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Report on the eighth workshop organised by CRL-Salmonella
Bilthoven (the Netherlands), 14-16 May 2003

H. Korver, K.A. Mooijman and A.M. Henken
(editors)

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

Abstract

Report on the eighth workshop organised by CRL-*Salmonella*

The eighth workshop organised by the Community Reference Laboratory for *Salmonella* (CRL-*Salmonella*) was held from 14 to 16 May 2003 in Bilthoven (the Netherlands).The representatives from the National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) of the Candidate Countries of the EU were present on the 14th and 15th of May 2003. The representatives of the NRLs-*Salmonella* of the EU Member States joined the workshop at 15 and 16 May. The candidate member states presented themselves with a presentation about research on *Salmonella* in their own countries. Presentations about the EU enlargement and the Zoonoses Directive were given as well as the results of two collaborative studies, organised by the CRL-*Salmonella*. On the third day of the workshop two sessions entitled: "Antibiotic Resistance" and "Standardisation of detection methods" were organised. An introduction on both subjects was given by experts, whereupon the content of the presentations was discussed into detail. Comparison of data on antimicrobial resistance testing is hampered by the use of different methods and interpretation criteria. The discussion held on this subject revealed that their is a need for standardisation of testing methods and harmonisation of international data. It was also discussed whether a separate method for the analysis of Salmonella in faeces should be written as an Annex to the present ISO 6579: 2002.

Contents

Samenvatting

Van 14 tot en met 16 mei 2003 is door het Communautair Referentie Laboratorium voor *Salmonella* (CRL-*Salmonella*) een workshop georganiseerd in Bilthoven, Nederland.
Op 14 mei en 15 mei 2003 waren de vertegenwoordigers van de referentielaboratoria van de kandidaat lidstaten van de EU uitgenodigd om deel te nemen aan de workshop. De deelnemers uit de kandidaat lidstaten kwamen uit Bulgarije, Tsjechische Republiek, Estland, Hongarije, Letland, Litouwen, Polen, Slovakije, Slovenië en Turkije.
De vertegenwoordigers van de Nationale Referentie Laboratoria voor *Salmonella* (NRLs-*Salmonella*) van de EU lidstaten hielden hun workshop op 15 en 16 mei 2003. In totaal waren er 58 deelnemers.

Het programma van de workshop bestond uit verschillende delen. De eerste dag werd besteed aan de presentaties van alle aanwezige kandidaat lidstaten, een historisch perspectief aangaande de vergroting van de EU en de zoonose-richtlijn, het "zoonoses reporting system" en ringonderzoeken welke worden georganiseerd door het CRL-*Salmonella*. Tevens werd een bezoek gebracht aan de verschillende CRL-*Salmonella* laboratoria.
De tweede dag werd gesproken over EU monitoring/control en epidemiologie van *Salmonella* spp. binnen de EU. Een aantal vertegenwoordigers van de NRLs hield presentaties over een verscheidenheid aan onderwerpen. Voorts werden de resultaten van twee ringonderzoeken, georganiseerd door het CRL-*Salmonella*, gepresenteerd en bediscussieerd.
Op de derde dag konden de deelnemers kiezen tussen twee verschillende sessies met als titels, respectievelijk: "Antibiotic Resistance" en "Standardisation of detection methods". Tijdens iedere sessie werd eerst een inleiding gegeven door experts op het desbetreffende gebied, waarna uitgebreid kon worden gediscussieerd over de inhoud. In een afsluitende gezamenlijke sessie werden de resultaten van de beide sessies uitgewisseld.
Vergelijking van gegevens aangaande antimicrobiële resistentie van verschillende landen is noodzakelijk, maar wordt belemmerd door het gebruik van verschillende methoden en verschillende interpretatie criteria. De discussie, gehouden over dit onderwerp wees uit, dat er behoefte bestaat aan standaardisatie van test methoden en harmonisatie van gegevens. Er werd ook gesproken over de vraag of er een aparte methode voor de analyse van Salmonella in feces geschreven zou moeten worden als een Annex bij de tegenwoordige ISO 6579:2002.

De presentaties, welke te vinden zijn in Appendix 4 tot en met 33 (bladzijden 52 – 241) zijn in dit rapport zwart/wit afgedrukt. Voor kleurweergave wordt verwezen naar de website van het CRL-*Salmonella*: http://www.rivm.nl/crlsalmonella/Publications or http://www.rivm.nl/crlsalmonella/Workshops

Summary

From 14 to 16 May 2003 the Community Reference Laboratory for *Salmonella* (CRL-*Salmonella*) organised a workshop in Bilthoven (the Netherlands).
At 14 and 15 May 2003 the representatives from the National Reference Laboratories of the Candidate Countries of the EU were invited to participate in the workshop. The participants of the Candidate Countries originated from Bulgaria, Czech Republic, Estonia, Hungary, Latvia, Lithuania, Poland, Slovak Republic, Slovenia and Turkey.
The representatives of the National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) of the EU Member States joined the workshop at 15 and 16 May. A total of 58 participants were present at the workshop.

The programme of the workshop consisted of several parts. At the first day all candidate members states presented themselves with a presentation about research on *Salmonella* in their own countries. Also, presentations about the EU enlargement and the Zoonoses Directive (a historical overview), the zoonosis reporting system in the EU and collaborative studies organised by the CRL-*Salmonella* were given. The participants of the Candidate Countries also visited the CRL-*Salmonella* laboratories.
The presentations of the second day were: EU monitoring/control and epidemiology of *Salmonella* within the EU. Furthermore, a number of representatives of the NRLs presented papers on a variety of subjects. The results of two collaborative studies, organised by the CRL-*Salmonella*, were also presented and were open for discussion.
On the third day the presentatives could take part in one of two sessions entitled: "Antibiotic Resistance" and "Standardisation of detection methods", respectively. An introduction on both subjects was given by experts, whereupon the content of the presentations was discussed into detail. At closure a final general session was organised where the results of the two parallel sessions were exchanged.
Comparison of data on antimicrobial resistance from different countries is needed, but is hampered by the use of different methods and different interpretation criteria. The discussion held on this subject revealed that their is a need for standardisation of testing methods and harmonisation of data. It was also discussed whether a separate method for the analyses of *Salmonella* in faeces should be written as an Annex to the present ISO 6579:2002.

The presentations which can be found in Appendix 4 till 33 (pages 52 – 241) are printed in black and white. For colour rendering see the website of the CRL-*Salmonella*:
http://www.rivm.nl/crlsalmonella/Publications or
http://www.rivm.nl/crlsalmonella/Workshops

1. Wednesday 14 May 2003: day 1 of the workshop

1.1 Opening and introduction

André Henken, Director CRL-*Salmonella*, Bilthoven, the Netherlands
(see Appendix 4)

Welcome

First of all I would like to sincerely welcome you all at this workshop held at the Dutch National Institute for Public Health and the Environment. I am very pleased that we were allowed by TAIEX (Technical Assistance Information Exchange Office/EU) to organise this workshop with you, the representatives of the NRLs-*Salmonella* of the candidate countries. Most candidate countries are present.
A special word of welcome for Jean-Charles Cavitte as the representative of the EU Commission amongst us. Also a special welcome to Anne Kaesbohrer from the CRL-Epidemiology of Zoonoses (Berlin).
At the Dutch National Institute for Public Health and the Environment about 1500 persons are working in 4 divisions, from Public Health Research, to Nutrition and Consumer Safety, to Environmental Risk and External Safety, to Environment and Nature Research.
Research on zoonoses has always been a major area of activity of this institute. This is true for zoonoses that are food borne but also for zoonoses that are transmitted to man by other routes. A major group working on this field is the Microbiological Laboratory for Health Protection, located in the division of Nutrition and Consumer Safety. This laboratory is hosting the EU Community Reference Laboratory for *Salmonella*.

Aims

What can we expect from this workshop?
- To learn about the activities of CRL-*Salmonella*
- To learn about what is expected from you when your country is going to participate in those activities from 1/1/2004 onwards
- To let CRL-*Salmonella* know what your needs and expectations are
- To learn from each other as NRLs-*Salmonella*
- To learn about what is stated in the zoonoses directive

The items of the workshop must be seen in relation to the functions and duties of the CRL-Salmonella according to the zoonoses directive:

- providing national laboratories with details of analytical methods and comparative testing;
- coordinating the application by national reference laboratories of the methods, referred to under the first mentioned point, in particular by organizing comparative testing;
- coordinating research into new analytical methods and informing national laboratories of advances in this field;
- conducting initial and further training courses for the benefit of staff from national reference laboratories and

- providing scientific and technical assistance to the Commission of the European Community.

The activtities of the CRL-*Salmonella* are:
- collaborative studies (2/yr): one on bacteriological detection and one on typing
- workshop (1/yr)
- research: related to analytical methods and reference materials that are used in the collaborative trials
- communication (newsletter (4/yr), website)
- ad hoc: own initiative or on request

Participants of the workshop (see Appendix 2)

- Representatives NRL-*Salmonella* of CCs (14-15 May)
- Representatives NRL-*Salmonella* of MSs (15-16 May)
- Representative CRL-Epidemiology Zoonoses Berlin
- Representatives CRL-*Salmonella*
- Representative of EU Commission
- Guest speakers

Workshop programme

14 May
- introduction CRL-*Salmonella*
- EU zoonosis directive and EU zoonoses reporting system
- presentations by CCs
- collaborative studies
- visit laboratories

15 May
- introduction and issues EU regulation
- CRL-Epidemiology
- Bacteriological collaborative studies
- Typing collaborative studies
- Various research contributions

16 May
- two parallel sessions:
    1. antibiotic resistance
    2. standardisation of detection methods
- presentation of results of the two parellel sessions and general discussion
- closing remarks

A detailed programme is presented in appendix 3.

1.2 The EU enlargement and the Zoonoses Directive: a historical perspective

Jean-Charles Cavitte, European Commission, Brussels, Belgium
(see Appendix 5)

Directive 92/117/EEC was adopted in the framework of achievement of single market and with the background of a large crisis in the UK, with *Salmonella* in eggs. It contains provisions on monitoring of certain zoonoses and control of *Salmonella* in flocks of breeding poultry. Two Member States (MS) were also granted additional trade guarantees for *Salmonella* when they joined the European Union.

Two Community Reference Laboratories were designated through Directive 92/117/EEC:

- CRL for the epidemiology of zoonoses: BfR in Berlin (Germany)
- CRL for *Salmonella*: RIVM in Bilthoven (the Netherlands)

Directive 92/117/EEC: monitoring

Four zoonoses are designated for compulsory monitoring: brucellosis and agents; tuberculosis (M bovis); trichinellosis; salmonellosis and agents.
Other zoonoses are for voluntary monitoring: campylobacteriosis; echinococcosis; listeriosis; rabies; toxoplasmosis; yersiniosis.
A Community report on trends and sources of zoonoses is produced every year. Its drafting is the task of CRL Berlin since 1995.
The data collection is usually not harmonised and therefore the report has to be carefully interpreted. The quality and quantity of data increased along the years, but comparability between countries is often not possible. Significant data are however collected and synthetised. For instance, the suspected sources of salmonellosis in humans are relatively well known. *Salmonella* Enteritidis (SE) and *Salmonella* Typhimurium (ST) are by far the most frequent serotypes in humans. The distribution of serotypes in humans is different from the serotypes in feedingstuffs but more similar to those serotypes found in different species of livestock.

Directive 92/117/EEC: Salmonella control programmes

MS *Salmonella* control plans in fowl were to be submitted for approval by EC, before 1.1.94. The requirement was later suspended, pending revision of the Directive.
A majority of MS have submitted; policy has been to approve plans when go further than minimum compulsory requirements laid down in the Directive for control of SE and ST breeding flocks. There is a possibility of EC co-financing for the elimination of breeding flocks found infected by the above serotypes. No financing is possible if the country does not eliminate these flocks. Plans were approved on the basis of Directive 92/117/EEC (*Gallus gallus*) in the following countries: DK, IRL, FIN, SWE, A, FR, NL (and NO). The scope varies : certain countries cover the whole/part of the production pyramid of *Gallus gallus* and/or cover other species and/or more *Salmonella* serotypes than SE/ST. The other Member States are obliged to control ST. and SE. in breeding flocks of *Gallus gallus*, (without the

obligation to destroy positive flocks). Certain countries also have control programmes in species other than poultry.

Sweden and Finland were granted additional guarantees for *Salmonella* in the context of the Act of Adhesion as from 1.1.1995. The additional guarantees were subject to an operational programme presented by Sweden and Finland and approved by the Commission (Decisions 95/50/EC and 94/968/EC).

Directive 92/117/EEC is at the final stage of revision, as an action foreseen in the "White Paper on food safety" and a legal requirement included in the Directive itself. This Directive is to be repealed and replaced by two separate pieces of legislation: a Directive on monitoring of zoonoses and zoonotic agents and a Regulation on control of specified food-borne zoonotic agents. These texts build on the existing legislation. There will be a transitional period between the repealing of Directive 92/117/EEC and the implementation of new control programmes for *salmonella* in poultry. During this period, the Member States, whether current or new Member States, will have to implement the minimum requirements contained in Directive 92/117/EEC (or where relevant the actions in their approved programmes) until corresponding programmes apply under the new legislation.

Discussion

Q: When the Candidate Member States become full members of the EU should they comply completely with the Directive 92/117 ?
A: Yes, the directive 92/177 must be fully implemented as a basic requirement. Later on they should comply with the new zoonosis directive. The NRLs of the present Candidate Members States may take part in the collaborative studies as soon as they are fully member of the EU.

1.3 Zoonosis reporting system in the EU

Annemarie Kaesbohrer, CRL-Epidemiology of Zoonoses, Berlin, Germany
(see Appendix 6)

On the basis of Council Directive 92/117/EEC, the Community Reference Laboratory for the Epidemiology of Zoonoses was established. One of the major tasks is to manage the compilation of the "Report on trends and sources of zoonotic agents in the European Union and Norway" which is published yearly by the European Commission (DG SANCO). This report summarises the information submitted by each country in the national reports. The report covers information on 11 zoonotic agents, which should be monitored along the food chain, from feedingstuffs (if relevant), animals, foods and in humans.
The National Report should contain a description of the national system in place, the results of it and a national evaluation of the situation. As a guide, a manual for reporting was prepared by the CRL-E and is yearly updated. Furthermore, a set of tables is distributed yearly where the results of the examinations should be filled in.
Currently, there is a lack of harmonisation of the monitoring activities applied in the individual countries. This makes the comparison and interpretation of the information

received from the countries difficult. For the surveillance of *Salmonella* in poultry breeding flocks certain sampling strategies are fixed in the zoonoses directive but it is not fully followed in all countries. In other countries, some additional sampling points are regularly included. Similarly, the measures applied in case of a confirmed finding of *Salmonella* is differing. Approved control programmes or monitoring schemes cover also the productive flocks of *Gallus gallus*, i.e. laying hens (for table egg production) and broilers or other poultry species (i.e. turkeys) in several countries. Some details were given in the presentation.
In humans, salmonellosis and campylobacteriosis are the most frequently reported zoonoses, both with an increasing tendency in 2001. The main serovars, which are involved in human disease, are *S.* Enteritidis and *S.* Typhimurium, followed to a lower degree by *S.* Infantis, *S.* Virchow and *S.* Hadar on the European Union level. In the individual country, the share of cases caused by *S.* Enteritidis and *S.* Typhimurium is varying in a wide range.
As soon as the new zoonoses directive has come into force, the reporting system has to be adjusted to the new requirements, i.e. additional zoonotic agents will be covered, monitoring requirements will be fixed and harmonised programmes can be implemented.
Another important step will be to integrate the "new" Member States of the EU in the zoonoses reporting system.

Discussion

Q: Do you expect the new member states to report you in the near future ?
A: Yes, we expect the candidate countries to report us starting with 2004.
A: Yes, as soon as possible. The Candidate Countries should report quickly on the basis of the existing directive.
Q: What should we do if there is a separation of human and animal cases in countries ?
A: You should make one report and send that report to the Commission.

1.4 Presentations by representatives of NRLs from CCs

1.4.1 Presentation NRL-Bulgaria

Ivan Kaloyanov, National Veterinary Research Institute, Sofia
(see Appendix 7)

Incidence and trends of Salmonella and Salmonellosis in humans, food animals and food animal origin

Occurence of *Salmonella* in food producing animals and food: in 2002 a total of 227 strains of *Salmonella* were isolated in the country.
Isolated from: food, predominant minced meat, poultry, eggs – 27; death bovines – 21; slaughtered bovines – 3; death pigs – 42; slaughtered pigs – 11; death ovins – 9; slaughtered ovins – 0; death poultry – 74; slaughtered poultry- 13; game – 4; feed – 5; environment (food chain) – 5.

Serovars *:*
Enteritidis – 38,23 % ; Typhimurium – 21,65 % ; Choleraesuis – 6,78 % ; London – 4,11 %; Agona – 4,03 % ; Gallinarum – 3,44 % ; Isangi – 3,43 % ; Anatum – 3,32 % ; Abortusovis – 2,89 %; Derby – 2,50 % ; Braenderup – 2,41 % ; Newcastle – 2,27 % ; Kentucky – 1,31 % ; Heidelberg - 1,13 % ; Give – 0, 44 % ; Haifa – 0,32 %.

Systems for food borne disease surveillance

Which laboratories are involved in food-borne pathogen surveillance in food producing animals and their products ?
In 1973 a general program was adopted for the country about the protection of people, animals and environment against *salmonellosis*. In result two reference laboratories were set up in Sofia – one for strains isolated from people, and another for strains isolated from animals, animal products, environment and feeds. Currently these are reference centers for salmonella strains isolated from all possible sources. Similar centers have been set up for *staphylococci, E.coli, Clostridium botullimum*. Food products of animal origin and feeds are tested in 2 local veterinary institutes, 8 accredited veterinary laboratory for food control and 21 regional veterinary laboratories. Tests for confirmation of antimicrobial resistance to strains are carried out only for therapeutic reasons and with relation to research programs.
Which of the above laboratories carry out, or have the technical capacity to carry out, sampling, isolation, identification and susceptibility testing of *Salmonella*?
Since the beginning of the century a wide network of mutually subordinated laboratories both as organization and as methods has been established for the testing of each batch of food products of animal origin and animal feeds produced in the country or imported. The test methods are complied with national and international standards – CEA and ISO. At present regional and reference laboratories are being equipped in accordance to the requirements of the EC under a Twinning Project with the Italian Veterinary Services.
Which internal and external quality assurance systems are used?
Good manufacturing practice, Good hygiene practice, HACCP - system are involved or are in way to be involved in the factories, producing food. For the lab - Good laboratory practice and accreditation according ISO 45001 are in way to be involved in the Salmonella reference laboratory.
What systems are used for collection and analysis of data (e.g. which computer software and hardware are used for acquisition, analysis and sharing of data) ?
No computer software were used till now to collect data and for analysis. In the beginning of this year in the veterinary system began to work the system VetInfo, including the collection of data from the laboratories
How often and by what means are results communicated and reported between the different laboratories and between laboratories and authorities (this includes between different public health laboratories as well as between the public health sector and the food control sector and veterinary services) ?
The obtained results are communicated suddenly by phone and by official letters when you have isolated serovar gallinarum, or in case of toxiinfection – to the health authorities. We have close relations between two national reference centers - on the national and local level.

Protection against foodborne infections and toxic infections is managed on a national level by a group of experts at the Ministry of Health and the Ministry of Agriculture and Forests, and in the same time attention is given to a wide range of zoonoses. Specific legislation is set

up in compliance with the EC directives for each individual disease. Each Ordinance is published in the State Gazette.

Systems for recording antimicrobials use in food animal production

Give a brief description of the animal husbandry practices in your country.
Give a description of the types and indications for antimicrobial use in food animal production in your country, this will include the use of growth promoters and antimicrobials used for therapeutic purposes.
The following antibiotics have been used in the country as therapeutic and prophylactic products and as growth stimulators for animals (produced in Bulgaria or imported):
Gentamycin; Streptomycin; Kanamycin and Amikacin; Amopen (amikacin); Apramycin (Amoxacin); Neomycin; Colistin (Polimixin); Bulaquindox; Spectan (Spectomycin); Tetracycline, Oxitetracyclin, Chlortetracylcin, Doxacyclin; Lincomycin; Ampicilin; Pephloxacin; Pleuromutilin (Tiamycin and Dynamutilin); Cephalexin; Cephalosporin; Cephamandol.

The following products are under ban by the Minister of agriculture and forests to be used in animal breeding: Chloramphenicol; Bayunox; Zincbacitracin; Furasolidon; Dimetridasol

Describe system for licensing, distributing and administering
For each medicine or growth stimulator the producer or importer submits detailed documentation and samples for analysis to the Veterinary Institute for Control of Medicinal Products. The results of their conclusions are presented to the State Commission for Registration of Veterinary Medicinal Products. The Commission has also been authorized to impose if necessary bans on their usage.

From where did you get the above information?
From the information system of National Veterinary Service

Produce a draft list of areas in need of strengthening to achieve a satisfactory food borne disease surveillance and antimicrobial resistance monitoring.
To be introduced to a uniform, united information system for exchange of data and all information
To be licensed according to the ISO 45001 the two reference national laboratories
To introduce and improve the antimicrobial resistance monitoring for strains of serovars Enteritidis and Typhimurium, isolated from the food chain.

Discussion

Q: Are there in your country suitable control systems ?
A: Yes, for over 15 years already. At present we are taking measures according to the EU rules.
Q: Do the institutes dealing with animal and human isolates communicate with each other ?
A: Yes.

1.4.2 Presentation NRL-Hungary

Zsuzsanna Sreter-Lancz and Zoltan Veres, National Food Investigation Institute, Budapest
(see Appendix 8)

Salmonella monitoring and control activity in Hungary

The official *Salmonella* monitoring and control activity in Hungary is based on the cooperation of the Veterinary and Food Control, and the Public Health Service. Broadly speaking, the part of the food chain from the farms to the food industry, as well as the products of animal origin at the retail is controlled by the former Service, the part from industry to the consumer (trade, catering), and the human infections are supervised by the latter one. According to the 92/117/EEC, a national *salmonella* eradication program for poultry was initiated in the middle of last year.
Our Reference Laboratory (VNRL), founded in the middle of the 1950s, is integrated to the Veterinary and Food Control Service. All the *Salmonella* strains isolated by the official examination (according to the standard MSZ EN ISO 6579: 2002) of food, feedingstuffs, and samples of animal origin, are sent (obligatory) to the VNRL for further characterisation, escorted with all important sampling notes. The implementation of the national *salmonella* eradication program caused a significant increase in the number of the isolates, expanding the work of the laboratory.

The main tasks of the VNRL are the following:

- serotyping of all the *Salmonella* strains isolated by the Veterinary Service
  (the laboratory uses commercial and own prepared antisera as well)
  6600 Salmonella isolates were serotyped in 2002, the dominant serovariants were:
  1. *S.* Infantis, (48%), showing an increasing rate in the last years,
  2. *S.* Typhimurium (14%)
  3. *S.* Enteritidis (10 %)
- phage-typing of *S.* Enteritidis (by the methods described by Maczierevicz),
  S. Typhimurium strains by the Felix-Callow scheme
- preparation of the yearly salmonella report on serotype and phage-type distribution
- antimicrobial susceptibility typing of *S.* Enteritidis and *S.* Typhimurium strains (by disc-diffusion method) and transmission of the data to the National Antibiotic Resistance Monitoring System
- scientific advisory role to support the policy development
- organising and taking part in inter-laboratory tests (FEPAS, Global Salm Surv)
- taking part in research programs and improve methods for the detection and typing of *Salmonella*
- cooperation with the *Salmonella* Reference Laboratory of Public Health.

Discussion

Q: According to which system do you interprete the phage type of *S.* Enteritidis or *S.* Typhimurium strains ?
A: *S.* Enteritidis according to the MacZierevicz system and *S.* Typhimurium according to the Felix-Callow system.

1.4.3 Presentation NRL-Slovak Republic

Alena Skarkova and Selma Jackova, State Veterinary and Food Administration, Bratislava
(see Appendix 9)

State Veterinary and Food Administration (SVFA) is operated under Ministry of Agriculture. Under the SVFA are operated Regional Veterinary and Food Administrations (RVFA) and six State Veterinary and Food Institutes (SVFI) or State Veterinary Institutes. The institutes perform the veterinary laboratory diagnostic tests and RVFA perform veterinary precautions in veterinary field in general. SVFI have investigated specimens from animals, feeding stuffs, foods and environment.
The Reference Laboratory is situated at the State Veterinary and Food Institute in Bratislava. The main activities of Reference Laboratory are isolation and serotyping of *Salmonella* from specimens investigated at own institute, pretentious serotyping of *Salmonella* from other veterinary institutes, evalution and application of laboratory results, advising and support for the workers of the veterinary carefulness, animal breeders and workers of the animal production, performance of antibiotic suceptibility of isolated strains, performance of surveillance of *Salmonella* from animals / 2 times annually/, elaboration and verification of new methods in laboratory diagnostic, participation on international external quality assurance of isolation and determination of *Salmonella* (FEPAS) and serotyping and antibiotic susceptibility testing (EQAS), contributing to quality control of methods by laboratories other veterinary institutes in particular by organising ring trials, collaboration with other institutions (Public Health Laboratories, Food Research Institute, Research Laboratory at Comenius University).
There are used international standards for isolation, serotyping and susceptibility testing of *Salmonella* (ISO 6579, EN 12824, NCCLS, OIE). For serotyping are used commercially produced antisera.
The most frequent serotypes of *Salmonella* from animals in the year 2002 were:
S.Enteritidis, *S*.Choleraesuis (the most frequent from pigs), *S*.Infantis, *S*.Saintpaul, *S*.Derby, *S*.Virchow. *S*.Typhimurium.

Discussion:
Q: From whom do you receive strains ?
A: From other veterinary institutes.

1.4.4 Presentation NRL-Latvia

Andra Utinane and Kristine Kraujina, State Veterinary Medicine, Riga
(see Appendix 10)

No abstract available

Discussion

Q: When you referred to clinical samples for *S*. Enteritidis. What was the reason for testing ?
A: Monitoring and when the poultry had symptoms
Q: Do you organise inter-laboratory activities ?
A: Yes, we do organise inter-laboratory activities but other institutions in our country too.

1.4.5 Presentation NRL-Czech Republic

Marketa Tomsickova and Iva Barnardyova, State Veterinary Institute, Prague
(see Appendix 11)

NRL for *Salmonellas* in the Czech Republic works at the Department of Bacteriology in the State Veterinary Institute. NRL is constituted by State Veterinary Administration. Main task of NRL are: serotyping of *Salmonella* strains, keeping of collection strains, arranging of ring test. Laboratory serotypes around 300 strains a year. Control of occurrence of *Salmonella* in the Czech Republic is organized by SVA. Laboratories report number of examined samples to the Information Centre of SVA. NRL is main coordinator of monitoring of *Salmonella* resistance. Monitoring has run since 2OOO. To the monitoring are filed strains isolated from pigs, cattle and poultry. Antimicrobial resistance is determined by disk diffusion method according to NCCLS. Laboratory collaborates with Veterinary Research Institute in Brno. From 2OOO laboratory has attended Quality assurance system EQAS organized by WHO.

Discussion

Q: You isolated *Salmonella* spp. arizonae. From what animals ?
A: From Zoo animals.
Q: Are private laboratories also included in your system ?
A: No, they are not.

1.4.6 Presentation NRL-Poland

Andrzej Hoszowski and Darius Wasyl, National Veterinary Research Institute, Pulawy

(see Appendix 12)

The National Veterinary Research Institute was established in 1945 as a scientific institution of the Ministry of Agriculture. The Institute has modern equipment and qualified staff numbering 115 scientific workers among 352 employees. The major mission of the Institute is applied research in veterinary medicine, particularly control of animal infectious diseases including zoonoses, and safety of food of animal origin and animal feedstuffs. The National Veterinary Research Institute provides the advisory and expertise service for the Veterinary Administration, supervises regional veterinary diagnostic laboratories, participates in medicine and vaccine licensing process, runs postgraduate training for veterinarians. It is also the State Reference Centre for infectious diseases in animals and safety of food of animal origin and animal feedstuffs.
National Veterinary Reference Laboratory for *Salmonella* was established in the National Veterinary Research Institute by the decree of Minister of Agriculture and Rural Development of 13 Feb 2003. The area of interests involves isolation, identification and susceptibility testing of *Salmonella* originated from animals, food and feedstuffs. The epidemiological survey covers *Salmonella* infections in animals and epidemiological typing of the isolates. The surveillance system is based on the data reported from the regional veterinary laboratories.

Salmonella monitoring and control in Poland: all bovine, swine and poultry salmonellosis cases should be notified and reported to General Veterinary Inspectorate. National salmonellosis control programme in poultry (chicken, turkey, geese, ducks) was introduced in 1999. It is based on 92/117/EEC Directive. Poultry flocks are monitored for *Salmonella* infections. *S*. Enteritidis, *S*. Typhimurium are controlled in lying and broiler flocks, and *S*. Enteritidis, *S*. Typhimurium and *S*. Gallinarum are controlled in rearing sector.
National cooperation:
National Veterinary Reference Laboratory for *Salmonella* cooperates with regional veterinary diagnostic laboratories. There is no official cooperation between human and veterinary laboratories.
International cooperation:

- 6FP Coordinated Action No QLK2-CT-2002-01146 "Antibiotic Resistance in Bacteria of Animal Origin – II"
- COST Action 920 " Research and Surveillance of Foodborne Zoonoses throughout Europe: a Co-ordinated Food Chain Approach"
- External Quality Assurance System organised by WHO Global Salm-Surv

Discussion

Q: What kind of epidemiological typing did you perform in your studies ?
A: Several DNA typing systems but also antimicrobial susceptibility testing.
Q: Does your country have regulations for the use of antimicrobial substances ?
A: Yes, we follow the EU regulations.

1.4.7 Presentation NRL-Estonia

Lea Rander and Toomas Kramarenko, Central Veterinary and Food Laboratory
(see Appendix 13)

The origin of food-borne pathogens are tested in Estonian Veterinary and Food Laboratory (VFL) and in Estonian Public Health Laboratories (PHL). The Public Health Laboratories are administratively attached to the Health Protection Inspectorate. These four laboratories are involved in in the safety surveillance of foodstuff transferred the final consumer and its handling and official surveillance of human health.
The Estonian Veterinary and Food Laboratory is the network of five laboratories. All of them are involved in the safety of foodstuffs from producer to wholesale trade and official surveillance of animal health. The Laboratory reports directly to the Ministry of Agriculture in Tallinn and is administratively and financially independent from Estonian Veterinary and Food Board (VFB). The Central Veterinary and Food Laboratory (CVFL), which also functions as a reference laboratory, is situated in Tartu. As a governmental institution the first priority of VFL is to carry out the statutory testing under various farm animal disease surveillance and food safety control programs, also laboratory testing of imported and exported animals and relevant goods. Along the statutory functions the VFL offers the laboratory service to private veterinarians and farmers for the diagnosis and control of animal diseases and to food processing industry for food safety and quality control. The Estonian food control is based on the principle of own-check programmes and control visits in food

manufacturers, restaurants, shops and markets. The samples for pathogenic bacteria are analysed by VFL.
With co-operation of VFB, the laboratory is participating in *Salmonella* monitoring program. This program includes the *Salmonella* monitoring not only in farms but also in manufacturers processing food of animal origin. In addition to this, the laboratory determines the antimicrobial susceptibility of isolated *Salmonella* strains.
The Laboratory has a good co-operation with other regional VFL-s, Veterinary and Food Board and Public Health system. The stuff of laboratory has got contacts with different specialists from Finnish National Veterinary and Food Reseach Institute (EELA) and Swedish Veterinary Institute in Uppsala.

Discussion

Q: Do laboratories in your country collaborate in ringtrials ?
A: Yes, twice or thrice a year.
Q: Do you also investigate human isolates in your institute ?
A: No, only veterinary isolates

1.4.8 Presentation NRL-Lithuania

Ceslova Butrimaite and Rasa Giceviciene, National Veterinary Laboratory, Vilnius
(see Appendix 14)

No abstract available

Discussion

No questions

1.4.9 Presentation NRL-Slovenia

Vojislava Bole-Hribovsek and Jasna Micunovic, Veterinary Faculty of University of Ljubljana (see Appendix 15)

Veterinary Faculty in Ljubljana performs teaching, research work, diagnostics, foodstuffs, feeding-stuffs and drug control of official samples and other expert activities like diagnostics and treatment for individual clients. Veterinary Faculty is divided in organisational units like clinics and institutes, one of them is National Veterinary Institute. NVI has 9 laboratories performing examinations for *Salmonella* of clinical and post-mortem samples, samples of foodstuffs, feeding-stuffs and environment. Quality assurance is provided by QA system according to SIS/EN ISI/IEC 17025, which includes external (VLA; FEPAS) and internal quality assurance. For isolation and identification of *Salmonella* accreditation was granted in December 2002 by RvA and SA. Besides 9 veterinary laboratories of NVI, examinations for *Salmonella* are performed also by Institute for Microbiology and Parasitology and Institute for Food Hygiene of VF and by 3 poultry company laboratories. In public health sector

examinations for *Salmonella* (including antibiotic susceptibility monitoring) are performed by 9 Public Health Institutes and Institute of Microbiology and Immunology of Medical Faculty. In Slovenia monitoring is established for foodstuffs and feeding-stuffs and for poultry production. Control includes import of foodstuffs and quarantines of poultry and some exotic animals. Results from veterinary and public health sectors are collected and published.

Discussion

Q: Why did you only use an ELISA test for *S*. Enteritidis ?
A: This ELISA is being used for monitoring outbreaks and is much more important in our country than *S*. Typhimurium, which is important in pig producing farms. The question that should be answered is whether the ELISA results are negative or not.
Q: How is your institute financed ? Where do you get your money from ?
A: The NVI in Slovenia is financed from two different sources.

1.4.10 Presentation NRL-Turkey

Kadir Kaya and Selahattin Sen, Cebtral Veterinary Control and Research Institute, Ankara
(see Appendix 16)

The Central Veterinary Control and Research Institute was established under the name of "The Rinderpest Serum Institution" in the Eskehir province. In 1921, the instute was transferred to Ankara as the "Serum Production Institute". In parallel of the developments in science, the development of the institute was continued, and today the institute consists of 7 departments and 26 laboratories. The duties of the institute are research, diagnosis and control, training and production.
Research: control and eradication of regional and general diseases of animals, the development of modern diagnostical and production technics, control and eradication of the disease of fish and honey bees, research on public health and research on genetics and breeding.
Diagnosis and control: isolation, identification and serological diagnosis of bacterial, viral and parasitic diseases, diagnosis by histopathological and immunopathological technics, diagnosis of the diseases of fish and honey bees, identification of serotypes of *Salmonella*, Antibiotic sensitivity tests, analysis of minerals and trace elements, doping analyses, blood typing. Microbiological, residue and hormon analyses of animal originated foods, serological control of foods; control of imported vaccines, hatcheries, and health control of imported animals.
Training: training of maidservants, staff from other institutes and of intern students, convey scientific meetings, panels and seminars
Production: bacterial vaccines: -Max-Sterne Anthrax vaccine; Paratuberculosis vaccine and Inactive sheep vibriosis vaccine.
Viral Vaccines: Rinderpest, Bluetongue, Kelev rabies, Semple rabies and others
Production of test materials like antisera for identification of immunosera of *Salmonella* and *Campylobacter*, *Leptospira* strains, antibiotic discs.

