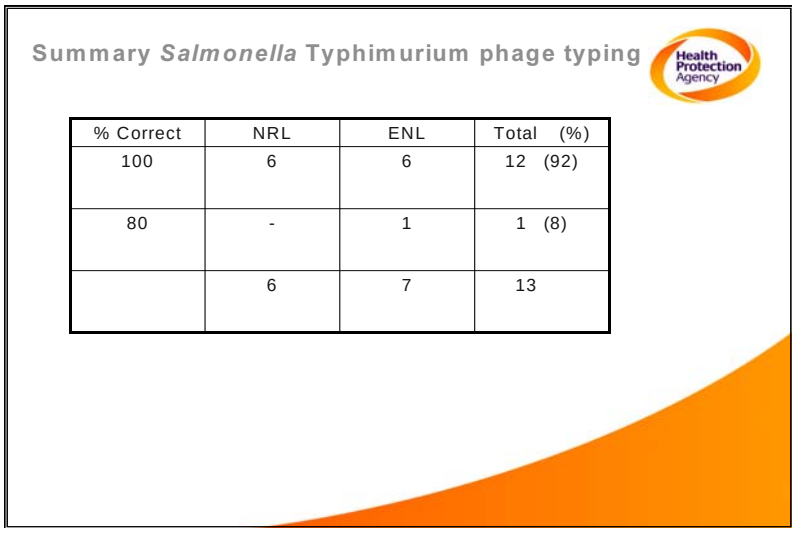
S. Enteritidis	*S.* Typhimurium
1b	41
1	1
14b	104
2	22
9b	9
	208
	136

Slide 9



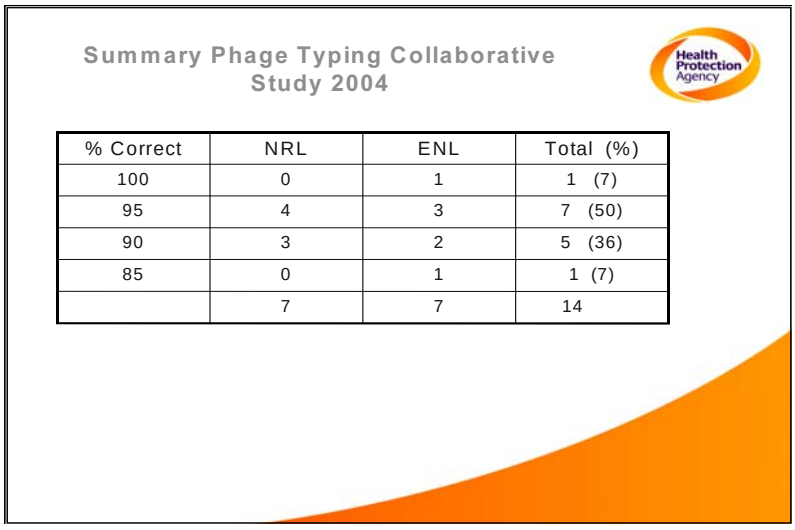
% Correct	NRL	ENL	Total (%)
100	-	1	1 (7)
90	4	4	8 (57)
80	3	2	5 (36)
	7	7	14

Slide 10



% Correct	NRL	ENL	Total (%)
100	6	6	12 (92)
80	-	1	1 (8)
	6	7	13

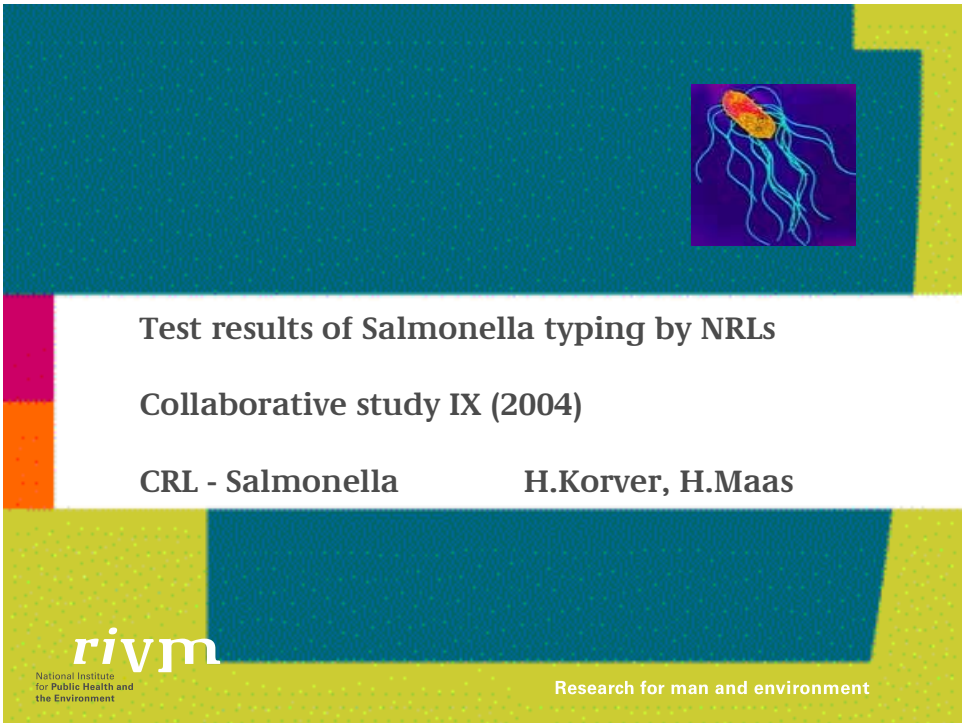
Slide 11



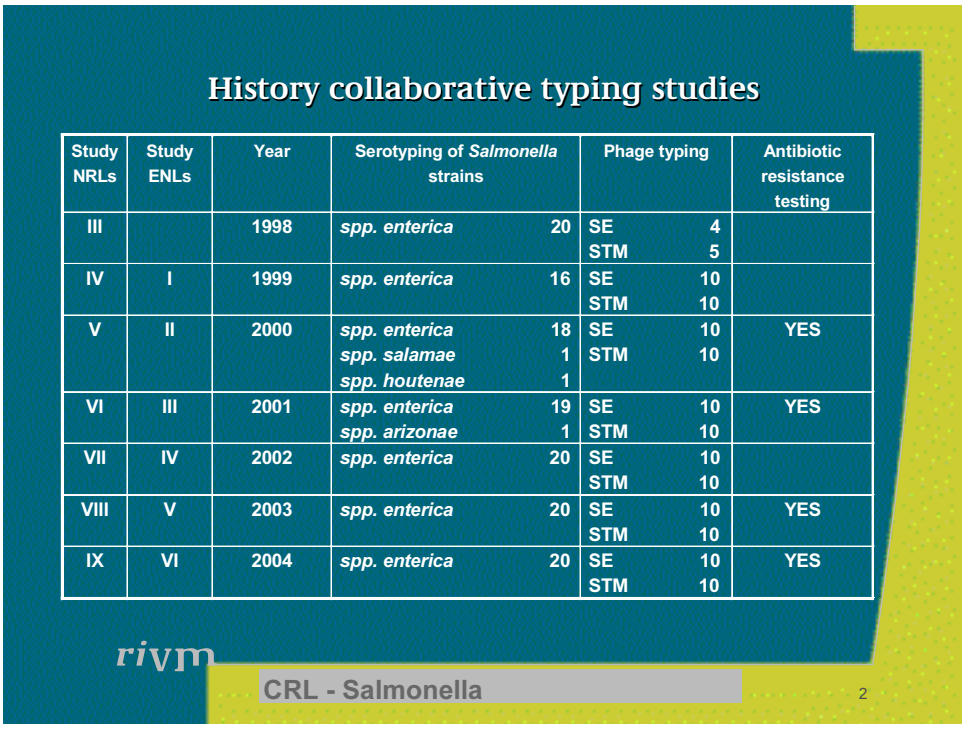
% Correct	NRL	ENL	Total (%)
100	0	1	1 (7)
95	4	3	7 (50)
90	3	2	5 (36)
85	0	1	1 (7)
	7	7	14

Annex 11. Slides of presentation 1.9

Slide 1



Slide 2



Slide 3

Salmonella strains for serotyping (1)

No	Serovar	O antigens	H antigens	Origin of strains
S1	*S.* Banana	1, 4, [5], 12	m, t : [1, 5]	Soy
S2	*S.* Paratyphi B var.Java	1, 4, [5], 12	b : 1, 2	Human
S3	*S.* Chester	1, 4, [5], 12	e, h : e, n, x	Swine
S4	*S.* Albany	8, 20	z_4, z_{24} : -	Human
S5	*S.* Blockley	6, 8	k : 1, 5	Human
S6	*S.* Enteritidis	1, 9, 12	g, m : -	Human
S7	*S.* Putten	13, 23	d : l, w	Animal feed
S8	*S.* Kivu	6, 7	d : 1, 6	Human
S9	*S.* Fresno	9, 46	z_{38} : -	Unknown
S10	*S.* Durban	9, 12	a : e, n, z_{15}	Human

rivm CRL - Salmonella 3

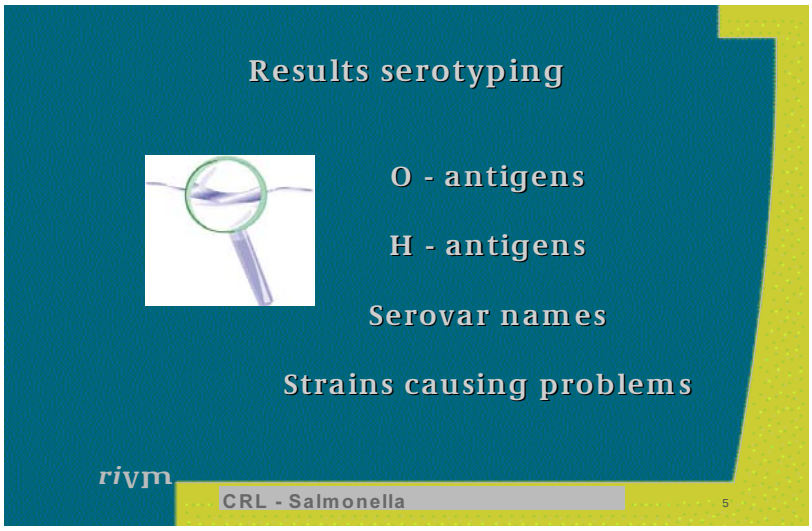
Slide 4

Salmonella strains for serotyping (2)

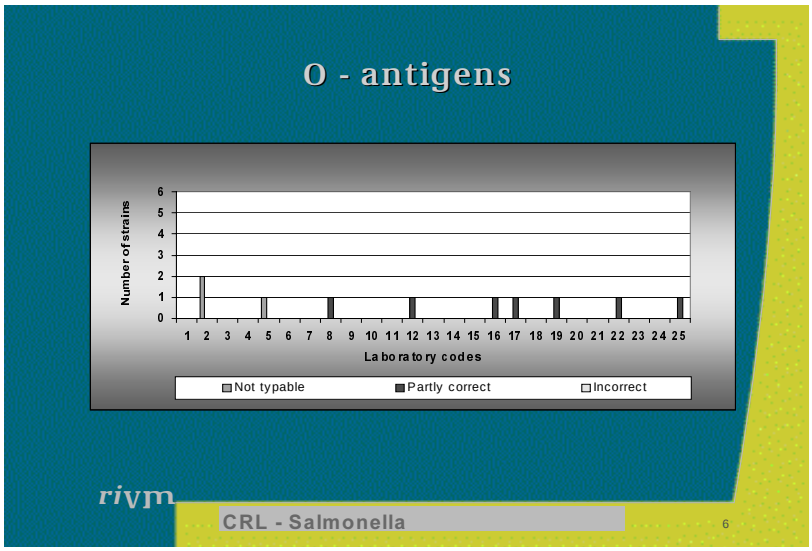
No	Serovar	O antigens	H antigens	Origin of strains
S11	*S.* Weltevreden	3, 10 [15]	r : z_6	Spices
S12	*S.* Amsterdam	3, 10 [15][15, 34]	g, m, s : -	Human
S13	*S.* Indiana	1, 4, 12	z : 1, 7	Chicken
S14	*S.* Livingstone	6, 7, 14	d : l, w	Human
S15	*S.* Typhimurium	1, 4, [5], 12	i : 1, 2	Human
S16	*S.* Alachua	35	z_4, z_{23} : -	Fish flour
S17	*S.* Liverpool	1, 3, 19	d : e, n, z_{15}	Chicken
S18	*S.* Virchow	6, 7, 14	r : 1, 2	Human
S19	*S.* Infantis	6, 7, 14	r : 1, 5	Human
S20	*S.* Hadar	6, 8	z_{10} : e, n, x	Human

rivm CRL - Salmonella 4

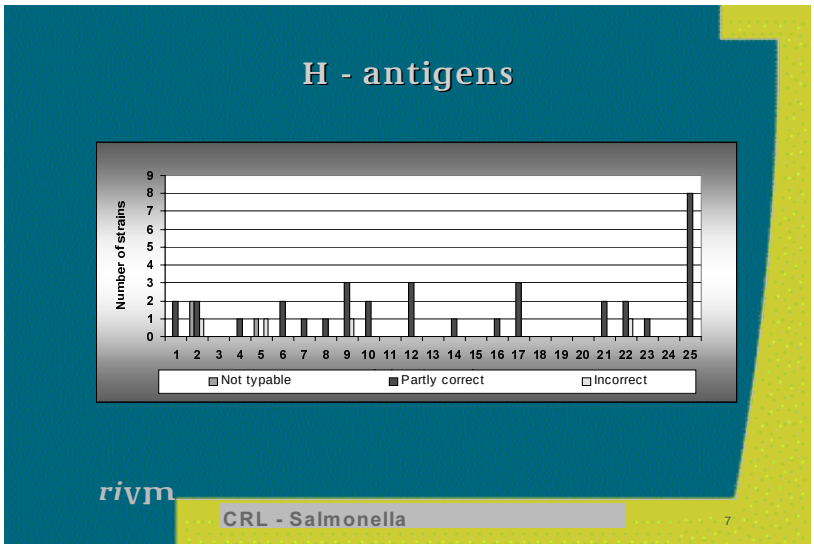
Slide 5



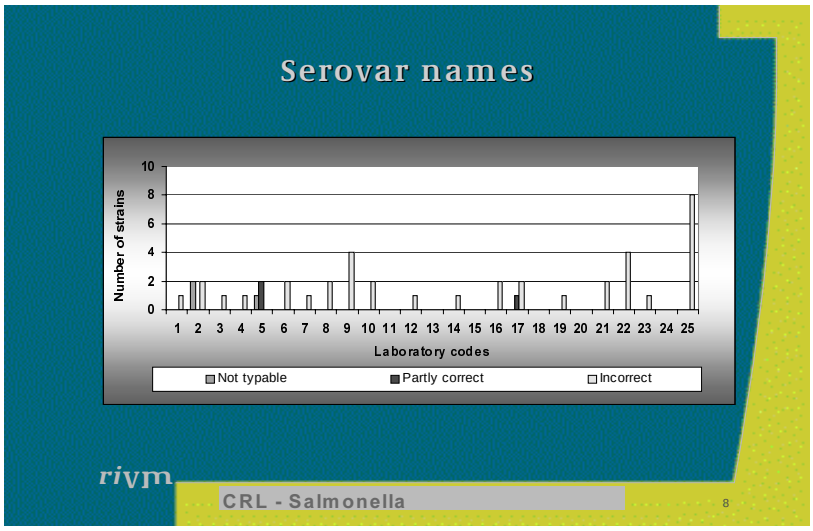
Slide 6



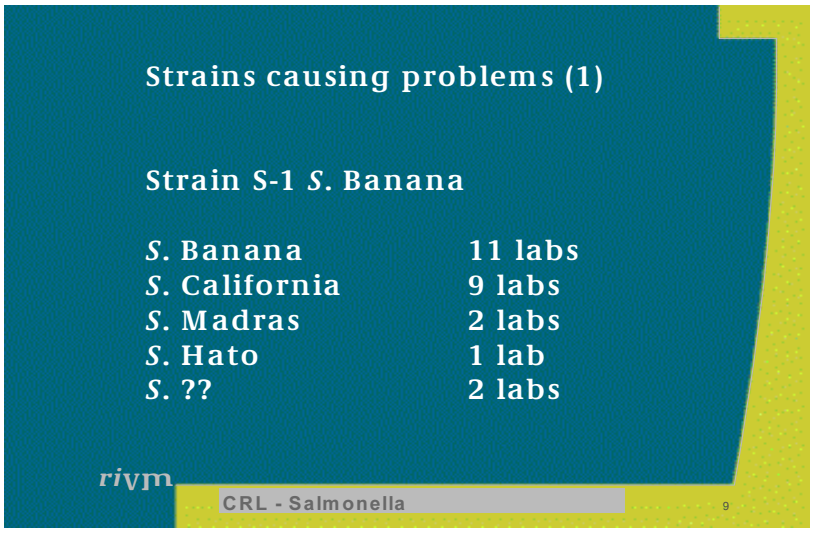
Slide 7



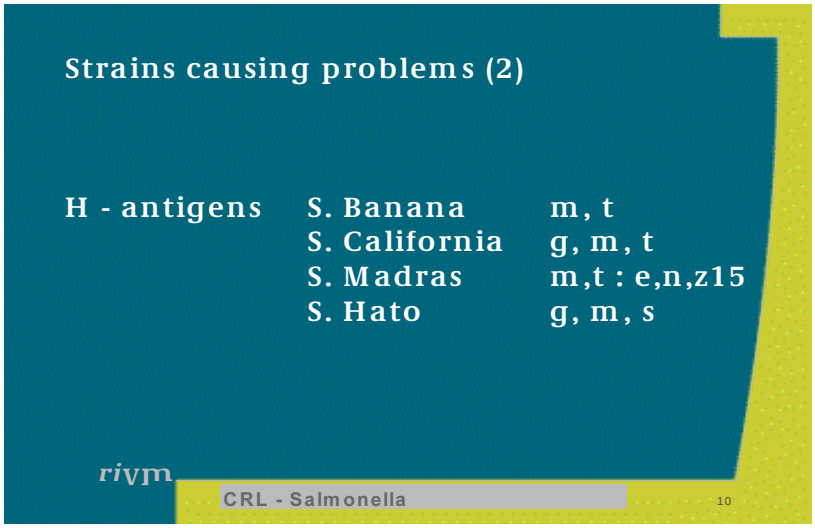
Slide 8



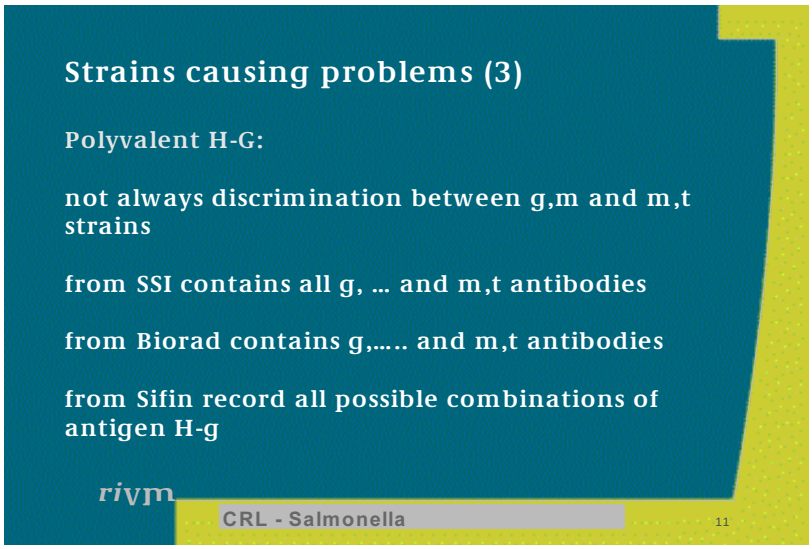
Slide 9



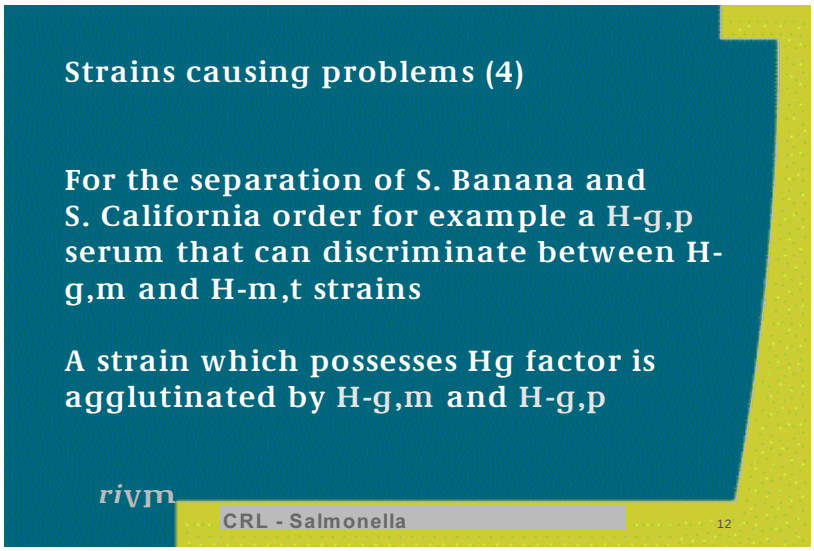
Slide 10



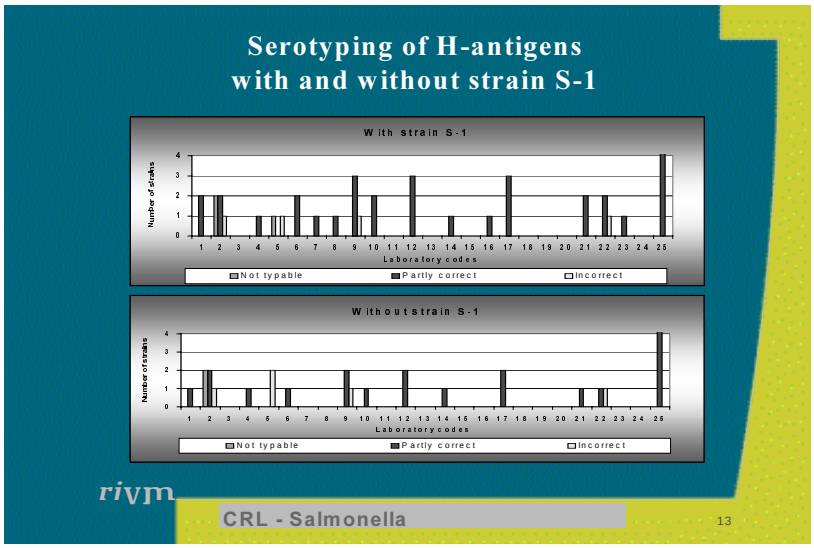
Slide 11



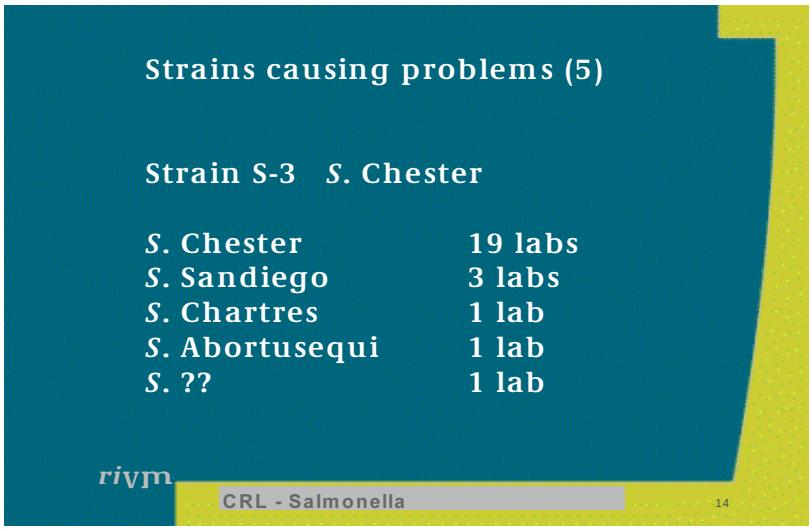
Slide 12



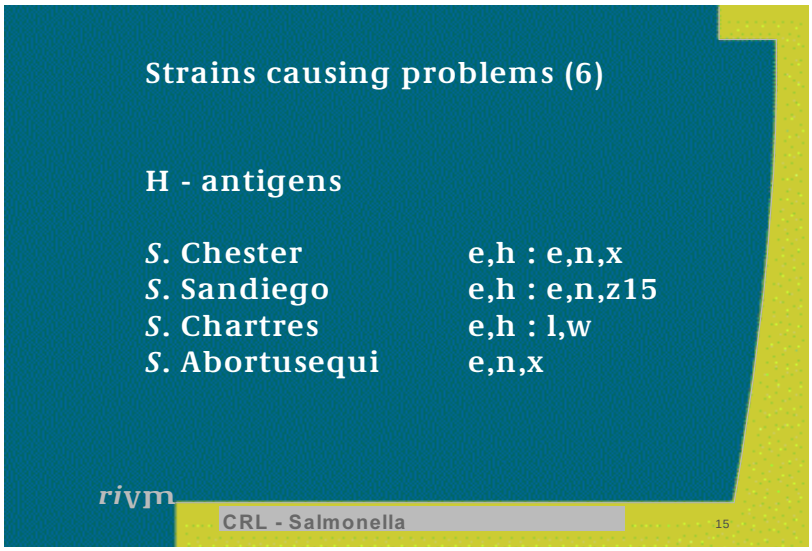
Slide 13



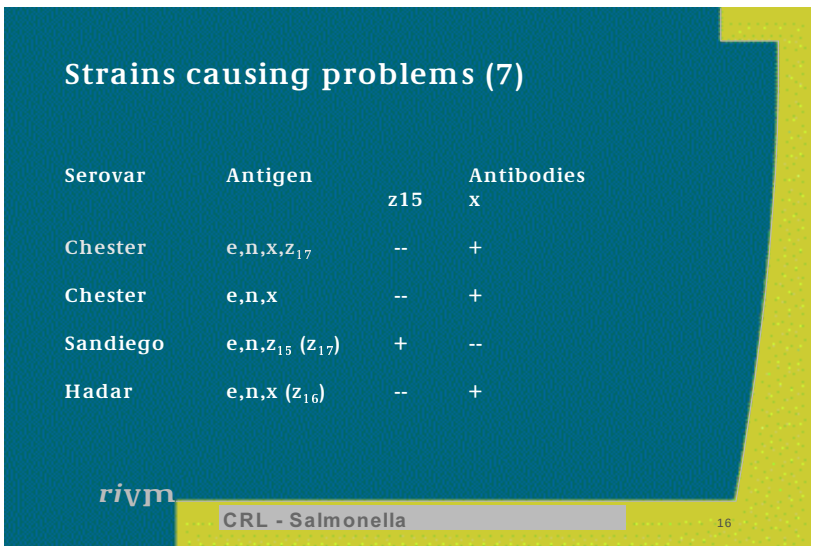
Slide 14



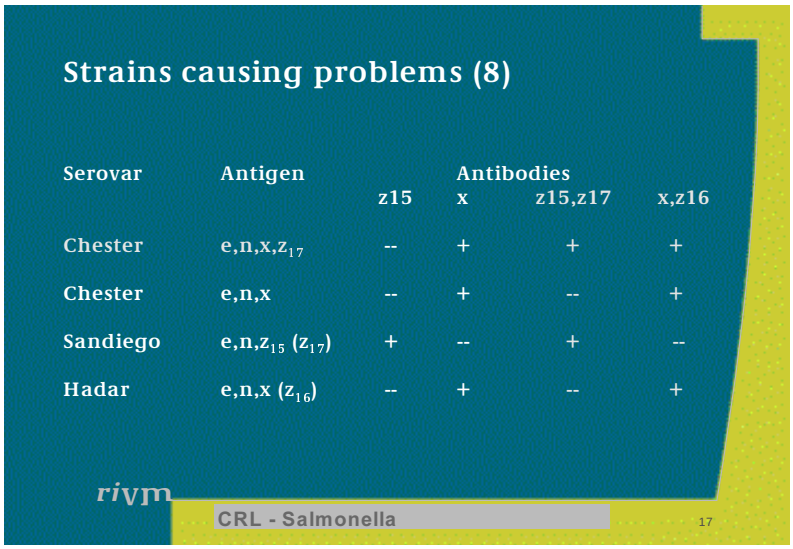
Slide 15



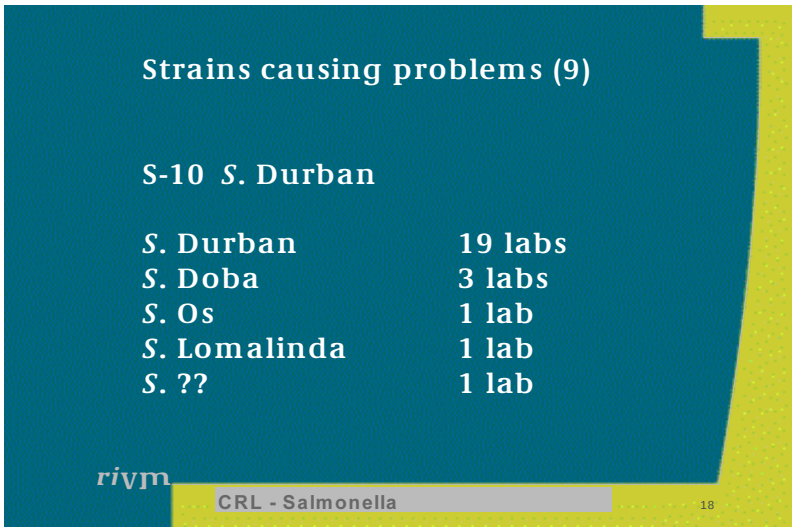
Slide 16



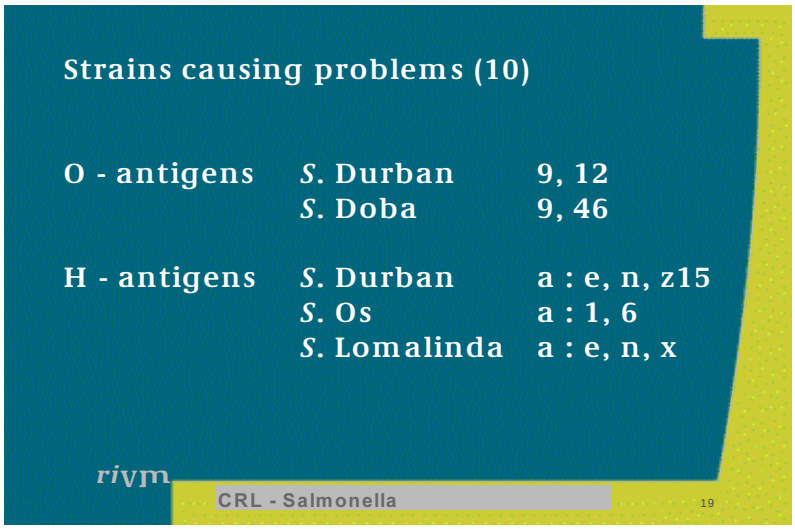
Slide 17



Slide 18



Slide 19



Slide 20

Quality control

Ask the manufacturer: Certificate of Analysis with

Name antiserum
Lot number
Immunisation strain (mention separate antigens)
Titer with the immunisation strain
Absorption strain(s)
Positive and negative controls (how many strains ?)
Dilution of antiserum for slide agglutination

rivm CRL - Salmonella 20

Slide 21

Studies and strains

Number of studies	Number of strans
9	Enteritidis, Typhimurium
5	Infantis, Virchow
4	Agona, Dublin, Hadar
3	10
2	18
1	48

rivm CRL - Salmonella 21

Slide 22

Correct identification in %

Serovar	2000	2001	2002	2003	2004
Enteritidis	100	100	94	94	92
Hadar	88	--	--	88	96
Infantis	100	--	--	100	96
Typhim.	100	100	100	100	100
Virchow	100	--	--	100	96

rivm CRL - Salmonella 22

Slide 23

Achievements in % correctness

	2001	2002	2003	2004
O-antigens	94	98	99	98
H-antigens	94	94	96	91
Serovar names	90	92	95	91
Number of labs	17	17	17	24

rivm CRL - Salmonella 23

Slide 24

Remarks

Quality control of antisera by manufacturer and by NRL

Some labs give serum formula; we ask for antigen formula (i.e. b : 2 versus b : 1,2 and m,s versus g,m,s) = sometimes interpretation problems

rivm CRL - Salmonella 24

Slide 25

Design typing study X (2005) concerning phage typing and serotyping

Phage typing: 10 STM and 10 SE strains

Serotyping: 20 strains

5 most important ones in EU

Strains causing problems

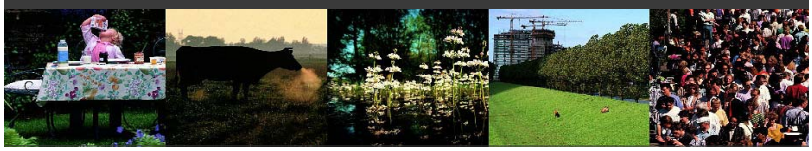
rivm CRL - Salmonella 25

Annex 12. Slides of presentation 1.10

Slide 1

Results typing study IX - 2004: antibiotic resistance testing

Dik Mevius



CIDC-LELYSTAD WAGENINGEN UR

Slide 2

Conclusions 2003

- CRL-Salmonella should develop EQAS (and IQAS)
- EU-should encourage quantitative testing
- Panel of strains selected based on phenotype
  - incl.: *S.* Java
- NCCLS is reference method
  - Participating lab's use routine method

CIDC-LELYSTAD WAGENINGEN UR

Slide 3

Recommended antibiotics

- Chloramphanicol
- Florfenicol
- Ampicillin/amoxicillin
- Cefotaxime
- **Amox-clavulanic acid**
- Enrofloxacin or ciprofloxacin
- Nalidixic acid
- Trim-sulphamethoxazole
- **Sulphonamide (sulphamethoxazole?)**
- Trimethoprim
- Streptomycin
- Gentamicin
- Kanamycin or Neomycin

CIDC-LELYSTAD WAGENINGEN UR

Slide 4

Strain selection 2004

CRL-1	S. Dublin	Cattle	AST-2
CRL-2	S. Enteritidis Pt 6a	Human	AST-8
CRL-3	S. Blockley	Human	AST-3
CRL-4	S. Typhimurium : Ft 508	Human	AST-9
CRL-5	S. Enteritidis Pt 4	Human	AST-4
CRL-6	S. Livingstone	Pig	AST-6
CRL-7	S. Hadar	Poultry	AST-1
CRL-8	S. Muenchen	Poultry	AST-10
CRL-9	S. Paratyphi B. var.Java	Poultry	AST-7
CRL-10	S. Kentucky	Human	AST-5

- Based on MIC profile obtained with broth microbilution using sensititre trays and CAMHB

CIDC-LELYSTAD WAGENINGEN UR

Slide 5

Strain selection

- Confirmation of MIC
  - Retesting with broth microdilution
  - Etest for amox-clavulanic acid and streptomycin
    - Amox S: 0.5 – 1 µg/ml and AMCL 0.5/0.25 – 1/0.5 µg/ml
    - Amox R: > 64 µg/ml and AMCL 16/8 µg/ml
    - (retested with broth microdilution (incl. kanamycin)
    - CIDC prefers to test Amp/amox and cefotaxime/ceftazidime
      – ESBL-pos strains confirmed with Etest

CIDC-LELYSTAD WAGENINGEN UR

Slide 6

ß-lactam MICs

			Sensititre		E-test		Sensititre
		AMOX		*AMCL*		*FOT*	
Sero/faagtype	**Code**	**MIC range**		**MIC range**		**MIC range**	
S. Dublin	CRL-1	</ 0.5	*S*	0,5/ 0,25	*S*	</ 0.12	*S*
S. Enteritidis pt 6a	CRL-2	> 64	*R*	1,5/ 0,75	*S*	</ 0.12	*S*
S. Blockley	CRL-3	</ 0.5 - 1	*S*	0,5/ 0,25	*S*	</ 0.12	*S*
S. Typhimurium : ft 508	CRL-4	> 64	*R*	16/ 8	*I*	> 16	*R* *
S. Enteritidis pt 4	CRL-5	> 64	*R*	2/ 1	*S*	> 16	*R* *
S. Livingstone	CRL-6	> 64	*R*	2/ 1	*S*	</ 0.12 - 0,25	*S*
S. Hadar	CRL-7	</ 0.5 - 1	*S*	0,5/ 0,25	*S*	</ 0.12	*S*
S. Muenchen	CRL-8	</ 0.5	*S*	0,5/ 0,25	*S*	</ 0.12	*S*
S. Paratyphi B. var.Java	CRL-9	</ 0.5 - 1	*S*	0,75/ 0,375	*S*	</ 0.12	*S*
S. Kentucky	CRL-10	> 64	*R*	4/ 2	*S*	</ 0.12 - 0,25	*S*

CRL-4: MIC ceftazidime/clav: > 4 µg/ml

CRL-5: MIC ceftazidime/clav: 0.25 µg/ml

CIDC-LELYSTAD WAGENINGEN UR

Slide 7

ß-lactam MICs

			Sensititre	Sensititre and Etest			Sensititre
		AMOX		*AMCL*		*FOT*	
Sero/faagtype	**Code**	**MIC range**		**MIC range**		**MIC range**	
S. Dublin	CRL-1	</ 0.5	*S*	0.5	*S*	</ 0.12	*S*
S. Enteritidis pt 6a	CRL-2	> 64	*R*	1,5 - 4	*S*	</ 0.12	*S*
S. Blockley	CRL-3	</ 0.5 - 1	*S*	0.5 - 1	*S*	</ 0.12	*S*
S. Typhimurium : ft 508	CRL-4	> 64	*R*	16 - > 16	*R*	> 16	*R* *
S. Enteritidis pt 4	CRL-5	> 64	*R*	2 - 4	*S*	> 16	*R* *
S. Livingstone	CRL-6	> 64	*R*	2 - 8	*S*	</ 0.12 - 0,25	*S*
S. Hadar	CRL-7	</ 0.5 - 1	*S*	0.5 - 1	*S*	</ 0.12	*S*
S. Muenchen	CRL-8	</ 0.5	*S*	0.5 - 1	*S*	</ 0.12	*S*
S. Paratyphi B. var.Java	CRL-9	</ 0.5 - 1	*S*	0.75 - 1	*S*	</ 0.12	*S*
S. Kentucky	CRL-10	> 64	*R*	4 - 8	*S*	</ 0.12 - 0,25	*S*

CRL-4: MIC ceftazidime/clav: > 4 µg/ml

CRL-5: MIC ceftazidime/clav: 0.25 µg/ml

CIDC-LELYSTAD WAGENINGEN UR

Slide 8

Aminoglycosides

	Sensititre		Sensititre		Sensititre
GEN		*NEO*		*Kana*	
MIC range		**MIC range**		**MIC range**	
</ 0,25 - 0,5	*S*	</ 1	*S*	4	*S*
> 32	*R*	32 - 64	*R*	> 16	*R*
0,5 - 4	*S*	128 - >128	*R*	> 16	*R*
> 32	*R*	</ 1	*S*	> 16	*R*
2	*S*	4	*S*	16	*S*
</ 0,25 - 2	*S*	> 128	*R*	> 16	*R*
0,5	*S*	</1 - 2	*S*	4	*S*
32 - > 32	*R*	</ 1	*S*	16	*S*
</ 0,25	*S*	</ 1	*S*	2	*S*
16 - 32	*R*	</ 1	*S*	4	*S*

CIDC-LELYSTAD WAGENINGEN UR

Slide 9

Aminoglycosides

	E-test		Sensititre
STREP		***Strep***	
MIC range		**MIC range**	
12	*S*	> 64	*R*
128	*R*	> 64	*R*
16	*S*	64	*R*
96	*R*	> 64	*R*
6	*S*	8	*S*
32	*R*	> 64	*R*
16	*S*	> 64	*R*
16	*S*	> 64	*R*
8	*S*	32	*R*
8	*S*	32	*R*

- Etest not reliable for styreptomycin.
  - diffuse endpoints!

CIDC-LELYSTAD WAGENINGEN UR

Slide 10

Tetracycline, quinolones

		Sensititre		Sensititre		Sensititre
	TET		***CIP***		***NAL***	
Code	**MIC range**		**MIC range**		**MIC range**	
CRL-1	2	*S*	</ 0,06	*S*	4	*S*
CRL-2	> 64	*R*	1	*S*	> 128	*R*
CRL-3	> 64	*R*	0,5	*S*	> 128	*R*
CRL-4	> 64	*R*	</ 0,06	*S*	16	*S*
CRL-5	2	*S*	</ 0,06	*S*	4	*S*
CRL-6	> 64	*R*	</ 0,06	*S*	4	*S*
CRL-7	64	*R*	0,25	*S*	> 128	*R*
CRL-8	1	*S*	</ 0,06	*S*	4	*S*
CRL-9	2	*S*	</ 0,06	*S*	4	*S*
CRL-10	> 64	*R*	8	*R*	> 128	*R*

CIDC-LELYSTAD WAGENINGEN UR

Slide 11

Fenicols

	Sensititre		Sensititre
CHL		***FFN***	
MIC range		**MIC range**	
128 - > 128	*R*	4	*S*
32	*R*	8 - 16	*S / I*
8	*S*	4 - 8	*S*
> 128	*R*	> 128	*R*
8	*S*	4	*S*
128	*R*	16	*I*
8	*S*	4	*S*
</ 4 - 8	*S*	4	*S*
8	*S*	4	*S*
8	*S*	4	*S*

CIDC-LELYSTAD WAGENINGEN UR

Slide 12

Trimethoprim, Sulphamethoxazole

		Sensititre		Sensititre		Sensititre
	TMP		***SXT***		***SMX***	
Code	**MIC range**		**MIC range**		**MIC range**	
CRL-1	</ 0,5	*S*	0,5/ 9,5	*S*	> 1024	*R*
CRL-2	2 - 4	*S*	2/ 38	*S*	> 1024	*R*
CRL-3	</ 0,5	*S*	</ 0,25/ 4,75	*S*	</ 8 - 16	*S*
CRL-4	1 - 2	*S*	2/ 38	*S*	> 1024	*R*
CRL-5	</ 0,5	*S*	</ 0,25/ 4,75	*S*	16	*S*
CRL-6	> 64	*R*	> 32/ 608	*R*	> 1024	*R*
CRL-7	</ 0,5	*S*	</ 0,25/ 4,75	*S*	16	*S*
CRL-8	</ 0,5	*S*	</ 0,25/ 4,75	*S*	> 1024	*R*
CRL-9	> 64	*R*	1/ 19	*S*	</ 8	*S*
CRL-10	</ 0,5	*S*	</ 0,25/ 4,75	*S*	> 1024	*R*

CIDC-LELYSTAD WAGENINGEN UR

Slide 13

Results

- 19 labs zone diameters
- 7 labs MICs
- some labs still missing

- Qualitative analysis
  - in the report quantitative analysis zone diameters

CIDC-LELYSTAD WAGENINGEN UR

Slide 14

Results Ampicillin (amoxicillin) (7 MIC, 19 mm)

Breakpoint MIC: S ≤ 8, R > 16

	AMOX		*Results*	
Code	**MIC range**		**MIC**	**Inh Zone**
CRL-1	</ 0.5	*S*	0	0
CRL-2	> 64	*R*	0	0
CRL-3	</ 0.5 - 1	*S*	0	0
CRL-4	> 64	*R*	0	0
CRL-5	> 64	*R*	0	0
CRL-6	> 64	*R*	0	0
CRL-7	</ 0.5 - 1	*S*	0	0
CRL-8	</ 0.5	*S*	0	0
CRL-9	</ 0.5 - 1	*S*	0	0
CRL-10	> 64	*R*	0	0
E. coli 25922		*2 -8*	*0*	
		16 - 22		*1*

CIDC-LELYSTAD WAGENINGEN UR

Slide 15

Results Amoxicillin clavulanic acid (6 MIC, 19 mm)

Breakpoint MIC: S ≤ 8/4, R > 16/8

	AMCL		Results	
Code	MIC range		MIC	Inh Zone
CRL-1	0.5	S	0	0
CRL-2	1,5 - 4	S	4/0	0/3
CRL-3	0.5 - 1	S	0	0
CRL-4	16 - > 16	R	0	0/1
CRL-5	2 - 4	S	2/1	0/3
CRL-6	2 - 8	S	4/0	1/4
CRL-7	0.5 - 1	S	0	0/1
CRL-8	0.5 - 1	S	0	0/1
CRL-9	0.75 - 1	S	0	0
CRL-10	4 - 8	S	4/2	4/11
E. coli 25922		*2/1 - 8/4*	*0*	
		18 - 24		*2*

CIDC-LELYSTAD WAGENINGEN UR

Slide 16

Results Cefotaxime (2 MIC, 16 mm)

Breakpoint MIC: S ≤ 8, R > 32

	FOT		Results	
Code	MIC range		MIC	Inh Zone
CRL-1	</ 0.12	S	0	0
CRL-2	</ 0.12	S	0	0
CRL-3	</ 0.12	S	0	0
CRL-4	> 16	R *	0	1/8
CRL-5	> 16	R *	0	0
CRL-6	</ 0.12 - 0,25	S	0	0
CRL-7	</ 0.12	S	0	0
CRL-8	</ 0.12	S	0	0
CRL-9	</ 0.12	S	0	0
CRL-10	</ 0.12 - 0,25	S	0	0
E. coli 25922		*.03 - .125*	*0*	
		29 - 35		*3*

CIDC-LELYSTAD WAGENINGEN UR

Slide 17

Results Chloramphenicol (7 MIC, 18 mm)

Breakpoint MIC: S ≤ 8, R > 16

	CHL		Results	
Code	MIC range		MIC	Inh Zone
CRL-1	128 - > 128	R	0	0
CRL-2	32	R	0/1	10/7
CRL-3	8	S	0	0
CRL-4	> 128	R	0	0
CRL-5	8	S	0	0
CRL-6	128	R	0	0
CRL-7	8	S	0	0
CRL-8	</ 4 - 8	S	0	0
CRL-9	8	S	0	0
CRL-10	8	S	0	0
E. coli 25922		*2 - 8*	*0*	
		21 - 27		*2*

CIDC-LELYSTAD WAGENINGEN UR

Slide 18

Results Florfenicol (5 MIC, 6 mm)

Breakpoint MIC: S ≤ 8, R > 16

	FFN		Results	
Code	MIC range		MIC	Inh Zone
CRL-1	4	S	0	0
CRL-2	8 - 16	S / I	8 - 16	0/5
CRL-3	4 - 8	S	0	0
CRL-4	> 128	R	0	0
CRL-5	4	S	0	0
CRL-6	16	I	16	1/1
CRL-7	4	S	0	0
CRL-8	4	S	0	0
CRL-9	4	S	0	0
CRL-10	4	S	0	0
E. coli 25922		*2 - 8*	*0*	
		22 - 28		*2*

CIDC-LELYSTAD WAGENINGEN UR

Slide 19

Results Nalidixic acid (6 MIC, 19 mm)

Breakpoint MIC: S ≤ 16, R > 16

	NAL		Results	
Code	MIC range		MIC	Inh Zone
CRL-1	4	S	0	0
CRL-2	> 128	R	0	1/0
CRL-3	> 128	R	0	0
CRL-4	16	S	8 - 16	6/10
CRL-5	4	S	0	0
CRL-6	4	S	0	0/3
CRL-7	> 128	R	0	0
CRL-8	4	S	0	0
CRL-9	4	S	0	0
CRL-10	> 128	R	0	0
E. coli 25922		*1 - 4*	*0*	
		22 - 28		*2*

CIDC-LELYSTAD WAGENINGEN UR

Slide 20

Results **Cipro/**enrofloxacin (4 MIC, 15 mm)

Breakpoint MIC: S ≤ 1, R > 2

	CIP		Results	
Code	MIC range		MIC	Inh Zone
CRL-1	</ 0,06	S	0	0
CRL-2	1	S	1/1	1/3
CRL-3	0,5	S	0/1	0/1
CRL-4	</ 0,06	S	0	0
CRL-5	</ 0,06	S	0	0
CRL-6	</ 0,06	S	0	0
CRL-7	0,25	S	0	0/1
CRL-8	</ 0,06	S	0	0
CRL-9	</ 0,06	S	0	0
CRL-10	8	R	0	0
E. coli 25922		*.004 - .016*	*0*	
		30 - 40		*1*

CIDC-LELYSTAD WAGENINGEN UR

Slide 21

Results Ciprofloxacin (2 MIC, 9 mm)

Breakpoint MIC: S ≤ 0.25, R > 1

	CIP		Results	
Code	MIC range		MIC	Inh Zone
CRL-1	</ 0,06	S	0	0
CRL-2	1	I/R	0	1/6
CRL-3	0,5	I	0/1	0/6
CRL-4	</ 0,06	S	0	0
CRL-5	</ 0,06	S	0	0
CRL-6	</ 0,06	S	0	0
CRL-7	0,25	I	0/1	0/7
CRL-8	</ 0,06	S	0	0
CRL-9	</ 0,06	S	0	0
CRL-10	8	R	0	0
E. coli 25922		*.008 - .03*	*0*	
		32 - 40		*1*

CIDC-LELYSTAD WAGENINGEN UR

Slide 22

Results Gentamicin (6 MIC, 17 mm)

Breakpoint MIC: S ≤ 4, R > 8

	GEN		Results	
Code	MIC range		MIC	Inh Zone
CRL-1	</ 0,25 - 0,5	S	0	0
CRL-2	> 32	R	0	0
CRL-3	0,5 - 4	S	0	0
CRL-4	> 32	R	0	0
CRL-5	2	S	0	0/1
CRL-6	</ 0,25 - 2	S	0/1	0/1
CRL-7	0,5	S	0	0
CRL-8	32 - > 32	R	0	0
CRL-9	</ 0,25	S	0	0
CRL-10	16 - 32	R	0	0/5
E. coli 25922		*0.25 - 1*	*1*	
		19 - 26		*1*

CIDC-LELYSTAD WAGENINGEN UR

Slide 23

Results Neomycin (5 MIC, 17 mm)

Breakpoint MIC: ?