Discussion

Q: Do you work together with regional laboratories ?
A: Yes, with eight regional labs and there is also collaboration with the human site.

1.5 Collaborative studies organised by CRL-Salmonella

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

Since 1995 the CRL-*Salmonella* organises yearly two collaborative studies:
1. study on typing of *Salmonella*;
2. study on bacteriological detection of *Salmonella*.

In these studies, 17 National Reference Laboratories (NRLs) for *Salmonella* and 18 Enternet Laboratories (ENLs, only in the typing studies) participate.
The aim of the studies is to determine whether examination of samples by the participating laboratories is carried out uniformly and that comparable results are obtained.

Typing studies

The type of samples used for the typing studies are pure cultures of different *Salmonella* serotypes, freshly cultured on a rich medium in a “mailing tube”.
Since 1995, eight typing studies have been organised. Each study included serotyping of different serotypes of subspecies of *Salmonella enterica*. Since 1998 also phagetyping of different phage types of *Salmonella* Enteritidis and *Salmonella* Typhimurium is included. In 2000 also testing for antibiotic resistance was added to the typing studies. The results of each study are reported in different steps:
1. checking of individual results by the participating laboratories on a print-out of the CRL;
2. presentation and discussion of (draft) results of all laboratories (using labcodes) at the (yearly) workshop;
3. summarising (final) results of all laboratories (using labcodes) of one study in a RIVM-report;
4. summarising several studies in an international publication.

Detection studies

The samples used for the detection studies exist of quantitative reference materials (RMs) and chicken faeces (negative for *Salmonella* as well as naturally polluted with *Salmonella*). Since 1995, six detection studies have been organised. In the first sudy only reference materials containing *Salmonella* Panama at a level of 5 cfp/capsule were analysed. In later sudies also RMs containing *Salmonella* Typhimurium (100 and 1000 cfp/capsule) were analysed with and without the presence of Salmonella negative chicken faeces. Since the third study (1998) also RMs containing *Salmonella* Enteritidis were added to the studies. In the last two studies also chicken faeces naturally polluted with *Salmonella* was analysed by the participating

laboratories. In all studies “prescribed” methods and “own” methods were used. The results of each study were reported in the same way as the typing studies.

Discussion

Q: When can the Candidate Countries join the collaborative studies from CRL-*Salmonella* ?
A: If you want to join the collaborative studies in 2003 the candidate countries have to pay the package and transport costs themselves
A: The expenses for 2004 will be financed by the EU
Q: Where do we send strains of possibly new serovars ?
A: Contact Dr. Popov (Institut Pasteur, Paris). You will receive the results in three months time. The strain representing a new serovar will also be sent to other reference laboratories for a double-check

2. Thursday 15 May 2003: day 2 of the workshop

2.1 Opening and introduction

André Henken, Director CRL-*Salmonella*, Bilthoven, the Netherlands
(see Appendix 18)

Opening and Introduction of the newly arrived participants of the Member States

First of all I would like to sincerely welcome also the Member States at this workshop. At this second day of the workshop both Candidate Countries and Member States are attending (see appendix 2 for participant list). I am very pleased that again this year Ms. Linda Ward from the Public Health Laboratory Services (Colindale, UK) is among us as during several years already now we co-operate together in the collaborative typing studies.
The functions and duties of the CRL-*Salmonella* according to the zoonoses directive have been presented yesterday (see page 9). The same holds for the aims of the workshop, but these can be extended a little bit now also the Member States are attending as to discuss also:

- general issues of relevance for CRL-/NRLs-*Salmonella*
    - EU level (e.g., zoonoses directive)
    - Reports of specific meetings/committees (e.g. ISO)
    - Organisational aspects of collaborative studies
- results of collaborative studies organised by the CRL-*Salmonella* with NRLs-*Salmonella*;
- results of collaborative studies within Member States;
- research activities of Member States;
- whether or not there are specific needs among NRLs-*Salmonella* and
- activities CRL - *Salmonella* 2003.

Opening website

As the representative of the EU Jean-Charles Cavitte was asked to open the new website of CRL-Salmonella. He stated the importance of such a website and asked the attendants of the workshop to send suggestions and remarks to CRL-*Salmonella*, Bilthoven, the Netherlands.
The website address is http://www.rivm.nl/crlsalmonella

2.2 Current issues in EU-regulation: monitoring and control

Jean-Charles Cavitte, European Commission, Brussels, Belgium
(see Appendix 19)

The main reasons for revision of the legislation were perceived needs to:

- decrease incidence of zoonoses in humans,

- improve the control of zoonoses in the primary production,
- strengthen the collection of relevant data, to support possibly risk assessment activities and risk management decisions

The proposals for revised zoonoses legislation were adopted by the Commission in August 2001. The Council common positions were adopted in February 2003 and the texts are now in second reading in the Council and the European Parliament, for co-decision. The Parliament is due to issue its position in its session of 18-19 June 2003. There may be agreement between EP/Council, which may lead to an entry into force of the new legislation before end of 2003.

The main features of the proposed Directive on the monitoring of zoonoses and zoonotic agents

- Surveillance throughout the food chain
- All food (animal and plant origin)
- Data in humans collected through the Community Communicable Diseases Network, apart from food-borne outbreaks
- Eight zoonoses and zoonotic agents to be compulsory monitored and other ones for voluntary monitoring
- Food-borne outbreaks required to be investigated and summarised data to be included in Member States reports
- Monitoring of antimicrobial resistance in zoonotic agents (*Salmonella*, *Campylobacter*, and possibly other agents in the future); in strains from animals (cattle, pigs, poultry) and food derived therefrom
- Monitoring based on the systems already in place in the Member States, but an procedure is established to harmonise in order to ensure comparability of data collected
- Competent authorities in animal/feed/food/human health sectors in the Member States required to co-operate
- Operators required to arrange for the keeping of strains of zoonotic agents isolated during own checks
- Co-ordinated monitoring programmes at Community level may be launched
- Member States report annually to the European Commission; the European Food Safety Authority (EFSA) prepares the Community report. Deadlines for reporting are end of May (of the following year) for member States and end of November for EFSA
- Reports made available to public without delay (EP)

Issues for future implementation of the Directive

- Need to consider schemes and methods for harmonised monitoring of zoonotic agents, antimicrobial resistance along the food chain
- Rules for keeping isolates
- Rules on foodborne outbreak investigations

The proposed Regulation on the control of salmonella and other specified foodborne zoonotic agents creates a framework for zoonoses control by setting targets for the reduction in

prevalence of pathogens (*Salmonella*), in animal populations essentially. Control measures will be defined more closely by Commission Decisions

The main features of the Regulation

- Pathogen reduction targets will be set, including the corresponding monitoring schemes to verify achievement of the targets.
- *Salmonella* serotypes considered to be with public health significance will be covered
- Progressive approach for the setting of targets: different targets set each year; national plans operational 18 months later
  Poultry breeding flocks: target set 1 year after entry into force (EIF)
  Layers: target set 2 years after EIF
  Broilers: target set 3 years after EIF
  Turkeys/slaughter pigs: 4 years after EIF
  Breeding pigs: 5 years after EIF
- When a target is established, each Member State will have to prepare and submit to the European Commission for approval, a national control programme
- Specific control methods used for controlling salmonella within the control programmes will be decided by Member States, unless certain control methods are restricted, banned or otherwise regulated by Commission decisions.
- responsibilities of food/feed businesses will be described and food/feed businesses may have own programmes agreed by the relevant Member State as part of the national programme
- Certain sampling requirements are defined
- Rules for trade in live animals and hatching eggs, including certification for intra-Community trade and for importation from third countries. Possibility to grant temporary additional guarantees
- Specific measures are defined:
  Fowl breeding flocks infected with SE/ST: slaughter/heat treatment/destruction
  Table eggs: have to originate from salmonella negative flocks (starting 6 years after EIF)
  Poultry meat: criterion of absence of salmonella in 25g or industrial heat treatment salmonella (starting 7 years after EIF)

Financial provisions are established, so that Community co-financing is foreseen for co-ordinated monitoring programmes, Community Reference Laboratories and implementation of new mandatory control measures (Commission report on financial arrangements due 3 years after entry into force)

Issues for future implementation of the Regulation

- Need to prepare for setting of first target(s): breeders (and laying hens); need to know before hand prevalence of serotypes in this/these animal populations and organise sufficiently harmonised sampling/testing schemes
- Consultation EFSA on different issues (specific control methods, target setting)

Laboratories

- Community Reference Laboratories (CRLs)/National Reference Laboratories (NRLs): to be appointed and tasks to be defined pursuant to the new legislation. There will be a need to reflect on CRLs/NRLs in conjunction with draft Regulation on Official Feed and Food Controls and the forthcoming revision of the Community microbiological criteria for food
- Laboratories involved in salmonella control required to be accredited within 2 years after EIF of the Regulation
- Laboratories ought to take part in ringtrials organised by NRLs
- Testing: methods recommended by International standardisation bodies as reference methods; possibility of using alternative methods validated in accordance with recognised protocols

Discussion

Q: There is a criterion which states that *Salmonella* should be absent in 25 g faeces of broilers. Is it allowed to perform decontamination with chemical agents ?
A: There is a procedure foreseen to allow some chemical agents, not yet excluded. It is under consideration for the moment.
Q: Can we meet all the goals within the period as indicated by the Commission ?
A: At certain moments in time the Commission will know the stages we all are in. Perhaps we have to reconsider our targets or have to speak about transitional guarantees.
Q: The costs of ringtrials are extremely high, we are doing more and more over the years. What can we do ?
A: Number of ringtrials is not fixed. Indeed it is costly. It is the responsibility of the Member State.
Q: We need to look for more realistic ringtrials which means less work.

2.3 Epidemiology of *Salmonella* spp. in the EU

Annemarie Kaesbohrer, CRL-Epidemiology of Zoonoses, Berlin, Germany
(see Appendix 20)

Two main issues

The Report on "Trends and sources of zoonotic agents in animals, feedingstuffs, food and man in the European Union and Norway in 2001 (Doc. SANCO/56/2003)" and the results of the workshop 2002, organised by the CRL-Epidemiology in Berlin.

In 2001, in animals, in general there seemed to be a decreasing trend in the *Salmonella* prevalence. In the countries with an approved control programme for several years now, the situation remained favourable. Reports from the individual countries have to be evaluated carefully. Besides differences in the monitoring schemes applied, the way (and content) of presenting the information might have been changed and thus may lead to a misleading interpretation. Some examples were given.

During the last workshop, the reporting of *Salmonella* serotypes and phagetypes was discussed. The representatives of the National Reference Laboratories were asked to provide the results of serotyping broken down at least by the main categories: humans, animal (poultry, cattle, pigs, others), food (poultry meat, eggs, beef, pork, other food) and feedingstuffs (animal derived feed materials, vegetable derived feed materials, compound feedingstuffs). More detailed information would be desirable, especially for the poultry sector (separated for egg and meat production line, breeders and productive flocks and the poultry species). A distinction of the isolates from monitoring programmes from those of diagnostic examinations would be desirable. Some examples of the information currently available in the zoonoses report and the way of presentation were given.
Another issue discussed was the reporting of the results of antimicrobial resistance monitoring. Compared to the previous year, more countries were able to follow the guidelines for reporting, i.e. as regards the animal species covered, the *Salmonella* serovars included in the programme and the antimicrobials tested. There is still some further work necessary to improve the comparability of the data between the laboratories and countries. Beginning in the report on the year 2002, the reporting system will also cover the reporting of quantitative data of antimicrobial resistance monitoring of *Salmonella* and *Campylobacter*.

Discussion

Q: What can you tell about the integration of human and animal data ?
A: The different countries should integrate human and animal data themselves on a national level and include the data in the national report.

2.4 Results bacteriological detection study VI

Hans Korver, CRL-*Salmonella*, Bilthoven, the Netherlands
(see Appendix 21)

A sixth bacteriological collaborative study was organised by the Community Reference Laboratory for *Salmonella* (CRL-*Salmonella*, Bilthoven, the Netherlands) in 2002. Seventeen National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) participated in the study.
Reference materials in combination with or without the presence of chicken faeces, as well as naturally contaminated faecal samples (containing *Salmonella* Infantis) 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 amended ISO 6579: 2002 [including 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 a high level of STM than, in declining order, from capsules with a high level of SE, from capsules

containing a low level of STM and from capsules with a low level of SE, analysed in the presence of (*Salmonella* negative) chicken faeces.
The overall results of all different capsules as well as the results of the naturally contaminated samples revealed better results for MSRV (with BGA and XLD as plating-out media) in comparison with the ISO 6579: 2002 method.

Discussion

The discussion was mainly focused on trying to find reasons for many negative results in collaborative study VI (2002) on the detection of *Salmonella*. The following points were discussed on their possible influences on the results:

1. transport time and transport temperature of the samples
   A correlation between long transport time and poor results was not found in this study. However, the information on transport temperature has been limited. For this purpose small electronic temperature recorders may be used in next studies
2. handling of capsules. Were the capsules completely dissolved?
   CRL-*Salmonella* will perform some extra tests to find the optimal time/temperature combination to dissolve the capsules completely
3. poor management in the laboratory during collaborative study
   Laboratories are asked to inform CRL-*Salmonella* if a reason for low results can be explained by the performance of the laboratory
4. quality of the media, possible differences between batches and/or manufacturers
   It is always important to check the quality of the media before use. It would be helpful if a standard test would be available for this purpose
5. cross contamination (positive blank controls).

The importance of finding reasons for poor results was also stressed by Jean Charles Cavittte. For this purpose trend analysis will be very important. Such an analysis can show whether poor performance of a laboratory is incidental or a trend.
During the study, the laboratories found more positive results when analysing naturally contaminated faeces than with the artificially contaminated faeces (using capsules). It was asked whether the contamination level of the naturally polluted faeces was known. MPN-results have shown an estimate level of *ca* 10^2 cfp/ 25 g faeces of *Salmonella* Infantis.

A final remark was made on the performance of the PCR methods. The present PCR methods work for food matrices, but are not yet optimised for a matrix like chicken faeces.

2.5 Discussion on design bacteriological detection study VI

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

Beside the proposal on the design of the bacteriological detection study also temperature recording during transport of samples was discussed. As elevated temperatures can have a negative effect on the mean number of *Salmonella* in the reference materials (RMs) as well as in faeces, it is important to obtain more information on the transport temperatures of the parcels. It was proposed to buy for this purpose small electronic temperature loggers, which

can be included in the parcels and can be re-used. As these loggers need to be programmed and read with special software, it is important that the loggers are returned to the CRL as soon as possible after receipt of the parcel. For this purpose full support was asked of the laboratories. The participating laboratories agreed to give support on the use of the temperature loggers, meaning that the CRL can start ordering some loggers as soon as possible.

For the design of the bacteriological detection study VII (2003) the following was **proposed and discussed**:

- date of the study: November 2003;
- same number and type of samples as study VI, but less methods.

Proposal:

Samples	Methods
5 STM10 + 10 g faeces (Salm neg) 5 STM100 + 10 g faeces (Salm neg) 5 SE100 + 10 g faeces (Salm neg) 5 SE500 + 10 g faeces (Salm neg) 5 Blank + 10 g faeces (Salm neg) 3 STM10 no faeces 3 SE100 no faeces 2 SPan no faeces 2 Blank no faeces 20 x 25 g Salmonella positive faeces	"Reference method": pre-enrichment in BPW selective enrichment in MSRV Plating-out on BGA and XLD "Own method": Preferable the (one) method routinely used

It was remarked that the availability of chicken faeces will depend on the situation concerning *Aviaire influenza* in the Netherlands. If no faeces is available at the time of the collaborative study, the study will most probable be organised without faeces.
Some other remarks were made concerning the number of methods. Most of the laboratories were in favour with less methods but were also concerned that the ISO method is not longer prescribed and it was questioned whether CRL should ask and/or advice some NRLs to use the ISO procedures as OWN method. This point on methods will be further discussed within the CRL-group. The NRLs will be informed on the final set up of the study later.
Some candidate countries already would like to participate in the study of 2003. The NRLs of these candidate countries were asked to inform Hans Korver if they would like to participate. CRL will next try to find out how and if this can be organised concerning the financial aspects.

2.6 Results typing study VIII: serotyping and antibiotic resistance

Hans Korver, CRL-*Salmonella*, Bilthoven, the Netherlands
(see Appendix 23)

In 2003 the eighth collaborative study on serotyping of *Salmonella* was organised by the Community Reference Laboratory for *Salmonella* (CRL-*Salmonella*, Bilthoven, the Netherlands) in collaboration with the Public Health Laboratory Services (PHLS, Colindale) in London and the Central Institute for Animal Disease Control – Section Infectious Diseases (CIDC, Lelystad, the Netherlands).
Laboratories that were interested had the possibility to perform phage typing and antimicrobial susceptibility testing. The main goal of this collaborative study was to compare the results among the National Reference Laboratories (NRLs-*Salmonella*) and among the EnterNet Laboratories (ENLs).
All NRLs-*Salmonella* of the Member States of the European Union (16) and NRL-Norway participated in the collaborative study. Seven of the 17 participating NRLs-*Salmonella* also performed phage typing. Fifteen ENLs participated of which 11 laboratories performed phage typing. Three of the NRLs-*Salmonella* are also ENLs. The results of these NRL/ENLs will only be mentioned with the NRLs-*Salmonella*. All three of these laboratories performed phage typing. 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. The laboratories were allowed to send strains for serotyping to another specialised laboratory in their country. Most problems were encountered when typing the H-antigens.
The PHLS selected 20 strains for phage typing, 10 were of the serovar *Salmonella* Enteritidis (SE) and 10 of the serovar *Salmonella* Typhimurium (STM). Two NRLs and four EnterNet Labs typed the SE strains correctly. Four NRLs and three ENLs typed the STM strains correctly.
In this study the results of antimicrobial susceptibility testing are also included. Ten strains of various *Salmonella* serovars had to be tested with a panel of twelve antibiotics. Three different kind of tests were used in this study namely minimal inhibition concentration (MIC), E-test and the disc diffusion test. For the MIC and E-test concentrations were recorded beside the notations sensitive, intermediate and resistant. For the disc diffusion inhibition zones in mm were asked beside the notations sensitive, intermediate and resistant. In this report deviations are recorded as minor and major deviations. Most problems occurred with the interpretation of the results obtained with antibiotic streptomycin.

Discussion

Q: Have the strains been checked out against various commercial sera?
A: No, they have only been identified by using the RIVM sera.
Follow up: The outcome of serotyping of e.g. a strain of *S.* Lexington may differ dependant on the commercial sera used.
Follow up: The same problem is recognized in Belgium: for certain reactions different results are obtained with different sera.

Follow up: If you have problems with the performance of your sera you should contact the manufacturer.
Q: Would it be a possibility to send our sera to Bilthoven to be checked out by CRL Salmonella?
A: In principle, this is a possibility.
Follow up: The real problem is the difference in antisera used; there is a need for a standard serotyping method.

2.7 Results typing study VIII: phagetyping

Linda Ward, Public Health Laboratory Service, London, United Kingdom
(see Appendix 24)

Ten *Salmonella* Enteritidis and ten *Salmonella* Typhimurium strains were selected for this study, from the salmonella collection of the National *Salmonella* Laboratory in England and Wales. They were recent isolates that had been typed during the previous year. So far, results have been received from four National Reference Laboratories (NRL) eight Enter-net Laboratories (ENL) and three laboratories that are both NRL and ENL laboratories. Five *S.* Enteritidis and five *S.* Typhimurium strains were typed correctly by all laboratories. The *S.* Enteritidis types giving most problems were PT5c and PT33 and for *S.* Typhimurium were definitive phage types (DT) 37 and DT 141. However, overall the results were satisfactory with twelve (80%) of the fifteen laboratories obtaining at least 90% correct typing results.

Discussion

Q: How can the Candidate Countries be supported to perform phage typing ?
A: The method of preparing the phages is difficult (identical preparation is done at PHLS) but the method itself is easy to perform.

2.8 Discussion on design typing study IX (2004)

Arjen van de Giessen, NRL-the Netherlands
(see Appendix 25)

A proposal for the design of the typing study in 2004 was presented by A.W. van de Giessen (NRL/CRL the Netherlands) followed by a discussion on this issue.

Proposal for design by A.W. van de Giessen
On behalf of the CRL-*Salmonella* mr. Van de Giessen proposed to include in typing study IX (spring 2004) the same three components as included in study XIII, i.e. serotyping, phagetyping and antimicrobial resistance. For serotyping, it was proposed to include 20 strains, selected by CRL, including serovars with public health significance, serovars with antigens similar to those of public health significant strains and serovars that have caused

typing problems in previous studies. For phagetyping, it was proposed to include 20 strains as well, selected by PHLS London, including 10 strains of *S.* Enteritidis and 10 strains of *S.* Typhimurium. For the antimicrobial resistance testing, it was proposed to test 10 strains, selected by CRL- *Salmonella*, as well as some control strains. Furthermore, a test panel for resistance testing was proposed based on the recommendations made at the EU-workshop on analytical methods held in Berlin in October 2002. Finally, mr. Van de Giessen referred to the methodological problems encountered with respect to resistance testing. Both different testing methods (qualitative versus quantitative; NCCLS versus other standard methods) and different interpretive criteria are used by the NRLs of the different Member States. Therefore, the need for harmonization of results was underlined and reference was made to the workshop on this issue scheduled for the next day.

Discussion

Q: Sometimes we test our strains with antisera that do not show the proper discrimination. What should we do about it ? The evaluation of the correctness of the antisera of various manufacturers should be checked.
A: You should check and evaluate your own system. This should be done at a national level.
Q: Do we need to test twenty strains for serotyping ?
A: This will be discussed during this session. It is decided to use 20 Salmonella strains for the next collaborative study. Among these twenty strains included the 5 most important serotypes.
Suggestion: Include Java in the next ringtrial.
Suggestion: Include again 10 SE and 10 STM strains for the next collaborative study.
It is decided to include 10 strains for antimicrobial susceptibility testing. Also add one or more control strains like *E. coli* (ATCC 25922). The number of antibiotics to be tested will be discussed on Friday 16 May 2003.

2.9 Interaction between the NRL and private laboratories in the Republic of Ireland

John Egan, NRL – Ireland, Dublin, Ireland
(see Appendix26)

No abstract available

Discussion

Q: When are private laboratories allowed to take part in monitoring programmes ?
A: The private laboratories have to join the ringtrial before that are allowed to take part in the monitoring programmes.
Follow up: Private laboratories tend to take over the workload of the National Institutes.

2.10 Salmonella in commercial egg layers in Northern Ireland: results of a prevalence survey

Stanley McDowell, NRL-Northern Ireland, Belfast, UK
(see Appendix 27)

Following increases in the number of *Salmonella* Enteritidis isolates from humans in Northern Ireland in 1998 and 1999[1], a survey was commissioned to establish the prevalence of infection in commercial egg laying flocks. Eligible flocks (those with >500 birds) were sampled by DARD staff during the latter part of 2000 and the first three months of 2001. Samples consisting of 6 composite dust and 6 composite litter or faecal samples were taken from a range of locations within each house and tested from *Salmonella*. In total 118 sites were sampled of which 106 had laying birds, 10 had birds in the rearing stage and 2 had both. Overall *Salmonella spp.* were isolated from 30 sites (25.4%), with *S.* Enteritidis isolated from 14 sites (11.9%), *S.* Typhimurium from 3 sites (2.5%) and other *Salmonella spp* from 15 sites (12.7%). More than one species of *Salmonella spp.* was isolated from two sites (1.7%). There was wide variation in the proportion of samples from infected houses which tested positive with only a single sample positive in 15 / 43 (35%) houses. Overall, dust samples were more likely to test positive that faecal samples, with a significantly greater number of houses positive only on dust compared to the number positive only on faecal sampling (P=0.0309). Preliminary univariate analysis has indicated that larger sites (>20,000 birds) were more likely to be infected with *S.* Enteritidis than smaller sites (<10,000 birds) (OR 8.17; P=0.005). Sites with a previous history of infection were also more likely to test positive (OR=8.89; P=0.002). Farms that tested positive in the survey were given advice by DARD staff on possible control options, which in the case of *S.* Enteritidis included advice on the use of vaccination. Since the survey was commissioned the number of isolates of *S.* Enteritidis from humans has decreased dramatically with the number of isolates in 2002 less than a quarter of that found in 1999[1].

References

1 Communicable Disease Surveillance Centre (Northern Ireland). Surveillance data on gastrointestinal infections 1992-2002. Accessible from http://www.cdscni.org.uk/surveillance/Gastro/default.asp.

Discussion

Q: Were the birds only sampled once ?
A: Yes

2.11 Salmonella in eggs: the saga continues ?

Linda Ward, Public Health Laboratory Service, London, UK
(see Appendix 28)

Since 1997 PT4 the most prevalent phage type in England and Wales has declined possibly as a direct result of the use of vaccines in poultry. In 2002 only 37% of *S.* Enteritidis infections were caused by *S.* Enteritidis PT4. Other phage types have become more prevalent and in late 2002, were being implicated in several outbreaks. The largest of these outbreaks was detected in September with an increasing number of human reports of *S.* Enteritidis PT14b, a phage type frequently linked with foreign travel, mainly to Mediterranean countries. However in September the majority of PT14b cases had not been abroad. A major outbreak investigation led to the possibility that raw shell eggs imported from Spain could be the source of infection. Batches of Spanish eggs were examined and *S.* Enteritidis was isolated. However, it was *S.* Enteritidis PT6a. Coincidentally an outbreak of PT6a was being investigated in a London Hospital, where eggs of Spanish origin were also being used. Samples of eggs collected from the hospital kitchen were also examined and six distinct strains of *S.* Enteritidis were found, including PT14b ! Eleven different strains of *S.* Enteritidis have been isolated from Spanish eggs and outbreaks have been caused by phage types 1, 6, 6a, 6d, 14b, and 58. Phage typing has been an invaluable tool in the detection and investigation of these outbreaks.

Discussion

Q: What is the follow up concerning the Spanish eggs ?
A: It is not prohibited to import the eggs to the UK.
Q: Is phage type 14b also present in other countries ?
A: Phage type 14b is more of a continental type of strain. In the UK you have more than one type of phage type 6.

2.12 Evaluation of pooled serum and meat juice in a *Salmonella* ELISA for pig herds.

Robert Davies, NRL-*Salmonella*, Addlestone, UK
(see Appendix 29)

Purpose: Monitoring for *Salmonella* in slaughter pigs is important to enable targetted control measures to be applied on problem farms and at the abattoir. Currently monitoring of pig herds for *Salmonella* is usually carried out by testing large numbers of individual tissue fluid samples for anti-*Salmonella* antibodies. The aim of this study was to determine whether mean optimal densities or sample/positive ratios obtained by testing pooled samples by ELISA could be a less expensive alternative test.
Methods: Samples of “meat juice”, serum, caecal contents and carcase swabs from 420 pigs on 20 commercial finishing or breeder finisher farms were tested. Additionally, pooled floor faeces were taken from the finishing pens on the farms of origin. *Salmonella* was cultured by

a BPW, Diasalm, Rambach Agar Method and ELISA tests were carried out using a commercial ELISA kit. Statistical analyses and correlations were carried out with Statistica software.
Results: *Salmonella* was found in samples from 19 of the 20 farms. 32.8% of pooled pen faeces and 24.3% of caecal samples were positive but Salmonella was only found in 1.7% of carcase swabs. 43.2% of "meat-juice" samples and 25.3% of serum samples gave positive ELISA results. None of the ELISA tests showed a statistically significant correlation with caecal carriage of *Salmonella* or contamination of carcases, although the percentage positive pen faeces did correlate significantly with caecal positives. Only serum mean optical density from pools of 5, 10 or 20 sera correlated significantly with *Salmonella* in pen faeces but all pooled serum and "meat-juice" optical density or sample/positive ratios correlated significantly with the percentage positive samples by individual ELISA.
Conclusions: The results suggest that a simple pooled sample of "meat-juice" or serum could substitute for 20 individual tests and allow a herd monitoring schedule based on regular tests of small numbers of pooled samples. Bacteriological examination of pooled pen faeces provides the best indication of herd *Salmonella* status and whether serotypes of major public health significance are present however.

Discussion

Q: Is there a good correlation between separate and pooled sera ?
A: Yes, a good correlation was found between separate and pooled sera.

2.13 Discrimination of *Salmonella* enterica subspecies enterica d-Tartrate fermenting and non-fermenting isolates by genotypic and phenotypic methods

Reiner Helmuth, NRL-*Salmonella*, Berlin, Germany
(see Appendix 30)

During the last decade multidrug-resistant *d*-tartrate-positive *Salmonella enterica* subspec. *enterica* serovar Paratyphi B (*S*. Paratyphi B dT+) isolates (formerly called *S*. Java) have increasingly been isolated from poultry and poultry products in Germany and in the Netherlands. Recent studies by Brown et al. strongly suggest that the same multiresistant clone found in German and Dutch poultry was responsible for a significant proportion of 10 human cases of *S*. Paratyphi B dT+ in Scotland. So a reliable easy test for d- (L+) Tartrate fermentation has become increasingly important.
A multiplex PCR and an improved lead acetate test were developed to discriminate *Salmonella enterica* subsp. *enterica* d-tartrate fermenting and non-fermenting strains. Both methods showed a concordance of 100% when 127 *Salmonella* strains belonging to 15 serovars were tested. Special emphasis was given to *Salmonella enterica* subsp. *enterica* serovar Paratyphi B isolates because of the clinical importance of its d-tartrate non-fermenting variant. The PCR assay was based on the genotypic difference of the presence

(d-tartrate fermenting strains) or absence (d-tartrate non-fermenting strains) of the ATG start codon for the gene STM 3356, encoding a putative cation transporter. Sequence data revealed a single nucleotide exchange within the ATG start codon of gene STM 3356 in the d-tartrate non-fermenting strains from G to A. In order to increase the reliability of the PCR assay, a positive control based on a *Salmonella* genus specific primer set for the detection of *Salmonella* DNA was included. The PCR-based discrimination needs only several hours versus 6 days using the improved lead acetate test to obtain the result. Consequently, the PCR d-tartrate assay should be the method of choice for the discrimination of d-tartrate fermenting and non-fermenting

Discussion

Q: What is the golden standard ? How did you know what was d-tartrate postive ?
A: Nowadays the PCR is our “standard” method to see what is going on.

2.14 Current issues in EU-regulation: analytical methods and antibiotic resistance testing

Jean-Charles Cavitte, European Commission, Brussels, Belgium
(see Appendix 31)

See abstract under 2.2 on pages 23-26

Discussion

The discussion about questions raised after the presentation were discussed on Friday 16 May 2003 during the two workshops

3. Friday 16 May 2003: day 3 of the workshop

3.1 Introduction to the workshops

André Henken, Director CRL-*Salmonella*, Bilthoven, the Netherlands

A special word of welcome to Dr. Dik Mevius (Central Institute of Disease Control, Lelystad, the Netherlands) and Dr. Henk Stegeman (RIKILT, Institute of Food Safety, Wageningen, the Netherlands). Both speakers are experts in the field of respectively antibiotic resistance testing and detection of Salmonella. This morning two separate workshops are held simultaneously about these two subjects. Our proposal is that one person per NRL cq. country will join one of the workshops, in this way all states will be represented in each workshop.

3.2 Introduction to workshop 1: antibiotic resistance

The workshop was opened by the discussion leader (A.W. van de Giessen, CRL/NRL, the Netherlands), who referred to the problems encountered with the interpretation of antimicrobial resistance data and expressed his hope to come one step further in the process to harmonisation of results. Subsequently, an introduction on this issue was given by Dr. Dik Mevius from the Central Institute for Animal Diseases Control in Lelystad, the Netherlands.

Abstract of the presentation of Dik Mevius entitled:
Problems in reporting resistance data from different locations (countries of laboratories)

While trying to implement international reportage of resistance data, amongst others the following problems will be encountered:

- different testing methods used; qualitative versus quantitative, NCCLS versus CRG, BSAC, SRGA etc...
- different interpretive criteria used
- different antibiotic panels used
- different selection criteria from strains (serotypes/phagetypes) used

It is tempting to try to **standardise** all these aspects before starting to report results of resistance data from different locations. However this is utopia and unrealistic. A lot of arguments, varying from very good to very bad ones, can and will be used by laboratories within and between countries to be unwilling to change existing methodologies. The result is that standardisation is a long term goal and long term will be sooner decades than years.

In order to improve the existing situation the first step should be an attempt to **harmonise** the results.
Harmonisation means that whichever method is used, the result are similar.
How can that be obtained, and how can that be controlled ?

The optimum methodologies to be used including inclusion or selection criteria for strains to be tested should be described. Within the EU 5th Framework Programme this was done in a concerted action entitled: "Antimicrobial Resistance in Bacteria of Animal Origin" (ARBAO, FAIR PL 97-3654) coordinated by AFSSA (Pascal Sanders). The recommendations of this concerted action are available at http://www.fougeres.afssa.fr/arbao/Recommendations/surveillance.htm.

In the Netherlands for resistance surveillance purposes in Hospitals and medical diagnostic laboratories for this problem a "Surveillance Standard" was published and distributed to all laboratories and hospitals in the Netherlands. In this publication quantitative methods are promoted and it includes the message that data can be harmonised by using internal quality assurance system with ATCC strains. If data comply with criteria for ATCC control strains then, whichever method is used, they are comparable in quality.

Following the above mentioned ARBAO project, recently a new concerted action with FP5 was started coordinated by Frank Aarestrup, ARBAO II. The purpose of project is to establish an External Quality Assurance System (EQAS) for susceptibility tests for a list of animal bacterial species (incl. *Salmonella*, *Campylobacter*, *E. coli* and animal pathogens). The result of EQAS will be that national summary resistance data (resistance proportions) will be improvingly comparable.

How is EQAS organised:

- a central laboratory organises a web page for downloading EQAS-results
- for each group of bacterial special one national veterinary reference laboratory acts as reference laboratory. Tasks are:
    - select a panel of well defined strains of certain species
    - define a list of antimicrobial agents to be included in EQAS for each species
    - determine the susceptibility with reference NCCLS method
    - have both identification and MIC's confirmed by another reference laboratory
    - send the strains (lyophilised or on charcoal swabs) on predetermined intervals and include a short questionnaire on methods used including breakpoints/interpretive criteria.
    - the strains should be tested by the methods routinely used in the laboratory.
        - E.g. Susceptibility testing of *salmonella* and *E. coli* is performed against as many as the following antimicrobials as possible: ampicillin, amoxicillin/clavulanic acid, ceftiofur, cefotaxime, chloramphenicol, ciprofloxacin, florfenicol, gentamicin, nalidixic acid, neomycin, streptomycin, sulphonamide, tetracycline, trimethoprim and the combination of sulphonamide and trimethoprim. However is not prescribed that all antibiotics mentioned are to be included.
        - Amoxicillin can be used instead of ampicillin, and another fluoroquinolones as substitute for ciprofloxacin.