	NEO		Results	
Code	MIC range		MIC	Inh Zone
CRL-1	</ 1	S	0	0
CRL-2	32 - 64	R	0	0/2
CRL-3	128 - >128	R	0	0
CRL-4	</ 1	S	0	0
CRL-5	4	S	0	0/1
CRL-6	> 128	R	0/1	0/1
CRL-7	</1 - 2	S	0	0
CRL-8	</ 1	S	0	0
CRL-9	</ 1	S	0	0
CRL-10	</ 1	S	0	0
E. coli 25922		*≤1 - 2*	*0*	
		NA		*16 - 24*

CIDC-LELYSTAD WAGENINGEN UR

Slide 24

Results Kanamycin (2 MIC, 14 mm)

Breakpoint MIC: S ≤ 16, R > 32

	Kana		Results	
Code	MIC range		MIC	Inh Zone
CRL-1	4	S	0	0/1
CRL-2	> 16	R	0	0
CRL-3	> 16	R	0	0
CRL-4	> 16	R	0	5/7
CRL-5	16	S	0	1/6
CRL-6	> 16	R	0	0
CRL-7	4	S	0	0/1
CRL-8	16	S	0	0/1
CRL-9	2	S	0	0/1
CRL-10	4	S	0	0/1
E. coli 25922		*1 - 4*	*0*	
		17 - 25		*1*

CIDC-LELYSTAD WAGENINGEN UR

Slide 25

Results Streptomycin (6 MIC, 17 mm)

Breakpoint MIC: S ≤ 16, R > 16

	Strep		*Results*	
Code	**MIC range**		**MIC**	**Inh Zone**
CRL-1	> 64	*R*	0	0
CRL-2	> 64	*R*	0	0
CRL-3	64	*R*	0	0
CRL-4	> 64	*S*	0	0
CRL-5	8	*S*	0/2	2/10
CRL-6	> 64	*R*	0	0
CRL-7	> 64	*R*	0	0
CRL-8	> 64	*R*	0	0
CRL-9	32	*R*	0/1	1/1
CRL-10	32	*R*	0/1	0/1
E. coli 25922		*4 - 8*	*0*	
		NA		*12 - 19*

CIDC-LELYSTAD WAGENINGEN UR

Slide 26

Results Trimethoprim (6 MIC, 15 mm)

Breakpoint MIC: S ≤ 8, R > 8

	TMP		*Results*	
Code	**MIC range**		**MIC**	**Inh Zone**
CRL-1	</ 0,5	*S*	0	0
CRL-2	2 - 4	*S*	0	1/4
CRL-3	</ 0,5	*S*	0	0
CRL-4	1 - 2	*S*	0	0/1
CRL-5	</ 0,5	*S*	0	0
CRL-6	> 64	*R*	0	0
CRL-7	</ 0,5	*S*	0	0
CRL-8	</ 0,5	*S*	0	0
CRL-9	> 64	*R*	0	0
CRL-10	</ 0,5	*S*	0	0
E. coli 25922		*0.5 - 2*	*0*	
		21 - 28		*1*

CIDC-LELYSTAD WAGENINGEN UR

Slide 27

Results Trimethoprim-sulphameth. (3 MIC, 16 mm)

Breakpoint MIC: S ≤ 2/38, R > 2/38

	SXT		Results	
Code	MIC range		MIC	Inh Zone
CRL-1	0,5/ 9,5	S	0	0
CRL-2	2/ 38	S	1/0	10/4
CRL-3	</ 0,25/ 4,75	S	0	1/0
CRL-4	2/ 38	S	1/0	7/7
CRL-5	</ 0,25/ 4,75	S	0	0
CRL-6	> 32/ 608	R	0	0
CRL-7	</ 0,25/ 4,75	S	0	0
CRL-8	</ 0,25/ 4,75	S	0	0
CRL-9	1/ 19	S	1/1	14/0
CRL-10	</ 0,25/ 4,75	S	0	0
E. coli 25922		*≤ 0.5/9.5*	*0*	
		23 - 29		*3*

CIDC-LELYSTAD WAGENINGEN UR

Slide 28

Conclusions

- EQAS provides valuable information
  - ß-lactams, aminoglycosides, quinolones, TmpS
- Reference values need to be 100% reliable
  - Confirmation by other lab??
- ATCC inclusion OK but more important for IQAS
- AMCL not reliable
- Strep only R reliable
- Use Chlor-breakpoints for florfenicol

CIDC-LELYSTAD WAGENINGEN UR

Slide 29

Suggestions for harmonisation:

- EQAS-reference MICs:
  - No more e.g. neomycin or kanamycin tested
  - Genetic profiles of ß-lactam resistance??
    - Or wait for EU-project results?
- Monitoring
  - Exclude
    - AMCL?
      - Instead: Ampi/amox, Cefotaxime and ESBL confirmation with Etest
    - Streptomycin?
    - Trim/Sulpha?
  - Include:
    - Sulphamethoxazole
    - Nal and ciprofloxacin (not enro)
    - Neomycin (not kanamycin)

CIDC-LELYSTAD WAGENINGEN UR

Slide 30

All the work was done by:

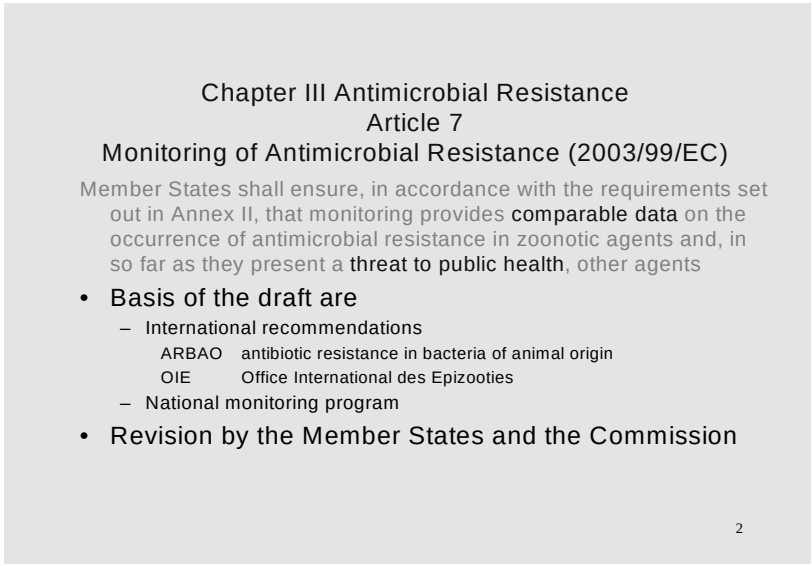
- Kees Veldman
- Marga Japing
- Jeanette Wup
- Hendrik-Jan Roest

CIDC-LELYSTAD WAGENINGEN UR

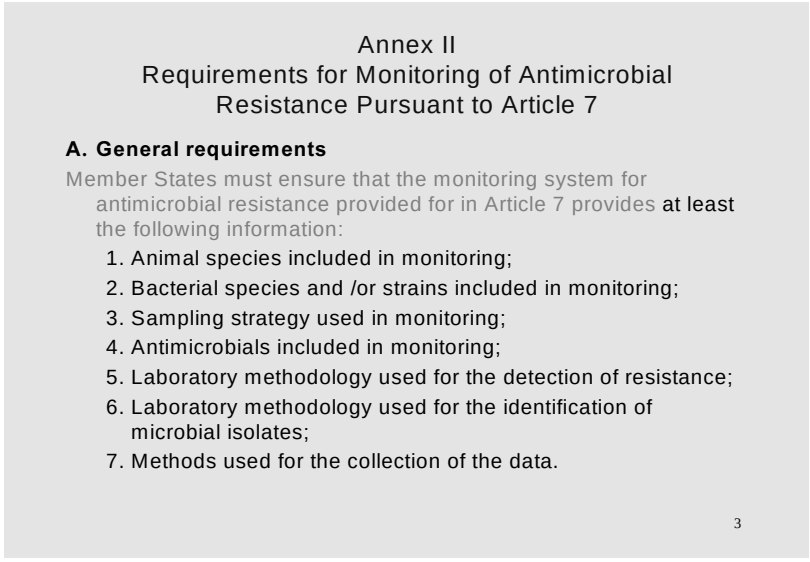
Annex 13. Slides of presentation 1.11

Slide 1

Slide 2



Slide 3



Slide 4

1. Animal Species Included in Monitoring

- Animal species
  - Cattle, pigs and poultry
    - Poultry, the main animal species and production levels should be covered: breeder, layer, broiler, turkey
- Food of animal origin
  - Beef, milk, pork, poultry meat and eggs
  - Poultry meat by the main animal species (fowl, turkey, ..)
- ~~Feed~~
  - ~~In the framework of monitoring of zoonosis, antimicrobial resistance monitoring in animal feed, including imported feed,may also be considered~~

4

Slide 5

2. Bacterial Species and /or Strains Included in Monitoring in Animals and Food

- Zoonotic bacteria
  - *Salmonella* spp.
    1. at least *S*. Enteritidis and *S*. Typhimurium
    2. most frequent salmonella serovars in human salmonellosis
    3. most frequent salmonella serovars in the animal species
    4. rare serovars
  - *Campylobacter jejuni* and *Campylobacter coli*
- Indicator bacteria
  - *E.coli* and ~~*Enterococcus faecium / faecalis*~~
- ~~Animal pathogens~~
  - ~~If they present a threat to public health~~
- ~~In feed~~
  - ~~Zoonotic bacteria at least *Salmonella* spp.~~

5

Slide 6

3. Sampling Strategy Used in Monitoring Kind of monitoring

Representative number of isolates should be tested

Active monitoring programme

- Zoonotic agents and indicator bacteria
- For several zoonosis and zoonotic agents monitoring programmes should be implemented. It may be considered that the antimicrobial resistance monitoring can be implemented by testing all or a subset of these isolates
- Indicator organisms could be collected from a subset of these samples

Passive monitoring could be used for the time period until an active programme can be started

- Representative isolates must be ensured

6

Slide 7

3. Sampling strategy used in monitoring Sample size

Animals

Zoonotic and indicator bacteria

Sampling :

- selected on statistical basis
- healthy animals: cattle, pigs and poultry
- one isolate per group of animals

• Sample Size :

- ~~385~~ 100 positive samples per year per bacteria and animal species (50% prevalence, 95% CI, ~~5%~~ 10 accuracy)

Specimen

- faecal samples from cattle and pigs
- caeca from poultry

7

Slide 8

Different options to collect a representative sample of isolates

- Active monitoring
- implemented for the purpose of collecting isolates for antimicrobial resistance testing
- Isolates from other monitoring activities
  - Directive 2003/99/EC
    - Harmonised monitoring schemes
    - Co-ordinated monitoring programmes
  - Regulation 2160/2003/EC
    - National control programmes
  - Programmes run by food business operators

8

Slide 9

Further investigations of zoonotic agents isolated in the framework of

- Directive 2003/99/EC
  - Harmonised monitoring schemes
    - one isolate from each epidemiological unit
  - Co-ordinated monitoring programmes
    - one isolate from each epidemiological unit
- Regulation 2160/2003/EC
  - National control programmes
    - food business operators
    - official controls (including sampling schemes) are required at feed, flock and/or herd level and other stages of the food chain
    - all or a representative subset of the isolates

9

Slide 10

Further investigations of zoonotic agents isolated in the framework of

- Programmes run by food business operators
  - Isolates from the self control
- Clinical isolates
  - Specimen from animals investigated for diagnostic purposes
    is not representative
    but new patterns of antimicrobial resistance could be detected

- Active monitoring is still needed for
  - Campylobacter in pigs and cattle,
  - Salmonella in cattle and products thereof
  - Indicator Bacteria

10

Slide 11

4. Antimicrobials included in Monitoring

		Zoonotic bacteria		Indicator bacteria	
Antimicrobial class		*Salmonella*	*Campylobacter*	*Enterococcus*	*E.coli*
Aminoglycosides	Streptomycin	+		+	+
	Neomycin	1 of the 2		(+)	+
	Kanamycin				
	Gentamicin	+	+	+	+
	Apramycin	(+)			(+)
	Spectinomycin	(+)			(+)
Amphenicols	Chloramphenicol	+		+	+
	Florfenicol	+			+
Beta-lactams	Ampicillin	+	+	+	+
Beta-lactams+ ß-lactam inhibitor	Amoxicillin/clavuanic acid	(+)	+	+	
Cephalosporins	Ceftiofur				
	Ceftriaxone	2 of the 3			2 of the 3
	Cephalothin				
	Ceftazidime	(+)			
	Cefotaxime	(+)			
Glycopeptides	Vancomycin			+	
Macrolides	Erythromycin		+	+	
Quinolones	Nalidixic acid	+	+		+
Fluoroquinolones	Enrofloxacin	1 of the 2			1 of the 2
	Ciprofloxacin		+		
Streptogramins	Virginiamycin			1 of the 2	
	Quinupristin/Dalfopristin				
Sulfonamides	Sulfonamide	+			+
	Trimethoprim –Sulfonamide	+			+
Tetracyclines	Tetracycline	+	+	+	+

(+) optional

11

Slide 12

5. Laboratory methodology used for the detection of resistance

Method

- Prerequisite of a European monitoring programme is the **comparability of the data**
- One aim of the programme is the **assessment of changes in the resistance pattern**
- Suitable methods are

Method	Statement	Unit of measurement
Broth Dilution Agar Dilution	quantitativ	µg/ml

12

Slide 13

Laboratory and analytical methods methodology used for the isolation of the bacteria and the detection of resistance

1. Draft fixed standardised methods:

- Reference methods for the isolation of bacteria:
  - EN/ISO 6579 (2002) *Salmonella*
  - ISO 10272 (1995) Thermophilic *Campylobacter*
  - ISO 21528-1 Enterobacteriaceae
  - ISO 4831 *E.coli*
- Reference methods for antimicrobial susceptibility testing:
  - NCCLS M31A for *Salmonella* and *E.coli*
  - NCCLS M100-S11 for *Enterococci*
  - NCCLS M31-A2 *Campylobacter*
- External controls
  - Community reference laboratories

revised

13

Slide 14

Laboratory and analytical methods used for the isolation of the bacteria and the detection of resistance

Current proposal:

- Appropriate methods for the
  - isolation of bacteria
  - antimicrobial susceptibility testing with internal controls
- External controls to guarantee the comparability of the results
  - Community Reference Laboratory/ies

advantage: national standards and ongoing monitoring programs remain untouched

prerequisite: Results from the ring trial must be combined with the submission of the data

14

Slide 15

7. Methods used for the collection and reporting of the data

- National collection and reporting system
- European collection and reporting system with the aim of the
  - Detection of the occurrence of the resistance patterns
  - Estimation of the prevalence of resistance to an antimicrobial substance
  - Assessing changes in the prevalence rate

15

Slide 16

Points of the revision
Approach to an agreement

Costs:

- Active monitoring programmes
  - Isolates for the antimicrobial resistance testing could be sampled as a subset of the zoonotic monitoring programmes covered by the Directive 2003/99/EC or the Regulation 2160/99/EC
- Sample size
  - Reduction of the accuracy to 10% leads to a reduction of the desired sample size to 100 isolates

National interests

- Methods
  - national standards and ongoing monitoring programs remain untouched, if only an external control is laid down

16

Annex 14. Slides of presentation 1.12

Slide 1

Design typing study X - 2005: antibiotic resistance testing

Dik Mevius

CIDC-LELYSTAD WAGENINGEN UR

Slide 2

Problems in comparability of resistance data are based upon

- Different
  - methodologies:
    - methods used for testing susceptibility
    - Interpretive criteria
    - antibiotic panels used
  - Selection criteria for strains

CIDC-LELYSTAD WAGENINGEN UR

Slide 3

Two options

- Standardisation of methodologies
  - long term perspective
- Harmonisation of results
  - EQAS, IQAS

CIDC-LELYSTAD WAGENINGEN UR

Slide 4

Recommended antibiotics (Reference values)

- **Conservative**
  - Ampicillin/amoxicillin
  - Chloramphenicol
  - Nalidixic acid
  - Trim-sulphamethoxazole
  - **Sulphamethoxazole**
  - Trimethoprim
  - Streptomycin (??)
  - Gentamicin
  - Kanamycin
  - Neomycin
  - **Tetracycline**
- **Newer generation**
  - Florfenicol
  - Cefotaxime
  - **Amox-clavulanic acid ??**
    - **Gene typing**
    - **EU-project**
  - Enrofloxacin
  - Ciprofloxacin

CIDC-LELYSTAD WAGENINGEN UR

Slide 5

Strain selection

- Based on resistance phenotype
- Relevant sero-, phagetypes to include??
- Not only highly R and S
  - Intermediate strains important to determine the precision of the methods used

CIDC-LELYSTAD WAGENINGEN UR

Slide 6

Reference values

- Preferably Reference MICs according to NCCLS
  - Broth or Agar Dilution
  - Etest only in exceptional cases (ESBL)
    - NCCLS breakpoints
- Always confirmed??
  - Budget??

CIDC-LELYSTAD WAGENINGEN UR

Slide 7

Report

- MIC Breakpoints and Interpretive criteria
- Reference MICs and categories R, I and S
  - Based on criteria for dilution and diffusion tests
- All results in tables incl. category assigned by ENLs and NRLs
- Qualitative analysis on R, I and S:
  - Discussion on each antibiotic
    - MICs and disc diffusion results
  - Numbers of minor, major and very major errors
- Quantitative analysis zone diameters
  - Mean and SD (Z-scores)
    - Indicate systematic differences in zone diamaters

CIDC-LELYSTAD WAGENINGEN UR

Slide 8

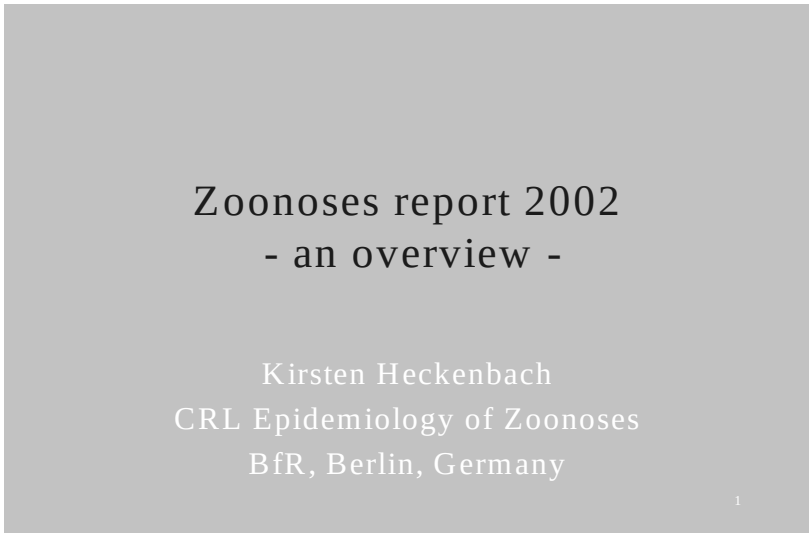
CRL-Antimicrobial resistance in Salmonella?

- Tasks:
  - Organize EQAS annually
  - Backup in case of problems at NRLs
    - phenotypical test
    - genetyping
  - Assist with IQAS
    - SOP, strains, antibiotics??
  - Stimulate harmonisation and standardisation of Eurpean Resistance Monitoring

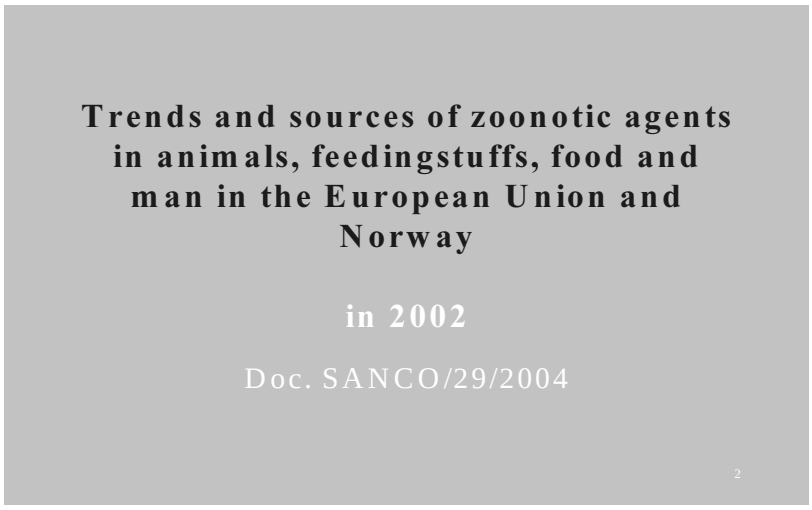
CIDC-LELYSTAD WAGENINGEN UR

Annex 15. Slides of presentation 2.1

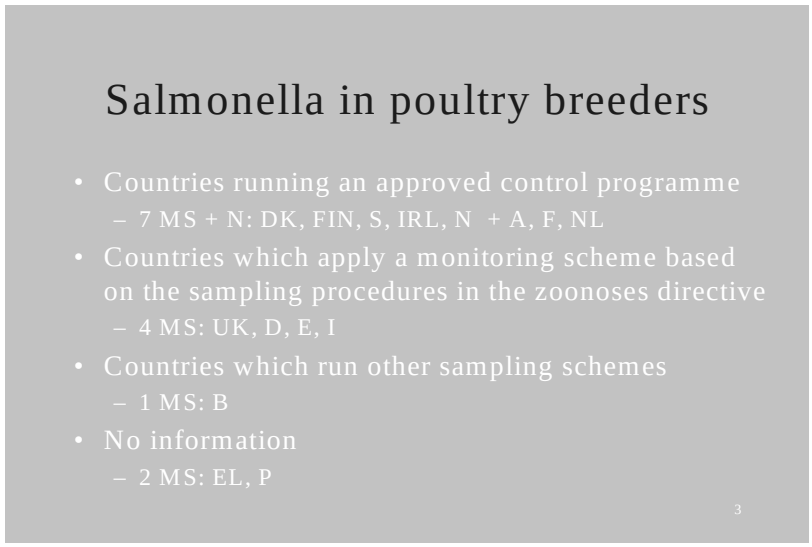
Slide 1



Slide 2



Slide 3



Slide 4

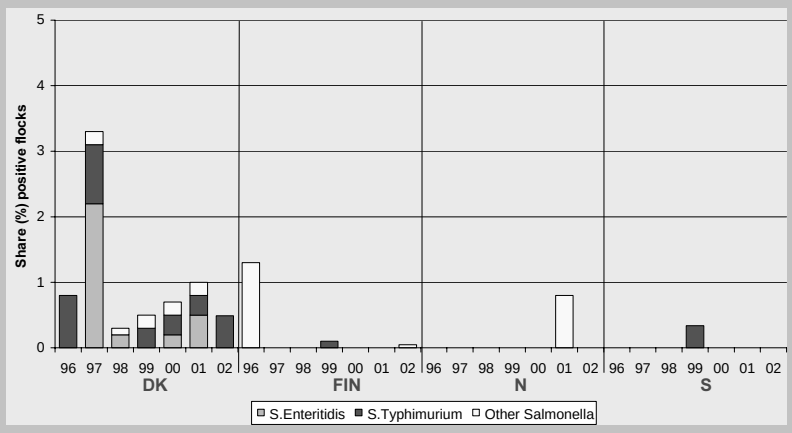
Salmonella in poultry breeders

- Serovars covered in the control programme
  - All Salmonella spp.
    - 6 countries: A, DK, FIN, N, NL, S
  - Restricted to S. Enteritidis and S. Typhimurium
    - 8 countries: B, D, E, EL, F, I, IRL, UK
    - sometimes the report covers also the other serovars

4

Slide 5

Approved control programme I Breeding flocks



5

Slide 6

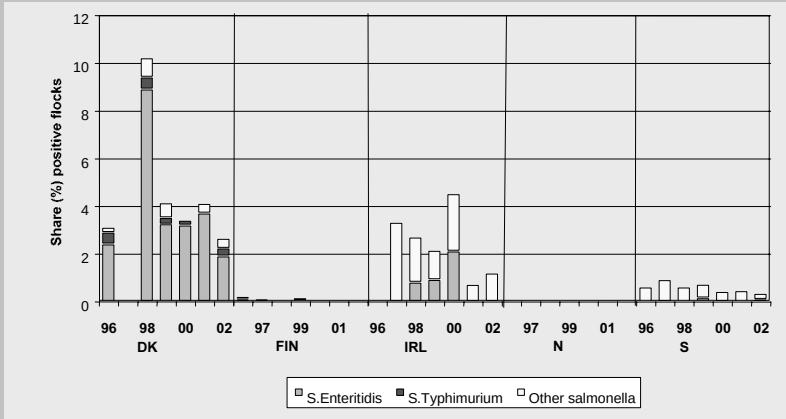
Salmonella in layers during production

- A surveillance scheme is in place in 6 MS + N
  - DK, F, FIN, IRL, N, NL, S
- Type of sample
  - Faecal samples / caecal droppings: all countries
  - Blood samples: NL
  - Egg samples: DK
  - Dust swabs: F, IRL
- Sample size: Involves each flock meeting the criteria
  - per flock: 60 samples / 2 pairs of socks
- Frequency
  - once to three times; not always at end of the lay

6

Slide 7

Approved control programme I Laying hens



7

Slide 8

Salmonella in broilers

At the farm: before slaughter

- Type of sample
  - Faecal samples / caecal droppings: A, B, DK, F, FIN, N, NL, S, UK
  - Caecum tissue: S
  - Dust swabs: F
- Sample size: 9 - 300 samples per flock

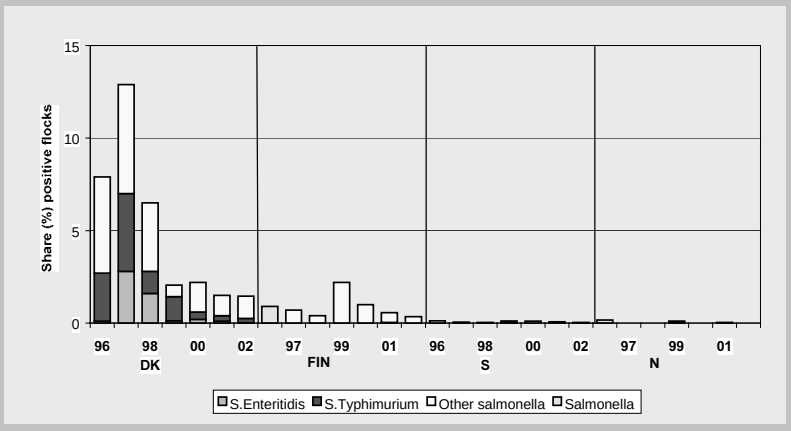
At slaughterhouse

- Type of sample
  - Neck skin sample: A, N, IRL, S
  - Carcass swabs: IRL, NL
  - Caecum: NL

8

Slide 9

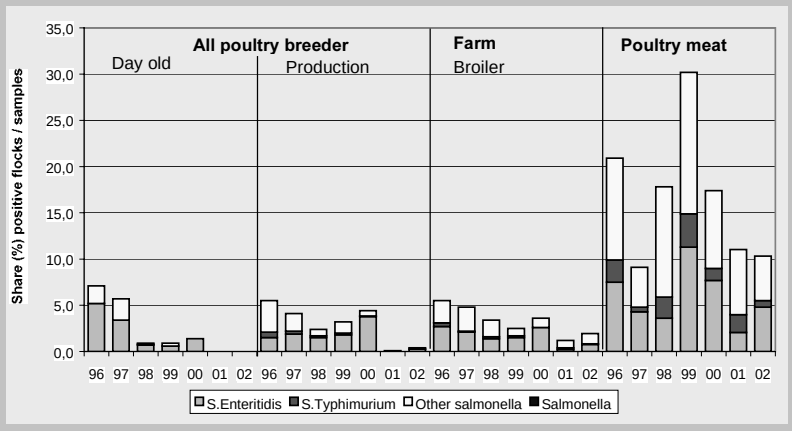
Approved control programme I Broilers



9

Slide 10

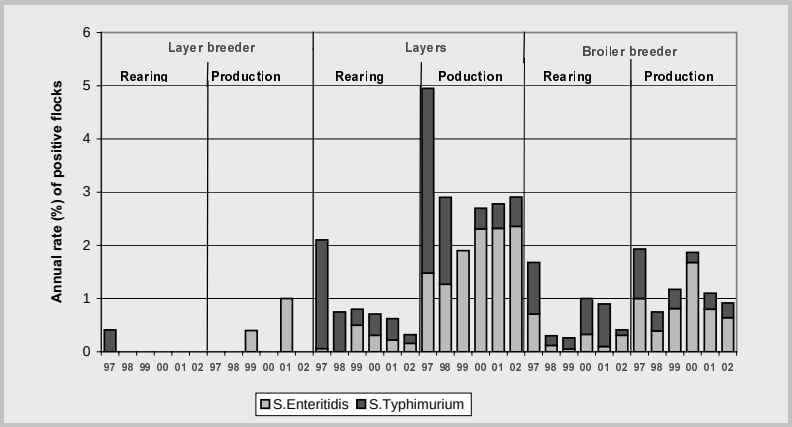
Approved control programme II Austria



10

Slide 11

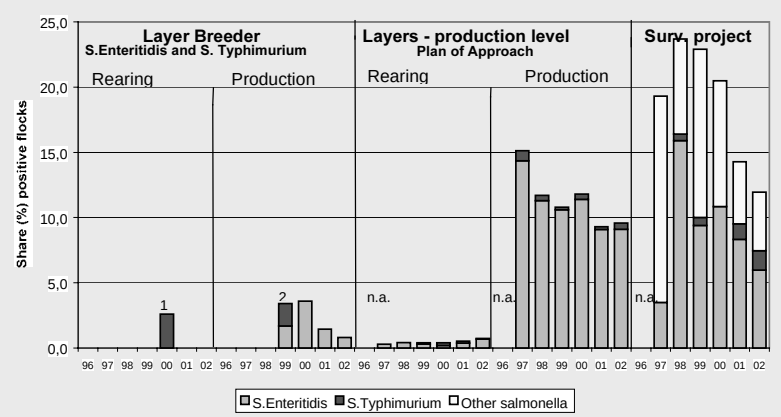
Approved control programme II France



11

Slide 12

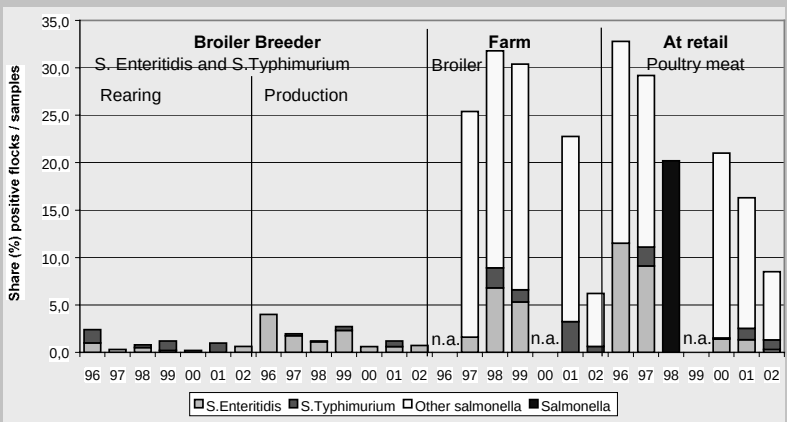
Approved control programme II The Netherlands



12

Slide 13

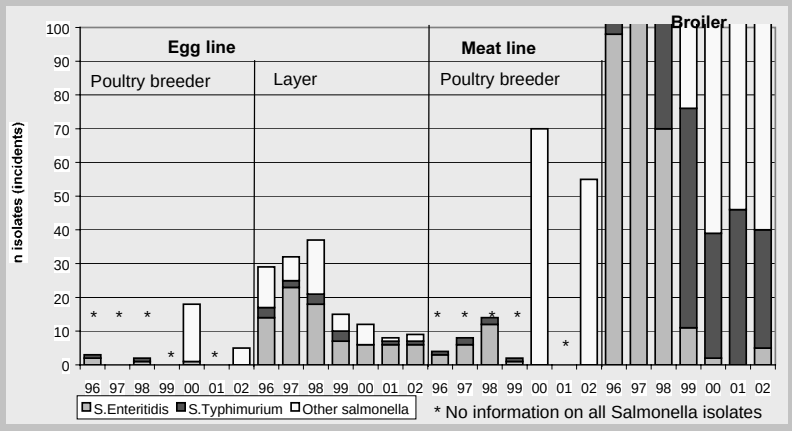
Approved control programme II The Netherlands



13

Slide 14

Monitoring programme acc Dir. Great Britain



14

Slide 15

Salmonella in eggs - UK studies

	Public Health investigation			London Public Health investigation		
Origin	N	Pos	% pos	N	Pos	% pos
Another Member State	468	24	5,1	45	0	0
UK (not Lion Quality mark)	74	1	1,3	200	1	0,5
UK (Lion Quality mark)	29	0	0	341	0	0
Country of origin unknown	60	11	18	140	6	4,28
US	60	0	0	0	0	0
Total	691	36	5,2	726	7	0,96

15

Slide 16

Salmonella in fattening pigs

At the farm: before slaughter

- Type of sample: Faecal samples : DK , NL, S
- Sample size: 1 - 60 samples

At slaughterhouse

- Type of sample: Meat juice: DK
  Lymph nodes: FIN, S, N
  Carcass swabs: B, DK, FIN, N, S,

At cutting / processing plant: B, FIN, IRL, N, S

At retail: B,D,DK,IRL,NL,S,GB

16

Slide 17

Salmonella in cattle and beef

At the farm:

- Type of sample: Faecal samples : NL
  Blood samples: DK (S.Dublin, S.Typhim.)

At slaughterhouse

- Type of sample: Faecal samples: DK
  Lymph nodes: FIN, S, N
  Carcass swabs: B, DK, FIN, N, S

At cutting / processing plant: B, FIN, N, S

At retail: B,D,DK,IRL,NL,S,UK

17

Slide 18

Salmonella in pigs

- Favourable situation: S, FIN, N
  - lymphnode samples 0.09 - 0.15%
  - carcass swabs 0.00 - 0.08%
- Varying rates in other countries - pigs
  - NL: 29.9% faecal sampling at farm level
  - DK: 3.2% level 2 or 3 by meat juice monitoring
  - D: 5.8% animals positive by meat juice ELISA
  - B: 15.4% carcass swabs
  - DK: 1.4% carcass swabs (4.1% pooled swabs)

18

Slide 19

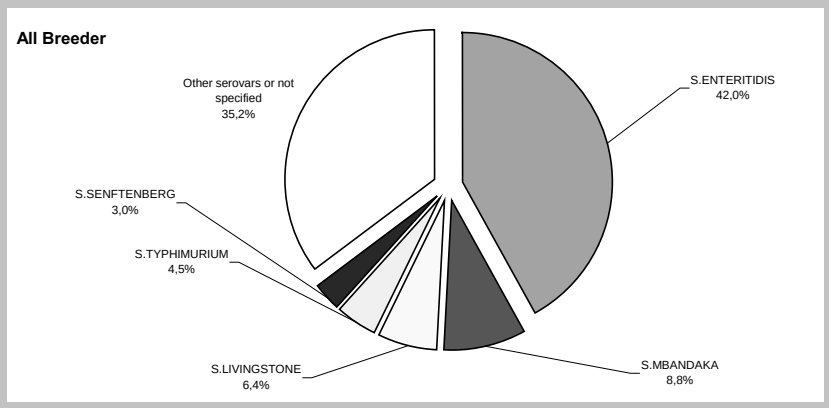
Salmonella in cattle

- Favourable situation: S, FIN, N
  - lymphnode samples 0.00 - 0.06%
  - carcass swabs 0.00 - 0.03%
- Varying rates in other countries - cattle
  - DK: 3.6% by bacteriological methods, farm level
  - NL: 5.6% by bacteriological methods, farm level
  - DK: 0.6% carcass swabs
  - B: 0.0% carcass swabs
- Contamination rate of beef is lower compared to poultry meat and pork

19

Slide 20

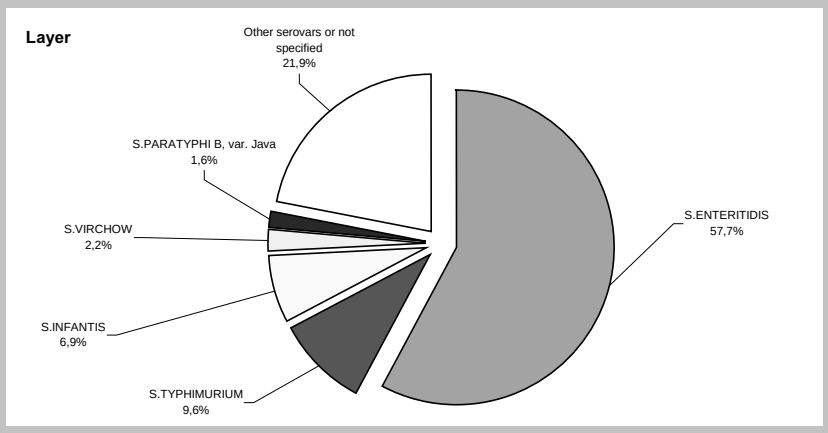
Five most frequent serovars - all breeder



20

Slide 21

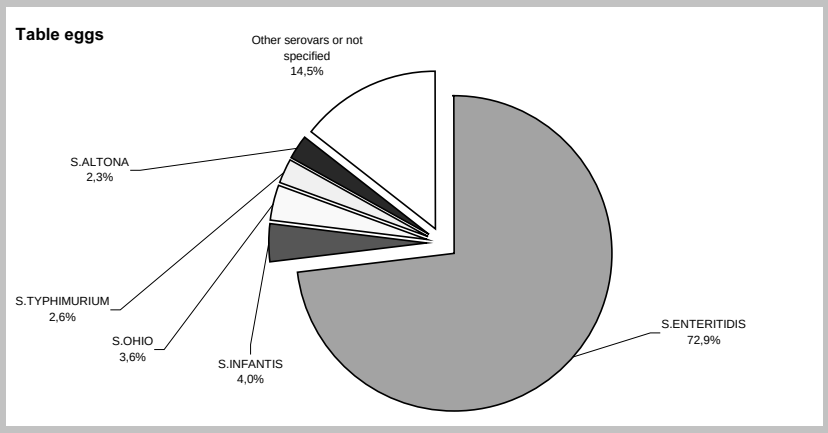
Five most frequent serovars - layers



21

Slide 22

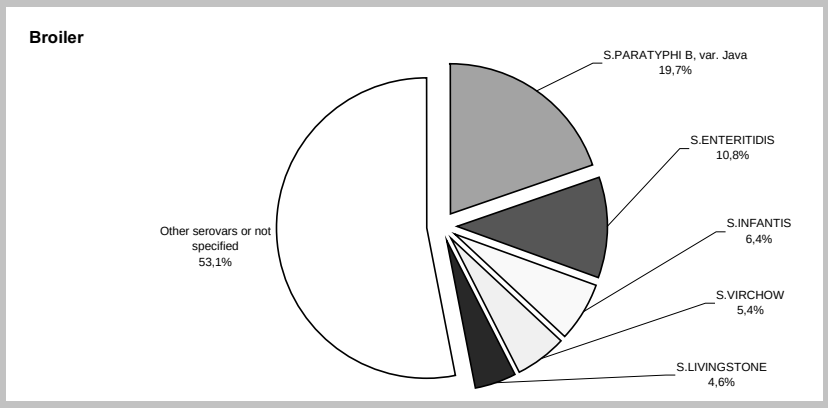
Five most frequent serovars - table eggs



22

Slide 23

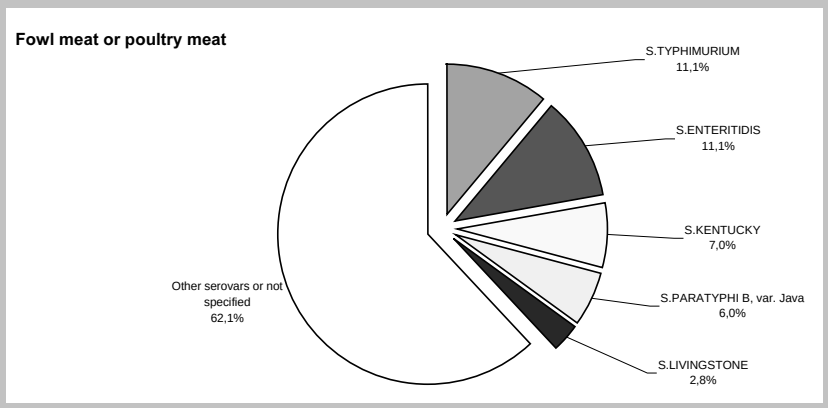
Five most frequent serovars - broilers



23

Slide 24

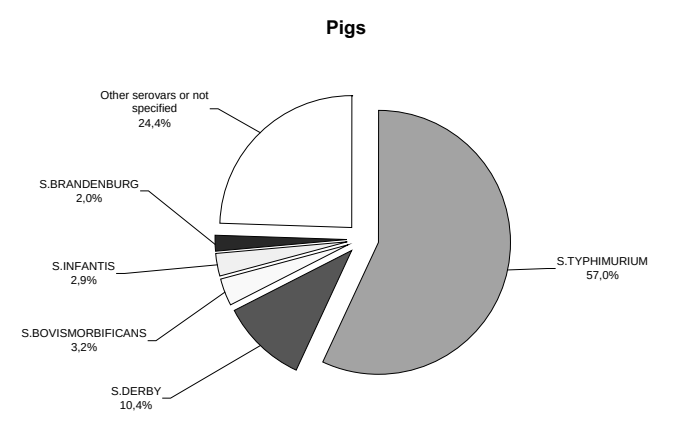
Five most frequent serovars - poultry meat



24

Slide 25

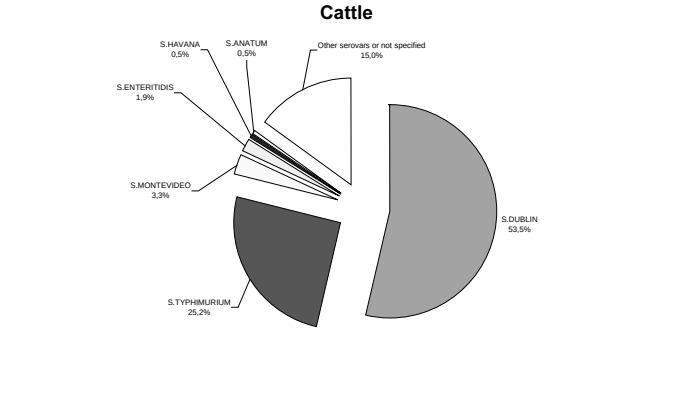
Five most frequent serovars - pigs



25

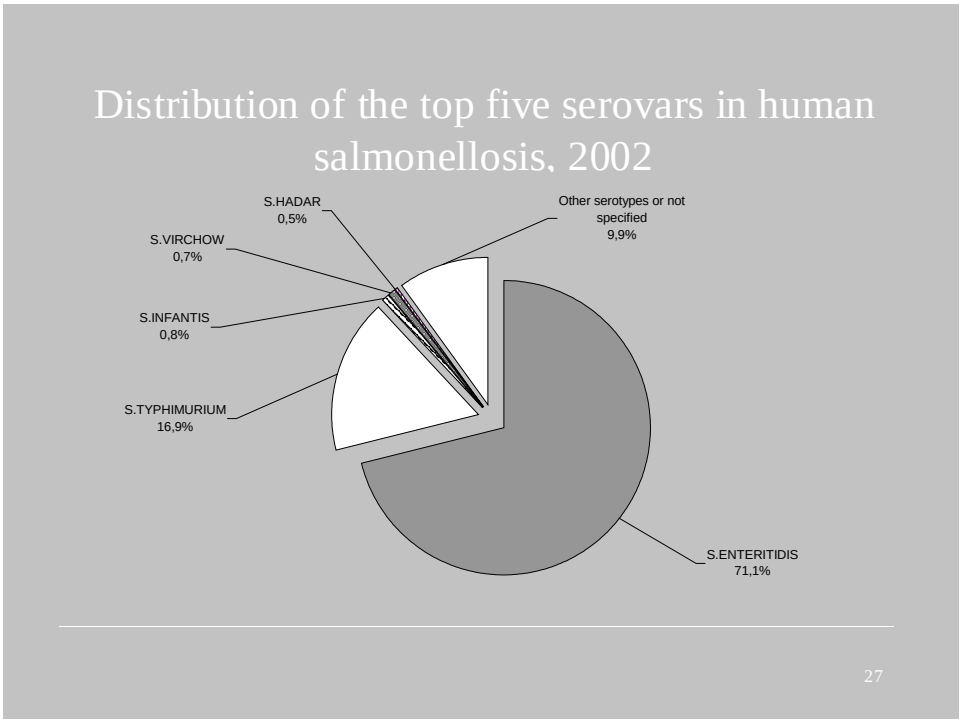
Slide 26

Five most frequent serovars - cattle

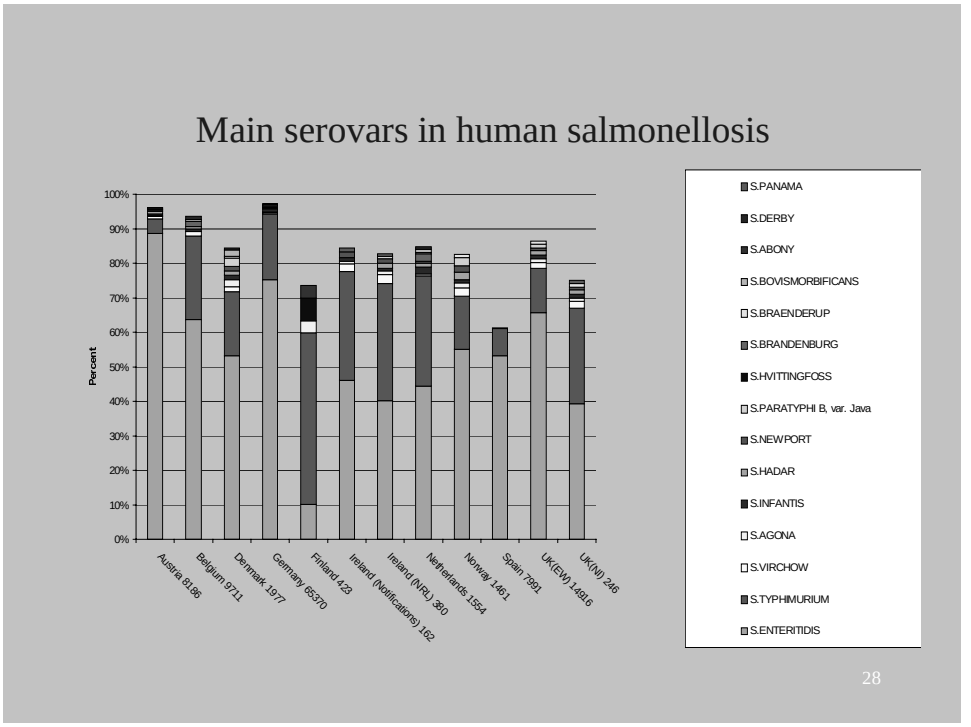


26

Slide 27

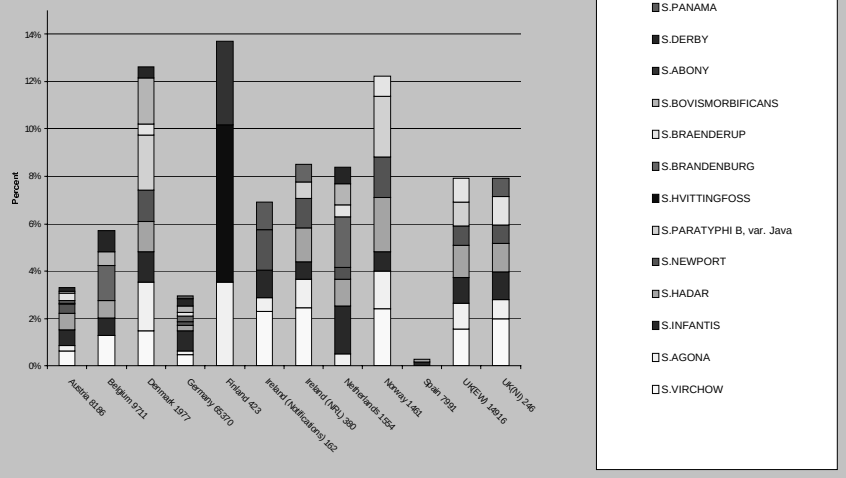


Slide 28



Slide 29

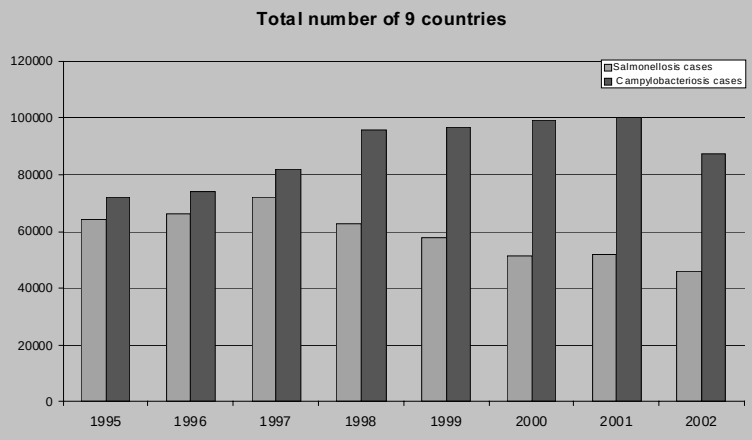
Main serovars in human salmonellosis



29

Slide 30

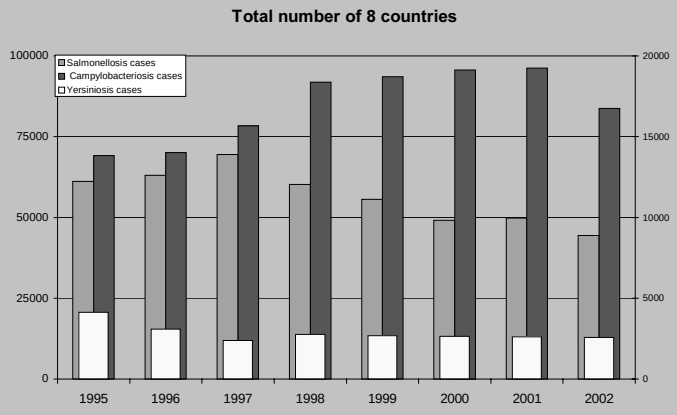
Trend in human salmonellosis and campylobacteriosis (9 MS)



30

Slide 31

The number of the different gastrointestinal cases over the years



31

Slide 32

Data on phagetyes of *S.*Enteritidis and *S.*Typhimurium

in 2002

32

Slide 33

Data basis
Sero- and phagetypes

- Mandatory
  - Serotypes
- all countries supplied the serotype distribution in different species

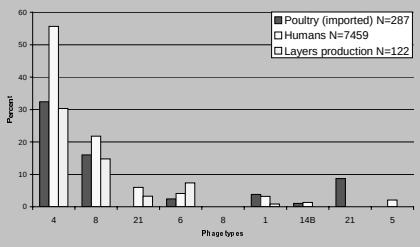
- Voluntary
  - Phagetypes
- 8 countries supplied data of the phagetypes
- 1 country delivered data of food isolates

33

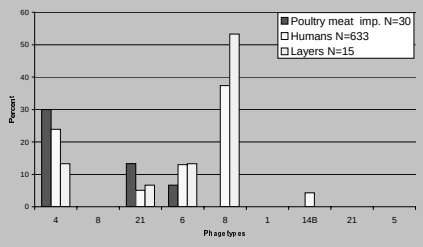
Slide 34

S. Enteritidis

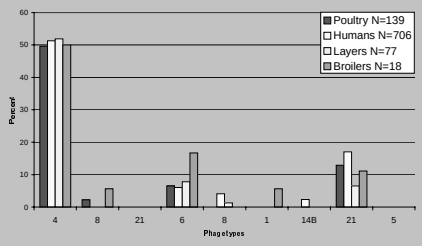
Austria *S.* Enteritidis



Denmark *S.* Enteritidis



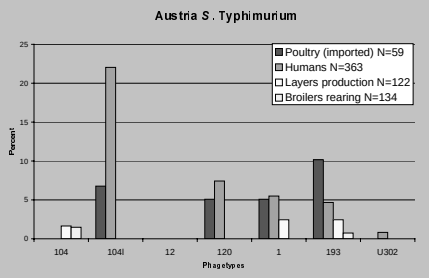
The Netherlands *S.* Enteritidis

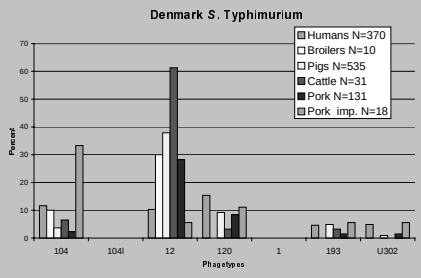


34

Slide 35

S. Typhimurium





35

Slide 36

Results - phagetypes

S. Enteritidis

- PT 4, PT 8, PT 21, PT1 and PT 6 are the main phagetypes in humans and also among the most frequent isolates in poultry

S. Typhimurium

- There is no common phagetype pattern in the countries
- All countries isolated DT 104

36

Slide 37

Antimicrobial resistance
in *Salmonella*
2002

37

Slide 38

Antibiotic resistance testing

- **Reporting countries**
  - 2000: 14 reports gaps: EL, L
  - 2001: 16 reports
  - 2002: 15 reports on *Salmonella*
    5 reports on *Campylobacter*

38

Slide 39

Antibiotic resistance testing

- Problem: Comparability of data
  - level of information on the source of the isolate
    - representiveness of the isolates
  - methods used
  - breakpoints used
  - antimicrobials tested

39

Slide 40

Antibiotic resistance testing

- Reporting the methods used
  - test method :
    - Agar diffusion 11 countries
    - Agar / Broth dilution 5 countries
  - testing standard used :
    - NCCLS 8 countries
    - VLA/ CASFM 1 /1
    - Microbiological Breakpoints 2 countries
    - Provider 1 country

40

Slide 41

Table AB 1. Test methods used for antibiotic resistance testing of Salmonella spp. 2002

Standard used for testing	Test method	Country	used for	further	Is the testing procedure subject of quality control
Salmonella					
NCCLS	Agar diffusion	Austria	Animal and food		yes
		Denmark	2 Humans	Used until 31 August	yes
		Finland	Animal and food		yes
		Greece	Animal and food		yes
		Italy	Animal and food		yes
		Portugal	Animal and food		yes
		Spain	1 Pigs		yes
		Spain	2 Poultry (Gallus		
NCCLS	Broth dilution	Denmark	3 Humans	since 1 September 2002	yes
		Denmark	1 Animal and food		yes
		Spain	3 Other animals		
		The Netherlands	Animal and food		yes
CASFM	Agar diffusion	France	2 Cattle	RESSAB and	yes
		France	1 Animal and food		yes
Microbiological Breakpoints	Broth dilution	Norway	Animal and food		yes
		Sweden	Animal and food		yes
n.a.	Agar diffusion	Luxembourg	Animal and food		no
Provider	Agar diffusion	Belgium	Animal and food	Rosco, Neo Sensitet	yes
VLA	Agar diffusion	England and Wales	Animal and food	adapted from BSAC 1991	yes

41

Slide 42

Antibiotic resistance testing

- **Monitoring frame**
  - 3 main species of food animals
    Countries reporting:

	cattle	pigs	poultry
- 2000:	11	12	11
- 2001:	13	12	12
- 2002:	13	14	12

42

Slide 43

Antibiotic resistance testing

- **Monitoring frame**
  - 5 most important Salmonella serotypes: S.Enteritidis / S.Typhimurium

- 2000:	6 countries	8 countries
- 2001:	6 countries	13/ 14 countries
- 2002:	5 countries	14/ 12 countries

43

Slide 44

Number of isolates tested in 2002

	>100	< 100
Austria	1042	
Belgium	230	
Denmark	4011	
Spain		70
Finland		65
France	1692	
Greece		43
Ireland	181	
Italy	1986	
The Netherlands	353	
Sweden		36
United Kingdom	3425	
England and Wales	2760	
Northern Ireland	600	
Norway		5
Number of countries	10	5