Together with the strains a test form for filling in results will be sent, and a password for entering the results into an interactive web database on the ARBAO-II homepage. When you enter the results via the Internet, you will be guided through all steps on the screen and you will immediately be able to get an evaluation report of your results. Annually results are discussed in a plenary meeting.

The expected results of ARBAO II are: comparable and quality controlled information on the resistance situation among food animals in the different EU-countries will be obtained.
Moreover summary data comparable in quality from different EU-member states can be loaded on a web page.

In conclusion:
For the purpose of **standardisation** a quantitative method for susceptibiltiy testing can be promoted. Preferably micro broth dilution using custom made panels of antibiotics.
Pro's: easy to standardise and control, easy to perform on large numbers. The method is used for this purpose in many countries which increases comparability of results.
Contra's: expensive, skilled technicians needed...

For the prupose of **harmonisation** of results:
- describe internal controle SOP with ATCC contol strains and interpretive criteria.
- organise EQAS

Summary data from different countries/labs are comparable in quality end and can be reported on a webpage, or in a report.

Do not be too prescriptive, that inhibits the will to participate!

3.3 Presentation and plenary discussion on workshop item 1

Following discussion in the subgroup attending this workshop, a summary report on this issue was given by dr. Reiner Helmuth (NRL Germany) addressing the following questions:
what do we know about antimicrobial resistance testing?
Worldwide, there is an increased awareness of developments in antimicrobial resistance.
In Europe, comparison of data on antimicrobial resistance from different countries is hampered by the use of different testing methods and different interpretive criteria.
The use of quantitative testing methods has – despite their diversity – generated important data relevant for public health. Quantitative resistance data have become an important epidemiological marker, e.g. in the spread of multiresistant *S.* Typhimurium DT104 and of a resistant clone of *S.* Paratyphi var. Java.
What do we do presently?
A discussion process has been initiated that should lead to standardization of testing methods and harmonization of data.
Within the EU 5th Framework Programme concerted actions (ARBAO I and II) have been launched to describe the optimum methodologies for resistance testing and establish an External Quality Assurance System (EQAS, see above).
What should be done?
- The EU should be encouraged to introduce quantitative testing (preferentially broth dilution MIC) as a standard (also WHO may play an important role in this respect).
- CRL-*Salmonella* should develop EQAS and IQAS (see above) for resistance testing of *Salmonella*. For this:

- a panel of well defined strains should be selected (the new Zoonoses Directive offers a great opportunity to get additional relevant isolates)
- NCCLS should be used as reference method.
- strains should be tested by the methods routinely used in the laboratory
- the following panel of antimicrobial agents is recommended:
  - Chloramphenicol
  - Florfenicol
  - Ampicillin
  - First 3rd generation Cephalosporin, e.g. Cefotaxim
  - Second 3rd generation Cephalosporin, e.g. Amoxicillin
  - Enrofloxacin or Ciprofloxacin
  - Nalidixic Acid
  - Sulfonamide/Trimethoprim
  - Sulfonamide
  - Trimethoprim
  - Streptomycin
  - Gentamicin
  - Kanamycin or Neomycin
  - Ampicillin

Q: Is it necessary to include nalidixic acid in the panel as well enrofloxacin or ciprofloxacin?
A: It is important to include nalidixic acid for getting qualitative results (if not using MIC testing)
Q: How to select strains for routine testing?
A: The most important goal is to harmonise the results from different countries. Leave it to the country which method is used and which percentage of strains is selected.
Q: Are the strains being retested before they are sent to the NRLs ?
A: Yes, the strains were retested before sending.
Q: Preferably the method to be used should be quantitative. Do you mean MIC or otherwise ?
A: We prefer MIC over the disk diffusion. Disk diffusion results are more difficult to compare.
Follow up: Until 2001 only qualitative data were collected. From 2002 also quantitative results are being collected.
Follow up: This means a lot of paper work.
Follow up: Yes, indeed, it is more complex to process MIC values.
Q: Have the CCs already started to investigate susceptibility of *Salmonella* strains ?
A: Yes, the CCs have already started.
Follow up: Sensititre prepares plates with special concentrations on request.
Follow up: The standard operation procedure/guidelines should be written by a small group of people.
Follow up: Kaesbohrer: This is the task of CRL-*Salmonella.*
Q: How should the selection of strains be arranged ?
Q: Which types do you send to the NRLs ?
A: Serotypes are the most important.
Follow up: Phenotypes are more important for the selection of the strains.
Follow up: Include type java for the AST as well as serotyping.

3.4 Introduction to workshop 2: detection methods

Henk Stegeman, RIKILT, Wageningen, the Netherlands
(see Appendix 33)

Standardized method for the detection of Salmonella in poultry matrices

Within the frame of the new Zoonoses Directive there is a need for a standard method for the detection of *Salmonella* in poultry matrices, especially poultry faeces. The present EN-ISO 6579 standard method is primarily intended for detecting this pathogen in foods and animal feeding stuffs. Therefore for the detection of *Salmonella* in poultry faeces modifications of this standard are most frequently used by the member states.
The Dutch control programme for *Salmonella* was started for broilers and layers in 1997 and turkeys in April 1999. The aim was to reduce *Salmonella* and *Campylobacter* in the poultry sector. For the detection of *salmonella* in the poultry chain a Dutch standard was developed.

The Dutch standard is based on a validation study of the Dutch Animal Health Service. In this study four detection methods for selective enrichment of *Salmonella* in poultry faeces were compared; MSRV (Modified Semi-Solid Rapport Vassiliadis), RV, RVS and Selenite Cystine Broth. It was found that approximately 95 % of the samples containing *Salmonella* would be detected by the combination of MSRV and RV (or RVS), followed by MSRV (91%) and RVS (69 %). A study of RIVM confirmed these results (faeces poultry layer flocks: RV + MSRV 95 %, MSRV 92 %, RV 41 %; faeces broiler flocks MSRV + RV 98 %, MSRV 93 %, RV 60 %). These studies show that the combination of MSRV and RV (S) is more suitable than ISO 6579: 1993.

Although with the combination of MSRV and RV more positive isolations were obtained for practical reasons the Dutch standard is based on the single use of MSRV. The combined procedure was much more labor intensive and the increase of positive samples was limited (2-3 %).

In a later study the Dutch standard was compared with a PCR-method (Probelia),using the protocol for the validation of alternative methods (EN-ISO 16140). This study showed that there was a good correlation between both methods. Both methods are now allowed for the control of *Salmonella* in the poultry chain.

3.5 Presentation and plenary discussion on workshop item 2

There is a need for a separate method for the analyses of *Salmonella* in (poultry) faeces. The present ISO method is intended for the analyses of food and feeding stuff and is not the best method for the analyses of faeces. From several studies (validation studies in the Netherlands, CRL collaborative studies, experiences of several laboratories) it can be concluded that a semi-solid medium gives good results in the selective enrichment step for the isolation of *Salmonella* from faeces. The only problem with the use of only semi-solid media is the fact

that non-motile *Salmonella* strains (like *Salmonella* Gallinarum) will not be detected. This can easily be solved by adding a broth as second medium in the selective enrichment step.

The procedure for analysing *Salmonella* in (poultry) faeces could exist of the following steps:

1. pre-enrichment in a non-selective medium, like Buffered Peptone Water (BPW, ISO 6579: 2002). It was remarked that good care should be taken with this medium concerning preheating of the medium (to which temperature?), incubation temperature and incubation time.
2. selective enrichment in:
   a. a selective semi-solid medium and
   b. a selective broth
   a. laboratories indicated to have good experiences with semi-solid media Diasalm and Modified semi-solid Rappaport Vassiliadis (MSRV). Diasalm as well as MSRV has some advantages and some disadvantages. Diasalm contains less inhibitory ingredients and more nutrients than MSRV and might therefore result in more positive results. Furthermore it is possible to perform serotyping directly on the colonies from Diasalm. MSRV is more worldwide used and accepted. It is possible to perform biochemical confirmation directly on the colonies of MSRV. The amount of disturbing background flora on MSRV is low. For both media some practical training will be necessary. Furthermore, special attention should be taken for manufacturer and batches variability and the consistency of the plates (if the agar is too "sloppy", the plates can not be transported).
   b. as selective broth Rappaport Vassiliadis (RV, ISO 6579: 1993) or Rappaport Vassiliadis with Soya (RVS, ISO 6579: 2002) were suggested.
3. plating-out.
   A wide variety of plating-out media is used in the European laboratories and no consensus on the 'best choice' could be made.

It was discussed whether it would be necessary to do a full validation study for a "new" method for the analyses of faeces or whether sufficient information can be obtained from the literature. J.C. Cavitte of EC DG-Sanco mentioned that for the new Zoonoses Directive a method will be needed soon. The fastest way to come to a "reference method" for the analyses of *Salmonella* in faeces should therefore be explored.
H. Stegeman mentioned that the next ISO meeting in which this subject can be discussed (ISO/TC34/SC9) will be organised in spring 2004. It would speed up the process if a proposal for a method will be ready by that time. An easy way forward could be to write an Annex to the present ISO 6579: 2002, in which it is indicated that for the analyses of *Salmonella* in (poultry) faeces one selective broth (e.g. MKTTn) should be replaced by a semi-solid medium (e.g. MSRV). H. Stegeman promised to discuss the subject with B. Lombard, the convenor of ISO/TC34/SC9.

3.6 Closing remarks

André Henken, Director CRL-*Salmonella*, Bilthoven, the Netherlands

Programme coming year

In the autumn of 2003 a seventh bacteriological collaborative study will be organised. This study will in principle have the same set-up as study VI held in the autumn of 2002. Due to the revised ISO 6579 small changes were introduced. It was agreed that the following methods will be used in the seventh study:

pre-enrichment in Buffered Peptone Water: BPW

selective enrichment in:
- Rappaport Vassiliadis medium with soya: RVS;
- Modified semi-solid Rappaport Vassiliadis medium: MSRV;
- Mueller-Kaufmann Tetrathionate-novobiocin broth: MKTTn.

plating-out on:
- Xylose lysine deoxycholate agar: XLD;
- Brilliant Green agar: BGA.

biochemical confirmation:
- Urea, TSI and LDC.

The number of capsules to be used in the seventh study will remain the same as the number used in study VI. Also the amount of faeces to be added to the capsules (10 g) will remain the same.

Next spring (2004) a 9th typing collaborative study will be organised including serotyping, phagetyping and antimicrobial susceptibility testing.
Serotypes selected will be ones that are important in terms of public health (5 most important serotypes in Europe will be selected) or ones that are easily confounded with those important ones. As in earlier studies again phagetyping will be included using 10 *S*. Enteritidis strains and 10 *S*. Typhimurium strains from PHLS/UK (now HPA/UK). Ten strains with various antibiotic resistance patterns will be provided by CIDC/Lelystad/Netherlands and have to be tested with a panel of antibiotics which was discussed during this workshop.

Evaluation of the workshop

The candidate countries of the EU were invited to join the workshop for the first time. Almost all candidate countries were represented by participants of their National Reference Laboratories. On the last day of the workshop two parallel session were held on the following two subjects: “Antibiotic Resistance” en “Standardisation of detection methods”. These sessions were organised to enable the participants to discuss the presented subjects into detail. Furthermore, the absence of suitable reference materials was discussed, but the capsules containing *S*. Typhimurium and *S*. Enteritidis are still not commercially available.

Did we succeed in reaching the aims of setting this workshop and are the plans of the CRL-*Salmonella* for the near future clear ? All participants agreed upon these two questions.

Farewell

The participants from the Member States and Candidate Countries were thanked for their active participation in the workshop programme. Every year participants step forward willingly to contribute and thus making the workshop a success. This is much appreciated. The EU commission and the TAIEX office is acknowledged for their support also in financial terms to make this workshop possible. The CRL-*Salmonella* team is acknowledged for their work of the previous year. The workshop organising team is thanked for their work making this workshop a success also in an organisational sense!

Appendix 1. Mailing list

01	European Commission, Director of Directorate D	P. Testori-Coggi
02	European Commission, Head of Unit D 2	E. Poudelet
03	European Commission	J.C. Cavitte
04	European Commission	P. Mäkelä
05	President of the Council of Health, the Netherlands	prof. dr. J.A. Knottnerus
06	Food and Consumer Product Safety Authority	ir. J. de Leeuw
07	Board of Directors	dr. M.W.J. Sprenger
08	Director Sector Nutrition and Consumer Safety	prof. dr. ir. D. Kromhout
09	Head of Microbiological Laboratory for Health Protection	dr. ir. A.H. Havelaar
10	Head of Laboratory for Analytical Residue Research	prof. dr. W.R. Stephany
11	Head of Technical Assistance Information Exchange Office of the European Commission (TAIEX)	B. Czarnota
12-75	Participants of the workshop	
76-78	Authors	
79	Dutch National Library for Publications and Bibliography	
80	SBC/Communication	
81	Registration agency for Scientific Reports	
82	Library RIVM	
83-87	Sales department of RIVM Reports	
88-100	Spare copies	

Appendix 2. Participants

European Commission	Jean-Charles Cavitte
CRL – *Salmonella*	André Henken Kirsten Mooijman Hans Korver Henny Maas
CRL – Epidemiology of Zoonoses	Annemarie Kaesbohrer
Guest speakers (the Netherlands)	Dik Mevius (CIDC, Lelystad) Henk Stegeman (RIKILT, Wageningen)

National Reference Laboratories for *Salmonella*

AUSTRIA	Christian Berghold Heimo Lassnig
BULGARIA	Ivan Kaloyanov Angel Petkov
BELGIUM	Hein Imberechts
CZECH REPUBLIC	Iva Bernardyova Marketa Tomsickova
DENMARK	Marianne Skov Jens Christian Jorgensen
ESTONIA	Toomas Kramarenko Lea Rander
FINLAND	Tuula Johansson Henry Kuronen
FRANCE	Marylène Bohnert Francoise Lalande
GERMANY	Reiner Helmuth Beatriz Guerra
GREECE	Maria Passiotou-Gavala Eleni Valkanou
HUNGARY	Zsuzsanna Sreter-Lancz Zoltan Veres

IRELAND	John Egan John Ward
ITALY	Antonia Ricci Denis Vio
LATVIA	Kristine Kraujina Andra Utinane
LITHUANIA	Ceslova Butrimaite-Ambrozeviciene Rasa Giceviciene
LUXEMBOURG	Joseph Schon
NETHERLANDS	Arjen van de Giessen Anjo Verbruggen
NORTHERN IRELAND	Stanley McDowell
POLAND	Andrzej Hoszowski Halina Sciezynska Darius Wasyl
PORTUGAL	Alice Amado Maria do Rosario Vieira
SLOVAK REPUBLIC	Selma Jackova Alena Skarkova
SLOVENIA	Vojislava Bole-Hribovsek Jasna Micunovic
SPAIN	Consuelo Rubio Montejano Christina de Frutos Escobar
SWEDEN	Ingrid Hansson Lena Falkenas
TURKEY	Kadir Kaya Selahattin Sen
UNITED KINGDOM	Robert Davies Linda Ward

Appendix 3. Programme of the workshop

14-16 May 2003, Bilthoven

General information

Hotels: The NRLs from the Candidate Countries stay in:
Hotel Park Plaza, Westplein 50, Utrecht, the Netherlands,
tel: +31 30 292 5200;
http://www.hotels.nl/utrecht/parkplaza
http://www.hotels-holland.com/utrecht/parkplaza.htm

The NRLs from the member states stay in:
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 14 May 2003.
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

Tuesday 13 May 2003

Arrival of representatives of NRLs from Candidate Countries (CCs) at Hotel Park Plaza in Utrecht

20.30 - 21.30 Social get together, bar Hotel Park Plaza

Wednesday 14 May 2003 (T007)

Morning chair: André Henken

8.15 Departure from hotel Park Plaza (Utrecht) to RIVM (Bilthoven)
9.00 - 9.30 Opening and introduction (André Henken)
9.30 - 10.00 The EU enlargement and the Zoonoses Directive: a historical perspective (Jean-Charles Cavitte)
10.00 – 10.30 Zoonoses reporting system (Annemarie Kaesbohrer)

10.30 - 11.00 Coffee/tea
Hand over PP-presentations to contact person; Payment of €100,- for lunches, diner, transport, etc to CRL; Give information on departure date and time.

11.00 - 12.30 Presentations by representatives of NRLs from CCs
- Presentation NRL Cyprus
- Presentation NRL Bulgaria
- Presentation NRL Hungary
- Presentation NRL Slovak Republic
- Presentation NRL Latvia
- Presentation NRL Malta

12.30 - 13.30 Lunch (hand over PP-presentations to contact person)

Afternoon chair: Arjen van de Giessen

13.30 – 15.15 Presentations by representatives of NRLs from CCs
- Presentation NRL Czech Republic
- Presentation NRL Poland
- Presentation NRL Estonia
- Presentation NRL Lithuania
- Presentation NRL Slovenia
- Presentation NRL Turkey
- Presentation NRL Romania

15.15 - 15.45 Coffee/tea

15.45 – 16.15 Collaborative studies organised by CRL-*Salmonella* (Kirsten Mooijman)
16.15 – 17.15 Visit to the laboratories of CRL-*Salmonella*

17.15 – 18.00 Cold drinks

18.00 Departure to Hotel Biltsche Hoek
18.30 Buffet at Hotel Biltsche Hoek, arrival of representatives from other MS
22.00 Departure to Hotel Park Plaza in Utrecht

Thursday 15 May 2003 (T007)

Morning chair: André Henken

7.45	Departure from Hotel Park Plaza in Utrecht to RIVM
8.40	Departure from hotel Biltsche Hoek in De Bilt to RIVM
9.00 - 9.30	Opening and introduction (André Henken)
9.30 - 10.00	Current issues in EU-regulation: monitoring and control (Jean-Charles Cavitte)
10.00 - 10.30	Epidemiology of *Salmonella* spp. in the EU (Annemarie Kaesbohrer)
10.30 - 11.00	*Coffee/tea*
11.00 - 11.45	Results bacteriological detection study VI (Hans Korver)
11.45 - 12.15	Discussion on design bacteriological detection study VII (Kirsten Mooijman)
12.15 – 13.30	*Lunch (during lunch: forms, copies of tickets, etc. Photograph)*

Afternoon chair: Kirsten Mooijman

13.30 - 14.00	Results typing study VIII : serotyping and antibiotic resistance (Hans Korver)
14.00 - 14.30	Results typing study VIII: phagetyping (Linda Ward)
14.30 – 15.00	Discussion on design typing study IX (2004) (Arjen van de Giessen)
15.00 - 15.30	*Coffee/tea*
15.30 - 15.50	Interaction between the NRL and private laboratories in the Republic of Ireland (John Egan)
15.50 - 16.10	Prevalence studies on *Salmonella* in Northern Ireland (Stanley McDowell)
16.10 - 16.30	*Salmonella* Enteritidis outbreaks linked to imported Spanish eggs (Linda Ward)
16.30 - 16.50	Evaluation of pooled serum and meat juice in a *Salmonella* ELISA for pigs (Rob Davies)
16.50 - 17.10	Discrimination of *Salmonella enterica* subspecies *enterica* d-Tartrate fermenting and non-fermenting isolates by genotypic and phenotypic methods (Reiner Helmuth)
17.10 - 17.30	Current issues in EU-regulation: analytical methods and antibiotic resistance testing (Jean-Charles Cavitte)
17.30 - 18.00	*Opportunity to provide the CRL-Salmonella team with your documents necessary for reimbursement of travel and subsistence costs*
18.00	Departure to Hotel Biltsche Hoek
18.00	Departure of NRL CCs to train station
18.45	Evening programme, departure to Utrecht
22.30	Departure to Hotel Biltsche Hoek

Friday 16 May (T007)

Chair: André Henken

8.30 Departure from hotel Biltsche Hoek to RIVM
9.00 - 9.10 Introduction to the workshops (André Henken)
9.10 - 10.30 Parallel workshops 1 and 2 with 15 min introduction of each item:

Item 1: Antibiotic resistance (T007)

Discussion leader:	Arjen van de Giessen
Introduction:	Dik Mevius (ID-Lelystad)
Reporter:	Reiner Helmuth

Item 2: Standardisation of detection methods (T019)

Discussion leader:	Kirsten Mooijman
Introduction:	Henk Stegeman (RIKILT)
Reporter:	Rob Davies

Of each NRL one member will participate in workshop 1, the other member in workshop 2

10.30 - 11.00 Coffee/tea

11.00 - 11.30 Presentation and plenary discussion on workshop item 1
11.30 - 12.00 Presentation and plenary discussion on workshop item 2
12.00 – 12.30 Closing remarks (André Henken)

12.30 - 14.00 Lunch
(Last opportunity to provide the CRL-Salmonella team with your documents necessary for reimbursement of travel and subsistence costs

14.00 Departure to train station

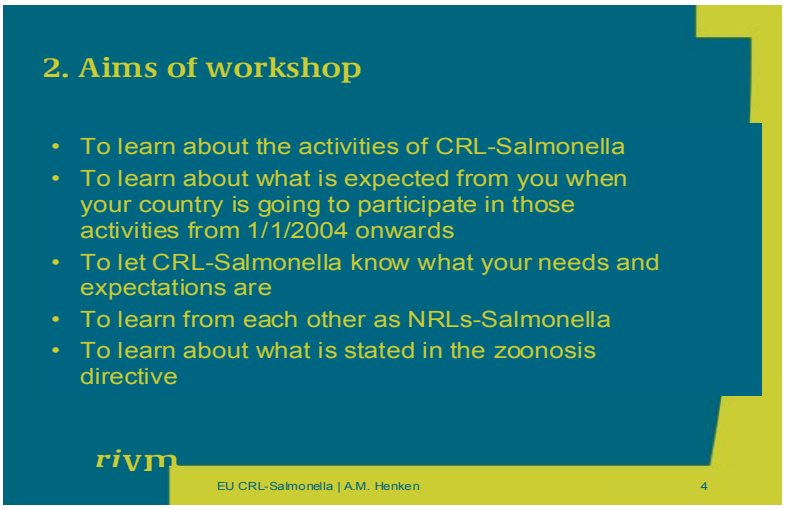
Appendix 4. Slides of presentation 1.1

Slide 1

Slide 2

Slide 3

Slide 4



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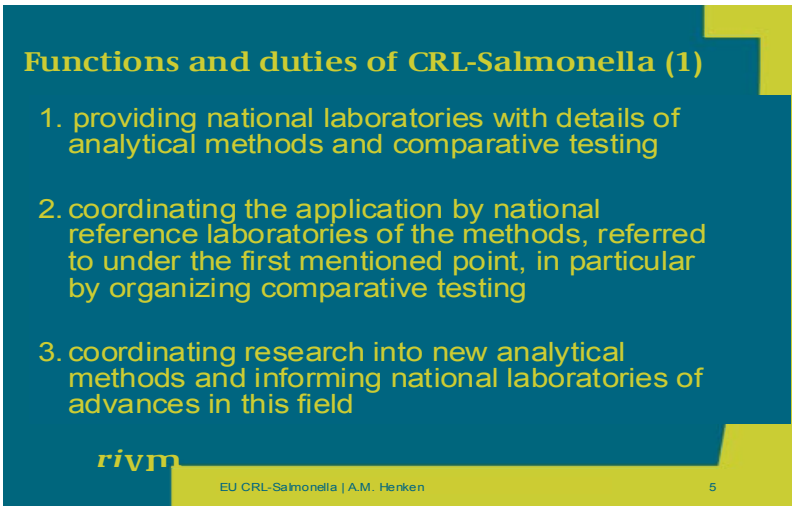

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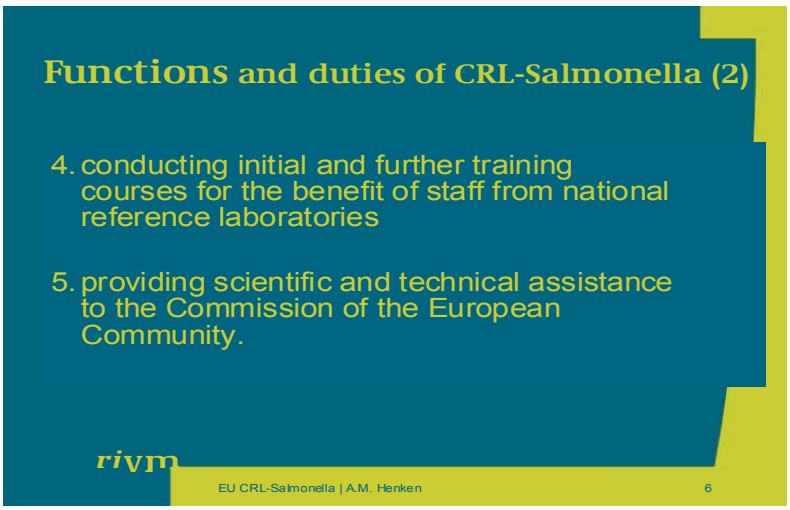

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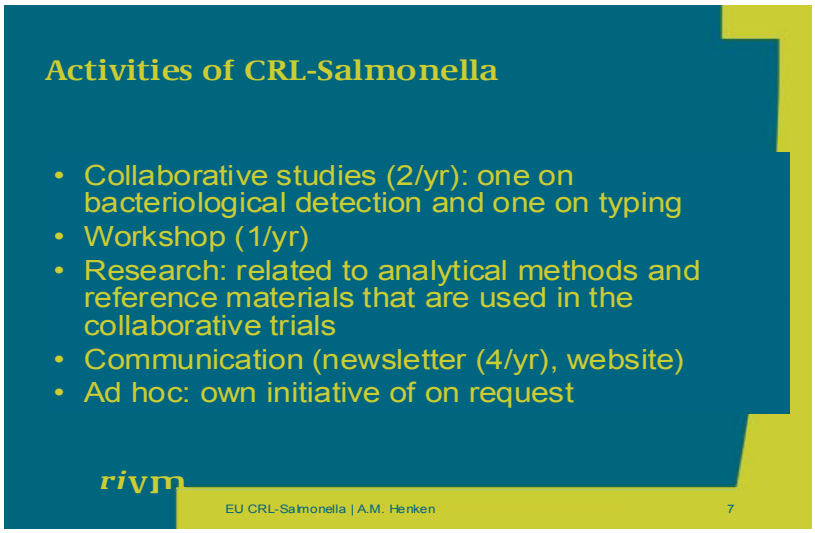
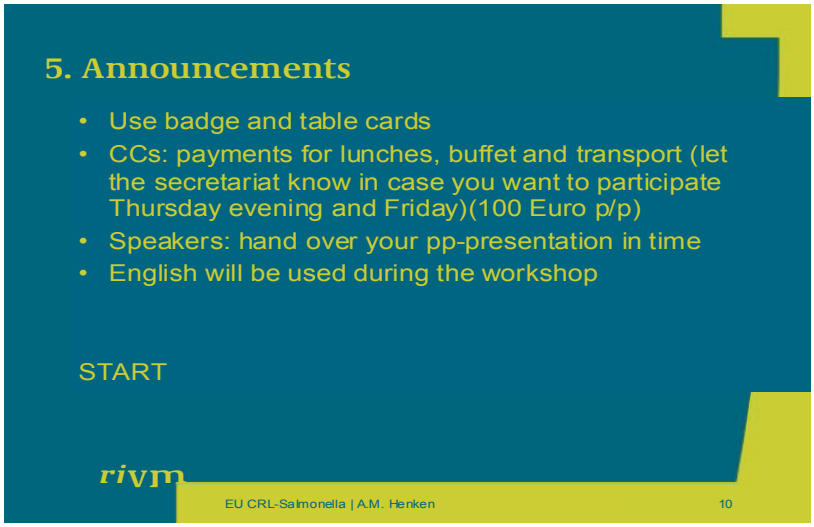


Slide 8

Slide 9

Slide 10


5. Announcements
• Use badge and table cards
• CCs: payments for lunches, buffet and transport (let the secretariat know in case you want to participate Thursday evening and Friday)(100 Euro p/p)
• Speakers: hand over your pp-presentation in time
• English will be used during the workshop
START
rivm
EU CRL-Salmonella | A.M. Henken
10

Appendix 5. Slides of presentation 1.2

Slide 1

Slide 2

Slide 3

Slide 4

Zoonoses
Directive 92/117/EEC
(and introduction on its revision)

- Monitoring of zoonoses under 92/117
- Salmonella control under 92/117
- Additional salmonella guarantees (Swe, Fin, (and No))
- Introduction to revision of zoonoses legislation

Slide 5

Directive 92/117/EEC concerning protection measures against certain zoonoses and zoonotic agents

- Background
  - In the framework of achievement of single market
  - Salmonella in eggs crisis in the UK

Slide 6

Directive 92/117/EEC concerning protection measures against certain zoonoses and zoonotic agents

- requires:
  - monitoring of certain zoonoses and zoonotic agents;
  - "control" of salmonella in fowl;
  - implementation of detailed minimum measures to eradicate of *S* Enteritidis and *S* Typhimurium in breeding flocks of poultry (*Gallus gallus*).

Slide 7

Directive 92/117/EEC concerning protection measures against certain zoonoses and zoonotic agents

- Two designated Community Reference Laboratories:
  - CRL for the epidemiology of zoonoses: BfR in Berlin (Germany)
  - CRL for salmonella: RIVM in Bilthoven (the Netherlands)

Slide 8

Dir 92/117/EEC: monitoring

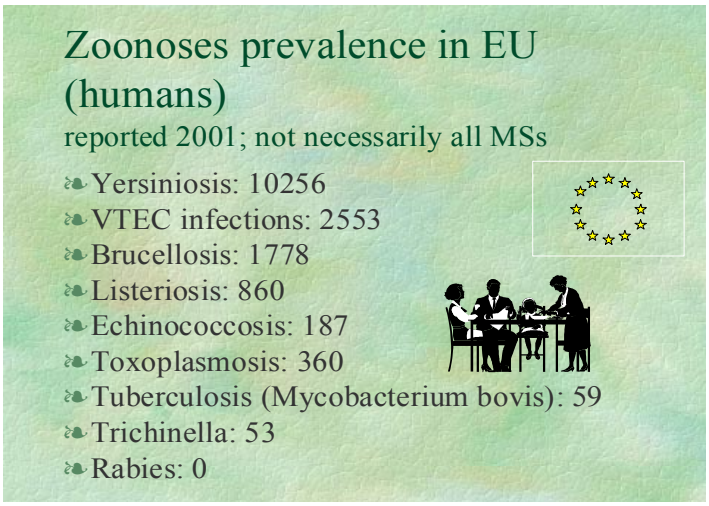
- 4 pathogens for compulsory monitoring: brucellosis and agents; tuberculosis (M bovis); trichinellosis; salmonellosis and agents
- voluntary: campylobacteriosis; echinococcosis; listeriosis; rabies; toxoplasmosis; yersiniosis,...

Slide 9

Community report on trends and sources of zoonoses:

- Task of CRL Berlin
- First report in 1995 (data 1994)
- Data collection usually not harmonised: the report has to be carefully interpreted;
- Quality and quantity of data increased along the years

Slide 10



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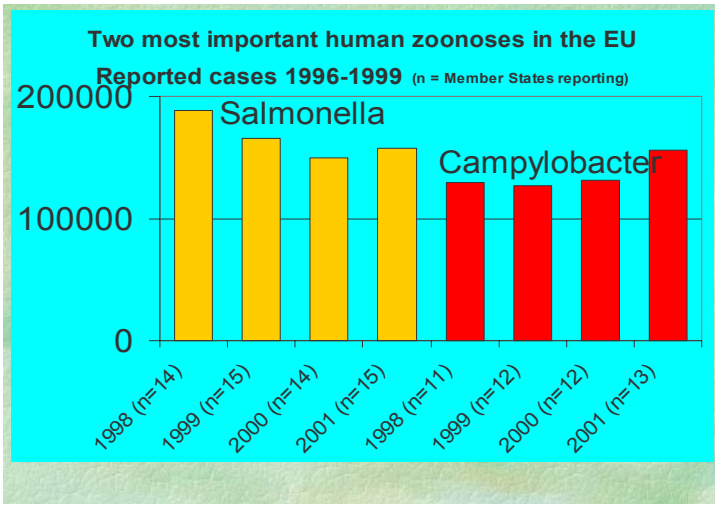

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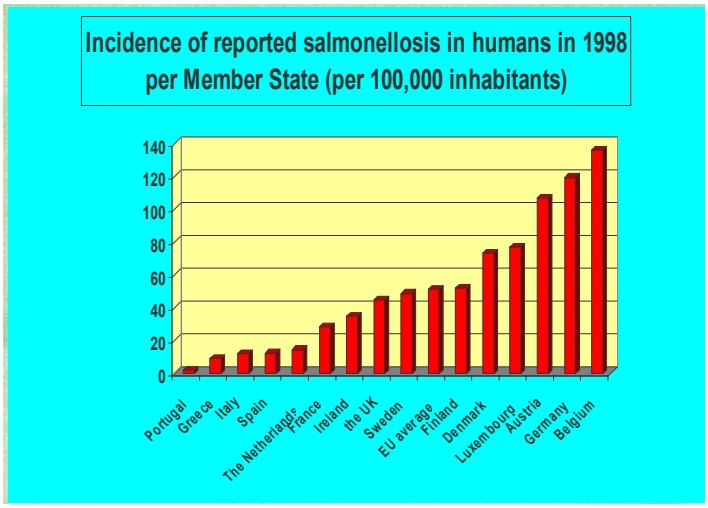


Slide 13

Sources of salmonella in humans

- *S*E and *S*T account for over 70% of the total salmonella serotypes isolated from humans
- The distribution of serotypes in humans is different from the serotypes in feedingstuffs but similar to those serotypes found in different species of livestock.

Slide 14

Sources of human salmonellosis

- Denmark 2001, estimated sources of human salmonellosis (DK report):

- 28-31% table eggs;
- 16-18% travel;
- 4.8-6.4% pork; 2.2-2.9% turkeys/ducks; 0.8-1.3% broiler; 0.7-1.1% beef;
- 5.9-8.3% imported poultry; 4-5.7% imported beef and pork;
- 6.5% outbreaks; 24-28% unknown.

Slide 15

Sources of human salmonellosis

- Netherlands, on the basis of salmonella isolates from 1994-98, it was estimated that 39.4% of human salmonellosis cases are related to eggs, 25.2% to pork, 21% to poultry meat, 10.7% to cattle.