44

Slide 45

Number of strains investigated along the foodchain

Animals	A	B	DK	E	FIN	I	N	NL	S	UK
Cattle	9	35	223		24	270	1	22	17	862
Pigs	67	99	2237	70	13	1506	4	38	16	309
Poultry			51							1580
Poultry (gallus gallus)	1325	83			25	1340		10	9	
Broilers			650							
Turkey	340				3	904				
Food										
Beef			151							
Pork			96							
Broiler meat			214							
Turkey meat			37							
Feed										
Feed										206

45

Slide 46

Antibiotic resistance testing

Antimicrobials investigated

		Cattle	Pigs	Poultry
Tetracycline	Tetracycline	13	14	12
Chloramphenicol	Chloramphenicol	12	12	11
Florfenicol	Florfenicol	6	5	4
ß-Lactam	Ampicillin	11	11	11
Cephalosporins				1
	Cephalothin CEP	2	2	2
	Cefotaxime CTX	5	5	6
	Ceftazidime CAZ	2	3	2
	Cefoperazone CFP	1	1	1
	Ceftiofur TIO	4	3	2
	Cefuroxime CXM	0	0	1
	Cefquinome CQN	1	0	0
	Ceftriaxone CRO	0	0	2
Fluoroquinolones	Ciprofloxacin	9	10	9
Quinolones	Nalidixic acid	12	13	11
Sulfonamides	Trimethoprim / Sulfonamide	8	10	9
	Trimethoprim	7	7	6
	Sulfonamide	9	10	11
Aminoglycosides	Streptomycin	11	12	10
	Gentamicin	8	11	11
	Neomycin	9	7	8
	Kanamycin	5	7	6

Ranking

	Cattle	Pigs	Poultry
Tetracycline	13	14	12
Chloramphenicol	12	12	11
Nalidixic acid	12	13	11
Ampicillin	11	11	11
Sulfonamide	9	10	11
Gentamicin	8	11	11
Streptomycin	11	12	10
Ciprofloxacin	9	10	9
Trimethoprim / Sulfonamide	8	10	9
Neomycin	9	7	8
Trimethoprim	7	7	6
Cefotaxime	5	5	6
Kanamycin	5	7	6
Florfenicol	6	5	4
Ceftiofur TIO	4	3	2
Cephalothin	2	2	2
Ceftazidime	2	3	2
Ceftriaxone CRO	0	0	2
Cefoperazone	1	1	1
Cefuroxime	0	0	1
Cefquinome	1	0	1 0

46

Slide 47

Antibiotic resistance *Salmonella*

mainly *S.*Typhimurium

	All	Cattle	Pigs	Poultry	Humans
common	TET AMP STR SU	TET CHL FLR AMP SU STR	TET CHL SU STR SXT TMP	TET AMP NAL SU STR	TET AMP STR SU
low		CE			

Exception: Fin, S, N have a very low resistance rate

Tetracycline (TET), Chloramphenicol (CHL), Florfenicol (FLR), Ampicillin (AMP), Nalidixic acid or other quinolone (NAL), Sulfonamide/Trimethoprim (SXT), Trimethoprim (TMP), Sulfonamides (SU), Cephalosporin (CE)

47

Slide 48

- The whole Report 2002 will be available soon at the webpage of the Commision
- The Report 2001 is available as a pdf version

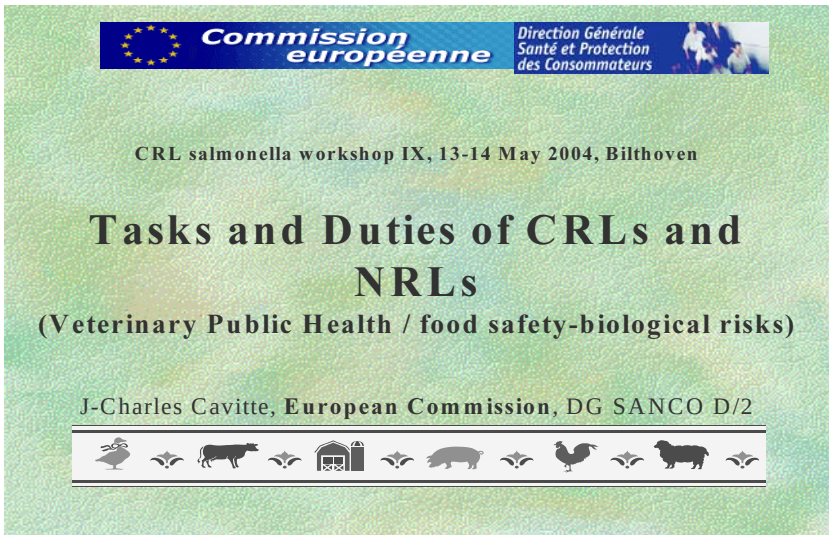
http://europa.eu.int/comm/food/food/biosafety/single chapters

Thank you!

48

Annex 16. Slides of presentation 2.2

Slide 1



Slide 2

Slide 3

Slide 4

Commission européenne
Direction Générale Santé et Protection des Consommateurs

Tasks for CRLs biological risks:

depend +/- on legislation

- ***provide NRLs with details of analytical methods (5/6)***
- ***coordinate application of methods by NRLs, by organising comparative testing in particular (5/6)***
- *co-ordinate research on new methods (4/6, +TSE)*
- *conduct training for NRLs (6/6)*
- *assistance to E Commission (4/6)*
- *cooperate with labs in third countries (2/6)*
- *help NRLs implement QA (2/6)*

Slide 5

Commission européenne
Direction Générale Santé et Protection des Consommateurs

Tasks of NRLs biological risks:

depend +/- on specific legislation

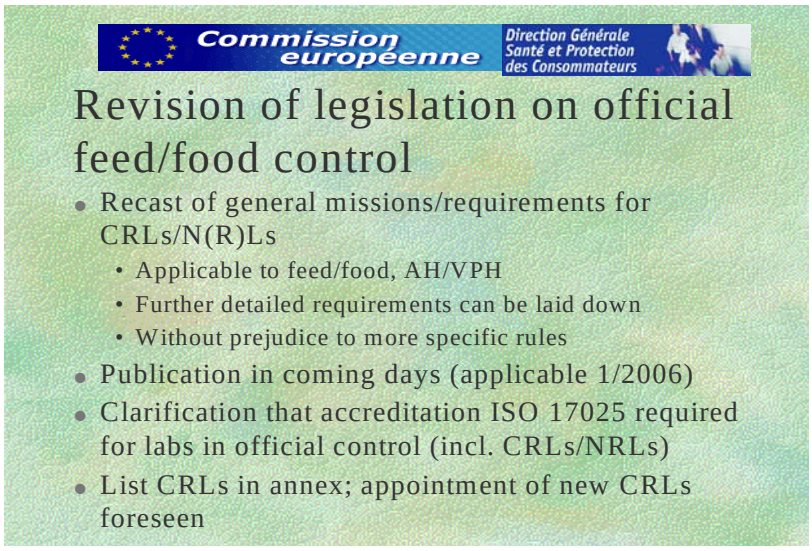
- ***co-ordination activities of NLs (4/6)***
- *assisting the national competent authorities (3/6)*
- ***organising on regular basis comparative tests between NLs (4/6)***
- *disseminating information supplied by the CRLs to authorities and NLs (3/6)*
- *collaboration with CRLs (3/6)*
- *...*

Slide 6

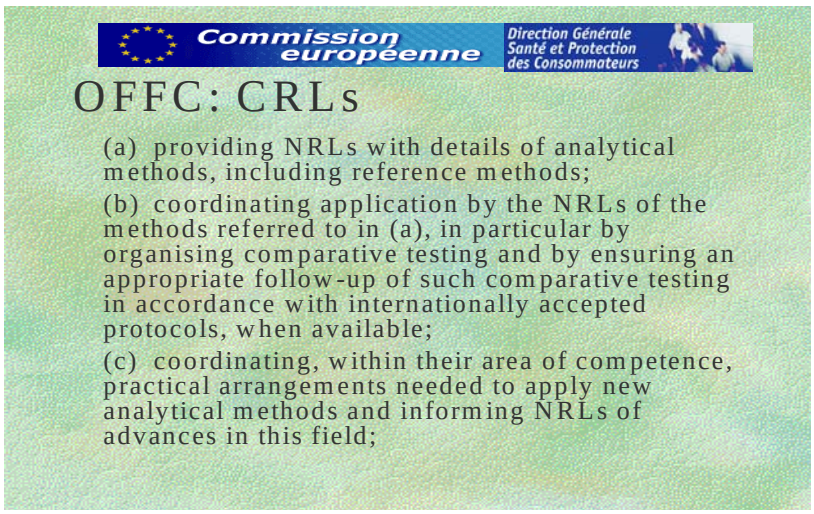
Commission européenne
Direction Générale Santé et Protection des Consommateurs

- Proficiency tests :
  - a core mission for CRLs;
  - most CRLs organise PTs regularly; CRLs usually produce RMs for their PTs
  - yearly workshops/meeting take place to discuss results and stress on particular issues;
  - unclear to what extent NRLs organise PTs periodically
- No precise overview on how NRLs perform their tasks

Slide 7



Slide 8

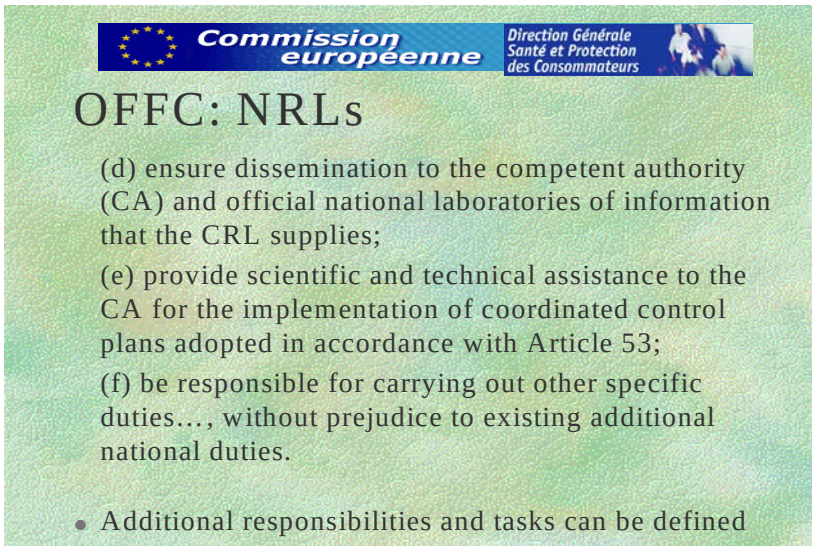


Slide 9

Slide 10

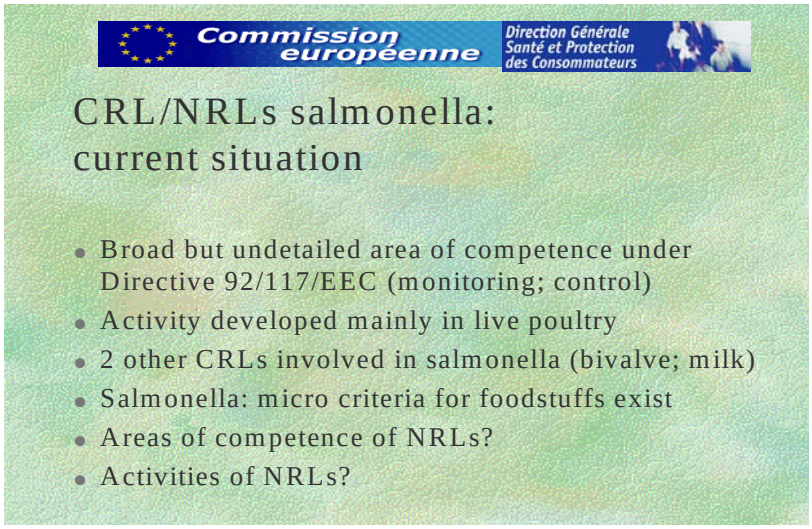
Slide 11

Slide 12



Slide 13

Slide 14

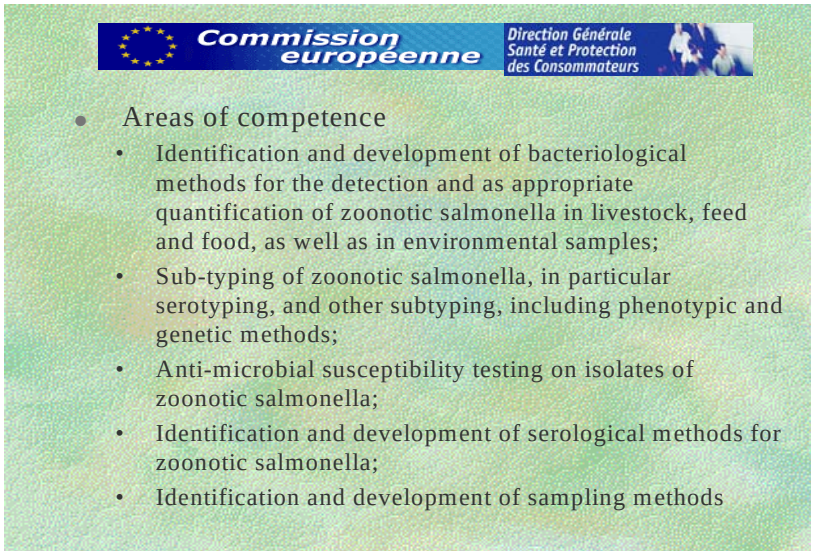


Slide 15

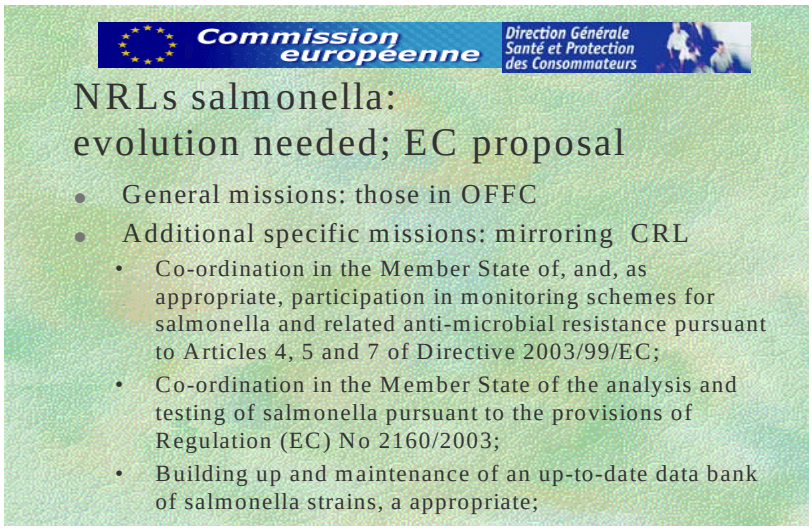
Slide 16

Slide 17

Slide 18



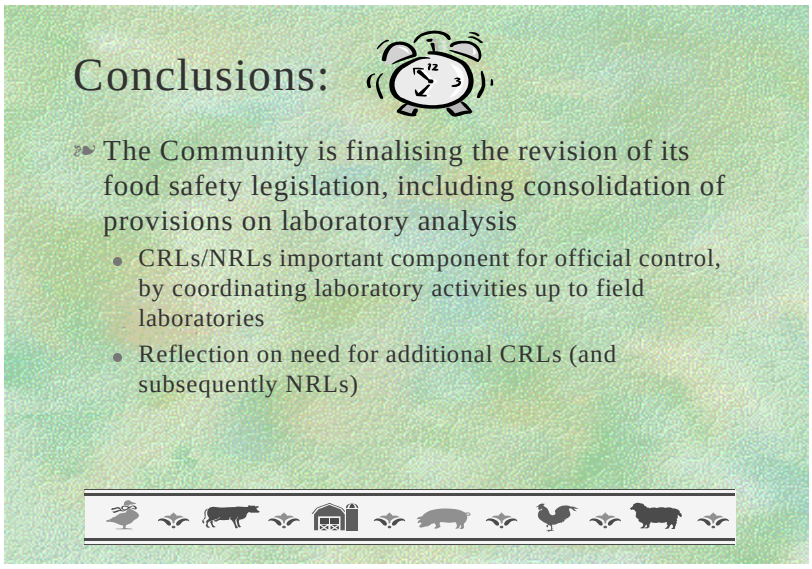
Slide 19



Slide 20

Slide 21

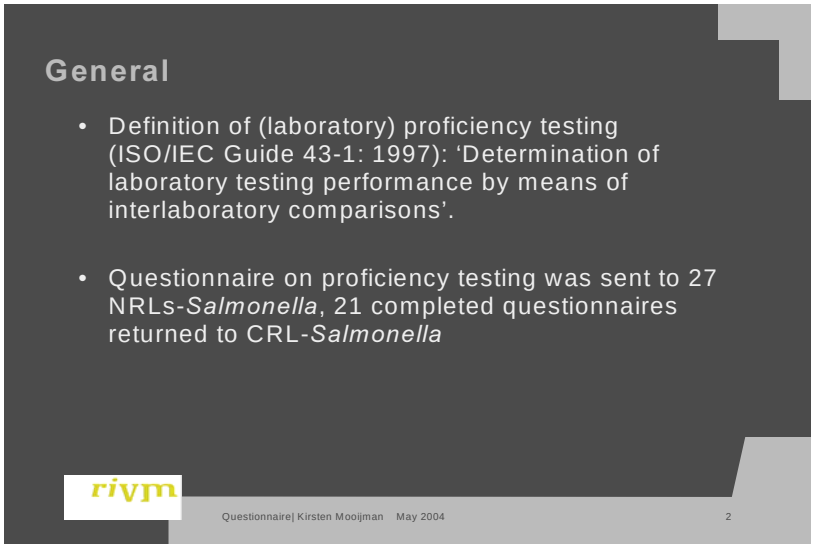
Slide 22


Conclusions:
The Community is finalising the revision of its food safety legislation, including consolidation of provisions on laboratory analysis
CRLs/NRLs important component for official control, by coordinating laboratory activities up to field laboratories
Reflection on need for additional CRLs (and subsequently NRLs)

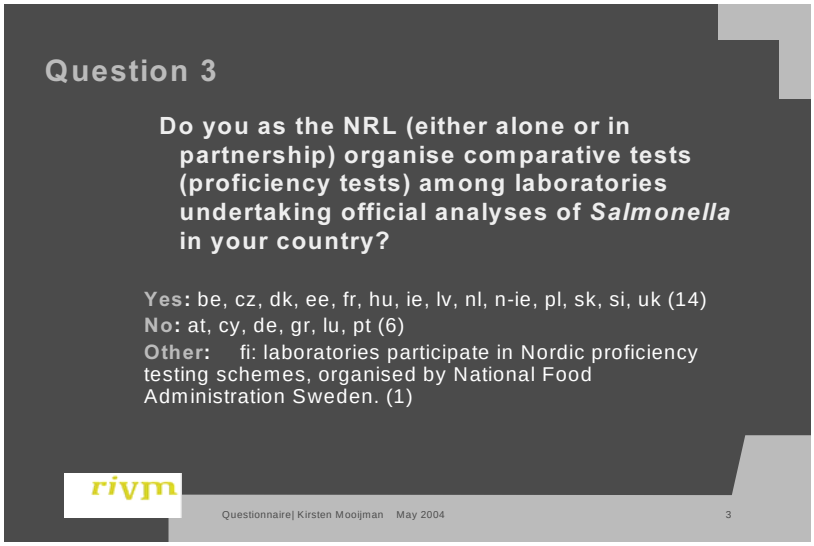
Annex 17. Slides of presentation 2.3

Slide 1

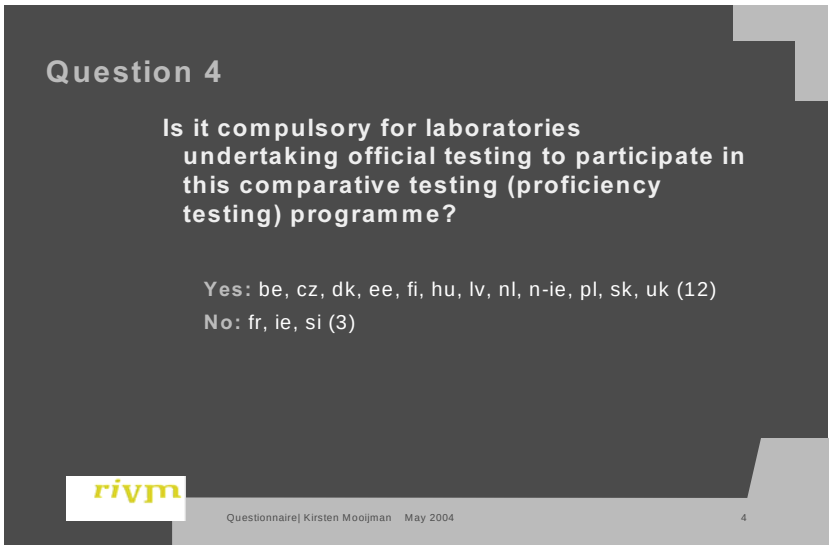
Slide 2



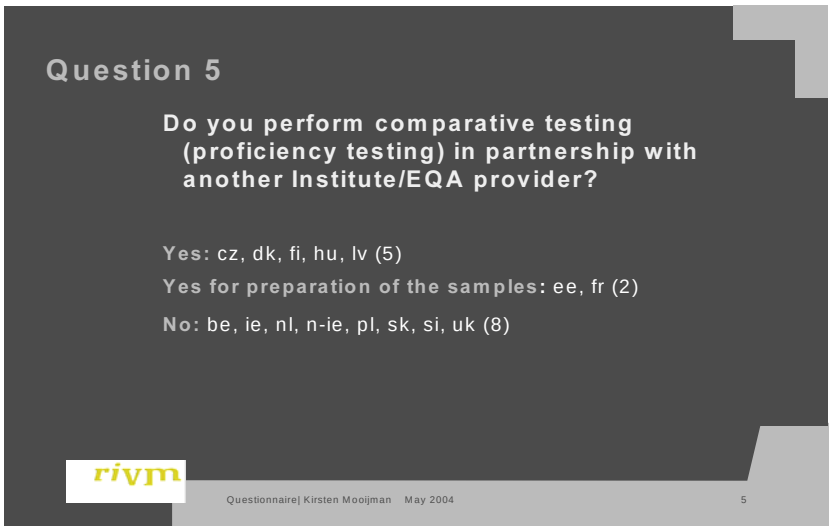
Slide 3



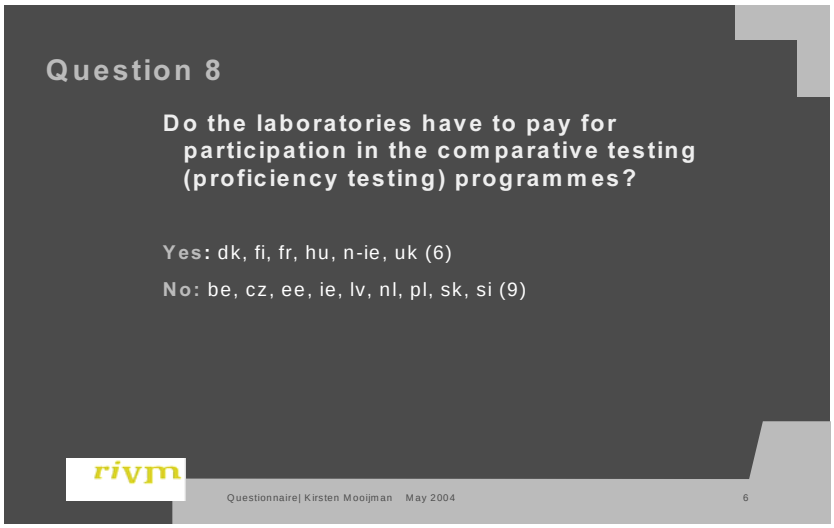
Slide 4



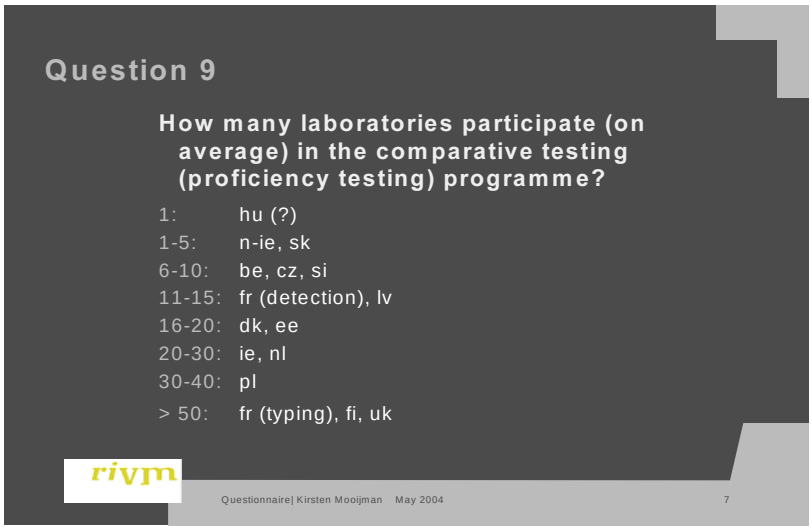
Slide 5



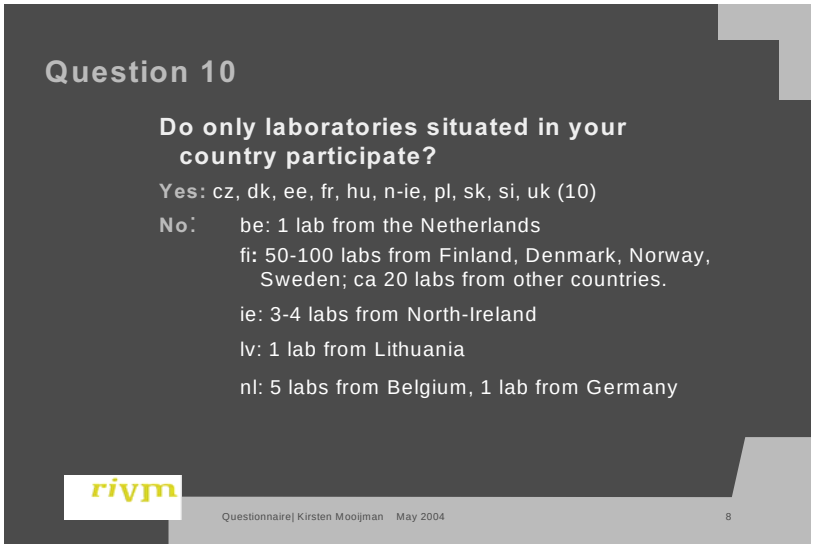
Slide 6



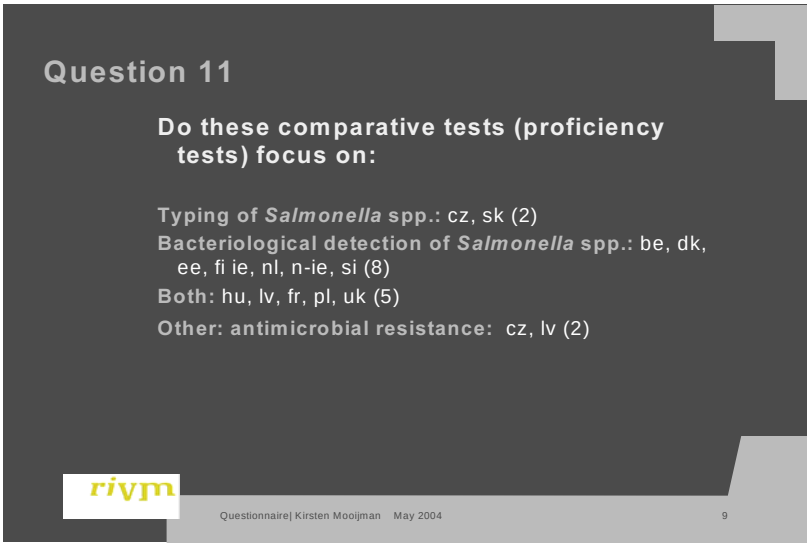
Slide 7



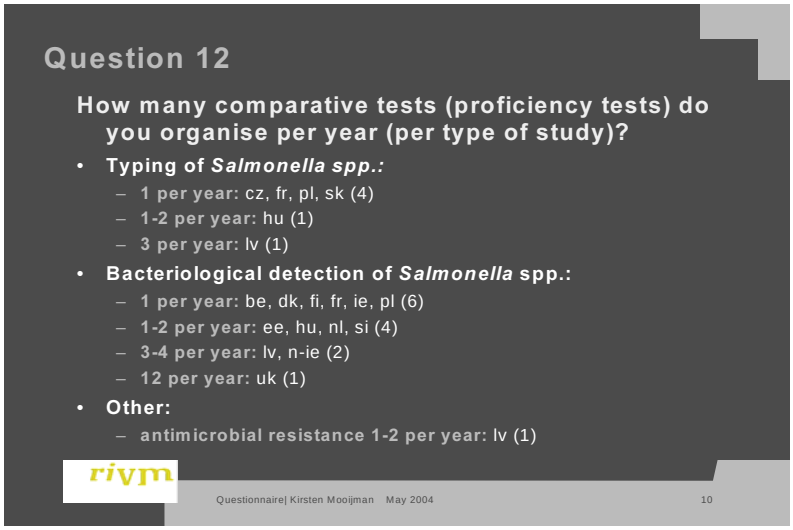
Slide 8



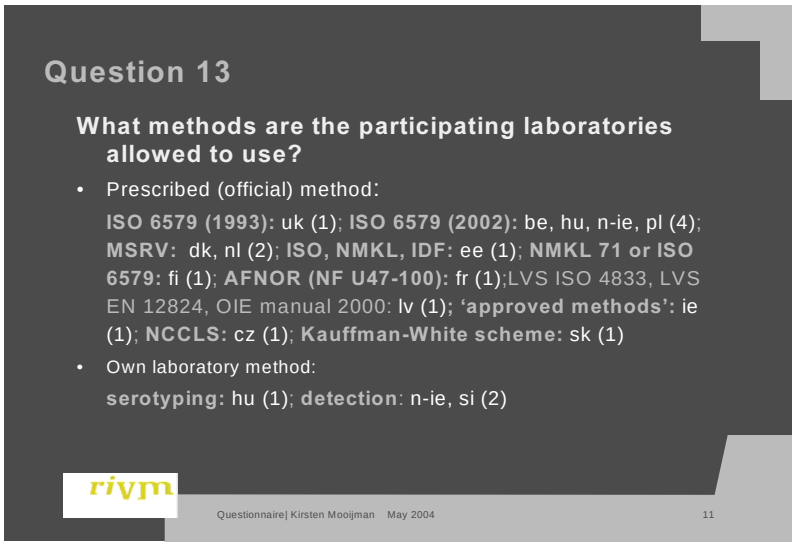
Slide 9



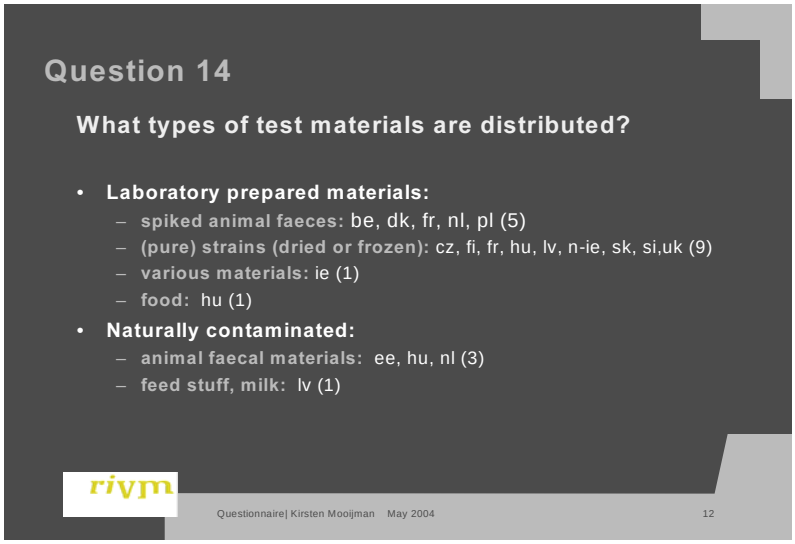
Slide 10



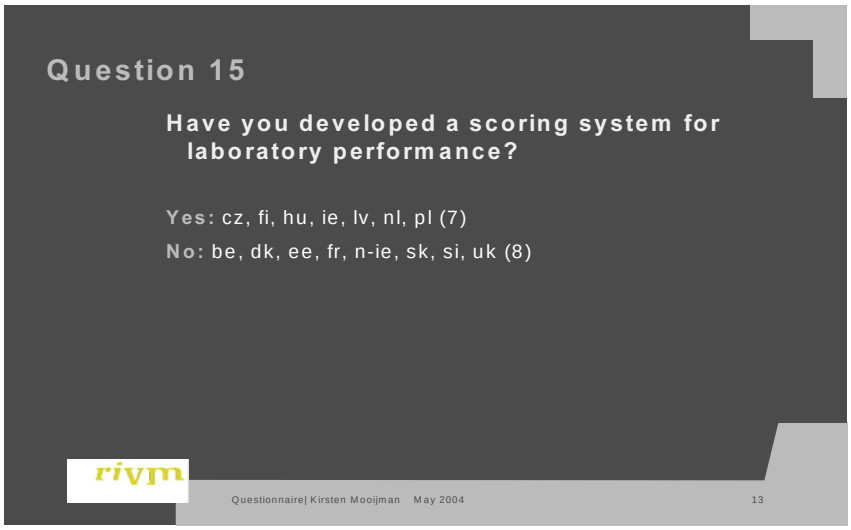
Slide 11



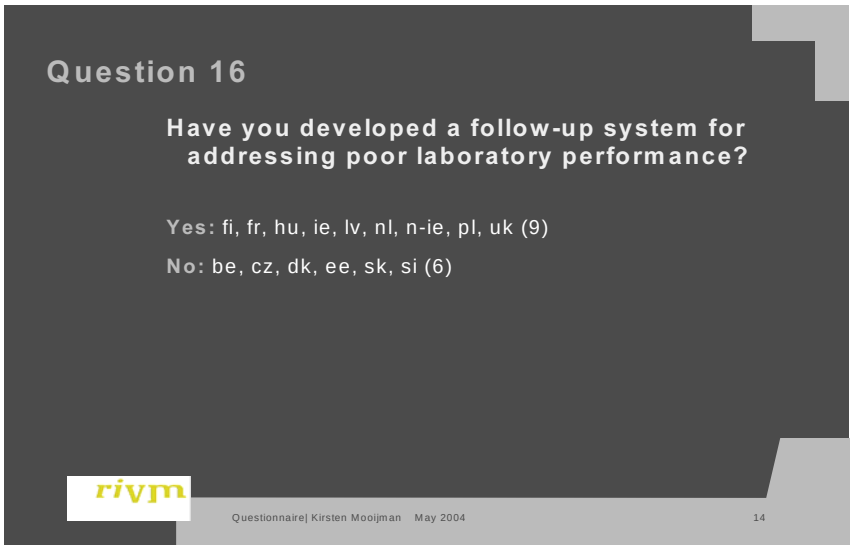
Slide 12



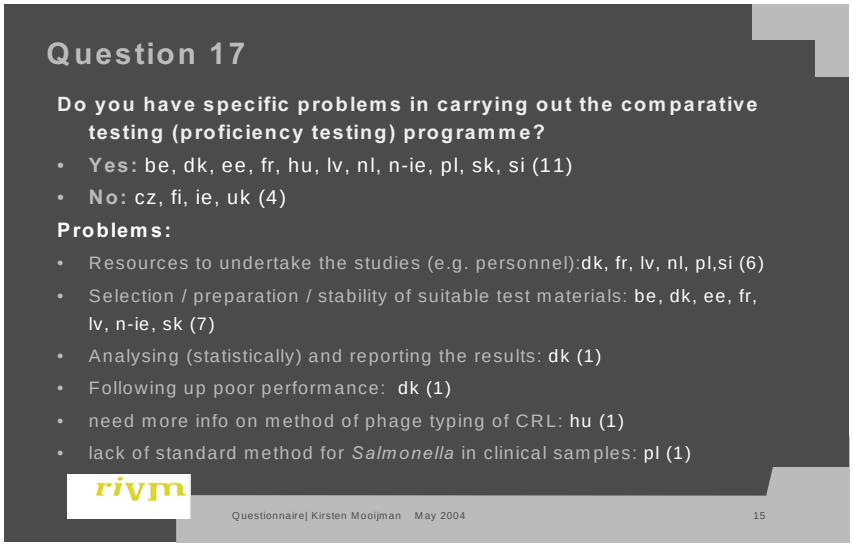
Slide 13



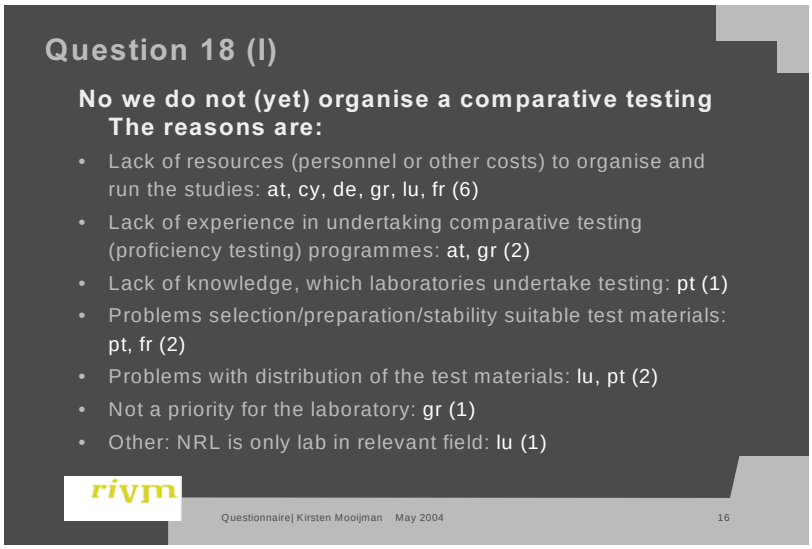
Slide 14



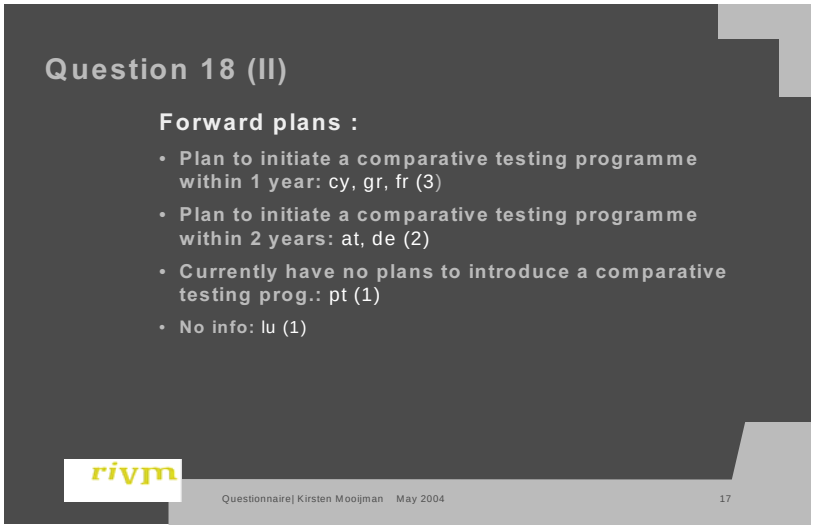
Slide 15



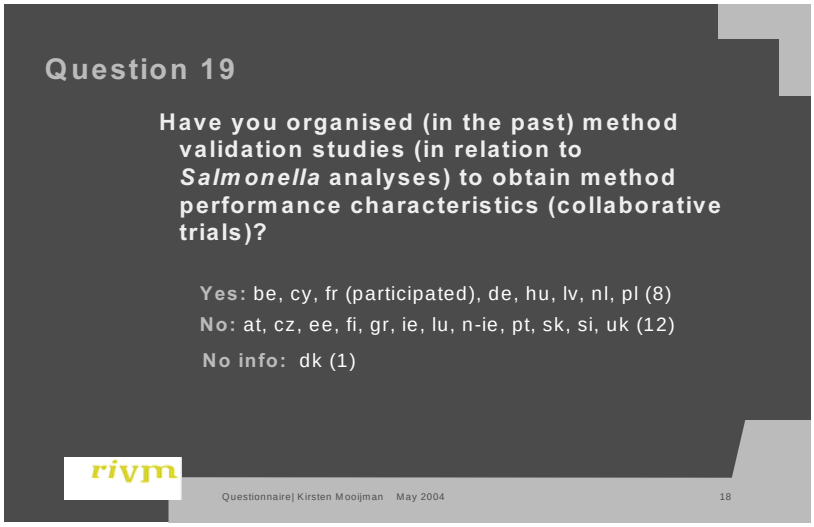
Slide 16



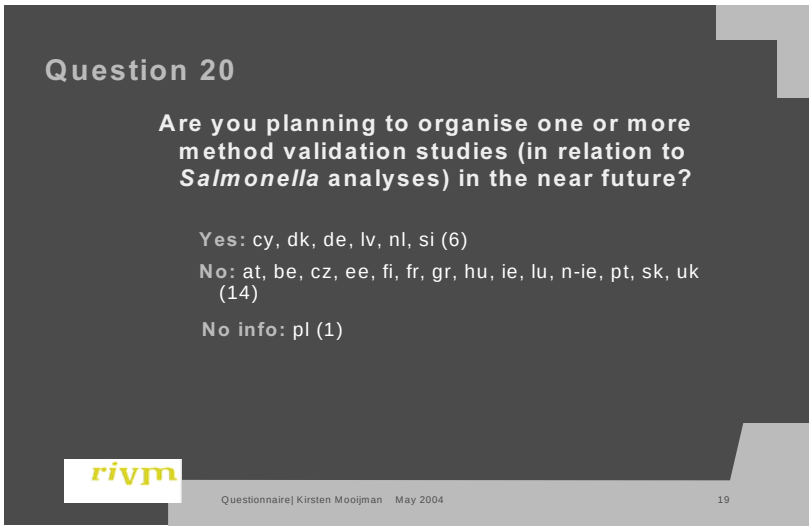
Slide 17



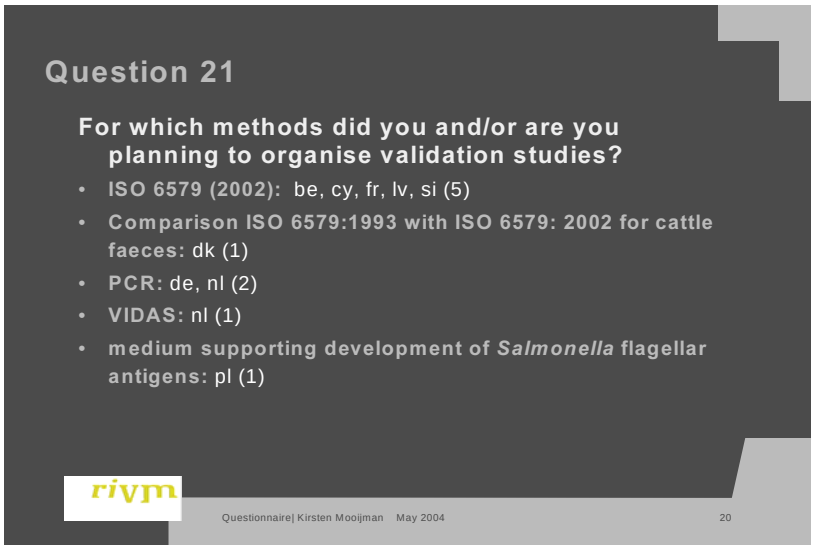
Slide 18



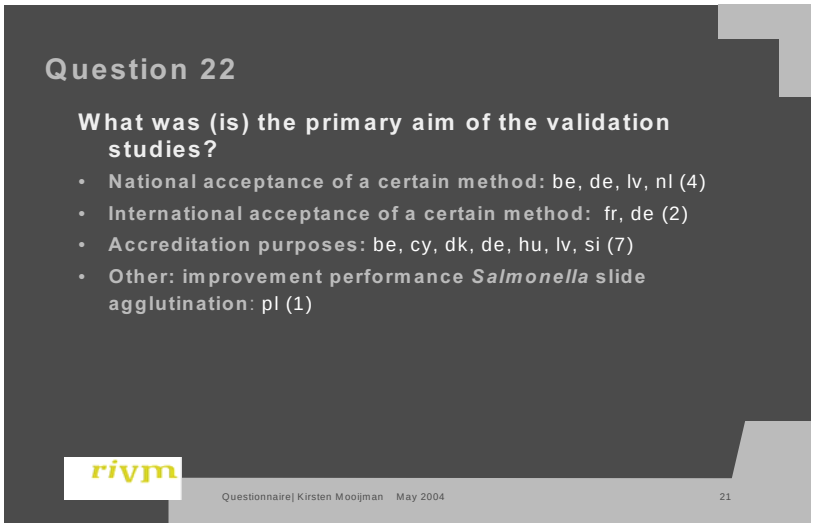
Slide 19



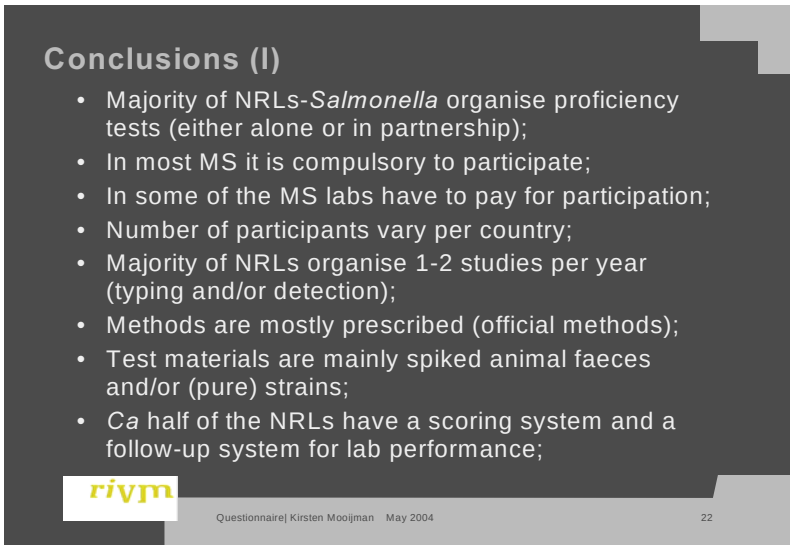
Slide 20



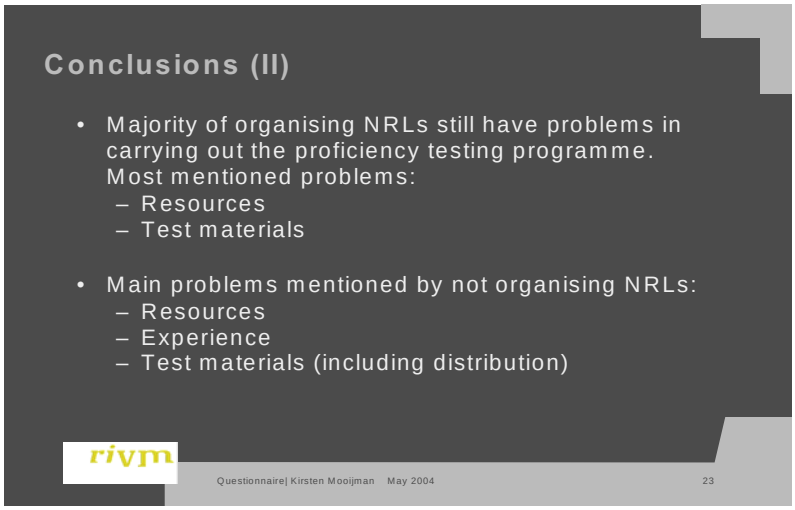
Slide 21



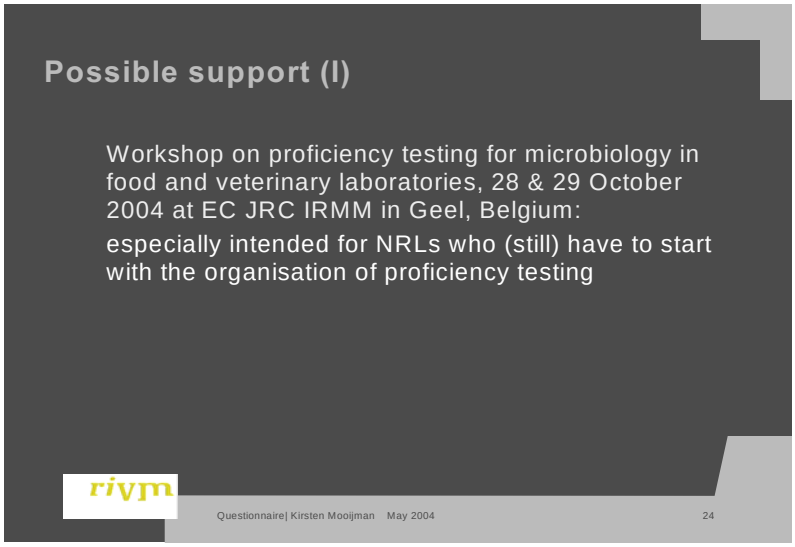
Slide 22



Slide 23



Slide 24



Slide 25

Possible support (II)

Availability of reference materials (lenticules) at Health Protection Agency (HPA) in Newcastle, UK:

- Price is *ca* 75 Euro (50 pound) per 25 lenticules (discount for large orders).
- The following can be discussed with HPA:
  - Number of lenticules required per distribution per year;
  - The strains involved and the target levels;
  - Need of single strains or mixtures per lenticule.
- Contact at HPA Newcastle: Danka Tharagonnet: danka.tharagonnet@hpa.org.uk

rivm

Questionnaire| Kirsten Mooijman May 2004 25

Slide 26

Reference materials

rivm

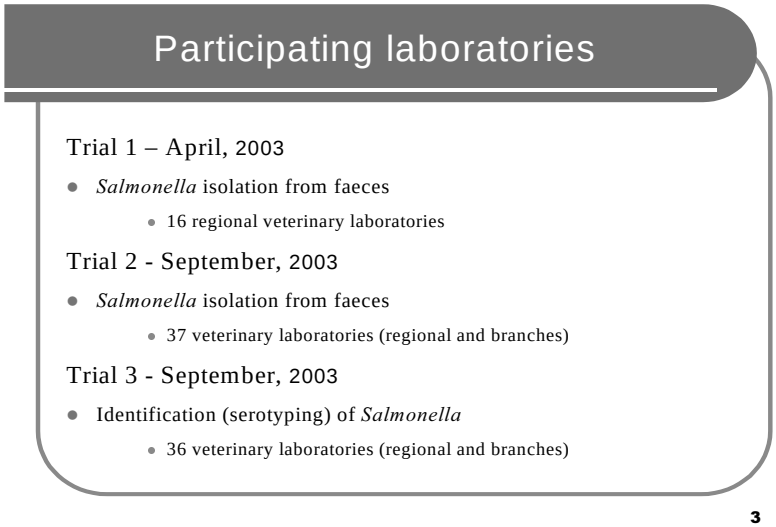
Questionnaire| Kirsten Mooijman May 2004 26

Annex 18. Slides of presentation 2.4

Slide 1

Slide 2

Slide 3



Slide 4

Interlaboratory proficiency tests

Objectives:

- To evaluate of diagnostic efficacy of regional veterinary laboratories on *Salmonella* isolation from samples of animal origin (faeces)
- To collect and analyse information concerning methods used by them

4

Slide 5

Trial 1

Salmonella isolation from faeces, trial 1 (April, 2003)

5

Slide 6

Trial 1

Materials and methods, trial 1(April 2003)

- 5 vials of lyophilised faeces
- 1g faeces (3 x 10^4 CFU of physiological flora)
- 4 artificially contaminated samples by *S.* Typhimurium at three levels:
  - 5 x 10^6CFU/sample – 1 sample
  - 3 x 10^3CFU/sample – 1 sample
  - 3 x 10^2CFU/sample – 2 samples
- 1 „blank” sample
- All samples were coded

6

Slide 7

Trial 1

Preparation of samples

- Salmonella-free bovine faeces
- 1:1 dilution with Skim Milk
- Dispersed into vials – ca. 1g
- Lyophilisation of blank samples
- Spiked samples:
  - Standardisation of S. Typhimurium density in saline solution
  - Inoculation of samples
- Lyophilisation of spiked samples
- Determination of the total number of Salmonella and competitive bacteria (decimal dilution method)

7

Slide 8

Trial 1

Transport samples and time of delivery results to NRL – *Salmonella* trial 1(April 2003)