Slide 16

Salmonella control programmes under Dir 92/117

- MS Salmonella control plans in fowl to be submitted for approval by EC, before 1.1.94 (requirement suspended):
  - Majority of MS have submitted; policy has been to approve plans when go further than min requirements for breeding flocks
  - Possibility of EC financing for breeding flocks
    (not if no eradication)

Slide 17

Minimum requirements for control of SE/ST in breeding flocks of *Gallus gallus*

- Applicable as from January 1998 (at latest)
- All breeding flocks (with 250 breeders or more) tested regularly
- Sampling of:
  - rearing flocks (day old chicks and pullets),
  - adult flocks (hatcheries and/or adult flocks)

Slide 18

Minimum requirements for control of SE/ST in breeding flocks of *Gallus gallus*

- Adult breeding flocks: sampling every 2 weeks/official sampling every 8 weeks
- Confirmatory step and measures of infected flocks
- Detailed sampling technique : feaces/meconium, dead animals
- Bacteriological testing, but laboratory method not defined

Slide 19

Approved salmonella control programmes in fowl

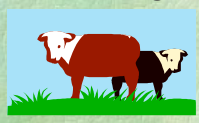
- Approved plans on the basis of Dir. 92/117/EEC (*Gallus gallus*): DK, IRL, FIN, SWE, A, FR, NL (and NO)
  - Scope varies
- other MSs also obliged to control *S*.T. and *S*.E. in breeding flocks of *Gallus gallus*, (but no obligation to destroy positive flocks)



Slide 20

Other salmonella control programmes

- **Other species**: in SWE, FIN (and NO) : pigs and cattle (mainly routine screening in slaughterhouses)



- There are also **voluntary** programmes/industry initiatives in poultry and other species

Slide 21

Additional salmonella guarantees

- Sweden and Finland were granted additional guarantees for salmonella in the context of the Act of Adhesion as from 1.1.1995. The additional guarantees were subject to an operational programme presented by Sweden and Finland and approved by the Commission (Decisions 95/50/EC and 94/968/EC).

Slide 22

Additional salmonella guarantees

- Detailed rules laid down for sampling/ testing:
  - **Fresh beef and pork** (Council Decision 95/409/EC)
  - **Poultry for slaughter** (Council Decision 95/410/EC)
  - **Fresh poultry meat** (Council Decision 95/411/EC)
  - **Breeding poultry and day-old-chicks** (Commission Decision 95/160/EC)
  - **Laying hens** (Commission Decision 95/161/EC)
  - **Table eggs** (Commission Decision 95/168/EC)

Slide 23

Additional salmonella guarantees

- **Either animals and / or products of animal origin are tested for salmonella in the country of origin, according to defined sampling and testing, or**
- **Animals and/or products are derived from holdings or establishments subject to a programme recognised as equivalent to the Swedish programme.**

- These additional provisions are referred to in the certificates to be used.
- NO programme has been recognised as equivalent.

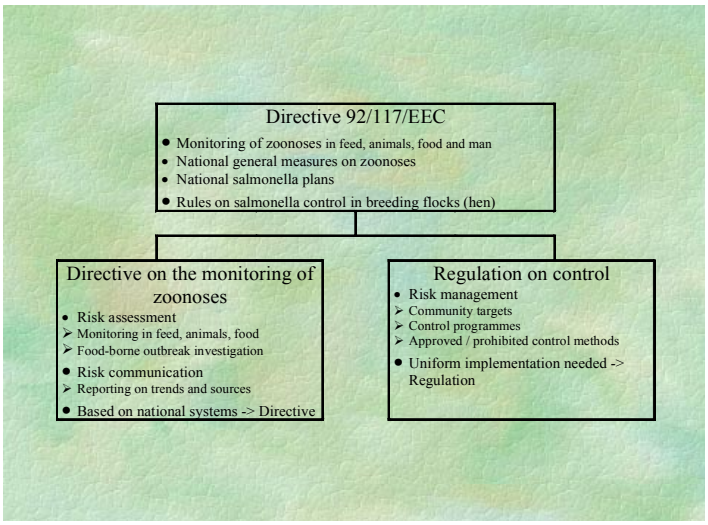
Slide 24

Revision of zoonoses legislation

- Framework: report and proposals foreseen in Dir 92/117/EEC; White Paper on food safety (high standards)
- Perceived need:
  - to decrease the incidence of zoonoses in humans and to improve the control of zoonoses in particular at primary production
  - to strengthen the collection of relevant data to support risk assessment activities and risk management decisions

Slide 25

Slide 26



Appendix 6. Slides of presentation 1.3

Slide 1

Zoonoses reporting system in the EU

Annemarie Käsbohrer
Community Reference Laboratory for the Epidemiology of Zoonoses,
BfR, Berlin, Germany

Slide 2

Current system

Legal basis

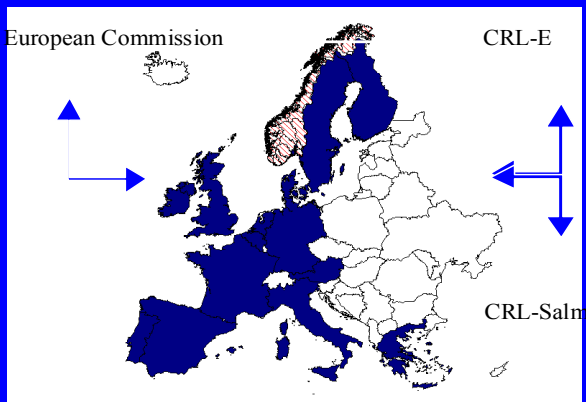
- Council Directive 92/117/EEC concerning protection measures against certain zoonoses and zoonotic agents

How does it work:

- Countries provide information based on their national systems
- Details are discussed in meetings

Slide 3

Zoonoses reporting system in the EU



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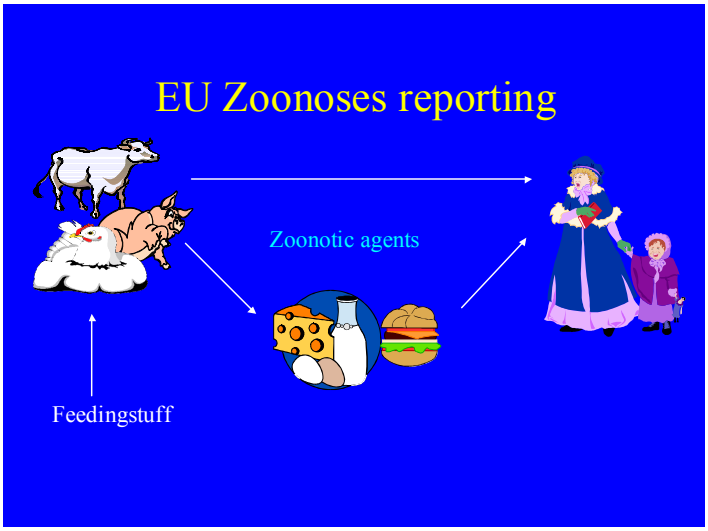


Slide 5

Zoonoses reporting system

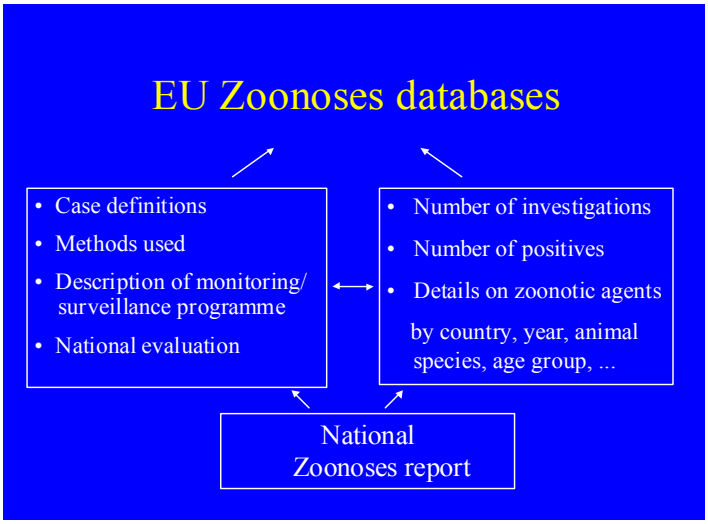
- Agents where control measures are implemented on Community level:
  Tuberculosis (M.bovis), Brucellosis, Trichinellosis, Salmonellosis
- Agents where control measures may be implemented on National level:
  Campylobacteriosis, Echinococcosis, Listeriosis, Rabies, Toxoplasmosis, Yersiniosis, other zoonoses
- Any other zoonosis not found in the Community

Slide 6

Data sources

- Compulsory routine programme
  ⇨Deviations from provisions of the legislation
- Voluntary routine programme / survey
  ⇨Number of samples taken
  ⇨Sample size, specimen collected
  ⇨Method used
- Diagnostic examinations
- Notification requirement

Slide 7



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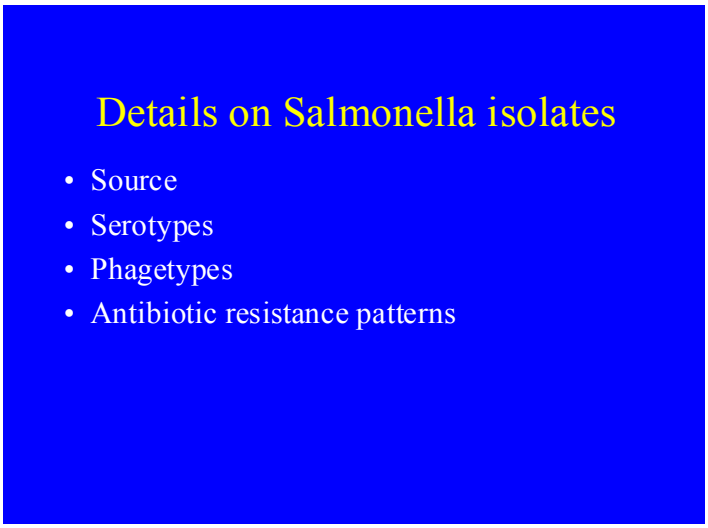
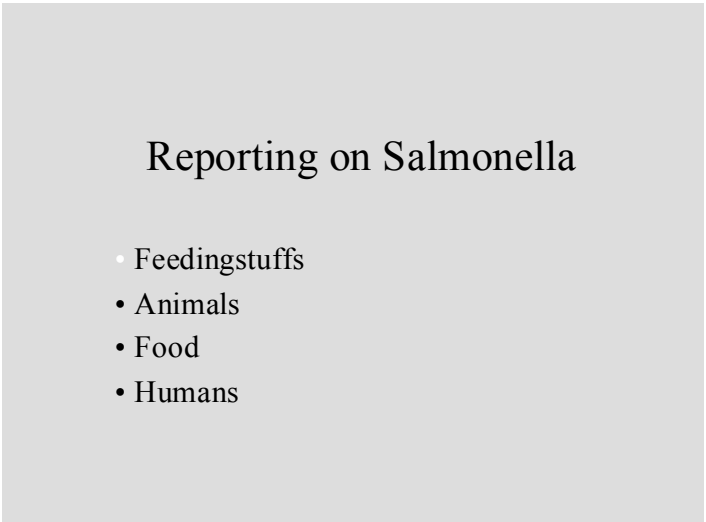


Slide 9



Slide 10

FEEDINGSTUFFS

COUNTRY:	Source of information	Remarks	Epidemiological unit [2]	Sample Weight	Units investigated	Units Salmonella detected	S.Enteritidis	S.Typhimurium
Categories [1]								
Milk products								
Land animal products								
Meat meal								
Meat and bone meal								

Slide 11

FEEDINGSTUFF

- Epidemiological unit:
  Batch or sample ?
- Sample size:
  1500g, 1000g, 500g, 200g, 100g, 25g
- Number of units investigated
  Pooled samples ?
  Several samples from one batch ?

Slide 12

POULTRY BREEDERS

COUNTRY:	Source of information	Remarks	Flocks tested	Flocks positive	S.Enteritidis [3]	S.Typhimurium [3]	4)	4)
Animal species								
Egg production line								
Elite								
Grandparents								
Parents								
Day-old chicks								
Rearing flocks								
Productive period								
Meat production line								
All breeders (production line not specified)								

Slide 13

POULTRY BREEDERS

- **Flocks investigated**:
  Is it the number of examinations (repeated) or the number of flocks under control ?
- **Production type and level:**
  - Egg and meat production line
  - All breeders together (parents and grandparents)
- **Overall rates:** how to calculate?
  - day old chicks
  - rearing flocks
  - productive period ?

Slide 14

HUMANS

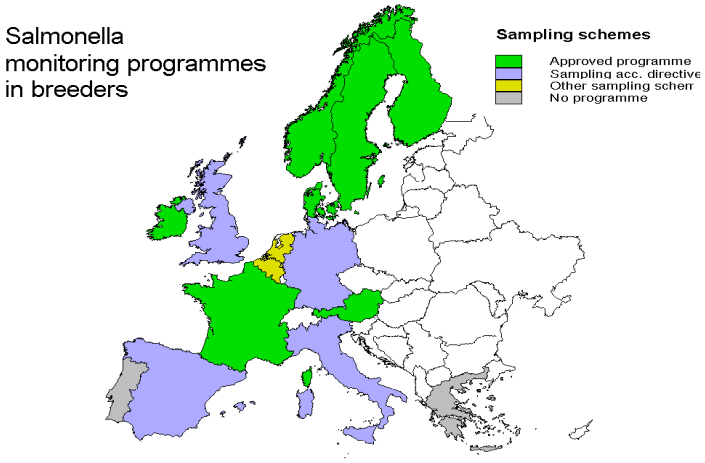
	Cases	Inc.	Autochtone cases	Inc.	Imported cases	Inc.
Salmonellosis						
S. Enteritidis						
*S.*Typhimurium						
of these: DT 104						
other serotypes						

Slide 15

Implementation of Dir 92/117

- MS Salmonella control plans in fowl to be submitted for approval by EC, before 1.1.94 (requirement suspended)
- Minimum requirements for *S*.E. and *S*.T. in breeding flocks to be implemented (at least) from 1.1.98

Slide 16



Slide 17

Salmonella - poultry breeders

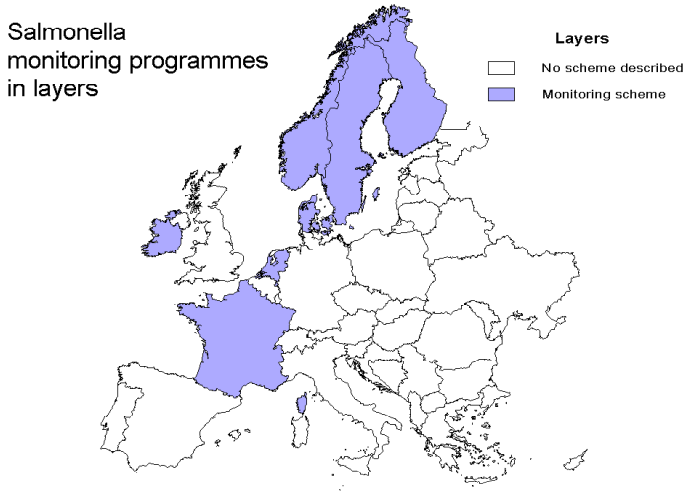
- Countries running an approved control programme for several years
  - DK, FIN, S, IRL, N
- Countries running an approved control programme since 1999 or 2000
  - A, F
- Countries, which apply a monitoring scheme based on the sampling procedures in the Zoonoses Directive
  - UK, D, E, I
- Countries, which run other sampling schemes
  - B, NL

Slide 18

Salmonella - poultry breeders Control measures

- Flock: Movement restrictions
- Animals: Treatment or slaughter or destruction
- Hatching eggs: destruction / heat treatment
- Feedingstuffs: heat treatment / destruction
- Manure: restrictions
- Building: cleaning and disinfection
- Epidemiological investigations

Slide 19



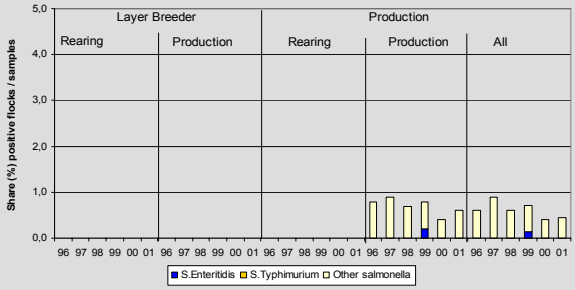
Slide 20

Monitoring in layers (table egg production)

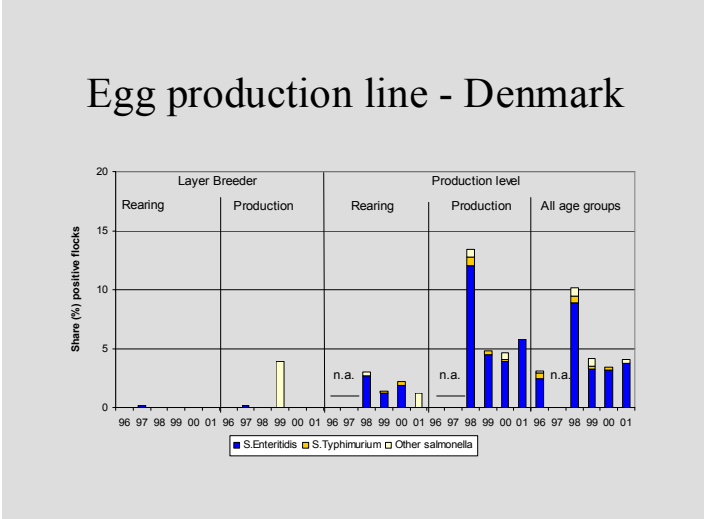
- Type of sample
  - Faecal samples or swabs, caecal droppings, blood samples, dust samples, **egg samples**
- Sample size
  - 24 - 60 ; 60 ; 60 - 90
- Frequencies
  - every 9 weeks; three times
  - 25 - 30 + 48 - 52 weeks
  - 24 + 40 + 55 weeks
  - Once max 9 weeks before delivery

Slide 21

Egg production line - Sweden



Slide 22



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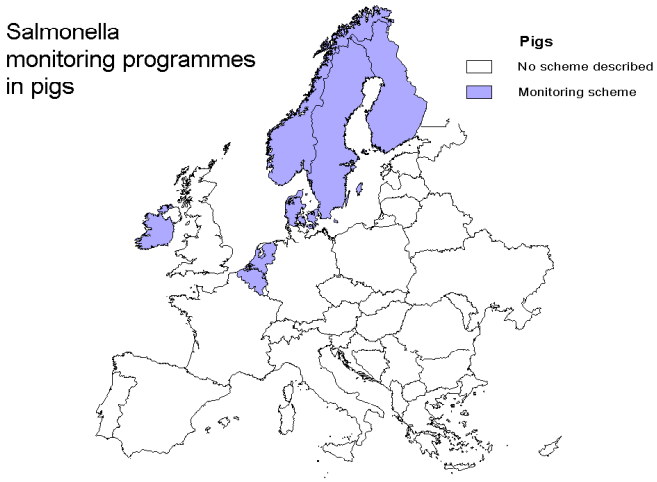


Slide 24

Monitoring in pigs

- Production level
  - Breeding / multiplying / fattening
- Type of sample
  - Faecal samples, blood samples, meat juice, lymph nodes, carcass swabs
- Sample size
  - per farm: 1, 20 , 60 (from a sample of the farms)
  - per production: 3000 samples

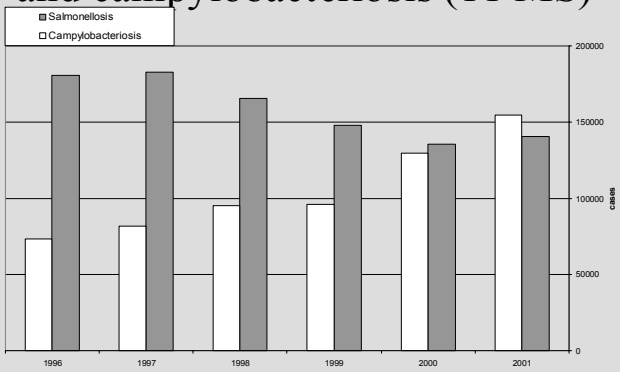
Slide 25

Monitoring of food (at retail)

- National / regional programme
- Survey
  - food product is exactly specified
  - method is exactly fixed
- Routine sampling
  - details are not given
- Monitoring by municipalities
- Sampling by industry (self-control)

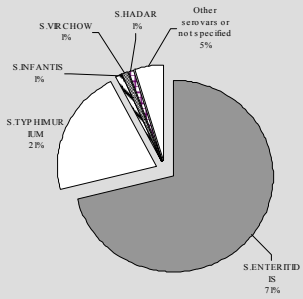
Slide 26

Trend in human salmonellosis and campylobacteriosis (11 MS)



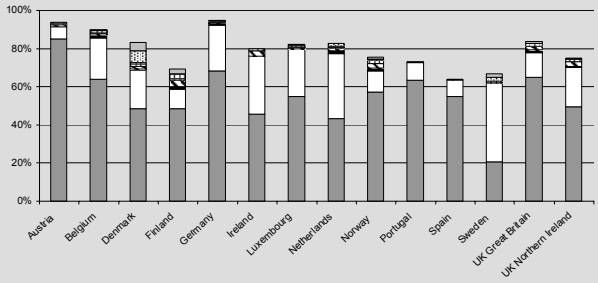
Slide 27

Main serovars in human salmonellosis



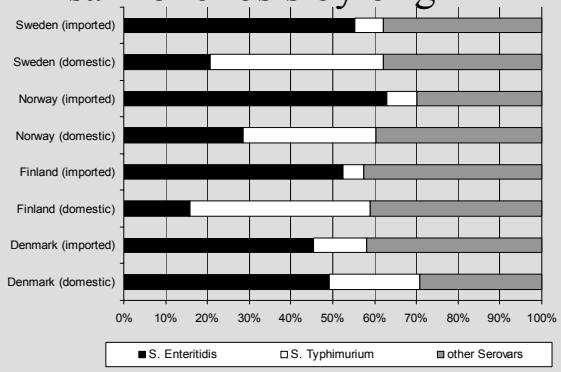
Slide 28

Main serovars in human salmonellosis



Slide 29

Main serovars in human salmonellosis by origin



Slide 30

The future

- Reporting will be adjusted when the new zoonoses directive will come into force
  - additional zoonotic agents
  - additional monitoring requirements
  - harmonised programmes
- The number of countries covered by the report will increase enormously

Appendix 7. Slides of presentation 1.4.1

Veterinary Reference Salmonella Laboratory to the National Veterinary Diagnostic Research Institute, Sofia

1. Occurence of Salmonella in food producing animals, food animal origin and food chain

In the last year (2002) total 223 strains of Salmonella were isolated in the country.

Isolated from:

- food, predominant minced meat, poultry, eggs – 31
- death bovines – 13;
- slaughtered bovines - 2
- death pigs – 34;
- slaughtered pigs – 8
- death ovins – 9;
- slaughtered ovins – 0
- death poultry – 94;
- slaughtered poultry- 24
- game – 2
- feed – 6
- environment (food chain) – 6.

Serovares :

Enteritidis – 43,04 %

Typhimurium – 14,34 %

Choleraesuis – 14,34

London – 5,38 %

Gallinarum – 4,93 %

Abortusovis – 4,03 %

Derby – 2,24 %

Kentucky – 2,24 %

Heidelberg - 1,80 %

Agona – 1,79 %

Anatum – 1,78 %

Isangi – 0,90 %

Newcastle – 0,90 %

Give – 0,90 %

Haifa – 0,90 %

Braenderup – 0,45 %, e.t.c.

2. Systems for food borne disease surveillance

In 1973 a general program was adopted for the country about the protec-tion of people, animals and environment against salmonellosis. In result two reference laboratories were set up in Sofia – one for strains isolated from people, and another for strains isolated from animals, animal products, environment and feeds. Currently these are reference centers for salmonella strains isolated from all possible sources.

Food products of animal origin and feeds are tested also in 2 local veterinary institutes, 8 accredited veterinary laboratory for food control and 21 regional veterinary laboratories. Tests for confirmation of antimicrobial resistance to strains are carried out only for therapeutic reasons and with relation to research programs.

Since the beginning of the century a wide network of mutually subordina-ted laboratories both as organization and as methods has been established for the testing of each batch of food products of animal origin and animal feeds produced in the country or imported. The test methods are complied with national and international standards – CEA and ISO. At present regional and reference laboratories are being equipped in accordance to the requirements of the EC under a Twinning Project with the Italian Veterinary Services.

Good manufacturing practice, Good hygiene practice, HACCP - system are involved or are in way to be involved in the factories, producing food. For the lab - Good laboratory practice and accreditation according ISO 45001 and ISO 17025 are in way to be involved in the Salmonella reference laboratory. The NVSRL participates in the WHO coordinated proficiency testing EQAS 2003.

3. What systems are used for collection and analysis of data (e.g. which computer software and hardware are used for acquisition, analysis and sharing of data)?

For collecting data and for analysis for all Salmonella strains we use the WHONET 5.1 program. In the beginning of 2002 in the National veterinary system began to work the system VetInfo, including the collection of data from all the Veterinary system.

The obtained results are communicated suddenly by phone and by official letters when you have isolated serovar gallinarum, or in case of toxiinfection – to the health authorities. We have cloth relations between two national reference centers - on the national and local level.

Protection against foodborne infections and toxic infections is managed on a national level by a group of experts at the Ministry of Health and the Ministry of Agriculture and Forests, and in the same time attention is given to a wide range of zoonoses. Specific legislation is set up in compliance with the EC directives for each individual disease. Each Ordinance is published in the State Gazette.

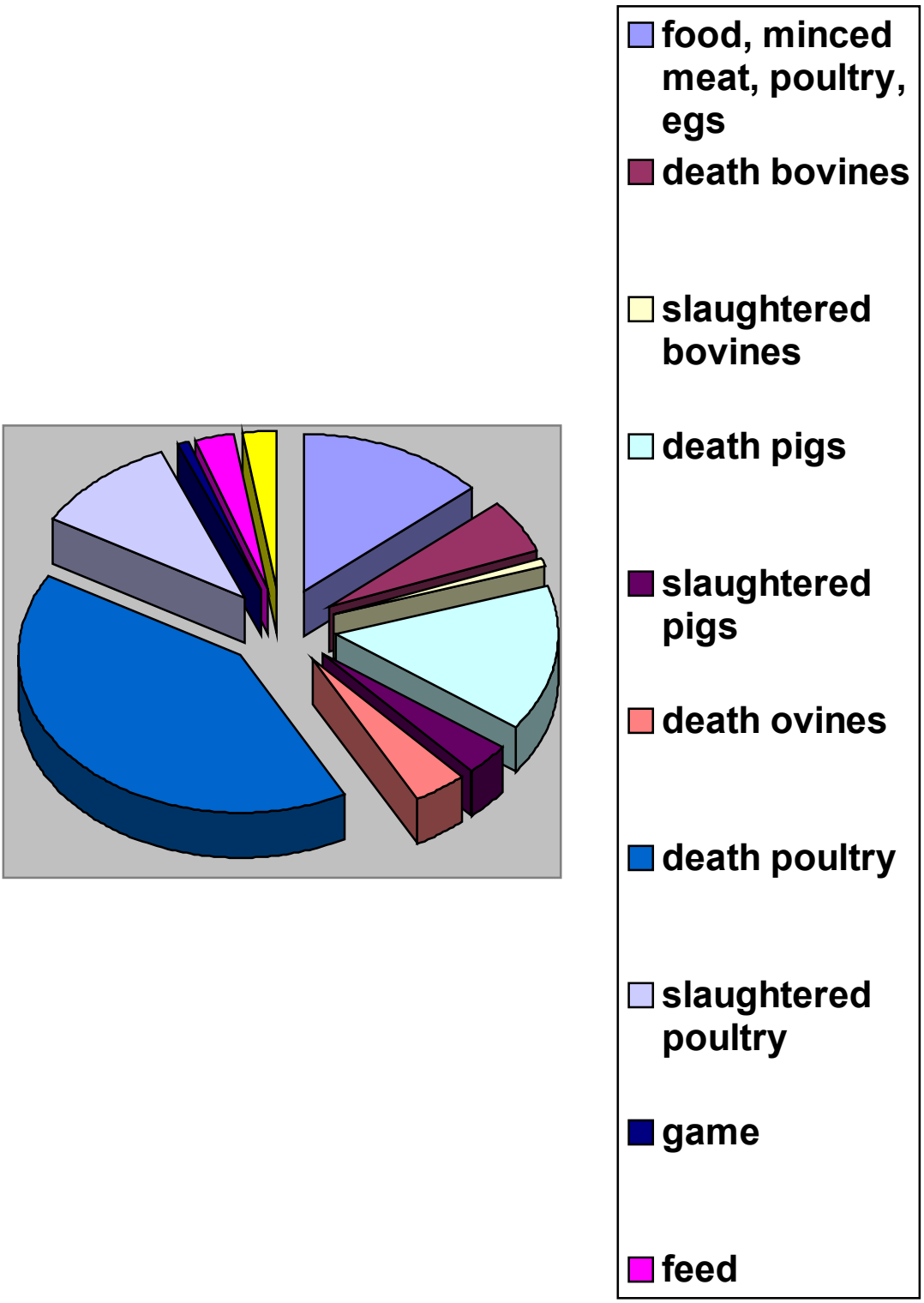


Figure 1

Occurrence of Salmonella in 2002

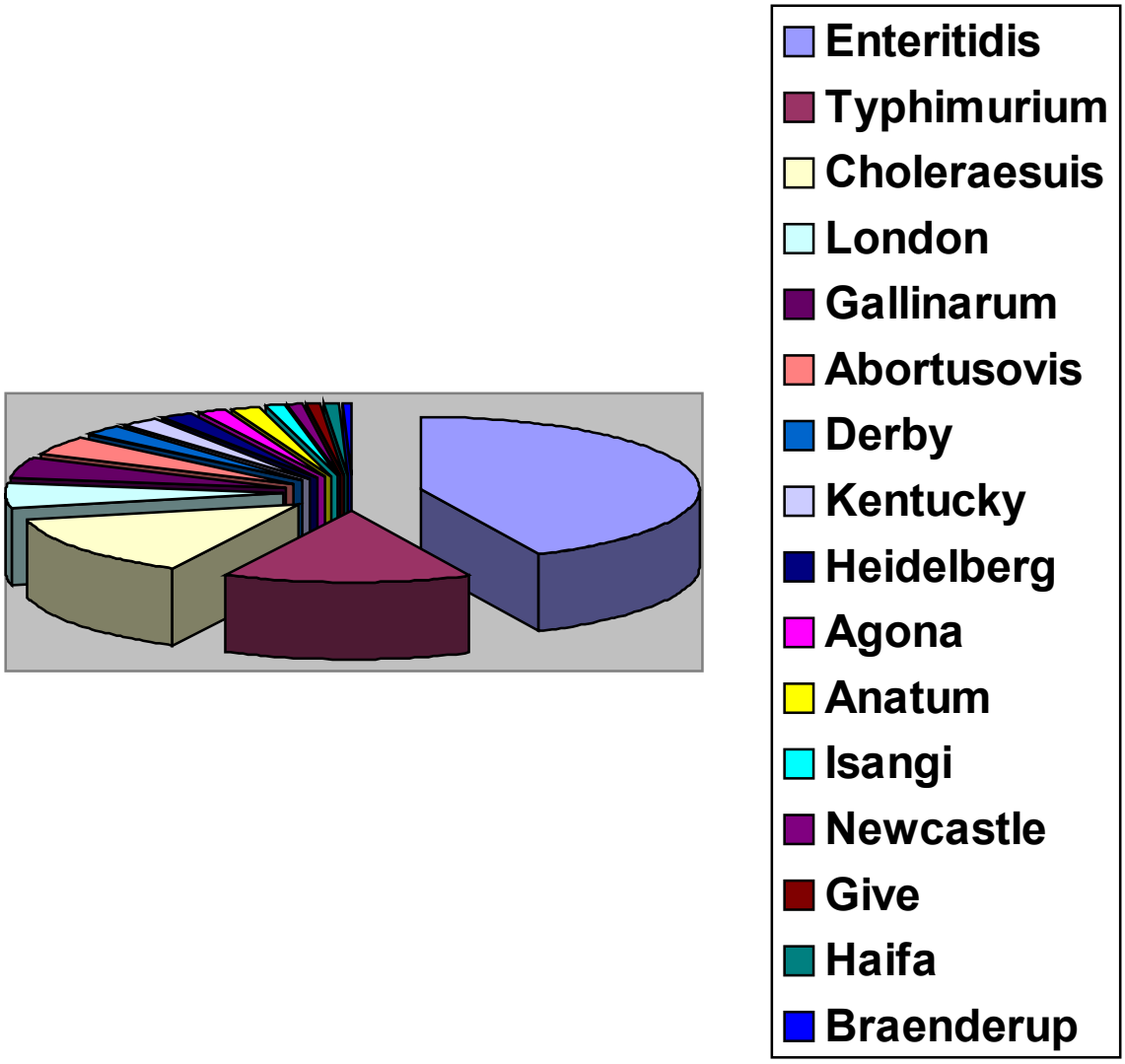


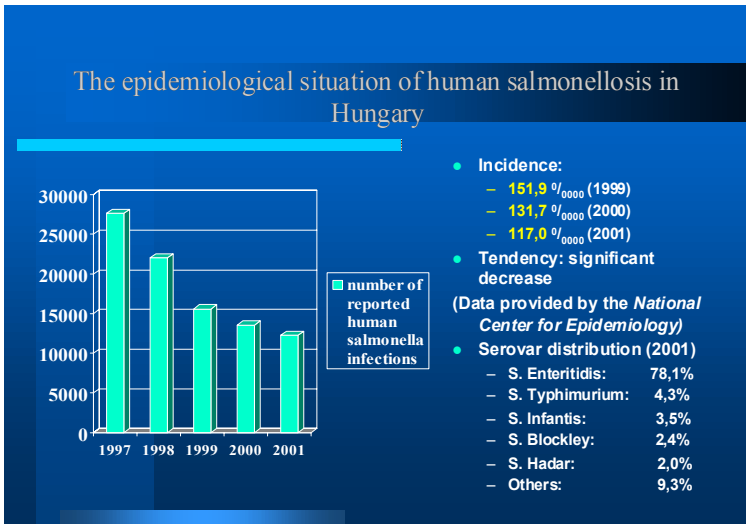
Figure 2

Salmonella serovares in 2002

Appendix 8. Slides of presentation 1.4.2

Slide 1

Slide 2



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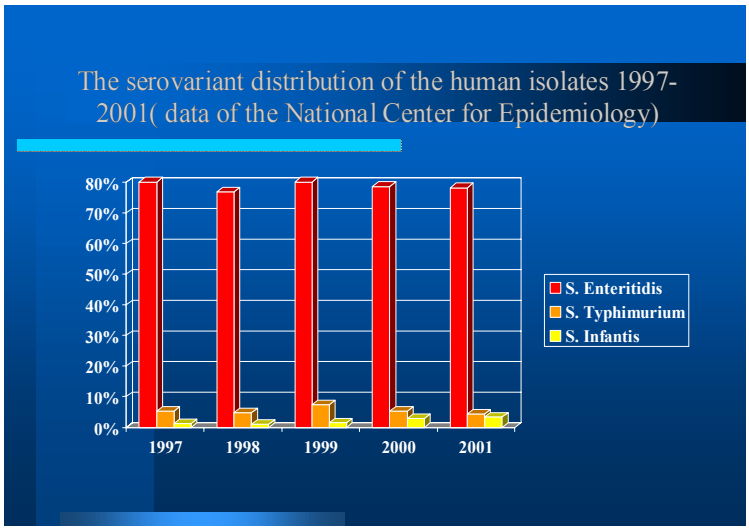

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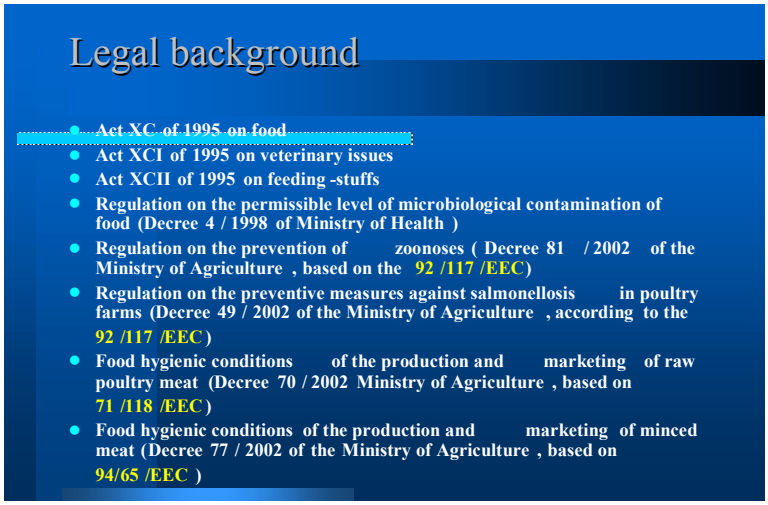

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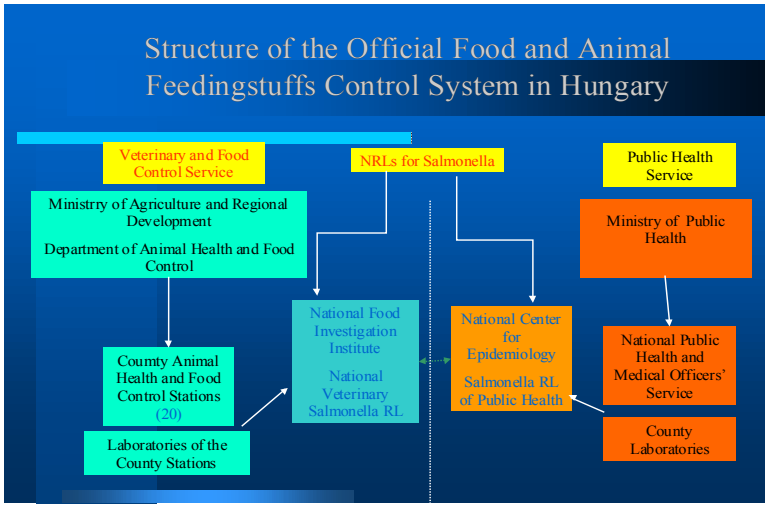

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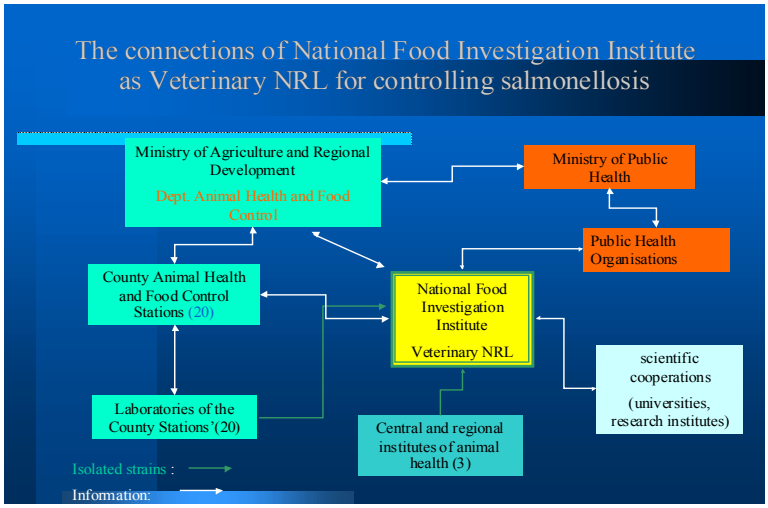

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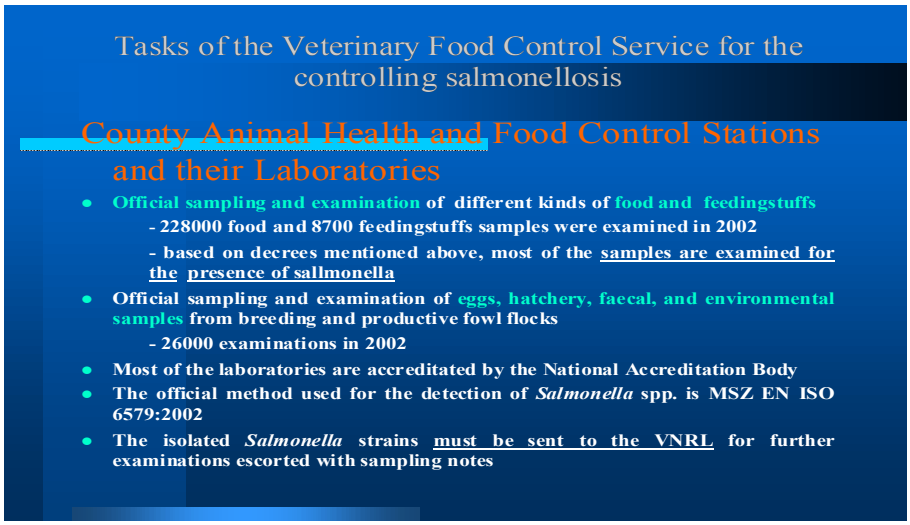

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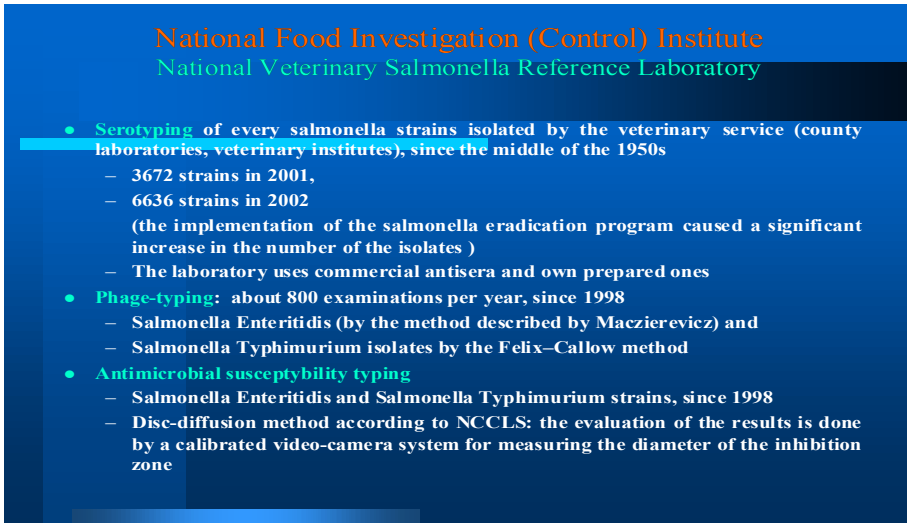

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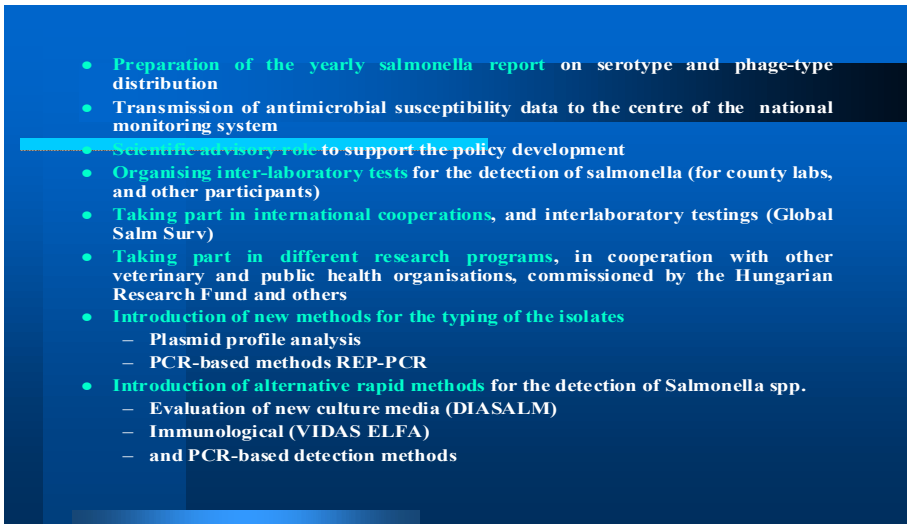

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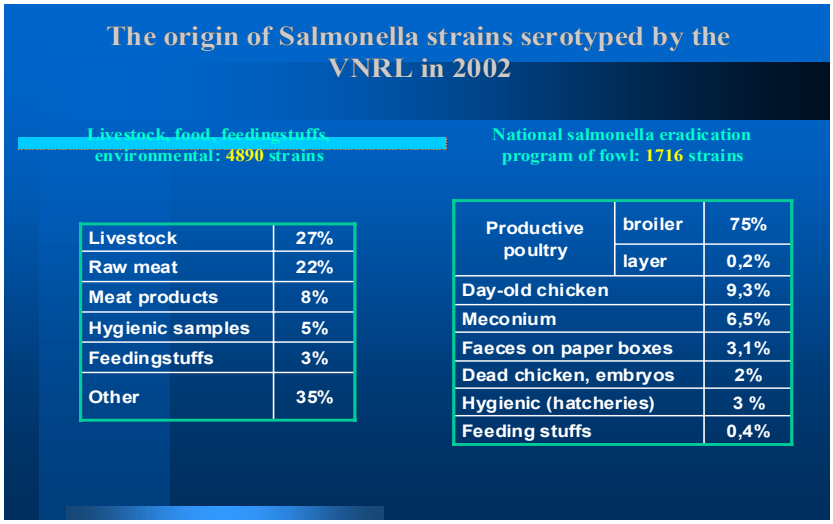

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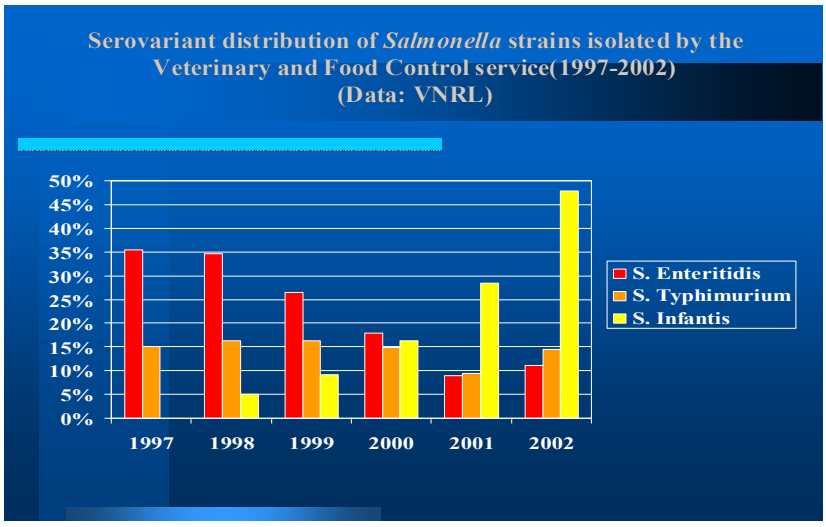

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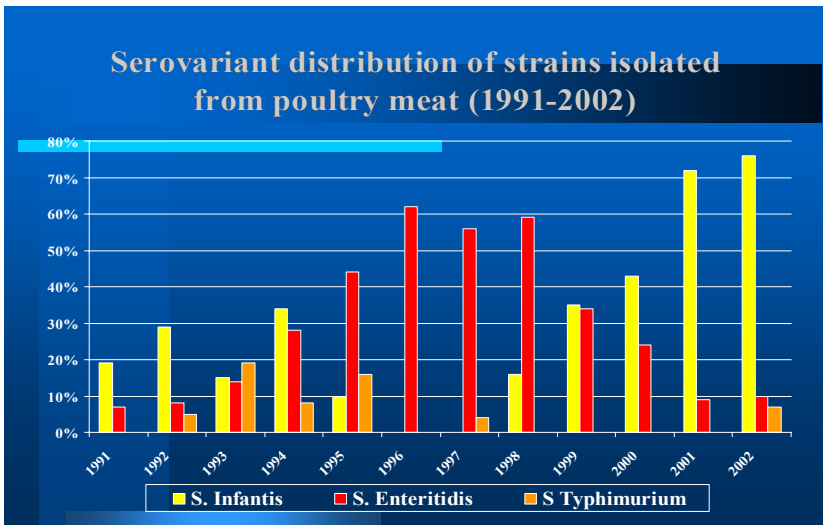

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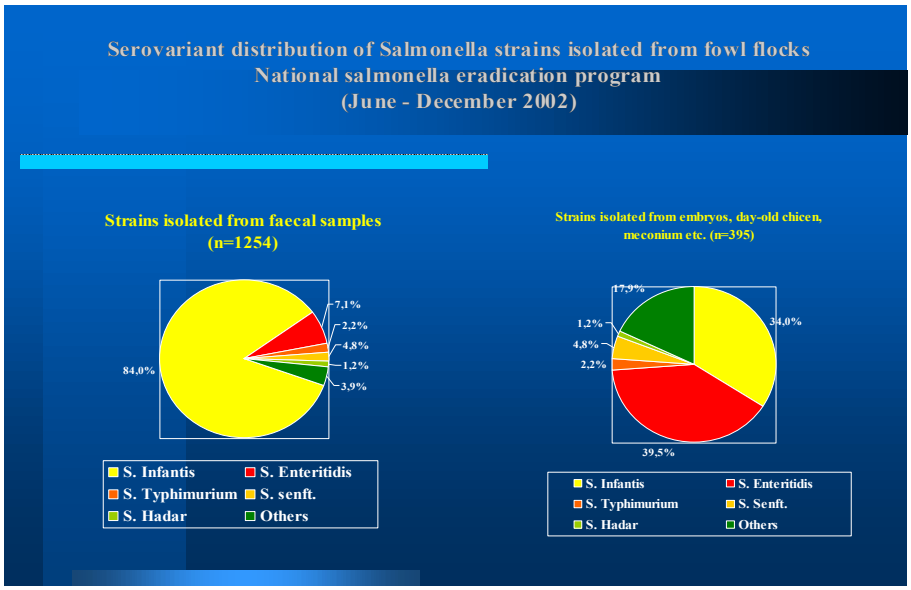
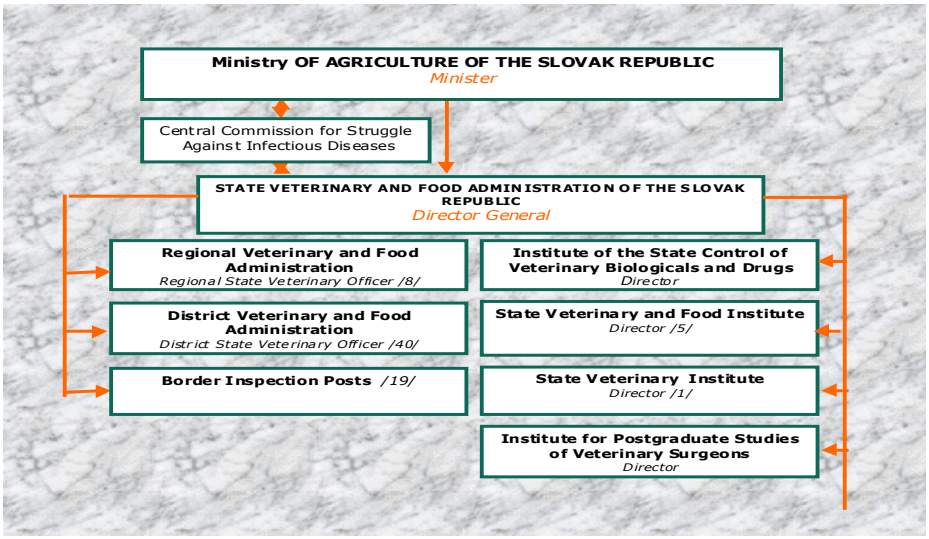

Serovariant distribution of Salmonella strains isolated from fowl flocks
National salmonella eradication program
(June - December 2002)
Strains isolated from faecal samples
(n=1254)
84,0%
7,1%
2,2%
4,8%
1,2%
3,9%
S. Infantis
S. Enteritidis
S. Typhimurium
S. senft.
S. Hadar
Others
Strains isolated from embryos, day-old chicen,
meconium etc. (n=395)
17,9%
34,0%
1,2%
4,8%
2,2%
39,5%
S. Infantis
S. Enteritidis
S. Typhimurium
S. Senft.
S. Hadar
Others

Appendix 9. Slides of presentation 1.4.3

Slide 1

Slide 2



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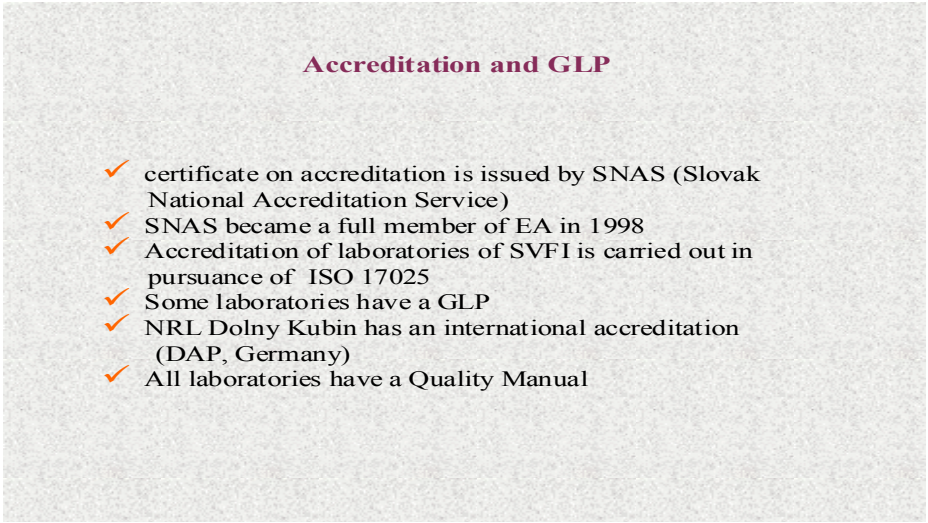


Slide 4

Actual situation in brief

Laboratory	Accreditation		GLP
	SNAS	DAP	
SVFI – Dolný Kubín	+	+	+
SVFI – Bratislava	+	-	+
SVFI – Košice	+	-	-

Slide 5

REFERENCE LABORATORY FOR SALMONELLA

STATE VETERINARY AND FOOD INSTITUTE

SLOVAK REPUBLIC
842 52 BRATISLAVA,
BOTANICKA 15

STATEMENT OF GLP COMPLIANCE
No: 6004
EN ISO / IEC 17025
ISO 9001: 1994

STATEMENT OF ACREDITATION
No: S 069
EN ISO / IEC 17025

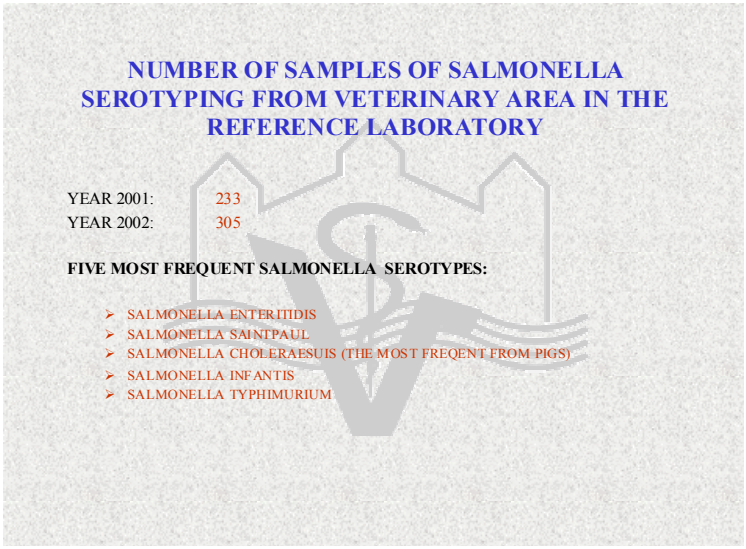
Slide 6

PERSONELL

1 Veterinary Doctor

1 Laborant

Slide 7



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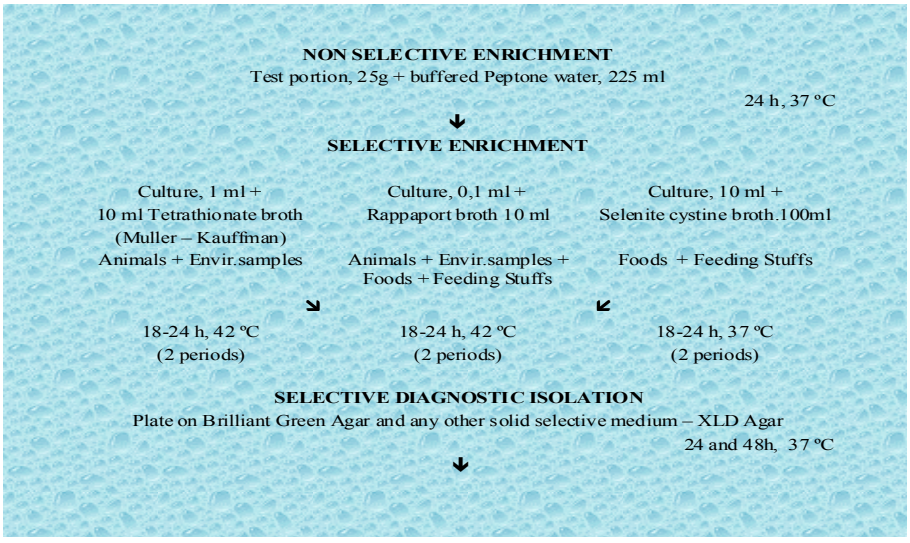
Slide 9



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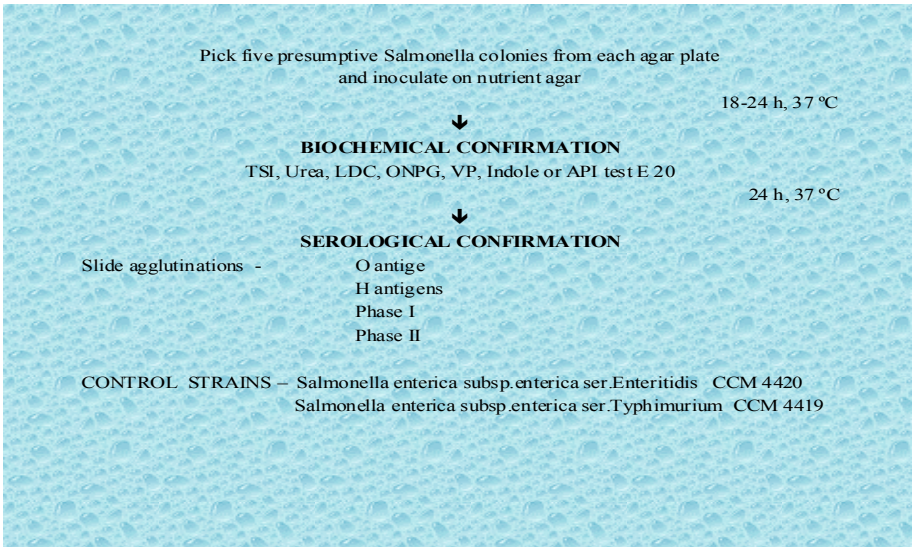

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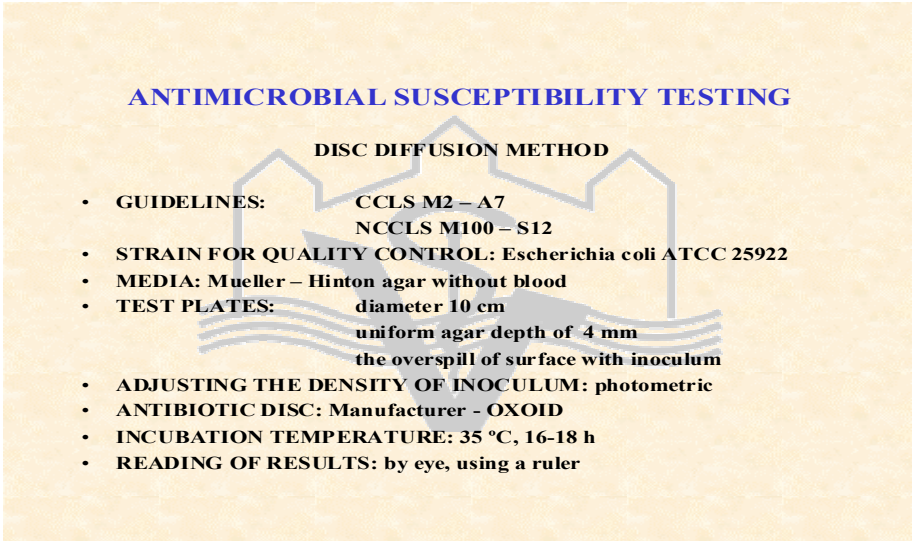

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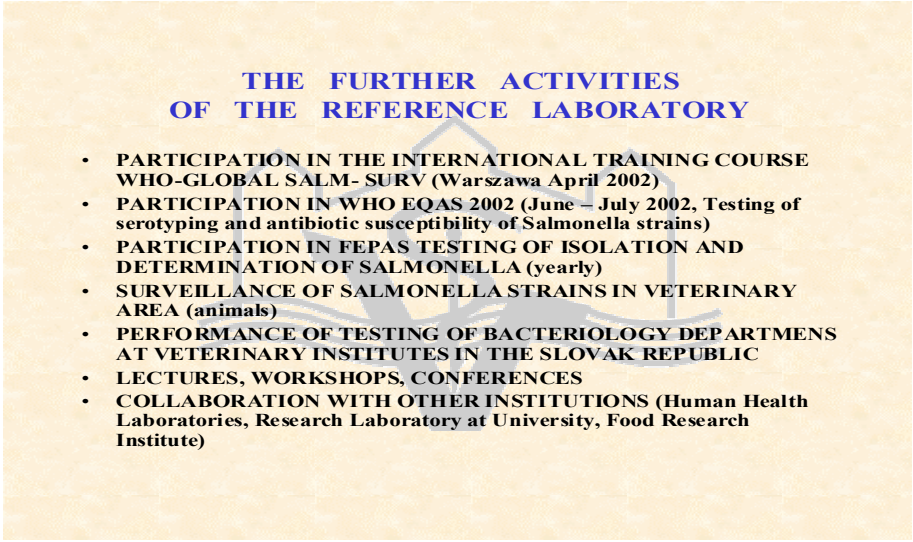
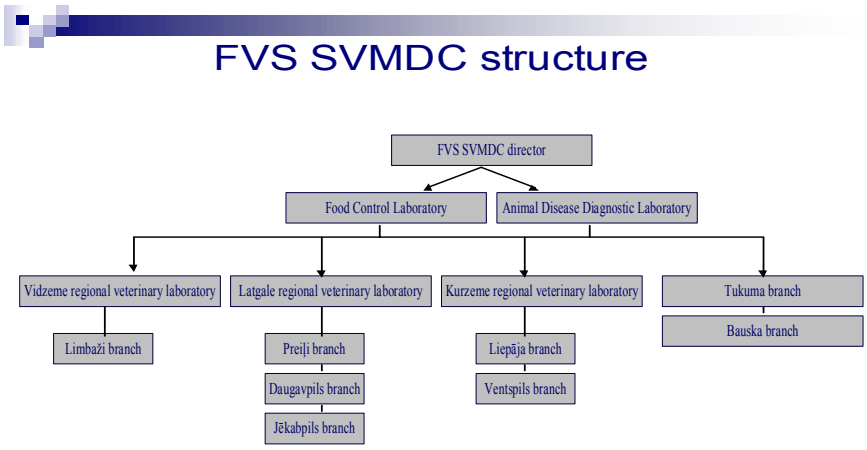


Appendix 10. Slides of presentation 1.4.4

Slide 1

Slide 2

Slide 3



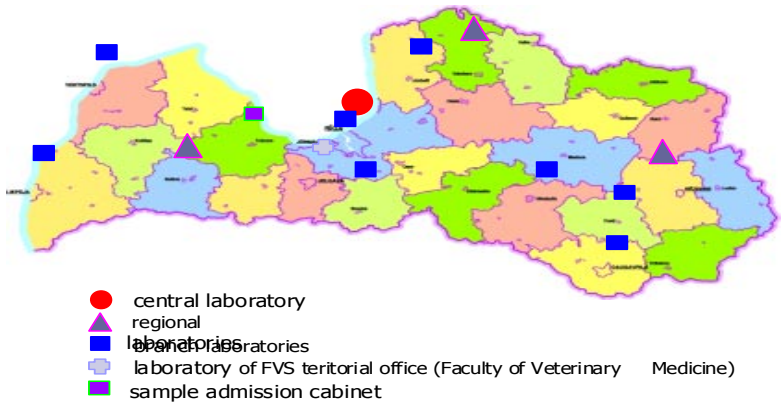
Slide 4

SVMDC functions

- ✓ Laboratory examination of animal diseases
- ✓ Testing of food products
- ✓ Central laboratories perform functions of national reference laboratory regarding:
  - □ animal infectious diseases
  - □ residue control in live animals and products of animal origin
  - □ Milk

Slide 5

Geographical location of laboratories



Slide 6

SVMDC Accreditation

SVMDC Food Control Laboratory:

- □ **Quality Assurance System according to standard (LVS) EN ISO/IEC 17025**
- □ **Accredited at:**
  - ■ **Latvian National Accreditation Bureau (LATAK) that is Member of European co-operation for Accreditation**
  - ■ **DAP, German Accreditation System**
  - ■ **GOST ROSSIJI (Russia Accreditation System)**

Slide 7

SVMDC Accreditation

SVMDC Animal Disease Diagnostic Laboratory:

Quality assurance system according to standard (LVS) EN ISO/IEC 17025

Accredited by Latvian national accreditation bureau (LATAK)

Slide 8

State Veterinary Medicine Diagnostic Centre

Animal Disease Diagnostic Laboratory (ADDL)

Slide 9

The Aim of ADDL

- To prevent the spread of animal disease and identify the status of animal health, thereby supplying safe and healthy food for the people and helping animal owners maintain productive herds

- **Main Directions:**

- Animal disease diagnostic work
- Determination of quality of animal feed

Slide 10

Animal Disease Diagnostic Laboratory

Performs the following investigations of pathological material:

- bacteriological
- virological
- mycological
- parasitological
- serological
- microanatomical

- Morbid anatomical sections of animal bodies
- Determination of quality of animal feed
- Clinical laboratory investigations of animals

Slide 11

State Veterinary Medicine Diagnostic Centre

Food Control
Laboratory (FCL)

Slide 12

FCL functions

- Chemical, microbiological, radiological, parasitological testing of food products of animal and vegetal;
- Estimation of hygienic condition of premises and surfaces;
- Calibration of laboratory dishes;
- Diagnosing of causes for microbiological perishability of food products;
- Taking samples;
- Transportation of samples;
- Interlaboratory comparative testing;

Slide 13

FCL functions

- Personnel training;
- Laboratory auditing;
- Identification of cultures of microorganism;
- Making of culture mediums and reagents and control;
- Giving consultations regarding laboratory equipment, methods, quality control, technological problems during food production processes etc.

Slide 14

Detection of Salmonella

- ✓ Milk and milk products
  LVS ISO 6785 : 1985
- ✓ Microbiology of food and animal feeding stuffs – Horizontal method for the detection of Salmonella (ISO 6579:1993 modified)
  LVS ISO 12824:1997
- ✓ Water quality – Detection of Salmonella species
  LVS ISO 6340:1995
- ✓ Animal disease - veterinary bacteriology methods

Slide 15

Salmonella monitoring

Food products 2002

Products of food	Number of samples	Number of positive samples
Fresh meat	161	11
Meat products	1381	2
Fresh milk	3	0
Milk products	359	0
Fish, fish products	314	0
Eggs, egg products	36	0
Other food products	662	0
Other	2935	0

Slide 16

Animal disease control 2002

Samples	Number of samples	Number of positive samples
Animal feed	155	8
Chich (72h old)	389	40
Faecalis from poultry	264	13
Eggs from incubator	30	0

Slide 17

Other service 2002

Type of samples	Number of samples	Number of positive samples
Fresh meat	1498	52
Meat products	966	5
Fresh milk	10	0
Milk products	869	0
Fish, fish products	264	0
Eggs, egg products	193	2
Other food products	350	5
Other	782	5
Animal feed	1177	20
Clinical materials	922	68

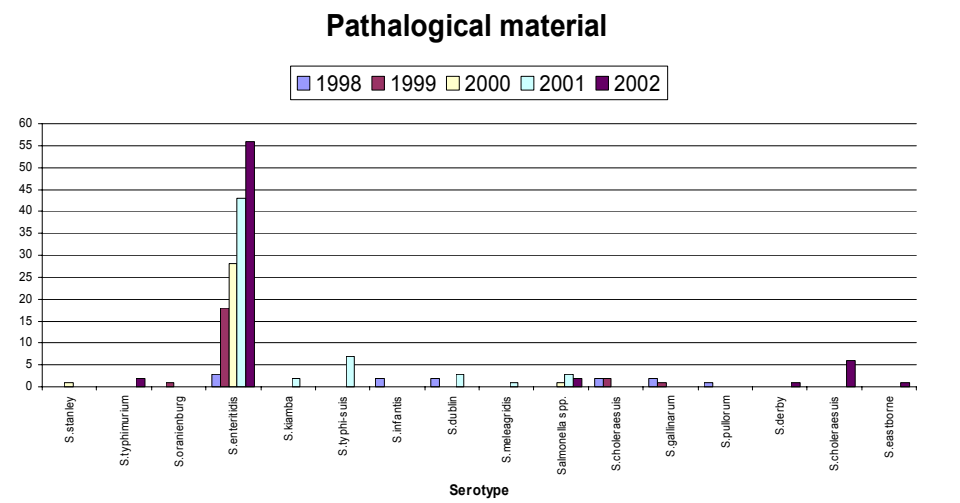
Slide 18

Salmonella strains

Food products 2002

Serotype	Fowl	Pork	Meat offal	Other food products
S.enteritidis	45			3
S.gallinarum	1			
S.indiana	2	1		
S.typhimurium	2			
S.infantis	2	2		
S.hadar				
S.heidelberg	1	1		
S.nhanga		1		
Salmonella spp.	5	5	4	1

Slide 19



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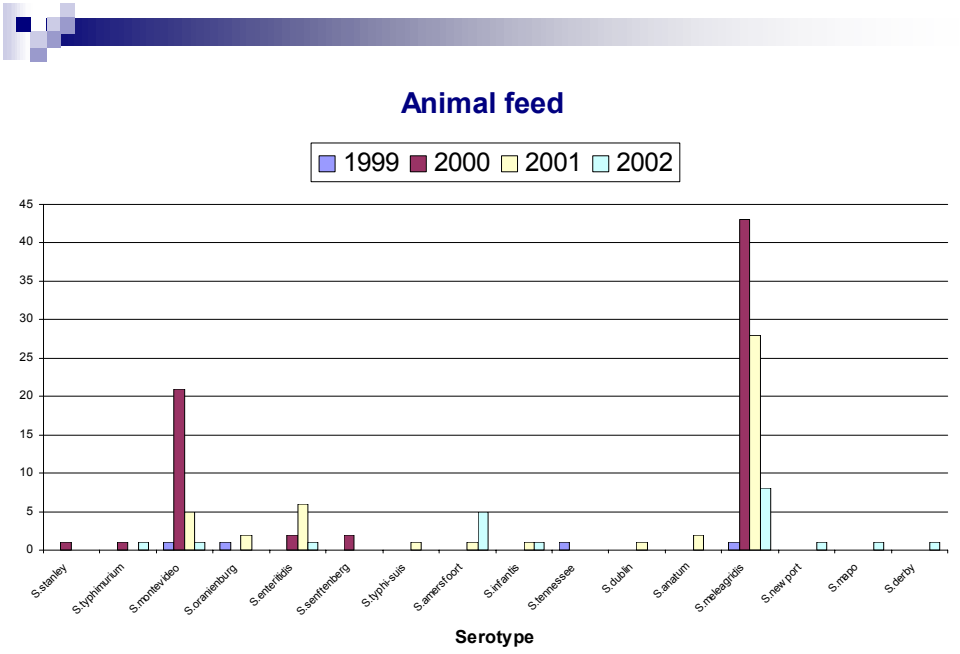


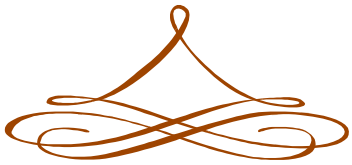
Slide 21

SVMDC cooperation with:

- Estonian Veterinary and Food laboratory
- National Veterinary laboratory of Lithuania
- PHLS Central Public Health laboratory
- Danish Veterinary laboratory
- Danish Veterinary Institute
- National Veterinary and Food Research Institute of Finland
- Van Hall Institute - Netherlands

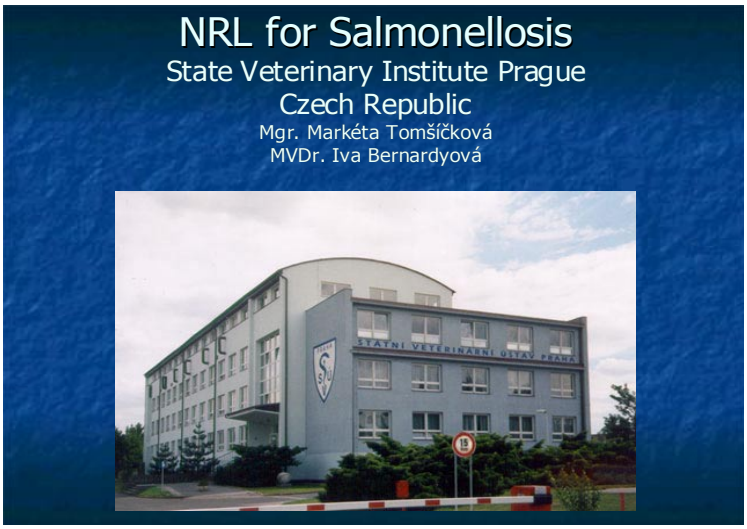
Slide 22

Thank you for your attention!