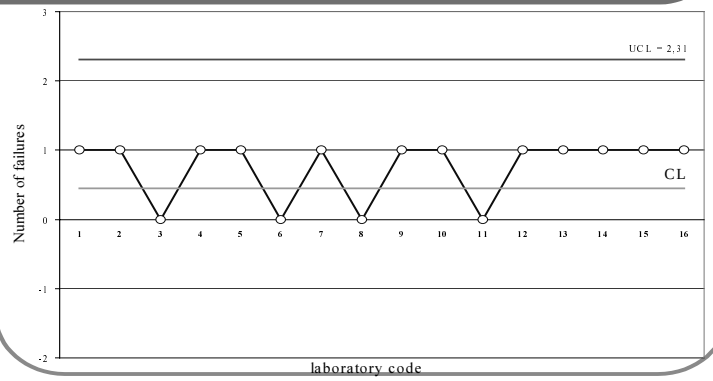
- All samples had been packed and transported as a dangerous goods
- Date of shipment : first week of April, 2003
- Results expected 10 days after arrival
- 13 laboratories sent results on time
- 3 laboratories sent results with 3 – 5 day delay

8

Slide 9

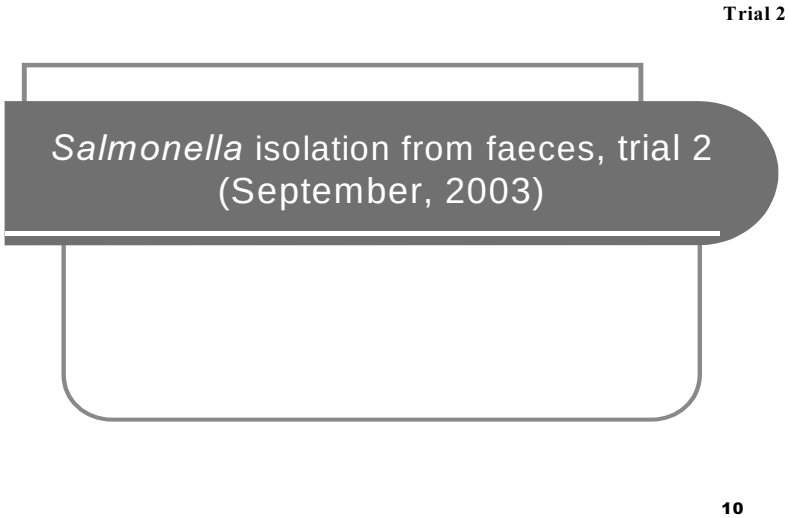
Trial 1

Proficiency of laboratories in the scope of *Salmonella* isolation (Trial 1, April. 2003)

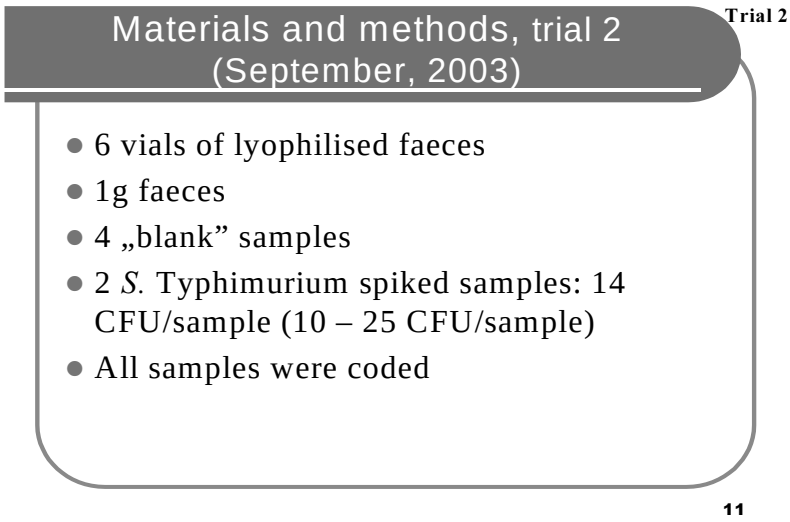


9

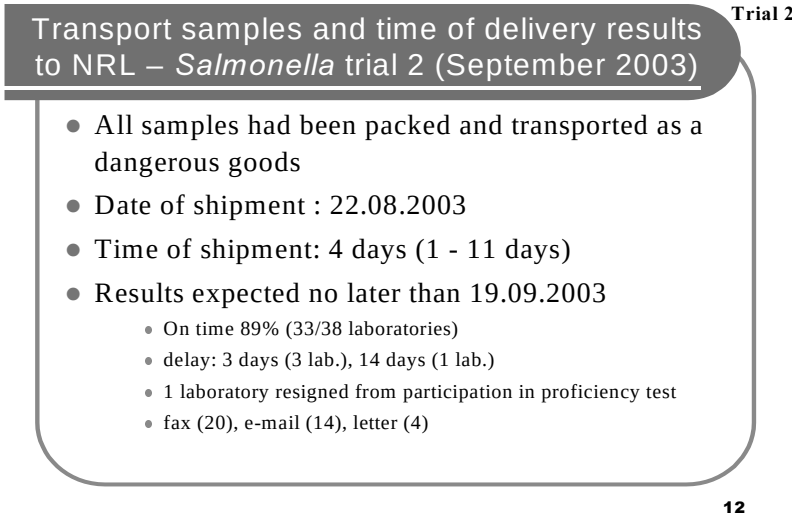
Slide 10



Slide 11



Slide 12



Slide 13

Trial 2

Isolation methods

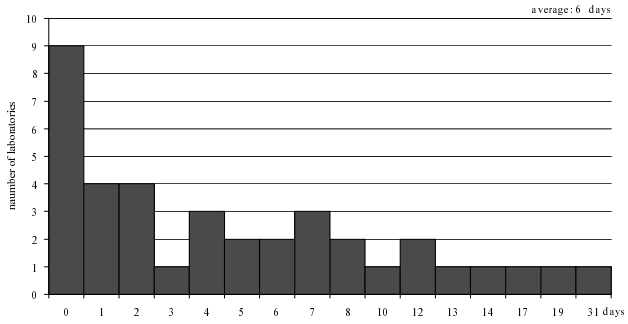
- Lab name,
- Date of the arrival, testing etc.
- Obtained results
- Method used
  - Media and reagents
  - incubation parameters

13

Slide 14

Trial 2

Starting time

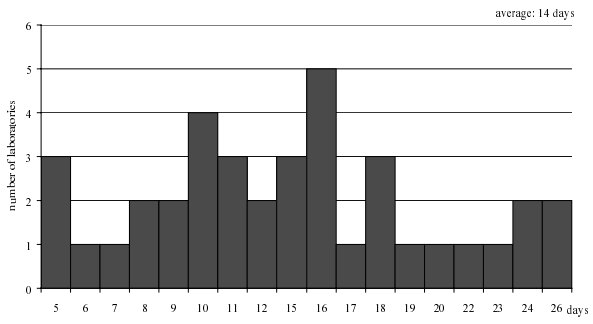


14

Slide 15

Trial 2

Testing time

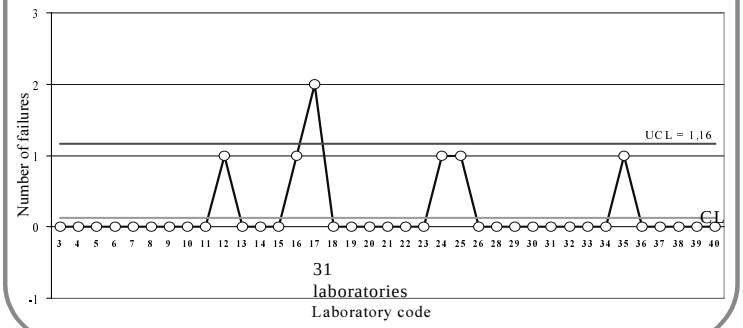


15

Slide 16

Trial 2

Proficiency of laboratories in the scope of *Salmonella* isolation (Trial 2, Sept. 2003)



16

Slide 17

Trial 2

Number (%) of correct results

	Positive samples	Negative samples	Total
Trial 1	60/60 (100%)	4/16 (25%)	64/76 (84%)
Trial 2	74/74 (100%)	141/148 (95%)	215/222 (97%)

17

Slide 18

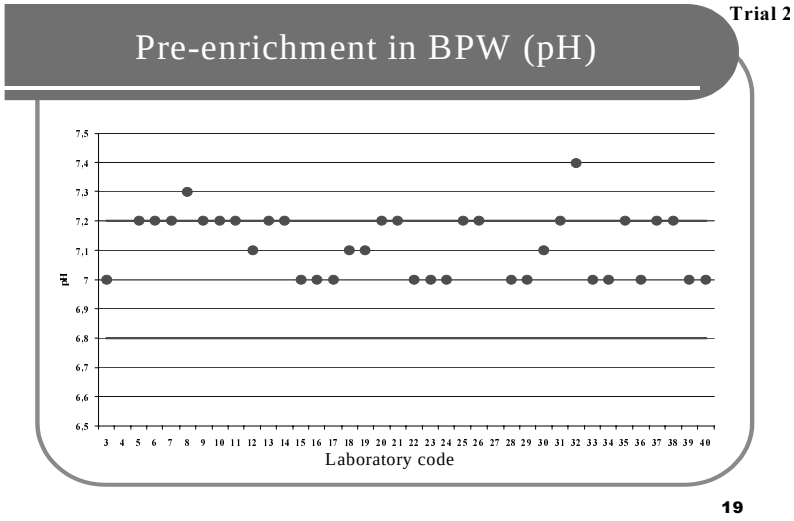
Trial 2

Isolation methods

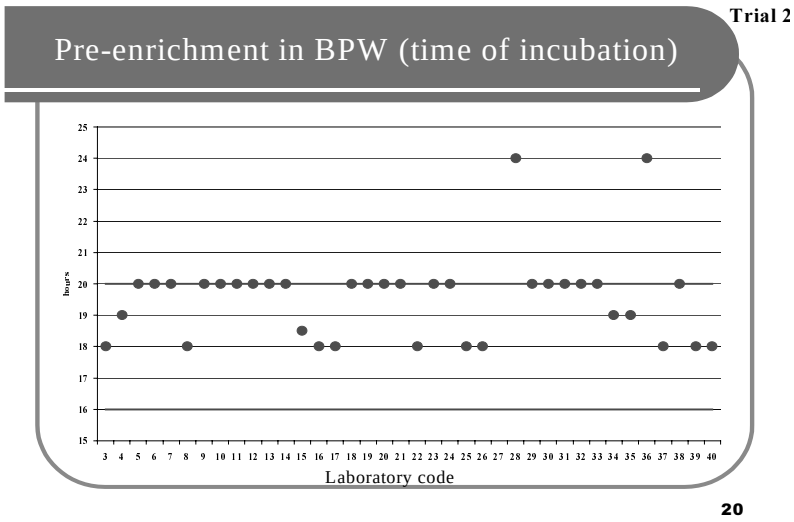
- pre-enirichment
- selective enrichment
- plating out on selective media
- the need for standardization isolation method

18

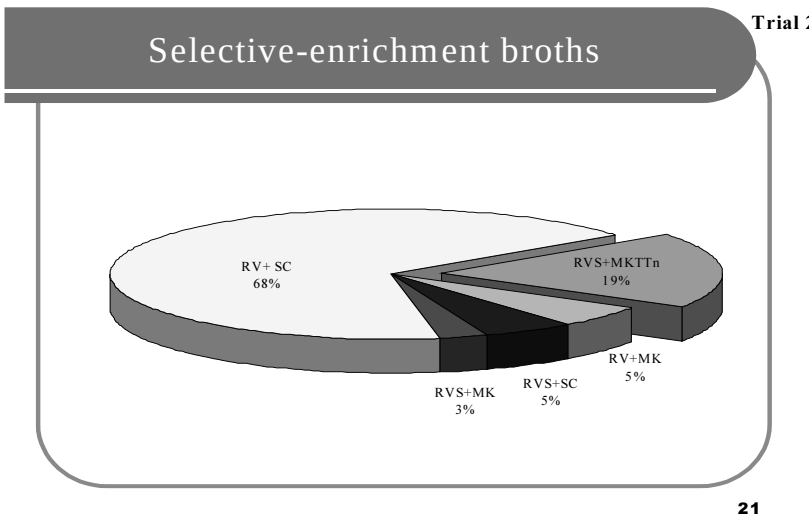
Slide 19



Slide 20



Slide 21



Slide 22

Trial 2

Selective-enrichment broths

pH

		N =			N =
RVS	**5,2**	**8**	**MKTTn**	**8,0**	**2**
(5,2 +/-0,2)	5,55	1	(8,2 +/-0,2)	**8,2**	**5**
RV	**5,2**	**26**	**MK**	7,4	1
(5,2 +/-0,2)	**5,15**	**1**	(7,0 +/-0,1)	8,2	2
	No data	**1**	**SC**	**6,95**	**1**
			(7,0 +/-0,2)	**7,0**	**18**
				7,1	**6**
				7,6	1
				No data	**1**

temperature

	41,5°C	**5**		**37°C**	**37**
	42°C	**32**			

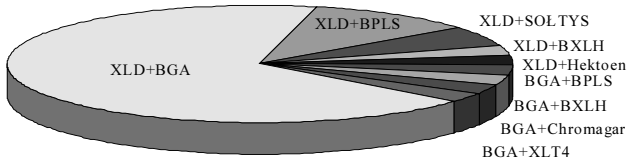
22

Slide 23

Trial 2

Selective plating media

XLD - 89%



23

Slide 24

Trial 3

Identification of *Salmonella*, Trial 3 (September, 2003)

24

Slide 25

Trial 3

Identification of *Salmonella*, trial 3 (September, 2003)

- 5 strains
- task: to define antigenic structure

Salmonella	Serological group	Antigenic structure
Enteritidis	DO	1,9,12: g,m:-
Gallinarum	DO	1,9,12: -: -
Typhimurium	BO	1,4,[5],12: i: 1,2
Agona	BO	1,4,[5],12: f,g,s: [1,2]
Derby	BO	1,4,[5],12: f,g,: [1,2]
Schwarzengrund	BO	1,4,12,27: d: 1,7
Dublin	DO	1,9,12: g,p:-

25

Slide 26

Trial 3

Determination of antigenic structure of *Salmonella*, trial 3 (September, 2003)

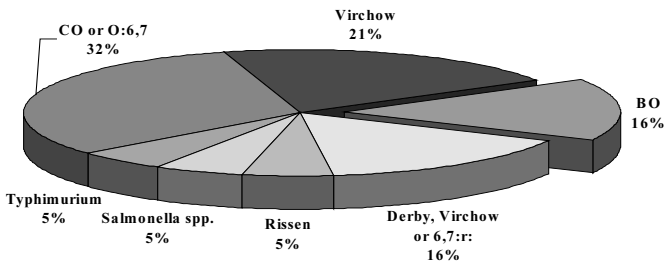
Salmonella	Tested strains	Correct results	
		Number	Percentage
Enteritidis	36	30	83%
Typhimurium	36	29	81%
Gallinarum	36	27	75%
Agona	19	13	68%
Schwarzengrund	18	7	39 %
Derby	19	0	0 %
Dublin	16	11	69 %
Total	180	116	64 %

26

Slide 27

Trial 3

Strains „with problems”: *S.* Derby



27

Slide 28

Trial 3

Determination of antigenic structure of *Salmonella*, trial 3 (September, 2003)

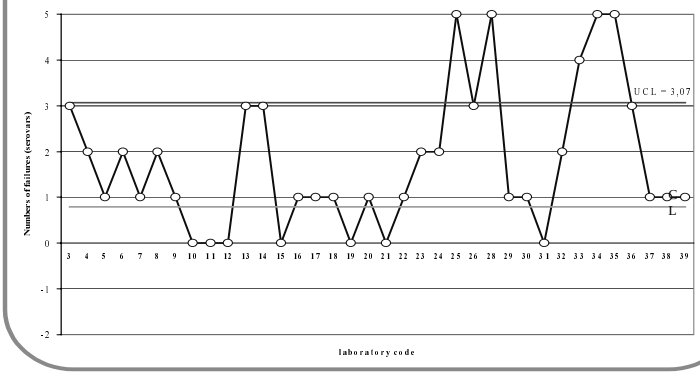
Number of errors	Number of laboratories	Percentage of laboratories
0	7	19%
1	13	36%
2	6	17%
3	5	14%
4	1	3%
5	4	11%

28

Slide 29

Trial 3

Determination of antigenic structure of *Salmonella*, trial 3 (September, 2003)

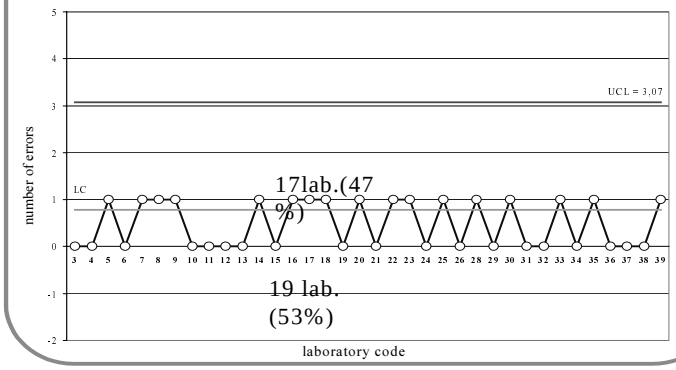


29

Slide 30

Trial 3

Determination of serological group of *Salmonella*, trial 3 (September, 2003)



30

Slide 31

Conclusions:

- Regional laboratories showed satisfactory efficacy in isolation of *Salmonella* and serotyping of significant serovars
- Standardisation and harmonisation of methods on *Salmonella* isolation from samples originating from animals is needed
- Participants expressed great benefit from participation in proficiency tests

31

Annex 19. Slides of presentation 2.5

Slide 1

NATIONAL VETERINARY INSTITUTE

PCR confirmation directly from MSRV agar plates

Erik Eriksson & Anna Aspán

Slide 2

Direct confirmation from BG & XLD agar plates

Since two years, direct confirmation on colonies from BG & XLD agar plates by real-time PCR is routinely used in analyses from feed samples in our laboratory.

- A suspected colony is picked by a touch of a loop on the agar plate, and transferred directly to the PCR-master mix. Lysis is performed in the PCR-machine before cycling starts.
- The master-mix is prepared in advance, and kept in freezer before use. Confirmation is completed within two hours.
- The method has been accredited by Swedac.

Slide 3

Real-time PCR assay of the invA gene

According to:

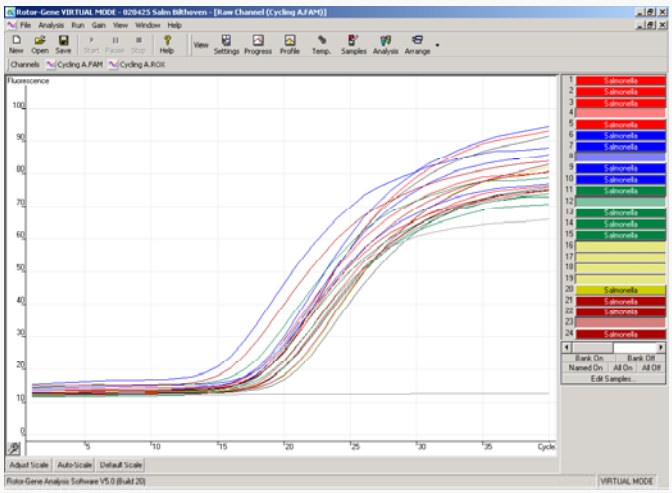
Hoorfar J, Ahrens P, Radstrom P.

Automated 5' nuclease PCR assay for identification of Salmonella enterica.

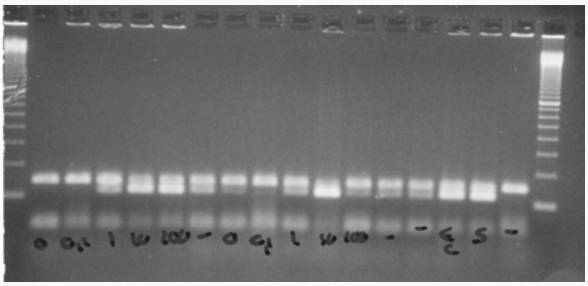
J Clin Microbiol. 2000 Sep;38(9):3429-35.

Slide 4

Real time PCR amplification of salmonella invA gene according to Hoorfar et al



Slide 5



Detection by agaros gel electrophoresis is equally robust and sensitive as detection by fluorescence.

150 bp is a specific salmonella product,

130 bp is an internal control molecule product.

Slide 6

Salmonella samples from the VII collaborative study

We wanted to test real-time PCR technique in the analyses on faecal samples from the collaborative study

Slide 7

Four different ways to detect salmonella by PCR, after pre-enrichment, were compared.

BPW from Oxoid was used for the pre-enrichments 16-20 h.

- 100 µl BPW was transferred to 900 µl BHI-medium, incubated at 37º C, 3h, and 10 µl BHI- medium was used as template for real-time PCR.
- MSRV agar plates after 2 days incubation, followed by real-time PCR.
- MSRV agar plates after 2 days incubation, transfer to 500 µl BHI-medium incubated at 37º C, 3h. 10 µl BHI- medium was used as template for real-time PCR
- Gel-BAX-salmonella according to the manufacturers instructions. (Lot:3143, Exp date 9/30/05)

Slide 8

	cfu/sample	BHI/PCR	MSRV/PCR	MSRV/BHI/PCR	GelBAX
C1	Blank	neg	neg	neg	neg
C2	SPan 5	pos	pos	pos	pos
C3	Blank	neg	neg	neg	neg
C4	SE 100	pos	pos	pos	pos
C5	STM 10	pos	pos	pos	pos
C6	STM 10	pos	pos	pos	pos
C7	STM 10	pos	pos	pos	pos
C8	SE 100	pos	pos	pos	pos
C9	SE 100	pos	pos	pos	pos
C10	SPan 5	pos	pos	pos	pos
C11	Non	neg	neg	neg	neg
C12	Non	neg	neg	neg	neg

Slide 9

Four different ways to detect salmonella by PCR, after pre-enrichment, were compared.

BPW from Oxoid was used for the pre-enrichments 16-20 h.

- 100 µl BPW was transferred to 900 µl BHI-medium, incubated at 37º C, 3h, and 10 µl BHI- medium was used as template for real-time PCR.
- MSRV agar plates after 2 days incubation, followed by real-time PCR.
- MSRV agar plates after 2 days incubation, transfer to 500 µl BHI-medium incubated at 37º C, 3h. 10 µl BHI- medium was used as template for real-time PCR
- Gel-BAX-salmonella according to the manufacturers instructions. (Lot:3143, Exp date 9/30/05)

Slide 10

cfu/sample	BHI/PCR	MSRV/PCR	MSRV/BHI/PCR	GelBAX
SE 100	neg	neg	pos	neg
Blank	neg	neg	neg	neg
STM 100	neg	pos	pos	pos
SE 100	neg	pos	pos	neg
STM 100	neg	pos	pos	neg
SE 500	neg	pos	pos	neg
SE 500	neg	pos	pos	neg
Blank	neg	neg	neg	neg
SE 500	neg	pos	pos	neg
SE 100	neg	pos	pos	neg
STM 10	neg	pos	pos	pos
Blank	neg	neg	neg	neg
SE 100	neg	pos	pos	neg
STM 10	neg	pos	pos	neg
STM 100	neg	pos	pos	pos
Blank	neg	neg	neg	neg
STM 100	neg	pos	pos	pos
STM 10	neg	pos	pos	neg
SE 500	neg	pos	pos	neg
STM 10	neg	pos	pos	neg
Blank	neg	neg	neg	neg
STM 10	neg	pos	pos	neg
SE 100	neg	pos	pos	neg
SE 500	neg	pos	pos	pos
STM 100	neg	pos	pos	neg

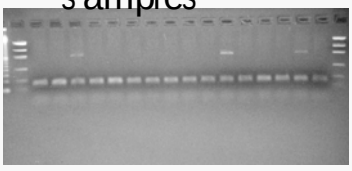
Slide 11

		BHI/PCR	MSRV/PCR	MSRV/BHI/PCR	GelBAX
N1	Yes (pos)	neg	neg	neg	neg
N2	Yes (pos)	neg	pos	pos	neg
N3	Yes (pos)	neg	pos	pos	neg
N4	Yes (pos)	neg	neg	neg	neg
N5	Yes (pos)	neg	neg	neg	neg
N6	Yes (pos)	neg	neg	neg	neg
N7	Yes (pos)	neg	neg	neg	neg
N8	Yes (pos)	neg	pos	pos	neg
N9	Yes (pos)	neg	neg	neg	neg
N10	Yes (pos)	neg	neg	neg	neg
N11	Yes (pos)	neg	neg	neg	neg
N12	Yes (pos)	neg	neg	neg	neg
N13	Yes (pos)	neg	neg	neg	neg
N14	Yes (pos)	neg	neg	neg	neg
N15	Yes (pos)	neg	neg	neg	neg
N16	Yes (pos)	neg	neg	neg	neg
N17	Yes (pos)	neg	neg	neg	neg
N18	Yes (pos)	neg	pos	pos	neg
N19	Yes (pos)	neg	neg	neg	neg
N20	Yes (pos)	neg	neg	neg	neg

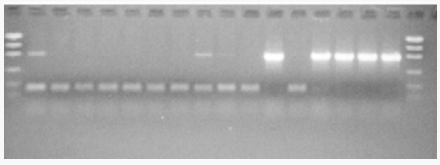
Slide 12

Gel-BAX salmonella;

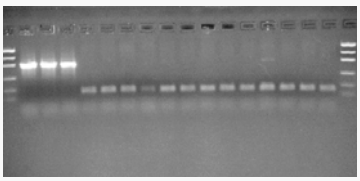
the IC molecule were amplified in all negative samples



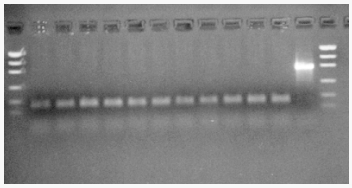
Sample 1-16



Sample 17-24, C1-C7



Sample C8-C12, N1-13



Sample N14-22, and controls

Slide 13

Conclusions

- Faecal samples from poultry are highly inhibitory to PCR
- A second enrichment step is not sufficient to overcome inhibition
- The results from PCR-confirmation of growth on MSRV-plates is promising as a sensitive and specific method to confirm salmonella directly from MSRV plates
- Gel-BAX salmonella is not suitable for these samples,
(Not validated for analyses of faecal samples)

Annex 20. Slides of presentation 2.6

Slide 1

MicroVal
European Validation and Certification

14 May 2004,
Pauline Kalkman, NEN, Delft,
MicroVal Secretariat

MICROVAL

1

Slide 2

Overview

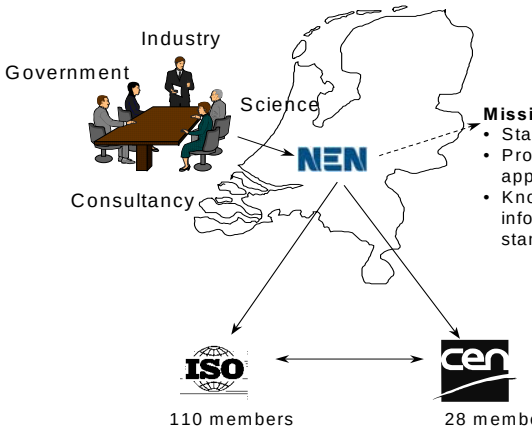
- NEN-CEN-ISO
- Trends and background validation market
- Introduction MicroVal
- EN/ISO 16140 - Validation
- MV Certification
- MicroVal Organisation
- Recent developments
- Assessment and conclusions

MV Secretariat
P.Kalkman, 14-05-2004

MICROVAL

2

Slide 3



Mission:
- Standards development
- Promoting standards application
- Knowledge and information center for standards

MV Secretariat
P.Kalkman, 14-05-2004

MICROVAL

3

Slide 4

Microbial detection market

- Shift from the traditional time-consuming validation methods
- Lack of international validation coordination of alternative methods
- Acceptance of alternative methods across markets often depends on multiple validations

Slide 5

Third Party Validation Systems

There is no one validation system that satisfies users throughout the world

- USA - AOAC International
- France - AFNOR Certification
- Nordic Countries - NordVal
- Europe - MicroVal

Slide 6

MicroVal European Certification organisation

- MV aims to provide a single accepted method validation and certification system in Europe
- It lowers the entry barrier to the European market
- Fulfills the requirements of European Legislation for rapid methods

Slide 7

Introduction MicroVal

MicroVal originally started as a Eureka project

- Validation: EN/ISO 16140 standard published
- Quality control of validation: MV Rules and Certification scheme.