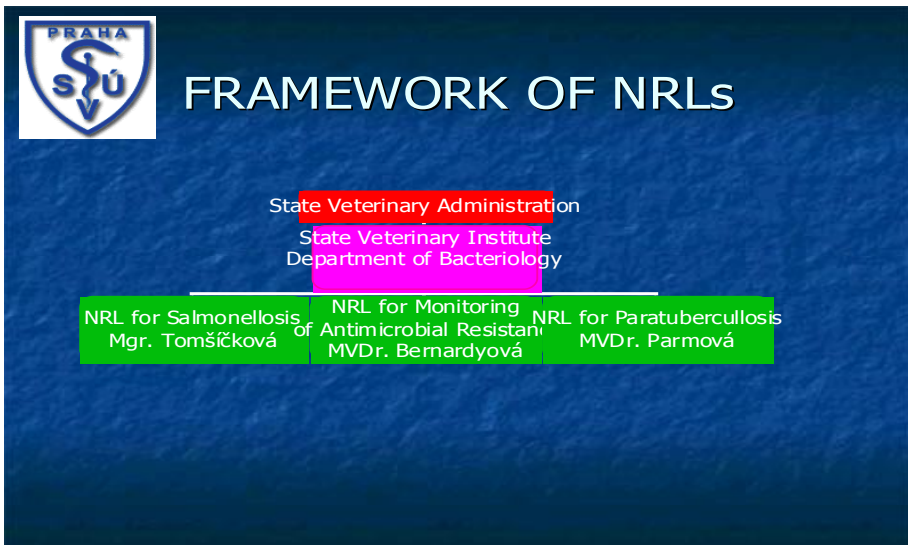
Appendix 11. Slides of presentation 1.4.5

Slide 1



Slide 2

Slide 3



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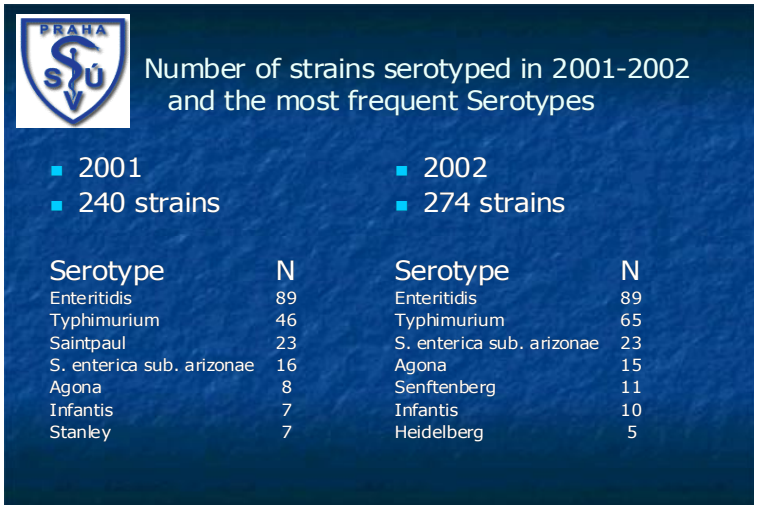
Slide 5



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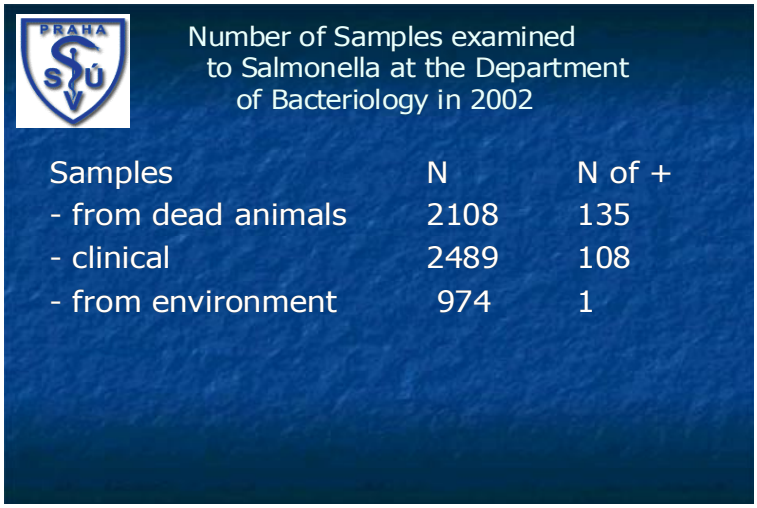

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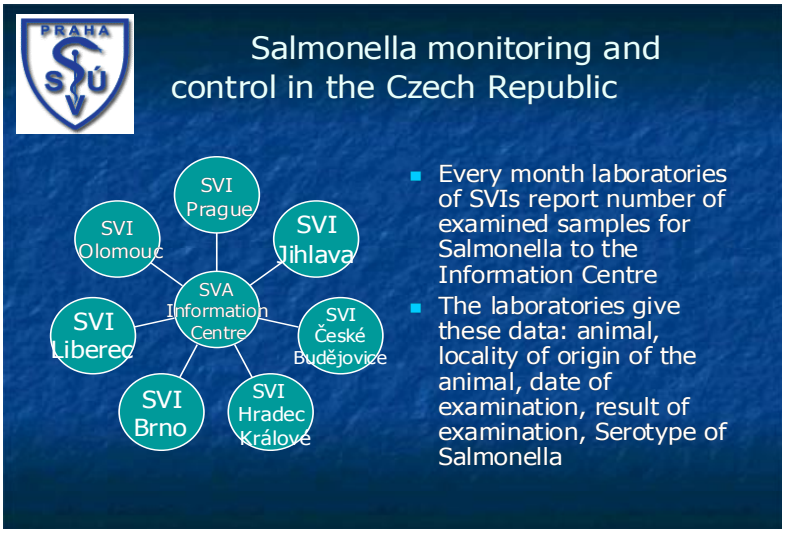

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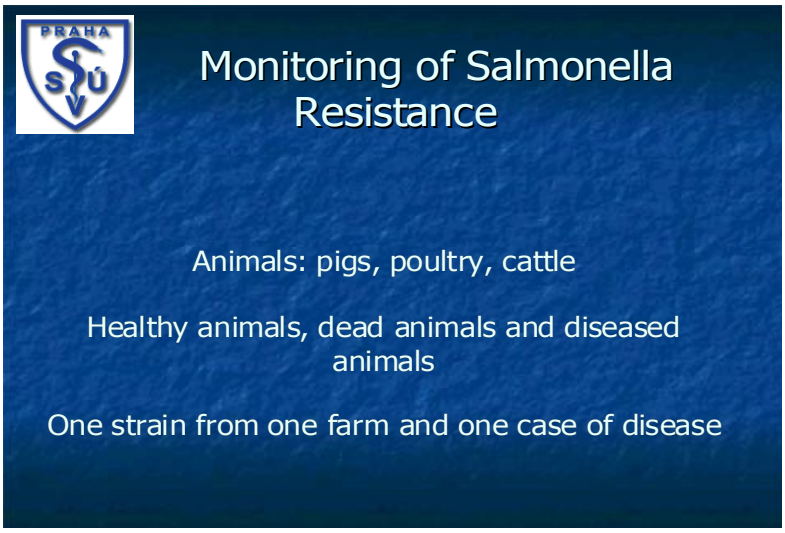

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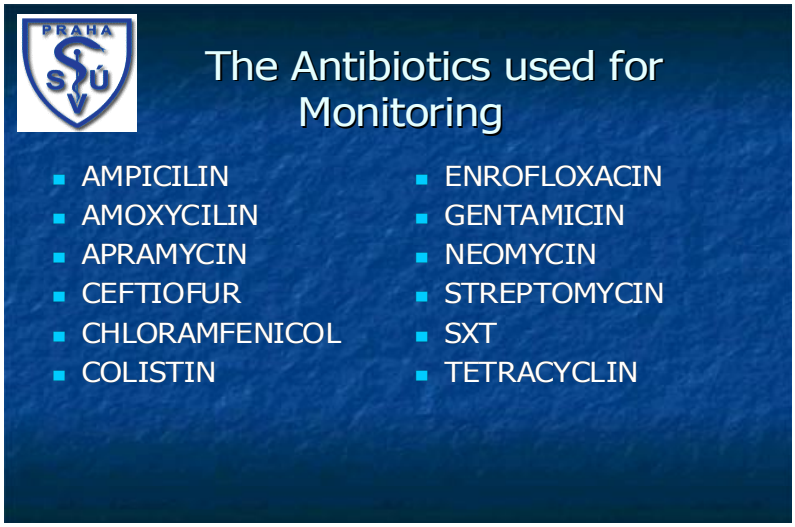
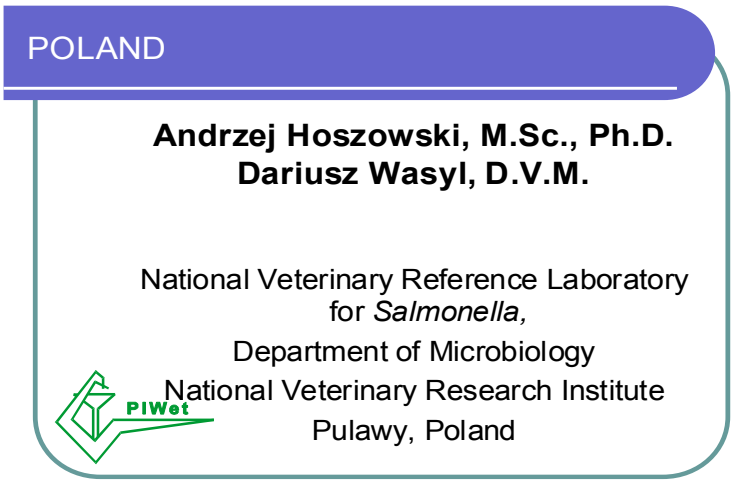


Slide 10

Slide 11

Appendix 12. Slides of presentation 1.4.6

Slide 1



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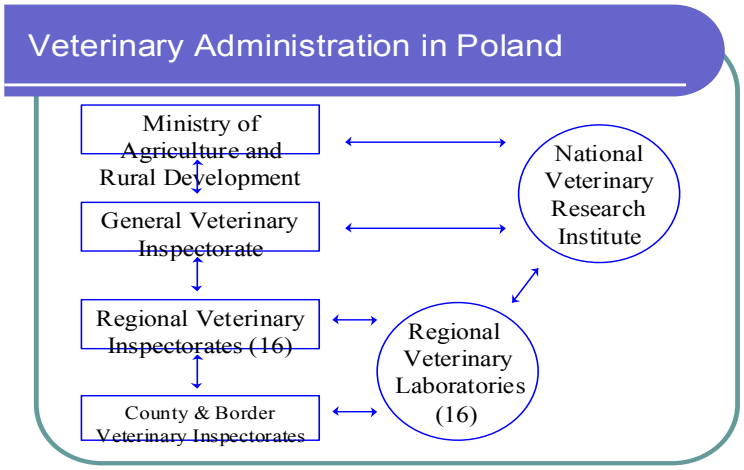
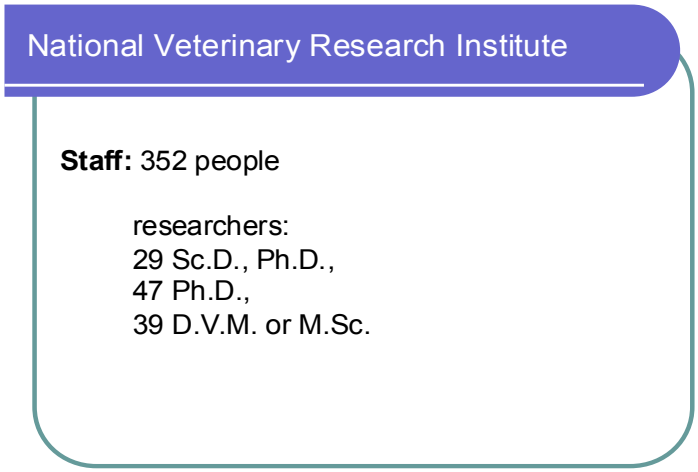


Slide 3

Slide 4

Slide 5



Slide 6

Slide 7

National Veterinary Research Institute

State Reference Laboratories for monitoring of infectious diseases in animals (13 Feb, 2003)

Slide 8

National Veterinary Reference Laboratory for *Salmonella*

Aim:

- isolation and identification
- susceptibility testing
- epidemiological typing
- surveillance

Slide 9

Salmonella in animals - scope of the problem

1999 (data reported to NVRI in 2000)

Samples tested		Number (%) of *Salmonella* positive samples
Origin	Number	
Poultry	124743	9157 (7.34)
Farms and hatcheries	18839	160 (0.85)
Food and feeding stuffs	154562	1107 (0.72)
Other*	4590	165 (3.59)
Total	302734	10589 (3.50)

* swine, pigeons, pheasants, fur and exotic animals, environment

Slide 10

Salmonella monitoring and control programmes

- Notifying and reporting to General Veterinary Inspectorate poultry, swine, bovine salmonellosis
- National salmonellosis control programme in poultry
  - since 1999
  - based on 92/117/EEC Directive
  - chicken, turkey, geese, ducks, (pheasants)
  - rearing, lying, broiler flocks
  - monitoring of *Salmonella* infections
  - control of *S*. Enteritidis, *S*. Typhimurium, *S*. Gallinarum

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Cooperation

- no official national cooperation of veterinary and medicine
- international cooperation
  - 6FP No QLK2-CT-2002-01146
    "Antibiotic Resistance in Bacteria of Animal Origin – II"
  - COST Action 920
    "Research and Surveillance of Foodborne Zoonoses throughout Europe: a Co-ordinated Food Chain Approach"
  - EQAS (WHO Global Salm-Surv)

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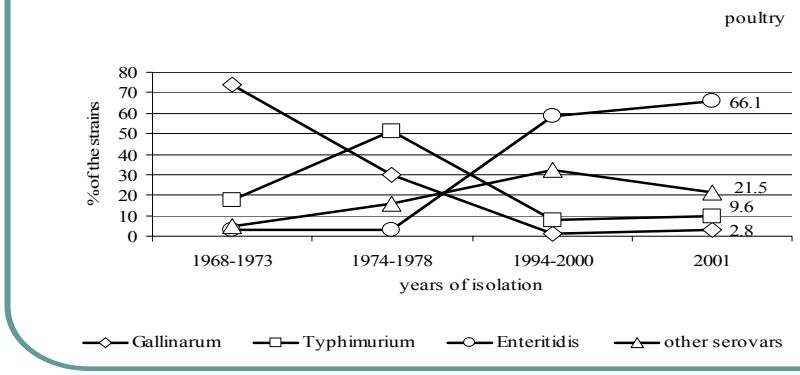
Salmonella serovars

Years 1994 - 2000		
Enteritidis	1592	(58.6)
Typhimurium	212	(7.8)
Mbandaka	116	(4.3)
Agona	98	(3.6)
Choleraesuis	72	(2.7)
Infantis	50	(1.8)
Hadar	43	(1.6)
Isangi	43	(1.6)
Gallinarum	41	(1.5)
Senftenberg	39	(1.4)
Other [b]	410	(15.1)
Total (n = 75)	2716	(100.0)

Year 2001		
Enteritidis	302	(53.3)
Typhimurium	60	(10.6)
Hadar	29	(5.1)
Agona	20	(3.5)
Choleraesuis	20	(3.5)
Derby	19	(3.4)
Infantis	13	(2.3)
Gallinarum	12	(2.1)
Mbandaka	11	(1.9)
Cubana	9	(1.6)
Other [a]	72	(12.7)
Total (n = 32)	567	(100.0)

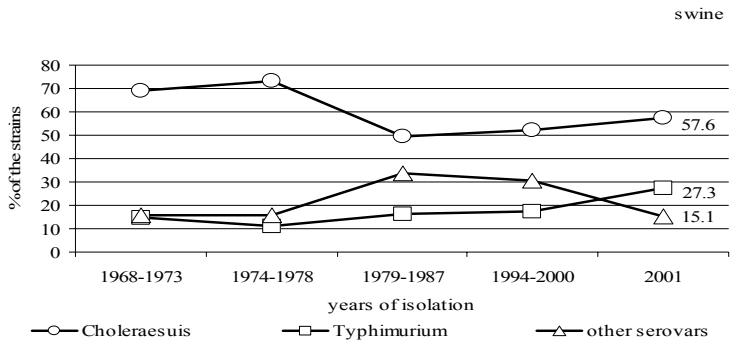
Slide 13

Salmonella in poultry



Slide 14

Salmonella in swine



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Salmonella in humans '2001 acc. National Institute of Hygiene

	2001	2002
Number of cases	19881	20688
Incidence rate	51.45	53.55

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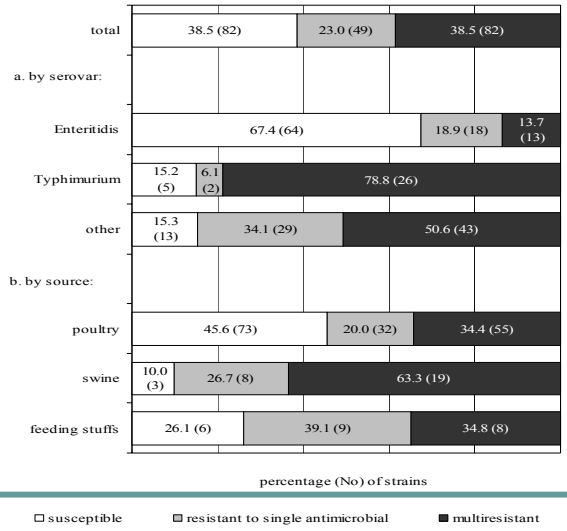
Salmonella in humans '2001 acc. National Institute of Higiene

Serovar	Percentage
Enteritidis	85.7
Typhimurium	4.23
Virchow	2.26
Hadar	2.16
Infantis	1.53
Oranienburg	0.49
Mbandaka	0.36
Agona	0.32
Newport	0.32
Thompson	0.26

Slide 17

Antimicrobial resistance in *Salmonella* '2001



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Antimicrobial resistance in *Salmonella* '2001

- increase in antimicrobial resistance and MDR
- genome-integrated determinants (*S.* Typhimurium DT104)
- Fluoroquinolone-resistance
- no ESβL-positive strains

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References:

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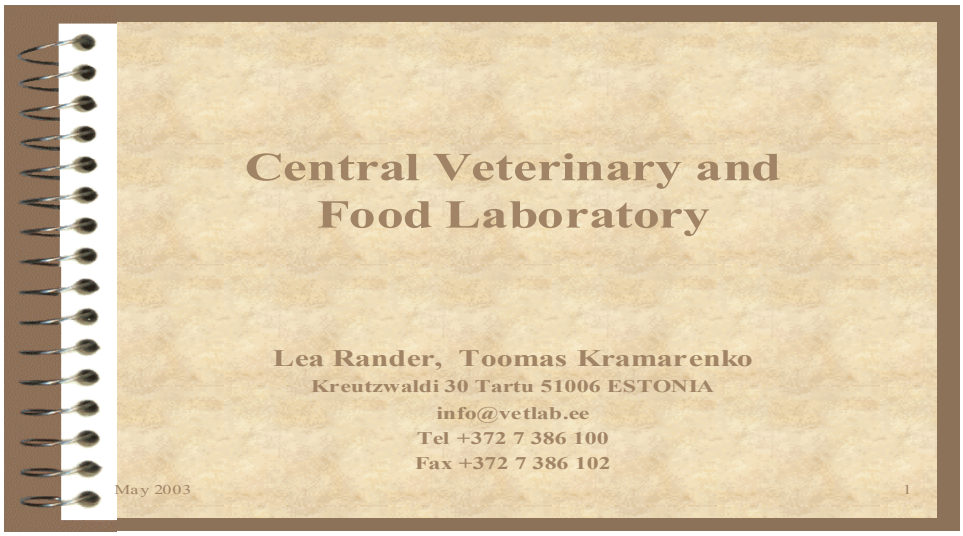
Appendix 13 Slide of presentation 1.4.7

Slide 1



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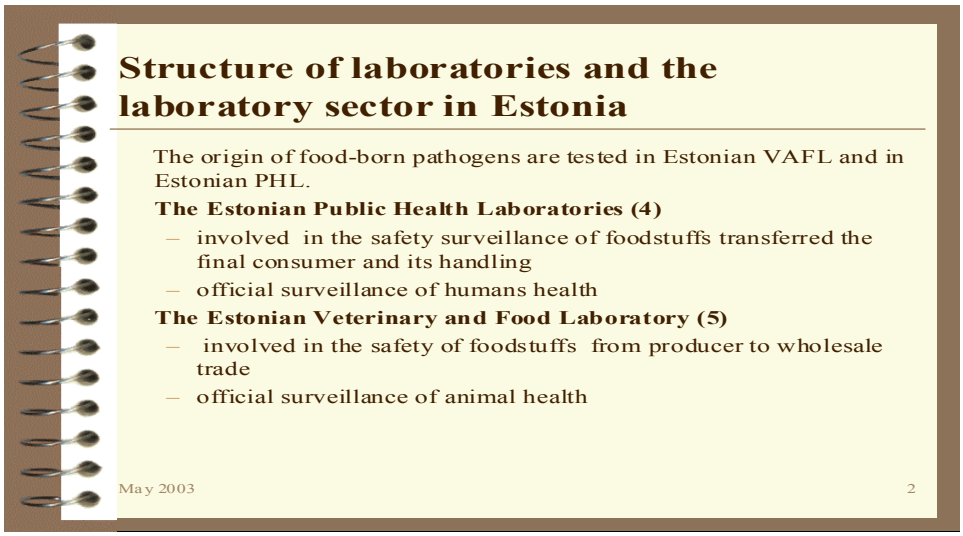
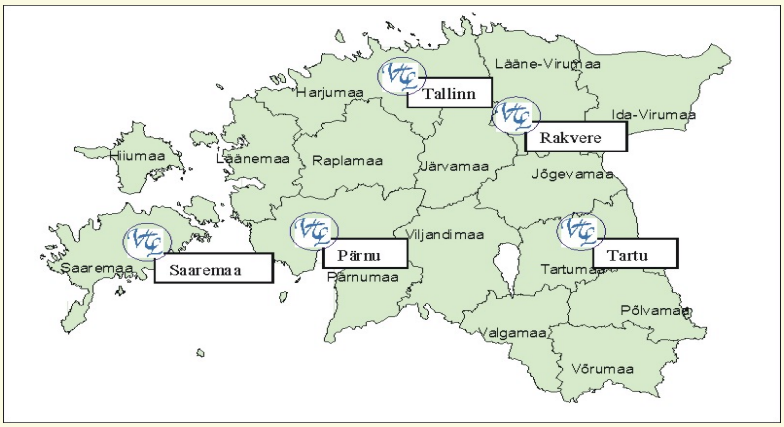


Slide 3

Geographical distribution of VAFL departments



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Slide 4

Salmonella control

- Food control is based on the principle of own-check programmes and control visits in food processing industry. The pathogenic bacteria are analysed by VAFL
- Veterinary and food control on borders
- Isolation and identification *Salmonella* from patological materials in domestic animals and pets
- Serotyping of *Salmonella* strains sent by others regional laboratories

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Salmonella monitoring programs

- *Salmonella* monitoring in animal origin food and feedstuffs
- *Salmonella* monitoring in farms of domestic animals and fawls
- Monitoring antimicrobial resistance of *Salmonella* strains isolated from food, feedstuff and animal

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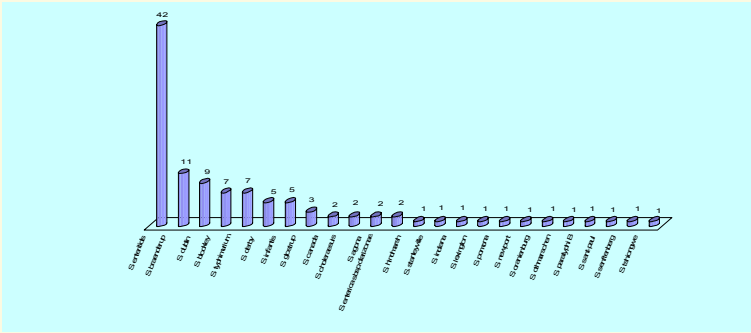
Isolated *Salmonella* serotypes in 2002



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Cooperation with other laboratories and authorities

- National
  - regional veterinary and food laboratories
  - Estonian Veterinary and Food Board
  - Estonian Health Protection Inspectorate and it´s laboratories
- International
  - Finnish National Veterinary and Food Research Insitute (EELA)
  - Swedish Veterinary Institute in Uppsala

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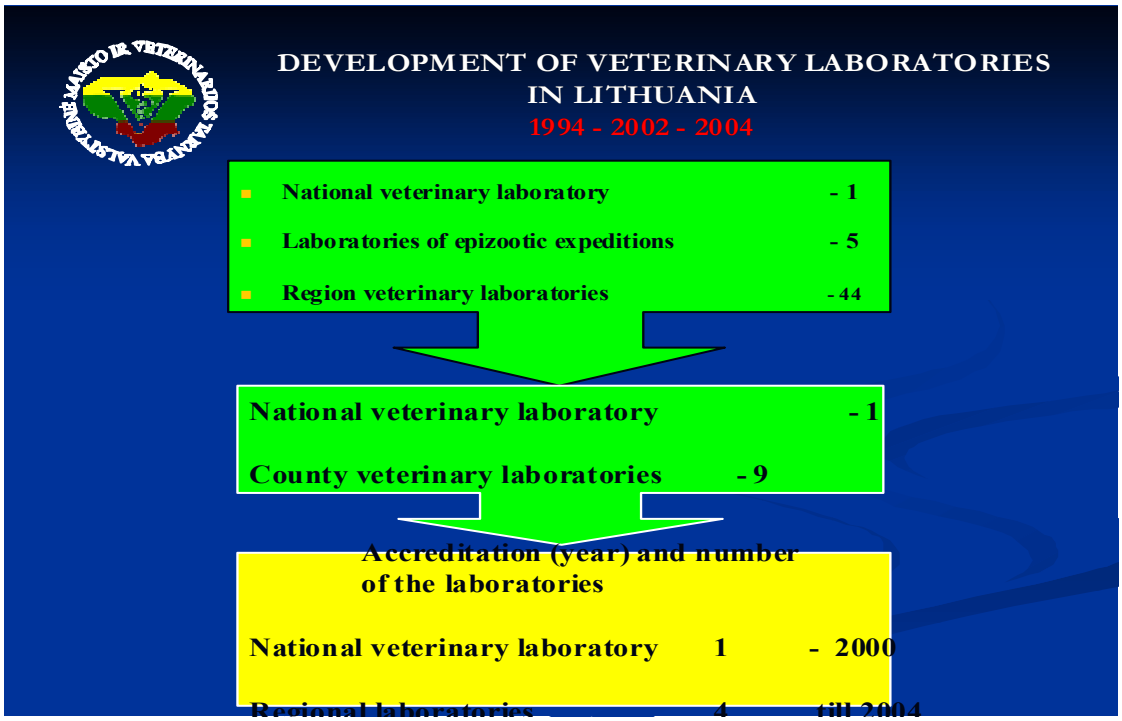
Appendix 14. Slides of presentation 1.4.8

Slide 1

Slide 2

Slide 3



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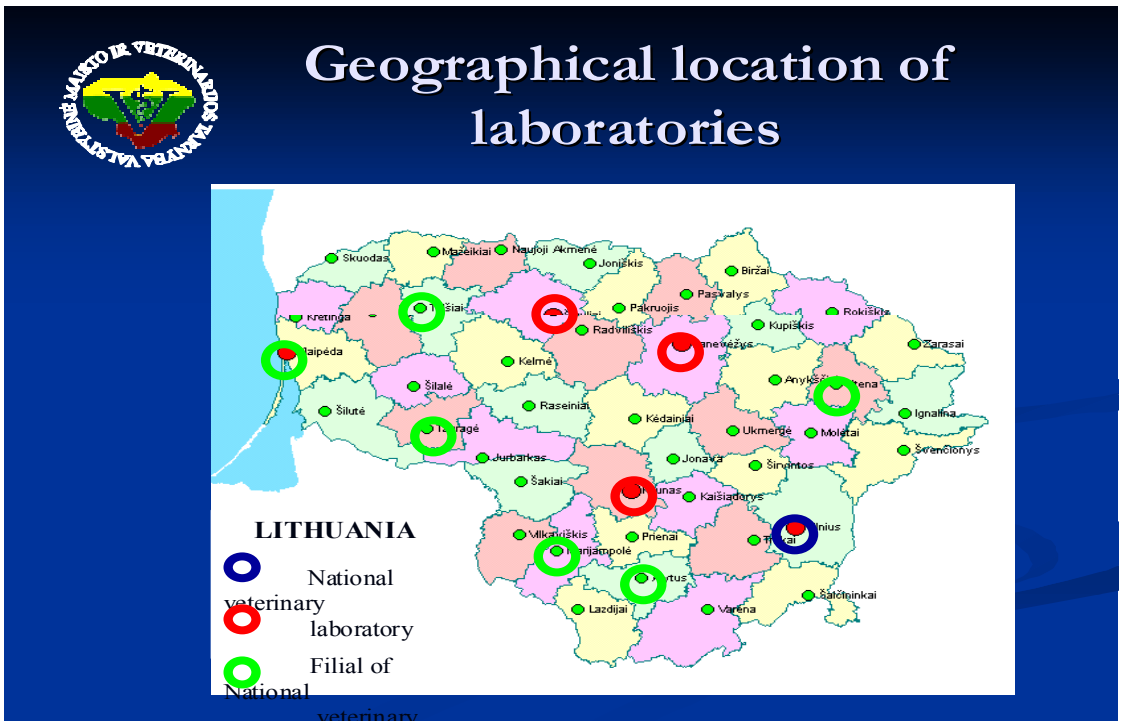

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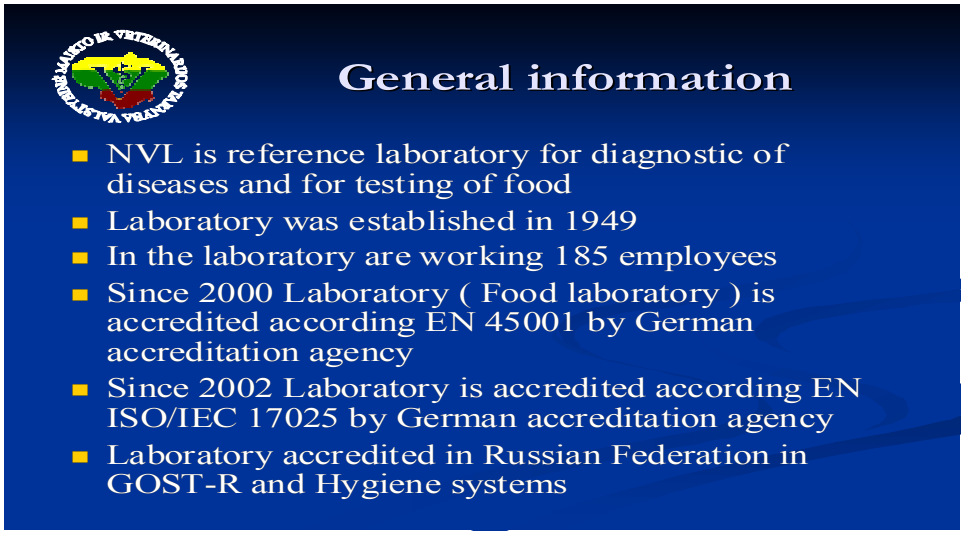

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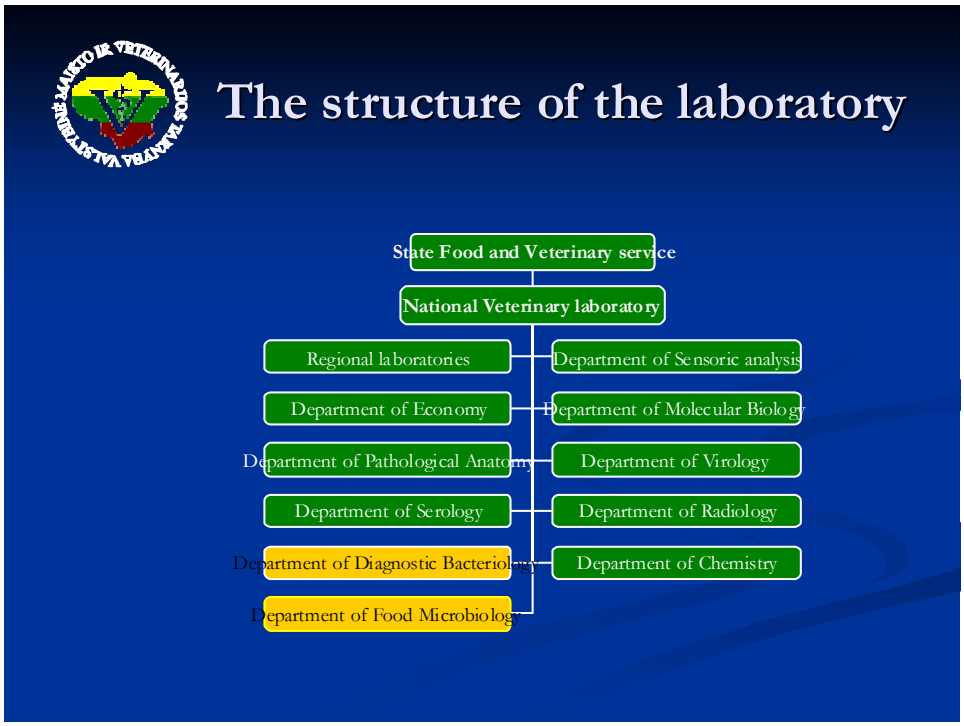

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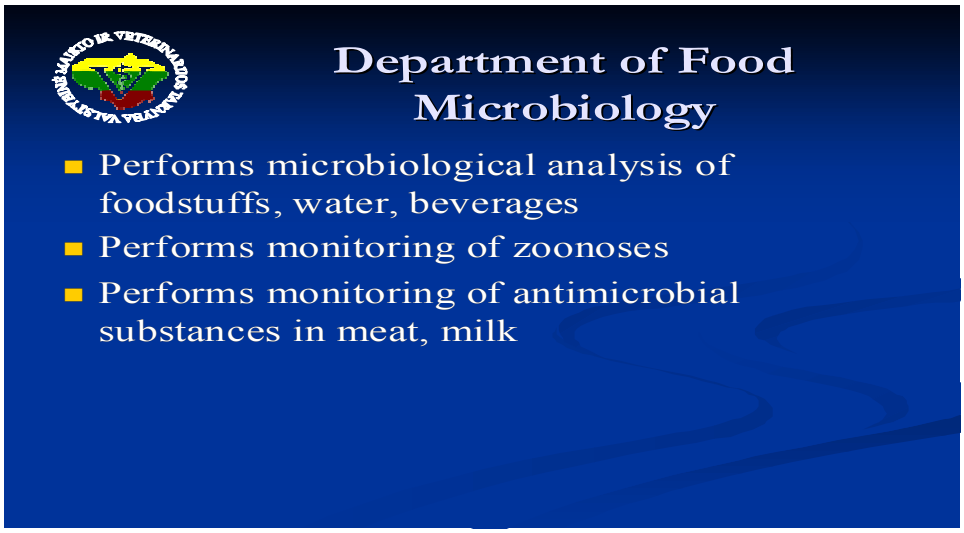

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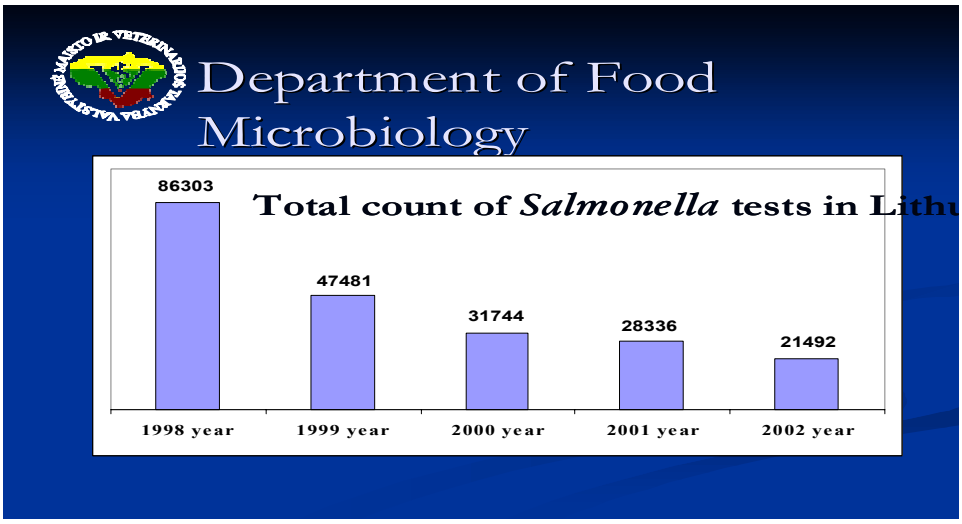

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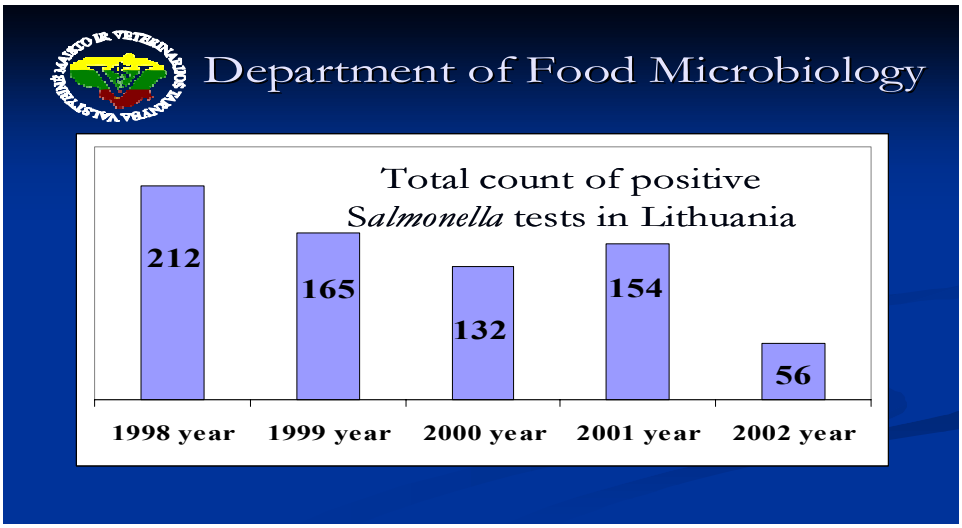

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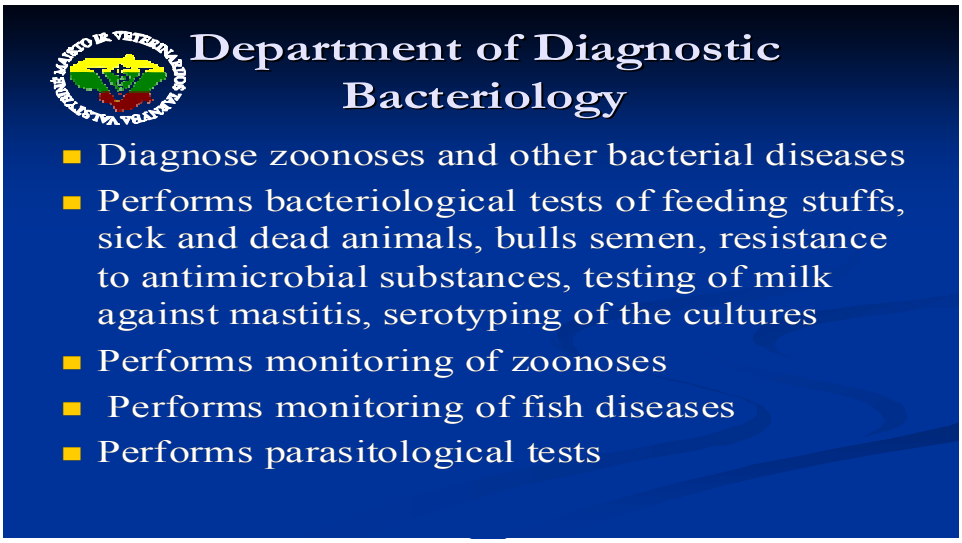

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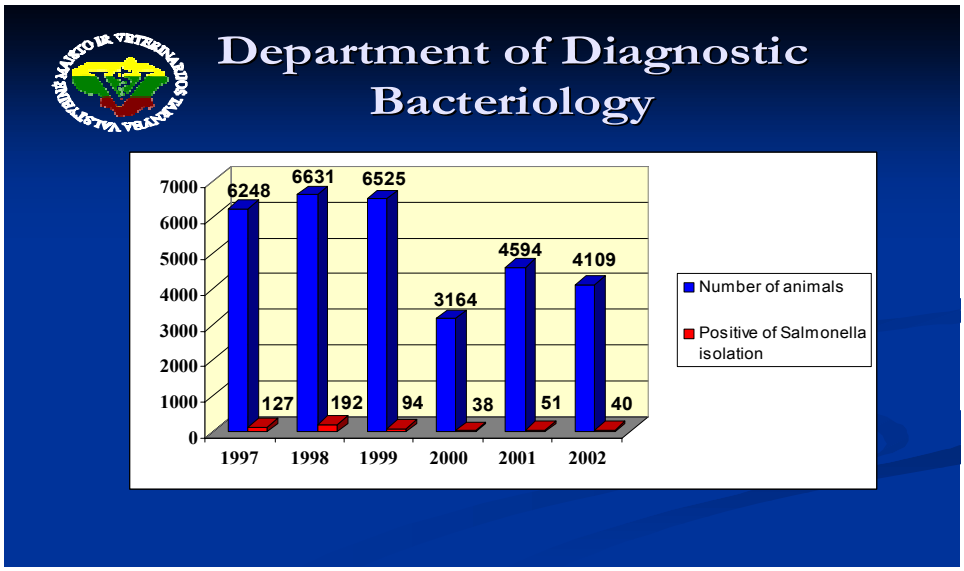

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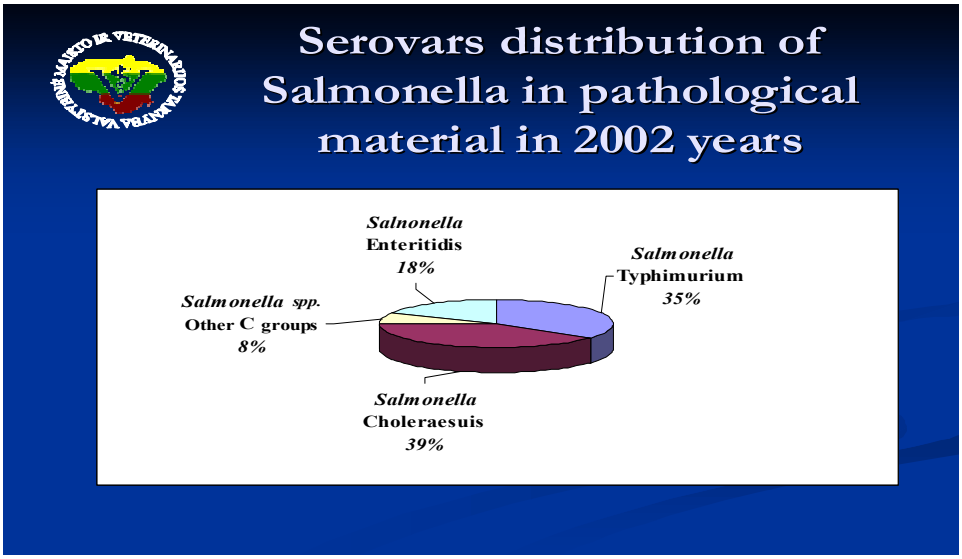

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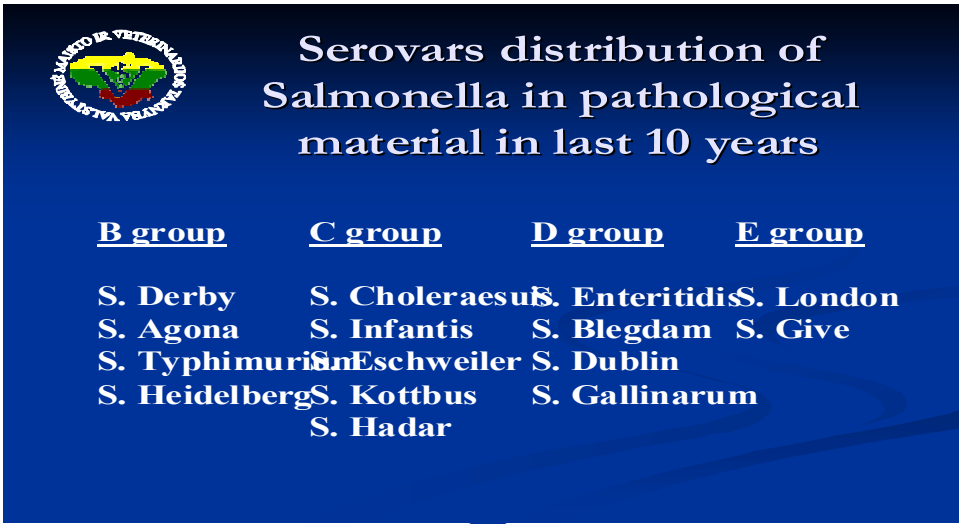

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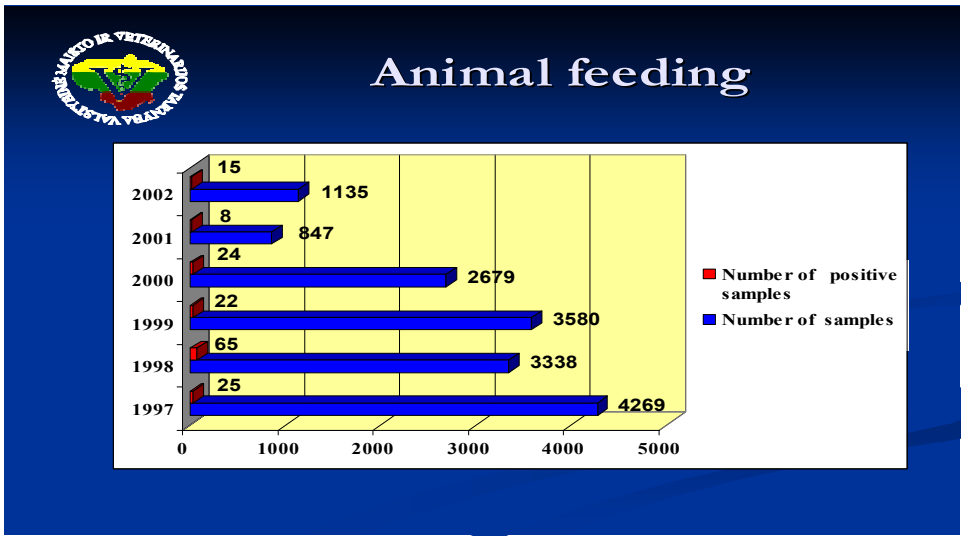

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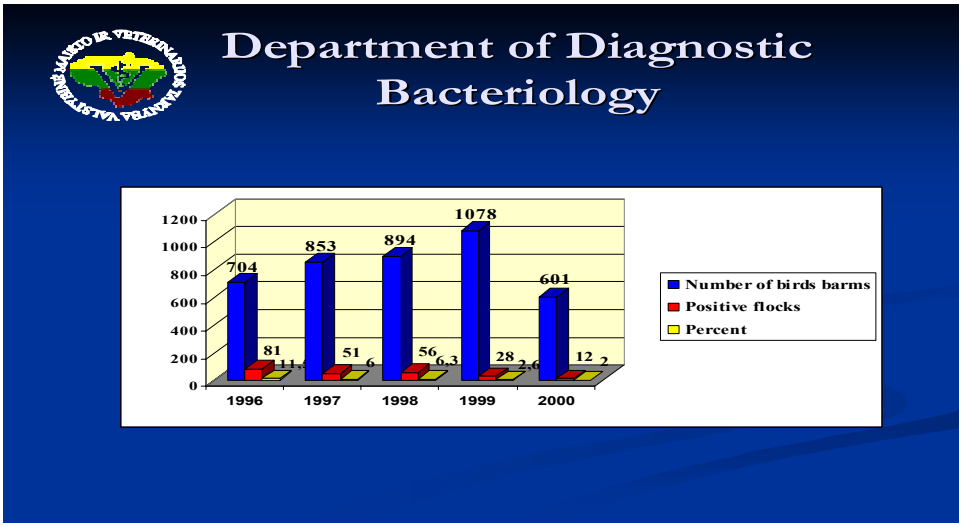

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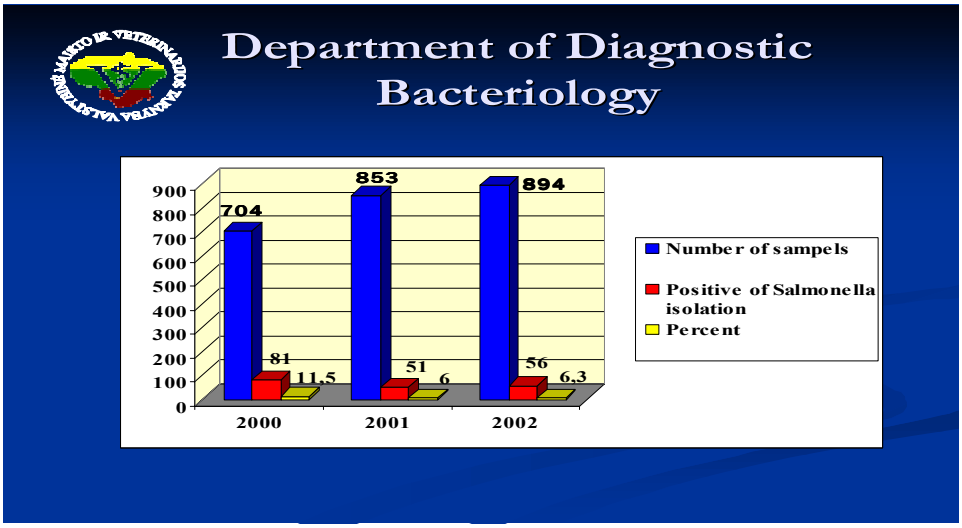

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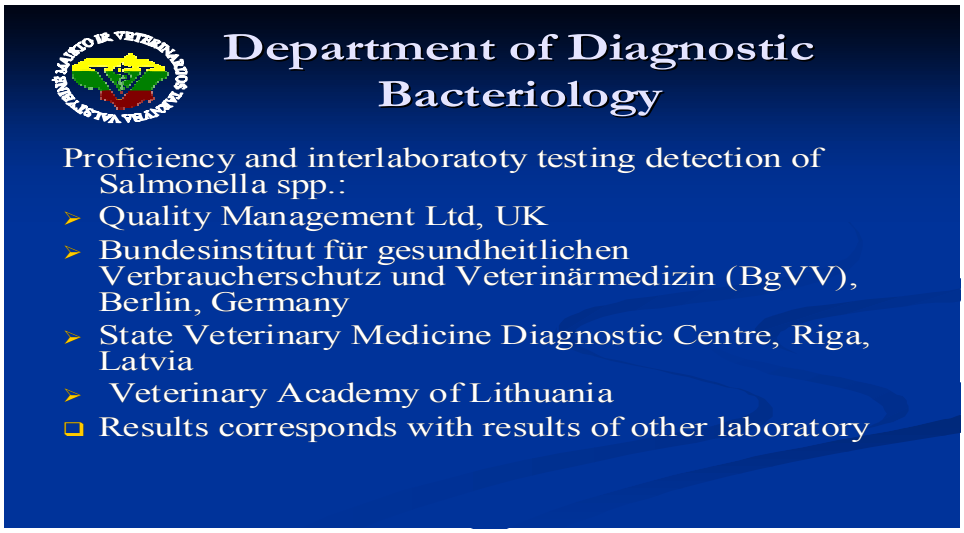

Slide 20

Appendix 15. Slides of presentation 1.4.9

Slide 1

Slide 2

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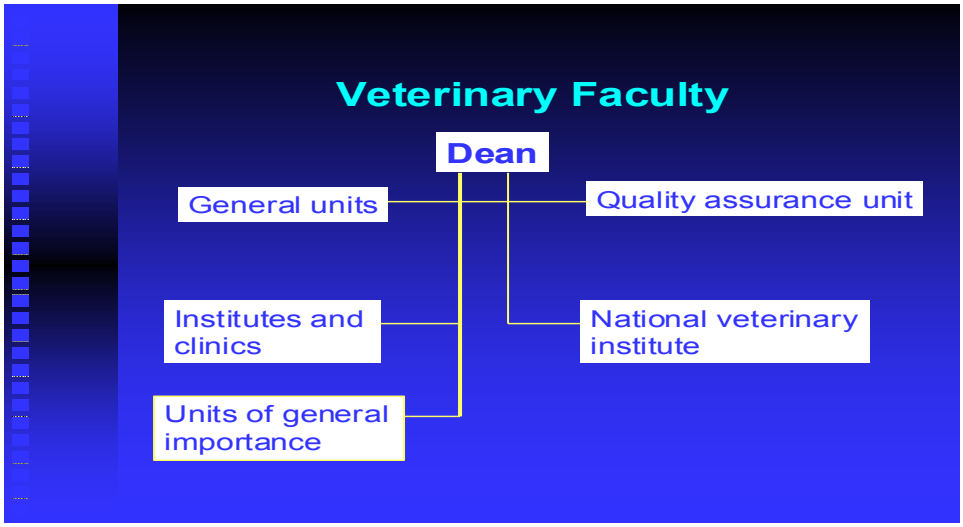

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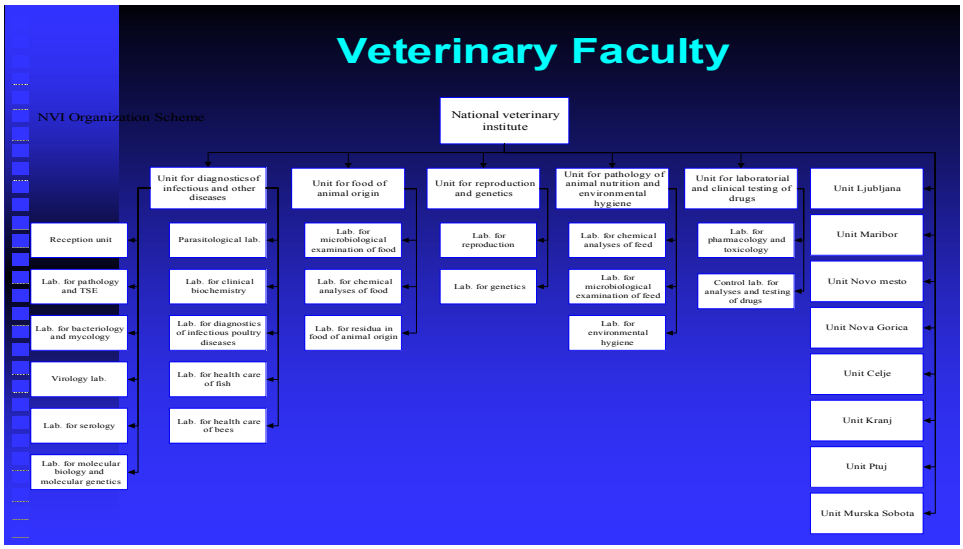

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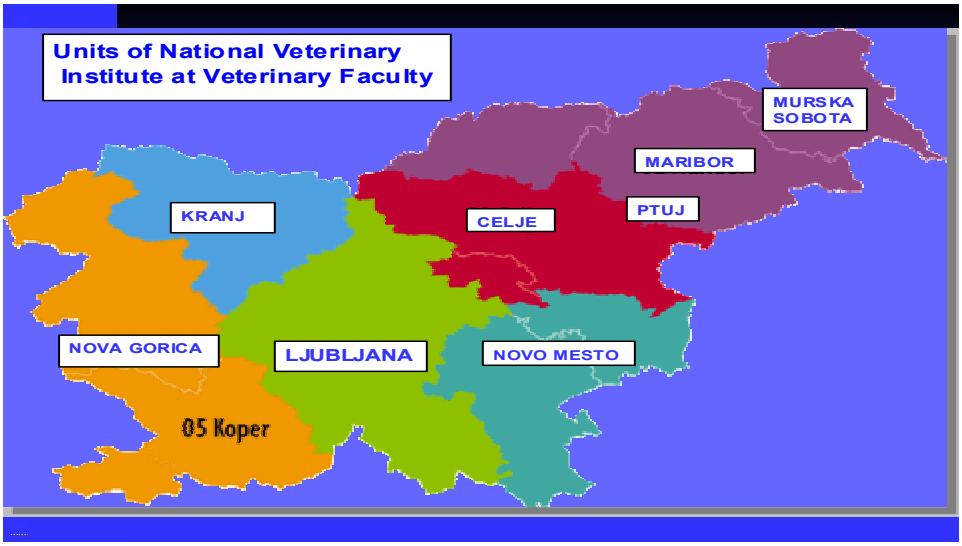

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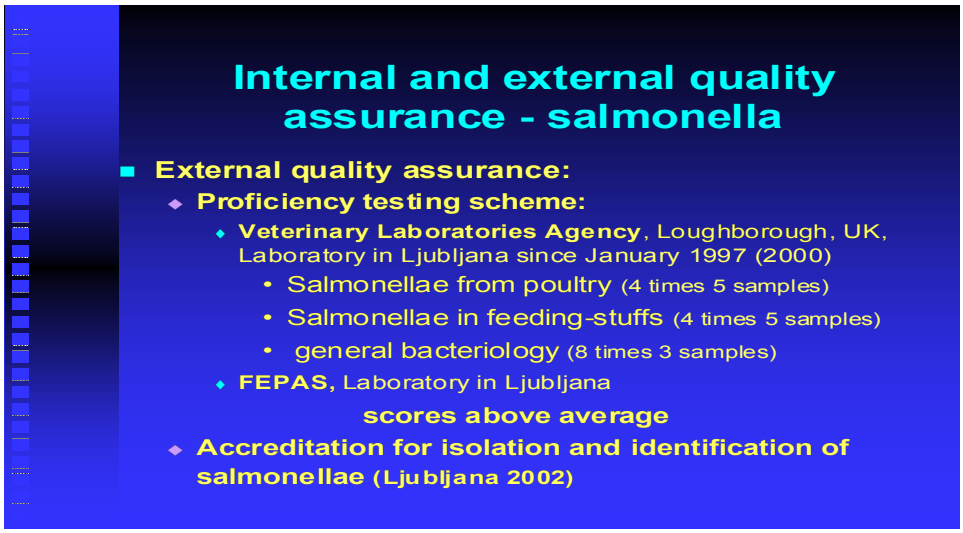

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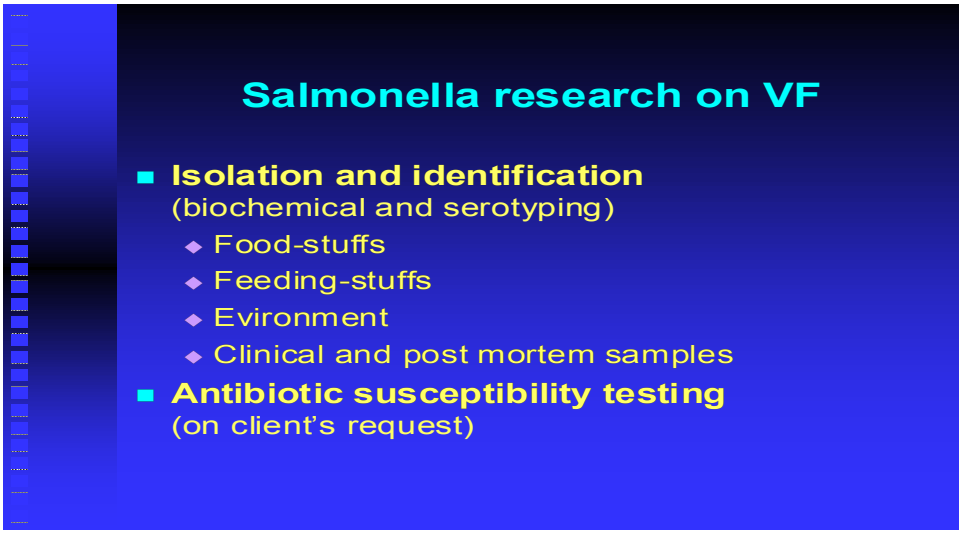

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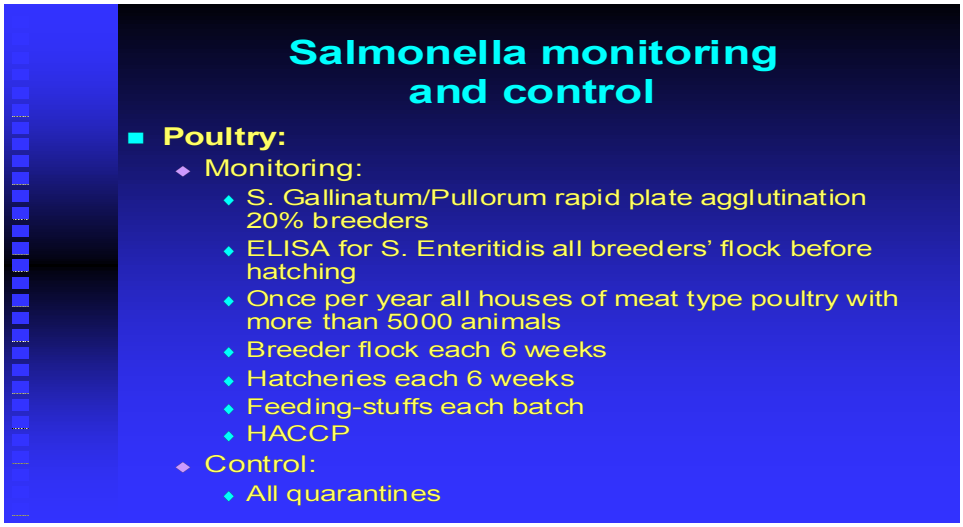

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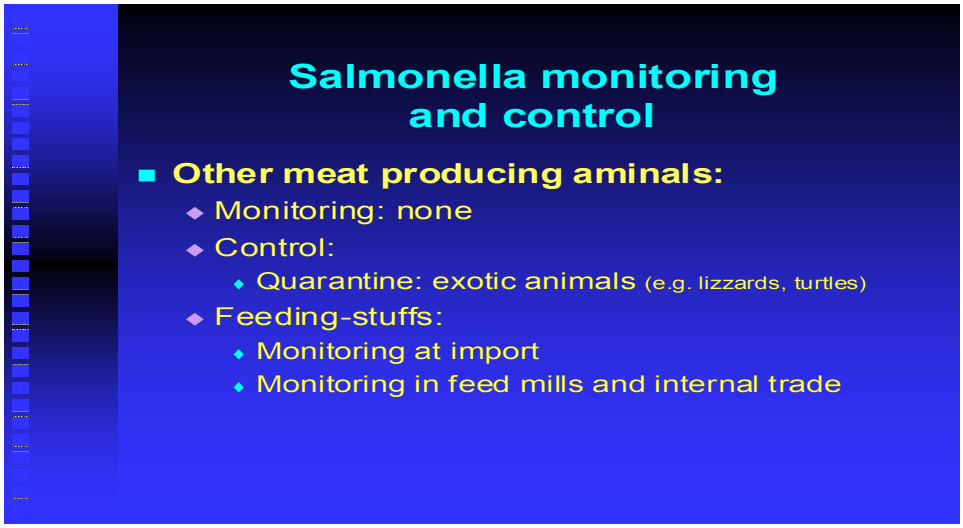

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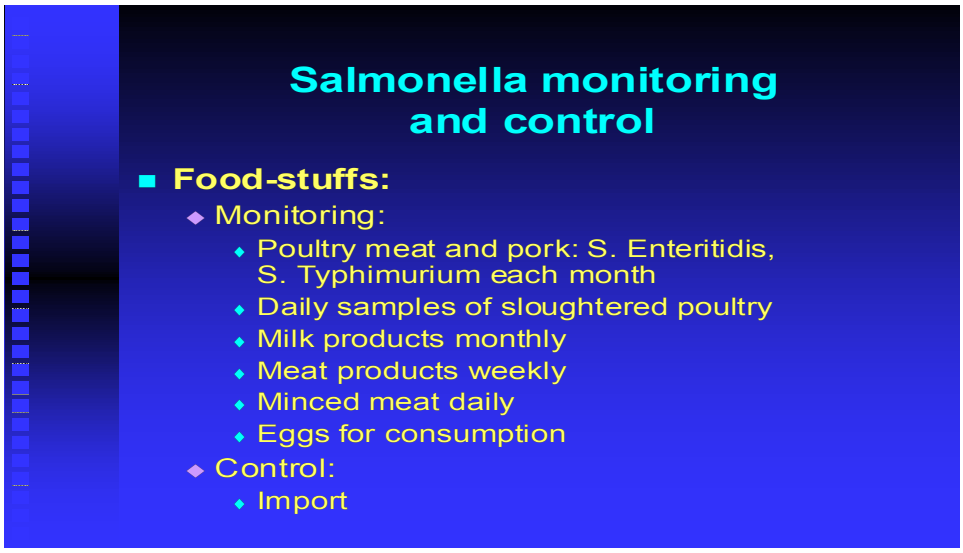

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Appendix 16. Slides of presentation 1.4.10

Slide 1

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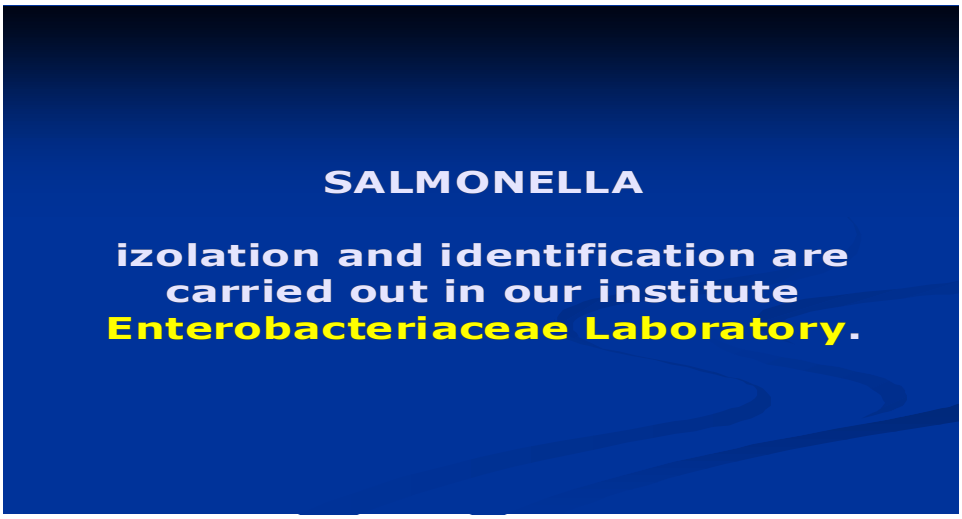

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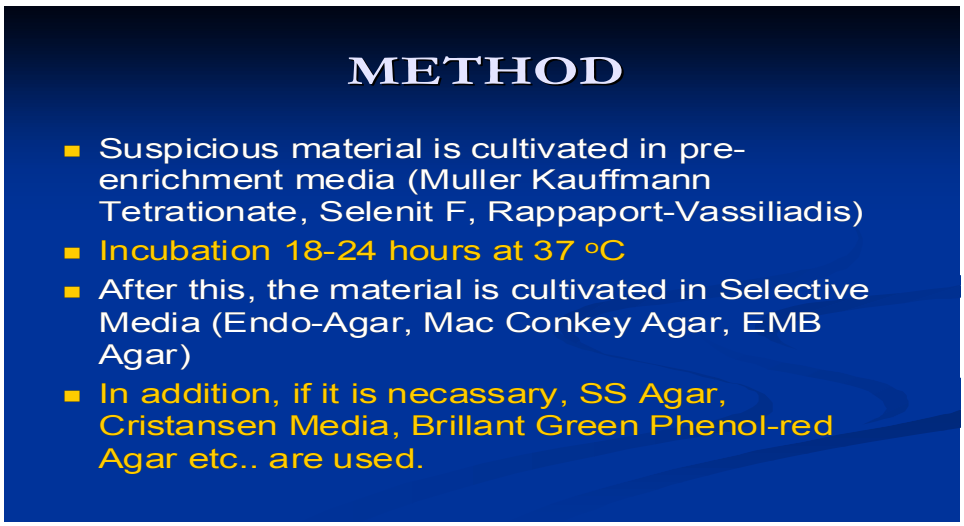


Slide 4

METHOD

- To examine the biochemical characters of lactose negative bacteria, tripple tube media is used (Tube 1: Glicose-Lactose-H_2S Media; Tube 2 : Mannithol-mobility Media and Tube 3 : Urea-Indole Media).
- Incubation 18-24 hours at 37 ºC
- After incubation, reaction is read, and lactose negative bacteria are identified to be Salmonella Spp. according to glicose, H_2S, Mannithol, mobility, Urea-Indol, ONPG and LDC results.