Slide 8

EN ISO 16140

- Microbiology of food & animal feeding stuffs - Protocol for the validation of alternative methods
- Scope
  - **Principle & technical protocol for validation of alternative methods**
  - **Food, animal feed, environmental and veterinary samples**
  - **Validation of methods for use in official control**
  - **International acceptance of results of the alternative method**
- If a method is used on a routine basis for internal laboratory use, a less stringent validation may be appropriate

Slide 9

Importance of EN ISO 16140

- Draft EU Microbiological Criteria Document (SANCO/4198/2001, rev. 9)
- Article 5 - Specific rules for testing & sampling
- Use of alternative methods is acceptable, when the method has been validated against the reference method & certified by a third party to EN/ISO 16140 (or other similar internationally accepted protocols).

Slide 10

Validation

- Qualitative alternative methods
- Quantitative alternative methods

Both require
- a method comparison study
- an interlaboratory study

to be performed by the MV Expert laboratories

Slide 11

Qualitative methods

Method comparison study
- Relative accuracy
- Positive and negative deviation
- Relative sensitivity and specificity
- Relative detection level
- Inclusivity & exclusivity

Method interlaboratory
- 8 sets of laboratory results

Slide 12

Quantitative methods

Method comparison study
- Linearity
- Relative accuracy
- Detection and quantification limits
- Sensitivity
- Inclusivity & Exclusivity

Method interlaboratory
- 10 sets of laboratory results

Slide 13

Additional requirements for manufacturers in 16140

- Quality system in place (EN ISO 9002)
- Verification of quality system is required regularly after certification

Slide 14

MicroVal Principles

The MicroVal certification procedure is based on three core principles.

- The **first** principle is to perform a method comparison study of the alternative method against the reference method, followed by an inter-laboratory method performance study of the both the alternative and the reference method.
- The **second** principle is that the quality organisation of the manufacturer where the materials are produced must be in conformity with quality assurance requirements. The frame of these requirements is the standard ISO 9002.
- The **third** principle is a regular verification of the quality of the certified methods, which is made after the certification is granted.

Slide 15

Certification

- Certification is granted by following the requirements of EN ISO 16140 whilst under the cover of a MicroVal certification body.

Slide 16

MicroVal Organisation

MV is a balanced organization representing the interests of all parties equally.

- MicroVal Certification Bodies (MCB)
- The MicroVal General Committee (MGC)
- Common MicroVal secretariat (NEN)
- European network of laboratories (expert, co-operative, collaborative)
- European network of reviewers and auditors

Slide 17

Composition of MicroVal General Committee (MGC) (I)

Public authorities:

- FLEP, Denmark
- SANCO Health and Consumer protection DG of the EC
- EFSA (to be appointed)

Manufacturers:

- 3M Sante, France
- R. Biopharm, Germany
- Diffchamb S.A., Sweden

Slide 18

Composition of MicroVal General Committee (MGC) (II)

Users:

- Unilever , UK
- UNIR, France
- (user being appointed)

MicroVal third parties:

- Lloyds Register QA and TNO Certification, NL
- AFNOR, France and DIN, Germany
- Campden & Chorleywood Food Res.Ass. UK

Slide 19

Becoming MicroVal certified

- Manufacturer presents request to any MCB
- The final decision is taken by the MCB
- If not granted the manufacturer can appeal to MGC
- If the method has already been validated and/or certified by an other organisation, specific rules apply in order to consider such results

Slide 20

Recent developments

- Two new MCBs entered MV: Lloyds Register QA and TNO Certification.
- AFNOR Certification decided to focus more on national certification, thus becoming a passive partner for the time being
- MV has performed a market survey to establish the need for MV certification

Slide 21

Recent developments (II)

- EC is supportive of European certification for reasons of transparency and openness
- FLEP indicated a need for a validation system accepted throughout Europe, for the benefit of manufacturers / official control laboratories, to avoid a situation of monopoly and to secure transparency

Slide 22

Results market survey

Common trends among manufacturers:

- Target market crosses national boundaries
- Multiple validations present burdensome administrative and financial overhead
- Harmonisation of EU validation/certification considered critical for business development
- Each manufacturer has multiple test kits which would qualify for MV certification, both in short and long term
- EU recognition is considered most important as an investment guarantee.

Slide 23

International perspective

Mutual recognition agreements need to be established with international organisations.

- NordVal has prepared a letter of cooperation which will be worked out for next MV meeting
- Discussions are ongoing with AOAC

Slide 24

Assessment present status

- Interested parties, e.g. support from both food industry and FLEP and recognized by EC
- Manufacturer market survey shows an obvious market need
- Currently negotiating with manufacturers to start MV certification

Slide 25

Conclusions

- A lack of uniformity in Europe as to which validation system should be used
- A number of national or regional validation systems exist
- These tend not to be accepted outside their particular region

MicroVal aims to address all these issues

For information: www.microval.org

Annex 21. Slides of presentation 2.7

Slide 1

Validation of alternative (rapid) methods at national or laboratory level

Henk Stegeman

RIKILT Institute of Food safety
Wageningen, The Netherlands

Slide 2

DEFINITIONS

Validation = determination of the performance characteristics of a method (detection limit, robustness, sensitivity, specificity, accuracy, repeatability, reproducibility)

Validation of an alternative method: Demonstration that adequate confidence is provided that results obtained by the alternative method are comparable to those obtained using the reference method.

Slide 3

Literature

-EN-ISO 16140:2003 Microbiology of food and animal feeding stuffs – Protocol for the validation of alternative methods
-NVN-ENV-ISO 13843:2001 Water quality – Guidance on validation of microbiological methods
-ISO/FDIS 17994 Water quality - Criteria for establishing equivalence between microbiological methods.
-AOAC International Guidelines for Validation of Qualitative and Quantitative Food Microbiological Official Methods of Analysis. J.of AOAC, 85, 2002,p1187.

Slide 4

ISO 16140 – Protocol for the validation of alternative methods

Technical protocol for validation has two parts:
- Qualitative methods (presence or absence tests)
- Quantitative methods (enumeration, MPN-technique)

RIKILT INSTITUTE OF FOOD SAFETY WAGENINGEN UR

Slide 5

Qualitative methods (ISO 16140)

- Comparison study between reference and alternative method (60 samples for one matrix)
- Detection level (5 strains, 3-5 levels) (pure and in foods)
- Inclusivity(sensitivity, > 50 strains) and exclusitivity (specifity, > 30 strains)
- Interlaboratory study (> 10 laboratories, 1 food matrix, 3 contamination levels)

RIKILT INSTITUTE OF FOOD SAFETY WAGENINGEN UR

Slide 6

Quantitative methods (ISO16140)

- Comparison study (linearity, accuracy,bias)
- Detection and quantification limits
- Relative sensitivity
- Inclusitivity (sensitivity) and exclusivity (specificity) (> 30 positive strains; > 20 negative strains)
- Interlaboratory study (> 8 laboratories, 1 matrix, 4 sub-samples)

RIKILT INSTITUTE OF FOOD SAFETY WAGENINGEN UR

Slide 7

Dutch Product Board for Livestock, Meat and Eggs (PVE)

Validation of a rapid method at national level:

- Reference method: MSRV method for Salmonella
- Alternative method: PROBELIA™ PCR method

Matrices: poultry fluff, faeces, skin or meat

RIKILT INSTITUTE OF FOOD SAFETY WAGENINGEN UR

Slide 8

Alternative method

Product Board requirement:
- validation data already available for an other matrix (sensitivity and selectivity data)

Needed (ISO 16140):
- method comparison study
- collaborative study

RIKILT INSTITUTE OF FOOD SAFETY WAGENINGEN UR

Slide 9

Samples

- Chicken fluff – naturally and artificially contaminated (S.Enteritidis)
- Chicken faeces – all natural contaminated
- scruff of the neck – all naturally contaminated

RIKILT INSTITUTE OF FOOD SAFETY WAGENINGEN UR

Slide 10

Expert laboratory

- Not one expert laboratory, but two commercially (accreditated) laboratories
- Each laboratory investigates 60 samples of each matrix, of which at least 20 positive samples.

RIKILT INSTITUTE OF FOOD SAFETY WAGENINGEN UR

Slide 11

Results: Specificity and Detectielimit

Data from AFNOR validation study for food products:

- Specificity: 52 Salmonella strains were detected and there was no cross-reaction with 51 non-Salmonella strains
- Detectielimit: between: 10 and 100 kve/ml of pure cultures of S. Montevido, S.Typhimurium, S.Enteritidis, S. Kottbus, S.Derby and S. Virchow

RIKILT INSTITUTE OF FOOD SAFETY WAGENINGEN UR

Slide 12

Detectielimit in poultry faeces

Number of positive samples of laboratory 1 in a RIVM ring trial with 50 samples				
without faeces 5 cfu S.Panama n = 5	without faeces 100 cfu S.T n = 5	with faeces blank n = 4	with faeces 100 cfu S.T n = 18	with faeces 1000 cfu S.T n = 18
5	5	0	17	18

RIKILT INSTITUTE OF FOOD SAFETY WAGENINGEN UR

Slide 13

Calculations

- Relative accurancy: AC = (PA+NA)/N x 100 %
- Relative specificity: SP = NA/N- x 100 %
  - norm for false-positive result
- Relative sensitivity: SE = PA/N+ x 100 %
  - norm for false-negative result

- PA= positive agreement NA=negative agreement
- N =total number of samples
- N-=total number of negative results with the reference method
- N+=total number of positive results with the reference method

RIKILT INSTITUTE OF FOOD SAFETY WAGENINGEN UR

Slide 14

Results of laboratory 1 - method comparison

Matrix	PA	NA	ND	PD	N	**AC %**	N+	**SE %**	N-	**SP %**
Faeces	27	52	2	2	83	**95**	29	**93**	54	**96**
skin	37	32	2	5	76	**91**	39	**95**	37	**86**
fluff	22	39	0	4	65	**94**	22	**100**	43	**91**
Total	86	123	4	11	224	**93**	90	**96**	134	**92**

RIKILT INSTITUTE OF FOOD SAFETY WAGENINGEN UR

Slide 15

Results of laboratory 2 - method comparison

Matrix	PA	NA	ND	PD	N	**AC %**	N+	**SE %**	N-	**SP %**
Faeces	36	58	0	8	102	**92**	36	**100**	66	**88**
skin	30	66	3	0	99	**97**	33	**91**	66	**100**
fluff	28	48	0	8	84	**91**	28	**100**	56	**86**
Total	94	172	3	16	285	**93**	97	**97**	188	**92**

RIKILT INSTITUTE OF FOOD SAFETY WAGENINGEN UR

Slide 16

Results of interlaboratory study

- Participation two times / year in RIVM ring trial
- Same results with reference and alternative method
  - 5 Blank samples
  - 5 Faeces samples with 100 cfu S.T
  - 5 Faeces samples with 1000 cfu S.T

RIKILT INSTITUTE OF FOOD SAFETY WAGENINGEN UR

Slide 17

Discussion

- For laboratory 1 no statistical difference between 2 methods (more positive with PCR)
- For laboratory 2 significant more positive samples for faeces and fluff with PCR method.
  - In faeces 5 of 8 MSRV-negative samples, but PCR-positive, Salmonella, were positive with an other method
- PCR is able to detect more serotypes. Method is not equivalent to the reference method

RIKILT INSTITUTE OF FOOD SAFETY WAGENINGEN UR

Slide 18

Results of interlaboratory study

- Participation two times / year in RIVM ring trial
- Same results with reference and alternative method
  - 5 Blank samples
  - 5 Faeces samples with 100 cfu S.T
  - 5 Faeces samples with 1000 cfu S.T

RIKILT INSTITUTE OF FOOD SAFETY WAGENINGEN UR

Slide 19

Conclusion

- ISO 16140 has no criteria for acceptance
- Product Board criteria: AC > 90 %, SE > 90 % (fluff > 95%)
- For SP no criterion (more positive with PCR)
- PCR is accepted as an alternative method for the MSRV method in the poultry production chain

RIKILT INSTITUTE OF FOOD SAFETY WAGENINGEN UR

Slide 20

Verification requirements for the alternative method

- Verification (secondary validation) demonstration by experiment that the validated method functions according to is specifications in the user's hand.
- Comparison method study with 5 to 10 positive and 5 negative samples for each matrices (poultry fluff, faeces, skin) with good results.
- Participation in the Proficiency testing programme of RIVM with good results

RIKILT INSTITUTE OF FOOD SAFETY WAGENINGEN UR

Slide 21

Other national studies of alternative methods based on ISO 16140

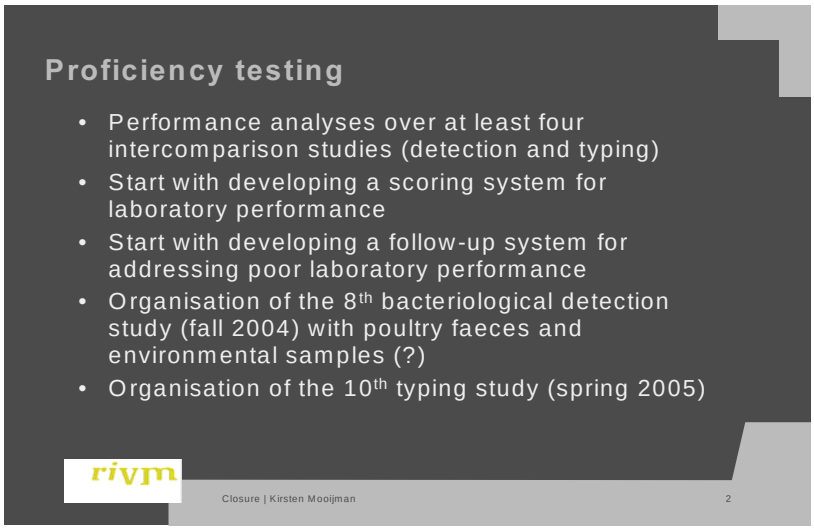
- Dutch Product Board for Livestock, Meat and Eggs
  - Comparison of MSRV and Immunological method (VIDAS)
  - Comparison of MSRV and IQ Check Salmonella real-time PCR
- Dutch Product Board Animal Feed
  - Salmonella ISO method with PCR for the matrix animal feed
  - Salmonella ISO method with immunological method for the matrix animal feed
- Dutch Food Authority (Food Inspection Service)
  - Salmonella ISO method with MSRV method for the matrix food stuffs

RIKILT INSTITUTE OF FOOD SAFETY WAGENINGEN UR

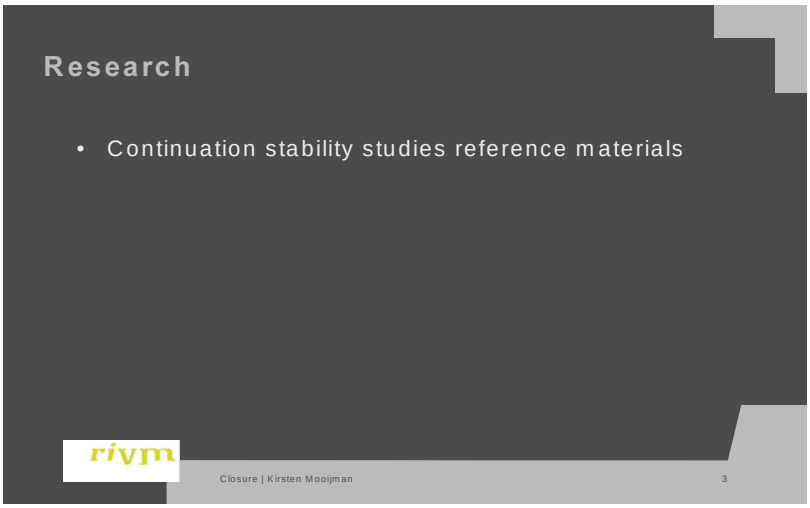
Annex 22. Slides of presentation 2.8

Slide 1

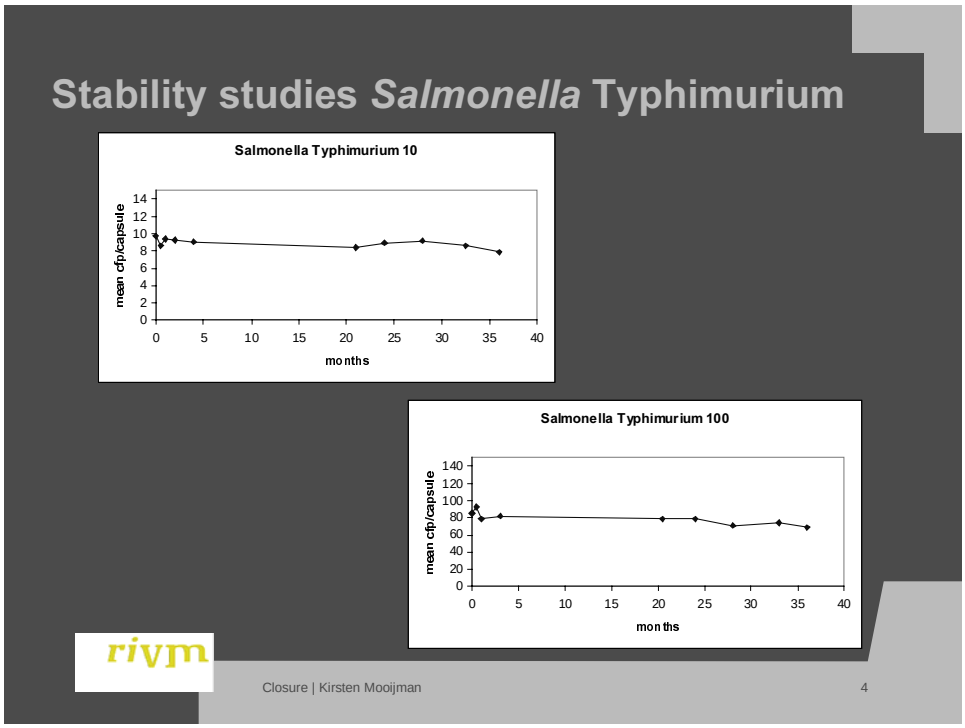
Slide 2



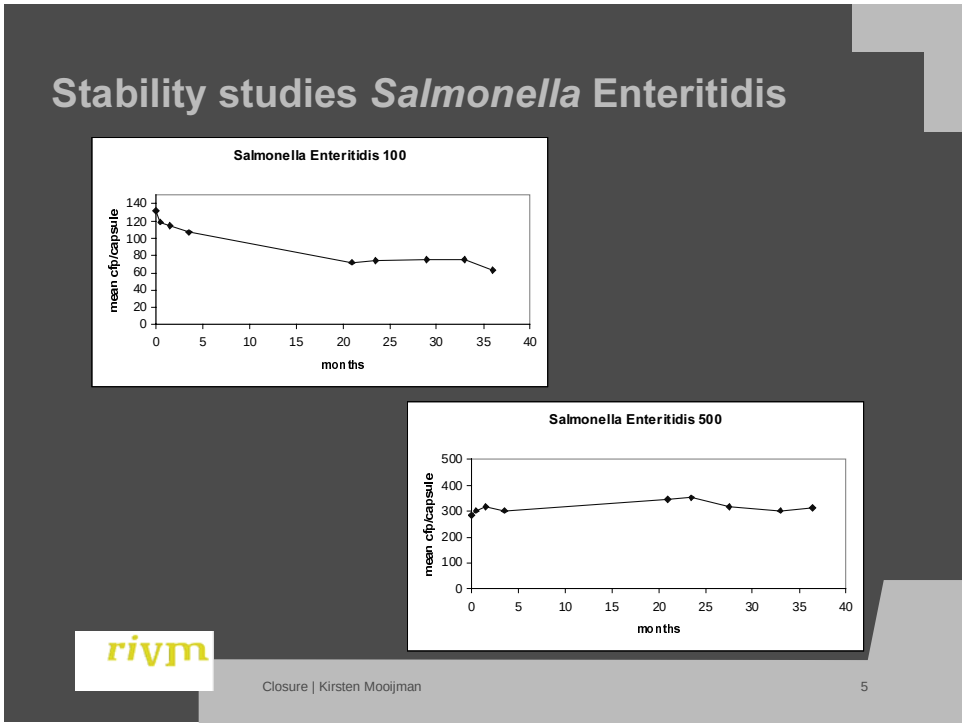
Slide 3



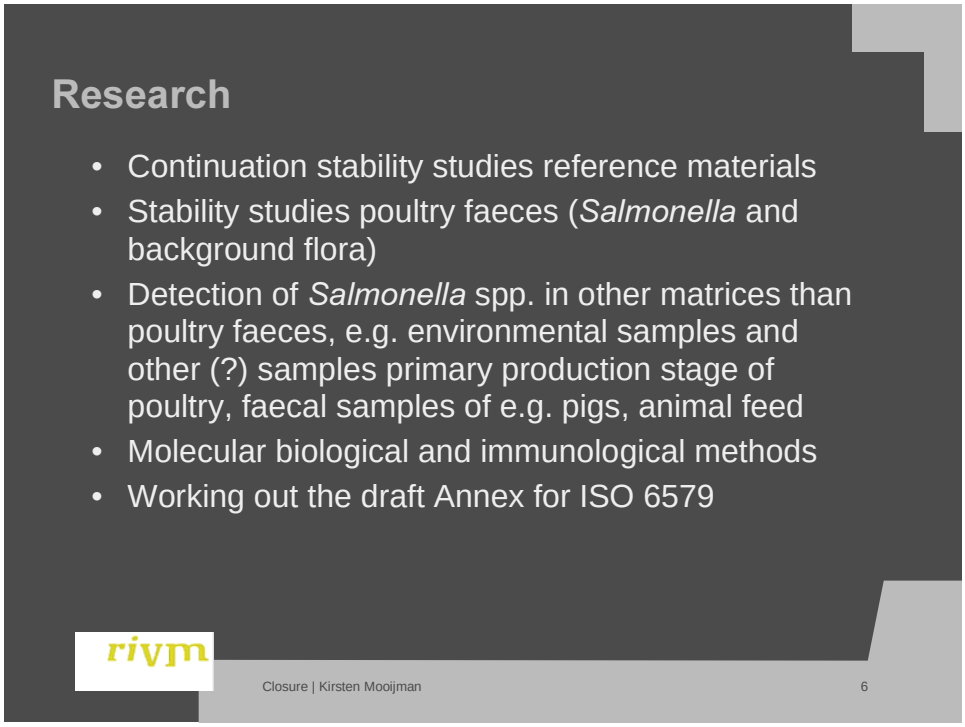
Slide 4



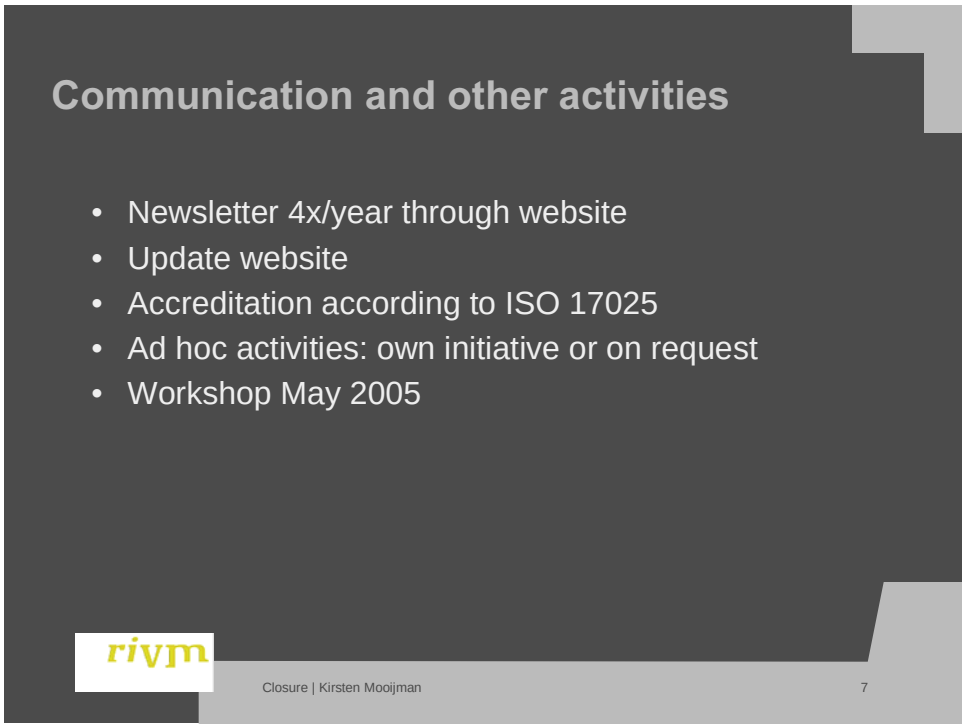
Slide 5



Slide 6



Slide 7



Slide 8

Closure

Thank you very much!

- European Commission
- Guest speakers
- Participants
- Organising committee

Please return your badges
Please send your abstracts asap

rivm

Closure | Kirsten Mooijman 8

Slide 9

Goodbye!

Save journey home!

See you next year!

rivm

Closure | Kirsten Mooijman 9