Slide 5

SEROTYPING

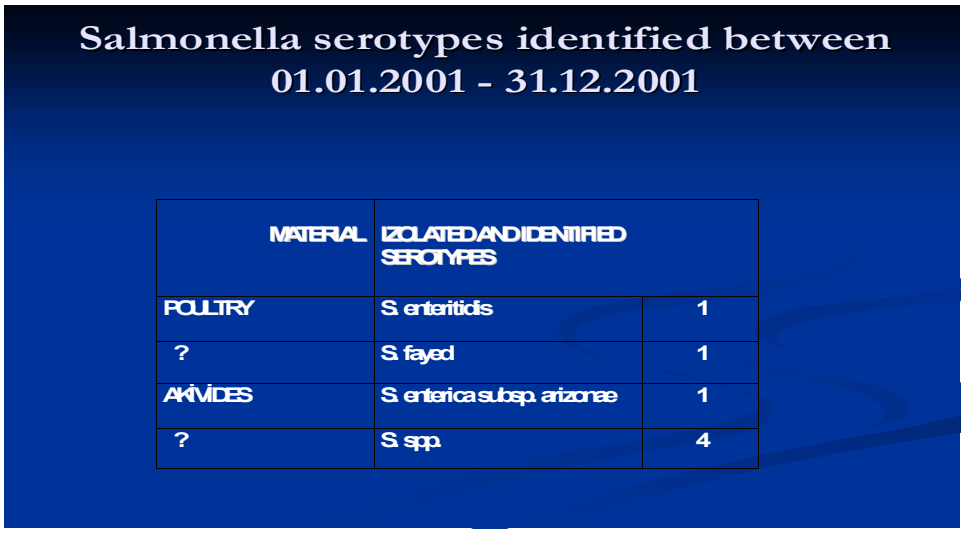
- To increase the mobility, it is cultivated to 0,2 % Craigei media from 24 hour pure buyyon culture of Salmonella.
- After 18-24 hour incubation at 37 ºC, this is cultivated in 0,2 % mild Agar (30 ml in a petri dish).
- After 18-24 hour incubation at 37 ºC, Sallmonella are serotyped by slayt agglutination test by using poly-group antisera, group antisera and H antisera.

Slide 6

Salmonella serotypes identified between 01.01.2000 - 31.12.2000

MATERIAL	IZOLATED AND IDENTIFIED SEROTYPES	
POULTRY	S. enteritidis	1
SHEEP ABORTION	S. thompson	1
?	S .thompson	2
?	S. typhimurium	6
AKİVİDES	S. tshiongwe	2
?	S. infantis	2
?	S. istanbul	6
?	S . B gruop	9
?	S. D gruop	1
?	S. C_2 gruop	9
?	S. poly A	1
?	S . poly C	2
?	S . poly D	3
?	S. poly G	1

Slide 7



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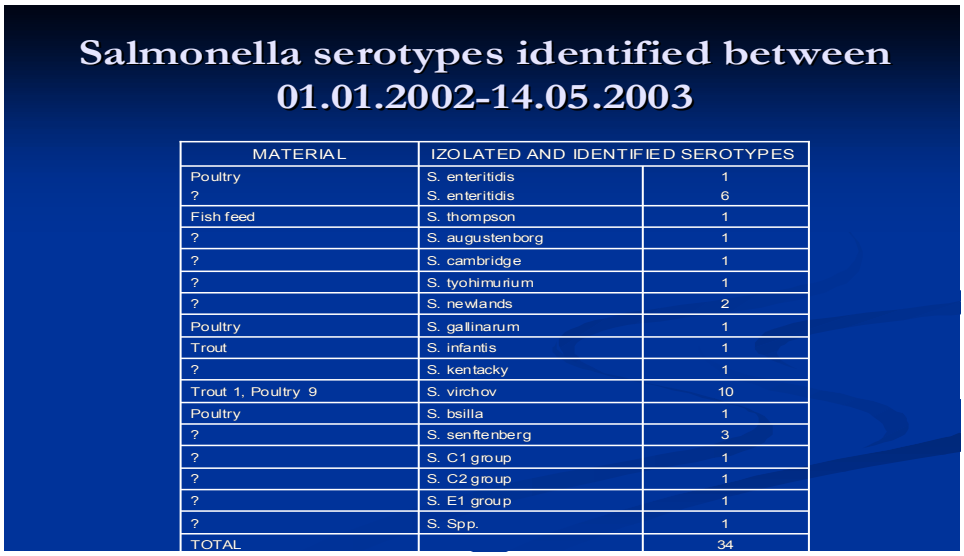

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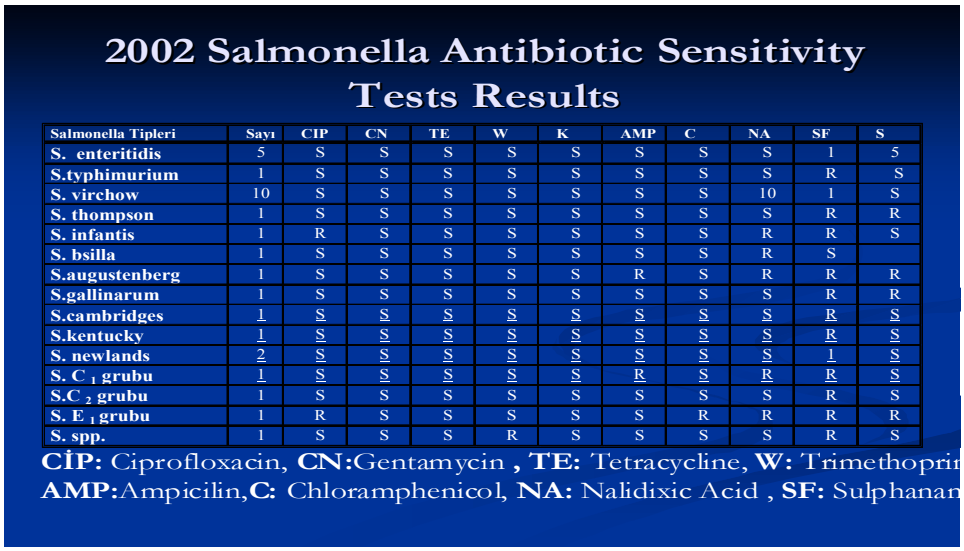

Appendix 17. Slides of presentation 1.5

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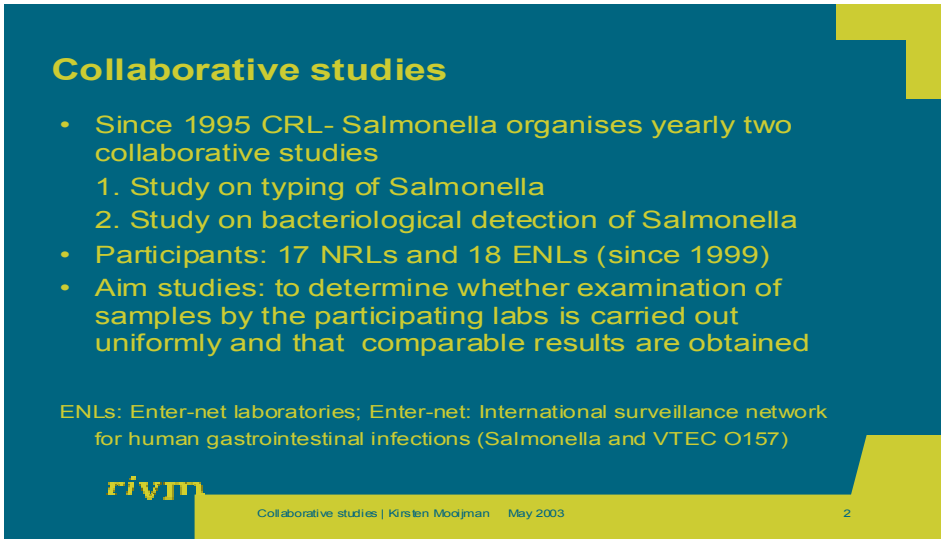

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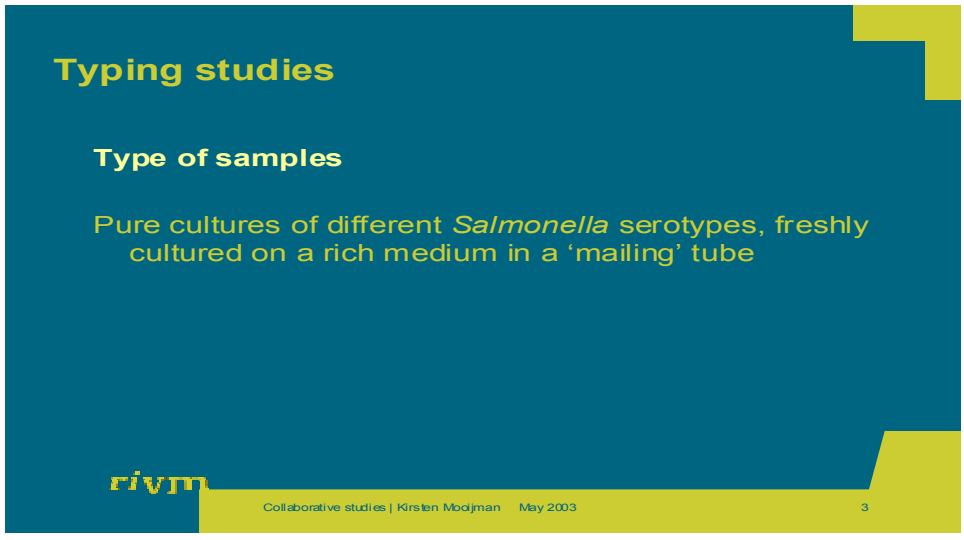

Slide 4

Typing studies, historical overview

Study NRLs	Study ENLs	Year	Serotyping of *Salmonella enterica* strains	Phage typing	Antibiotic resistance testing
I		1995	*spp. enterica* 18 *spp. salamae* 1 *spp. houtenae* 1		
II		1996/1997	*spp. enterica* 20		
III		1998	*spp. enterica* 20	SE 4 STM 5	
IV	I	1999	*spp. enterica* 16	SE 10 STM 10	
V	II	2000	*spp. enterica* 18 *spp. salamae* 1 *spp. houtenae* 1	SE 10 STM 10	YES
VI	III	2001	*spp. enterica* 19 *spp. arizonae* 1	SE 10 STM 10	YES
VII	IV	2002	*spp. enterica* 20	SE 10 STM 10	
VIII	V	2003	*spp. enterica* 20	SE 10 STM 10	YES

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Typing studies, set-up study 2003

Week	Date (2003)	Topic
5	27-31 Jan	Mailing of protocol & test report 2003 (to NRLs and ENLs)
8	17-21-Feb	Information to NRLs concerning airway bill number
9 9-?	24-28 Feb 24 Feb-?	CRL send strains for serotyping & AR to NRLs (extra set to PHLS). All send as dangerous goods to nearest airport. PHLS send strains for phagetyping to NRLs PHLS send strains for serotyping, phagetyping & AR to ENLs After arrival at lab subculturing and storage of the strains until the performance of the typing.
10	3-7 March	Starting with the identification of the strains.
12	17-21 March	Completion of the test report. Sending of the complete report to the CRL and sending results of the phage typing also to PHLS. **Deadline for NRLs: End of March 2003** **Deadline for ENLs: End of April 2003**
13-?	24 March-?	Printed version of individual results send to all NRLs and ENLs by CRL. NRLs and ENLs check their results for correctness and inform CRL.

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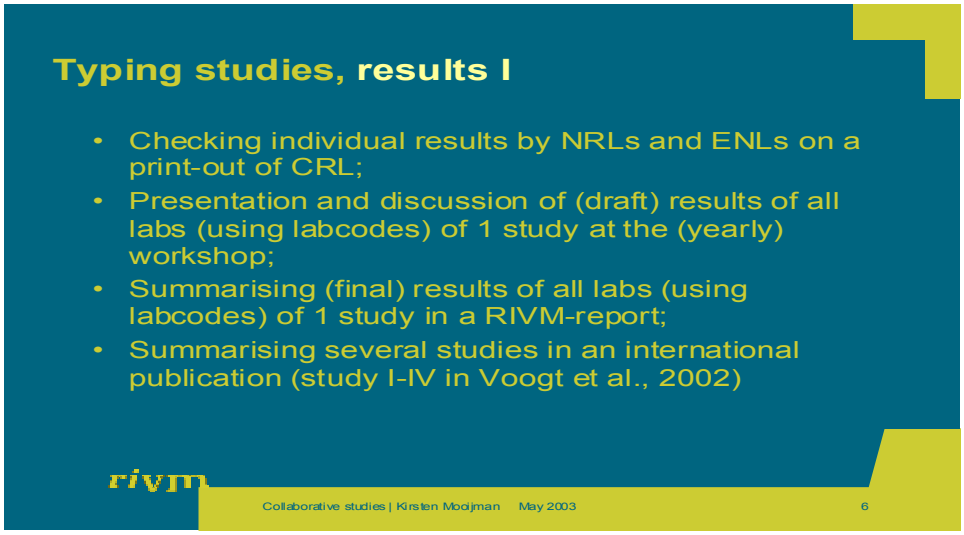
Slide 6



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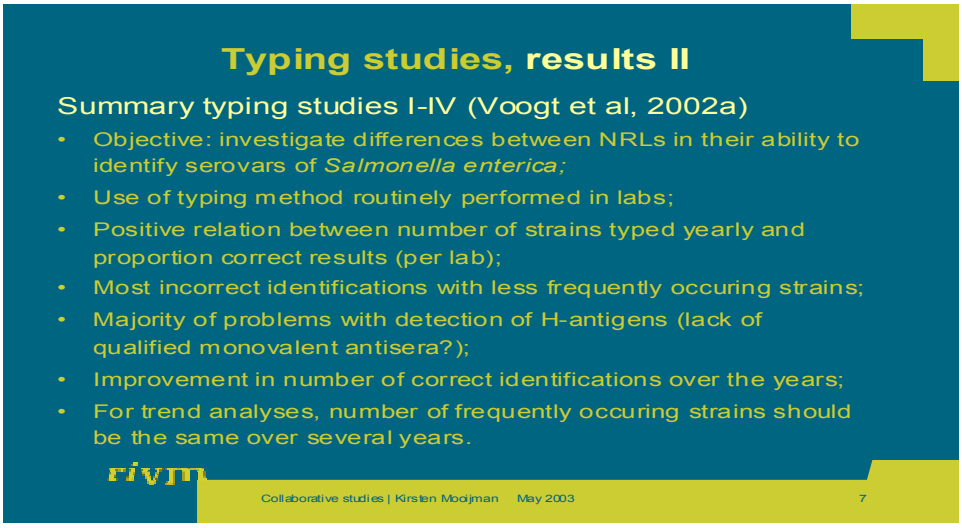

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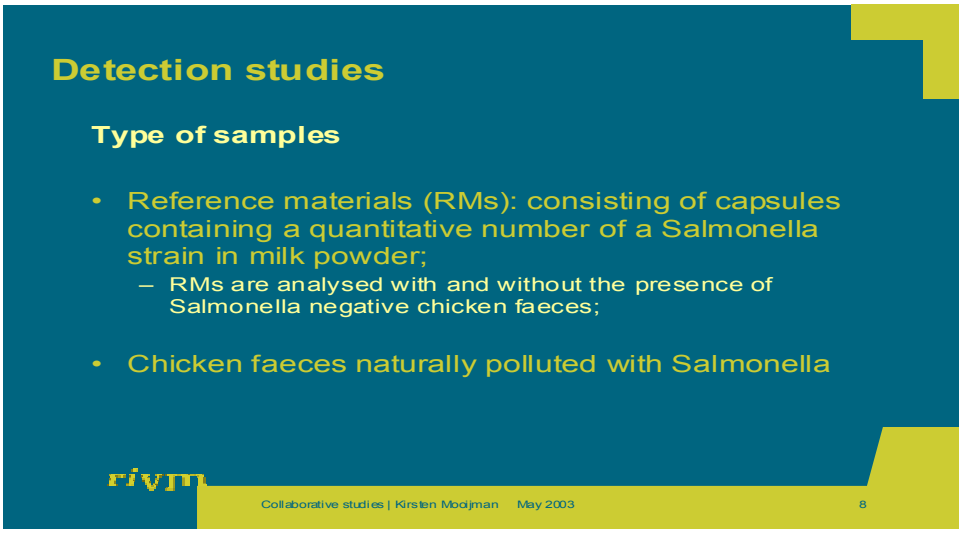

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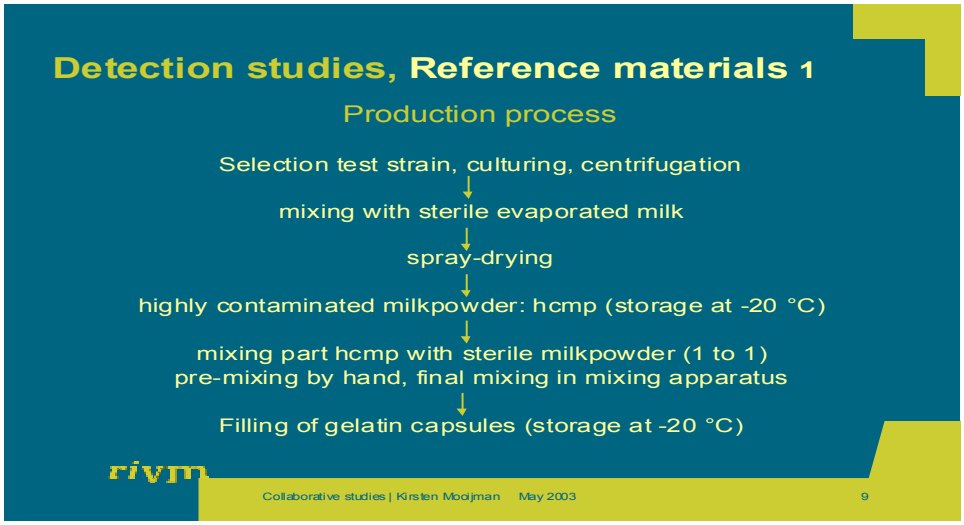

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Slide 12

Slide 13

Slide 14

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Slide 17

Slide 18

Detection studies, historical overview I

Study	Year	No of samples	Capsules aimed level	Capsules mean level	Faeces added (- *Salmonella*)	Selective enrichment	Plating-out
I	1995	26	STM5	6	No	RV and SC	BGA and own
		4	Blank	0	No		
II	1996	15	STM100	116	1 gram	RV, SC and own	BGA and own
		15	STM1000	930	1 gram		
		2	SPan5	5	No		
		1	STM100	116	No		
		1	Blank	0	No		
III	1998	14	STM10	11	1 gram	RV and own	BGA and own
		14	STM100	94	1 gram		
		7	STM100	94	1 gram (+AB)		
		14	SE100	95	1 gram		
		4	STM10	11	No		
		2	SPan5	5	No		
		5	Blank	0	No		

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Detection studies, historical overview II

Study	Year	No. of samples	Capsules aimed level	Capsules mean level	Faeces added (- *Salmonella*)	Selective enrichment	Plating-out
IV	1999	5	STM10	4	10 gram	RV or RVS, MSRV and own	BGA and own
		5	STM100	210	10 gram		
		5	SE100	61	10 gram		
		5	SE500	220	10 gram		
		5	Blank	0	10 gram		
		3	STM10	4	No		
		3	SE100	61	No		
		2	SPan5	5	No		
		2	Blank	0	No		
V	2000	5	STM10	4	10 gram	RV or RVS, MSRV and own	BGA and XLD
		5	STM100	47	10 gram		
		5	SE100	63	10 gram		
		5	SE500	450	10 gram		
		5	Blank	0	10 gram		
		3	STM10	4	No		
		3	SE100	47	No		
		2	SPan5	5	No		
		2	Blank	0	No		
		20	None		25 g (*Salm +*)		
VI	2002	5	STM10	11	10 gram	RVS, MSRV, MKTTn and own	BGA, XLD and own
		5	STM100	139	10 gram		
		5	SE100	92	10 gram		
		5	SE500	390	10 gram		
		5	Blank	0	10 gram		
		3	STM10	11	No		
		3	SE100	92	No		
		2	SPan5	5	No		
		2	Blank	0	No		
		20	None		25 g (*Salm +*)		

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Detection studies, set-up study 2002

Week	Date (2002)	Topic
40	30 Sept - 4 Oct	Mailing of protocol, SOPs and test report to the NRLs
44	28 Oct - 1 Nov	Information to NRLs concerning airway bill number
45	4 - 8 November	Mailing of parcels (packed with ice packs) as dangerous goods to nearest airport of the NRLs. NRLs collect the parcel at the airport (bring own cooling box with cooling devices or ice). Check parcel at the airport for damage. Immediately after arrival at the lab store materials at (-20 ± 5) °C. Preparation of media (according SOP).
46	11 - 15 Nov	Performance of the study
48	25 - 29 Nov	Completion of test report and faxing or e-mailing it to CRL.
50	9 - 13 Dec	Printed version of individual results send to all NRLs by CRL. NRLs check their results for correctness and inform CRL.

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Slide 21

Detection studies, results I

- Checking individual results by NRLs on a print-out of CRL;
- Presentation and discussion of (draft) results of all labs (using labcodes) of 1 study at the (yearly) workshop;
- Summarising (final) results of all labs (using labcodes) of 1 study in a RIVM-report;
- Summarising several studies in an international publication (study I-IV in Voogt et al., 2002)

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Slide 22

Detection studies, results II

Summary detection studies I-IV (Voogt et al, 2002b)

- Objective: evaluate results of NRLs of detection of different quantities of Salmonella in presence of competitive flora;
- Prescribed method and lab-own method(s);
- Content capsules unknown;
- No significant differences between prescribed and own methods;
- Best results in study I (no faeces), lowest no. of positive isolations in study III (1 g of faeces and SE100 added);
- No criteria yet what minimum % of Salmonella positive samples must be detected by a lab;
- For trend analyses, the design of the studies, including method of detection, should remain the same over several years.

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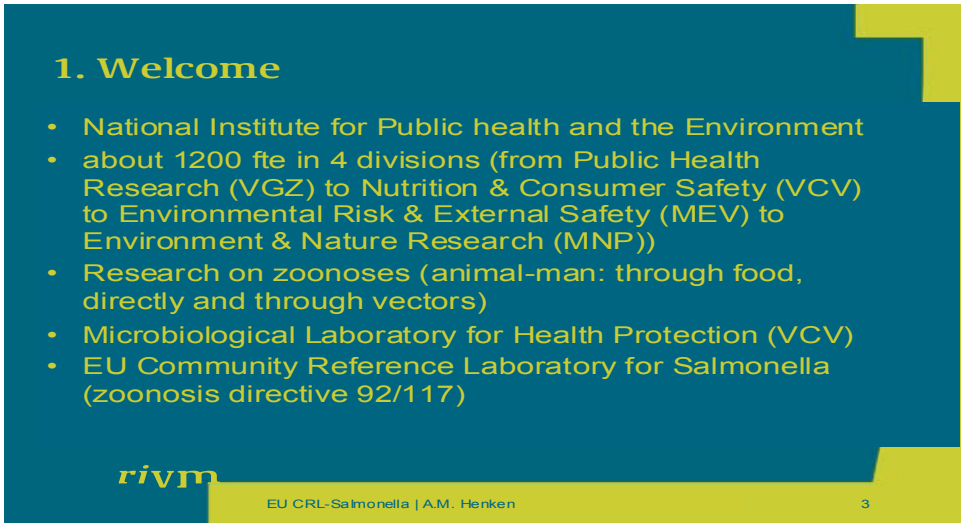
Appendix 18. Slides of presentation 2.1

Slide 1

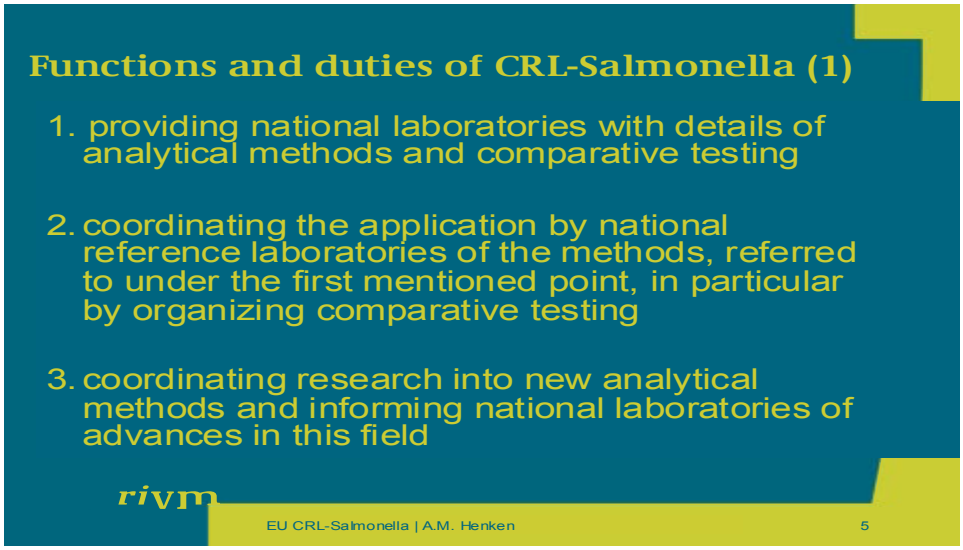
Slide 2

Slide 3



Slide 4

Slide 5



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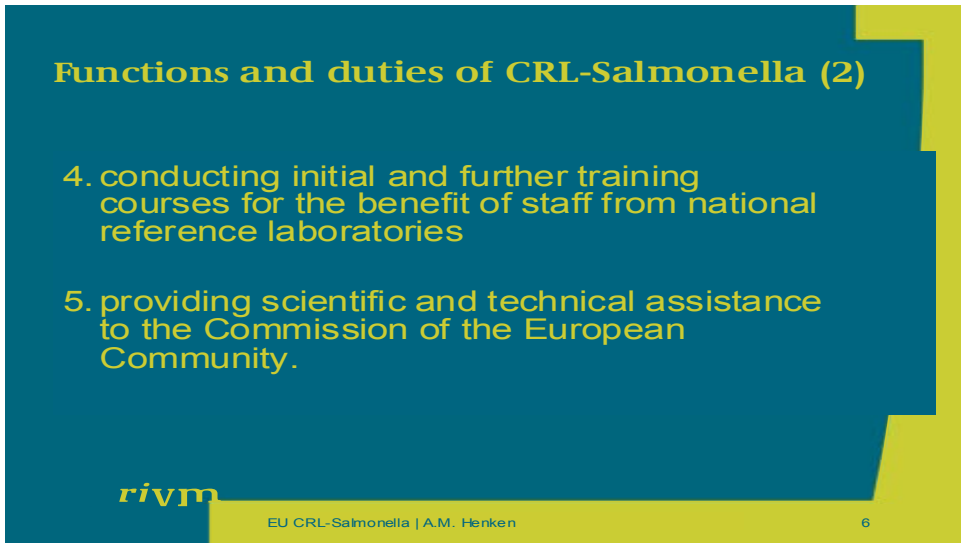

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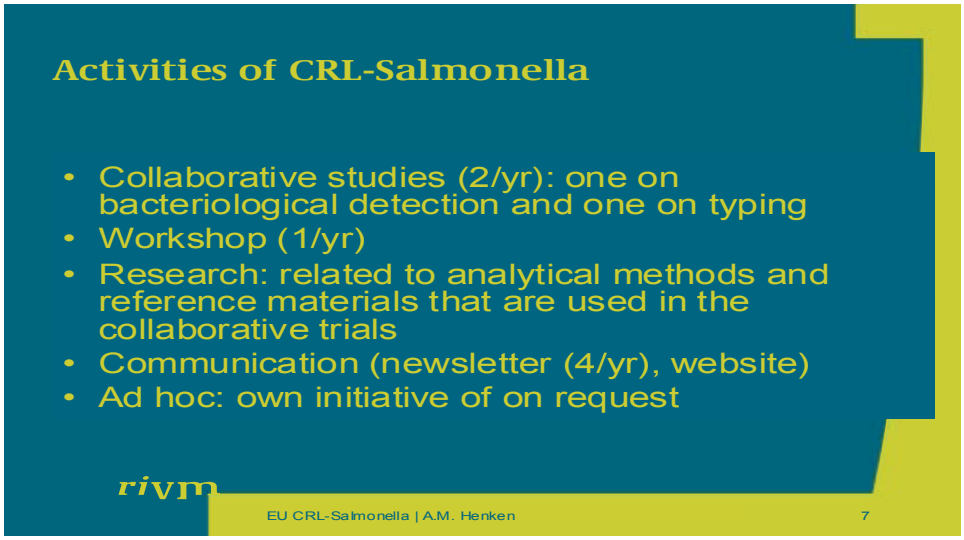
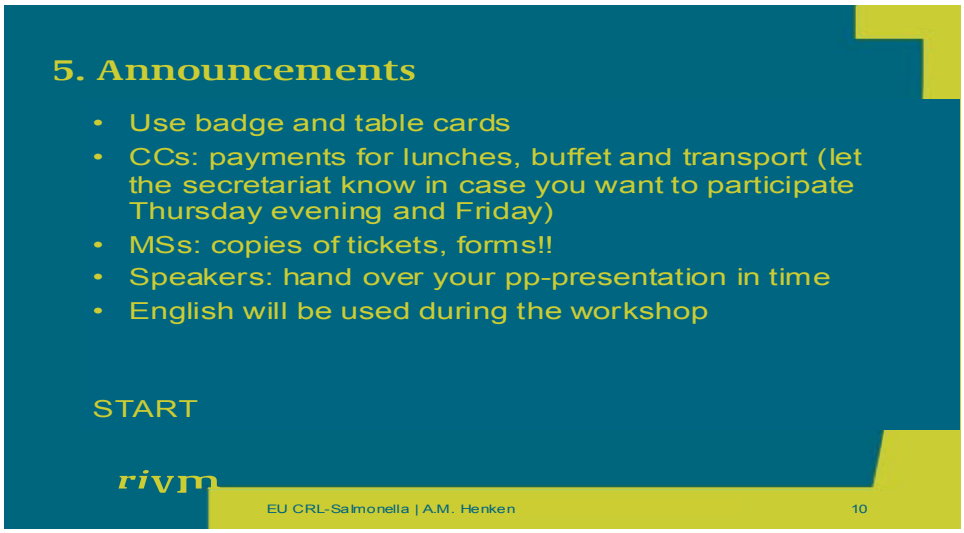


Slide 8

Slide 9

Slide 10


5. Announcements
• Use badge and table cards
• CCs: payments for lunches, buffet and transport (let the secretariat know in case you want to participate Thursday evening and Friday)
• MSs: copies of tickets, forms!!
• Speakers: hand over your pp-presentation in time
• English will be used during the workshop
START
rivm
EU CRL-Salmonella | A.M. Henken
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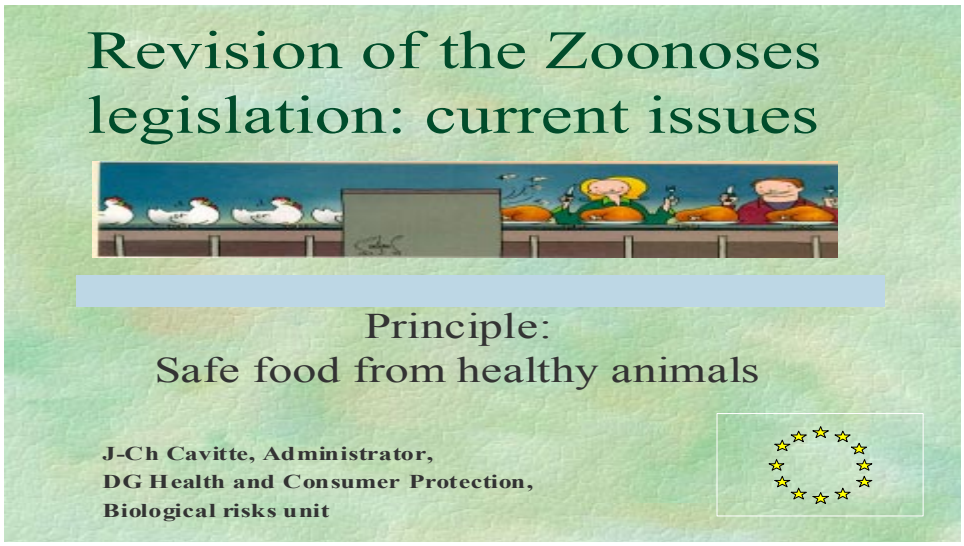
Appendix 19. Slides of presentation 2.2

Slide 1



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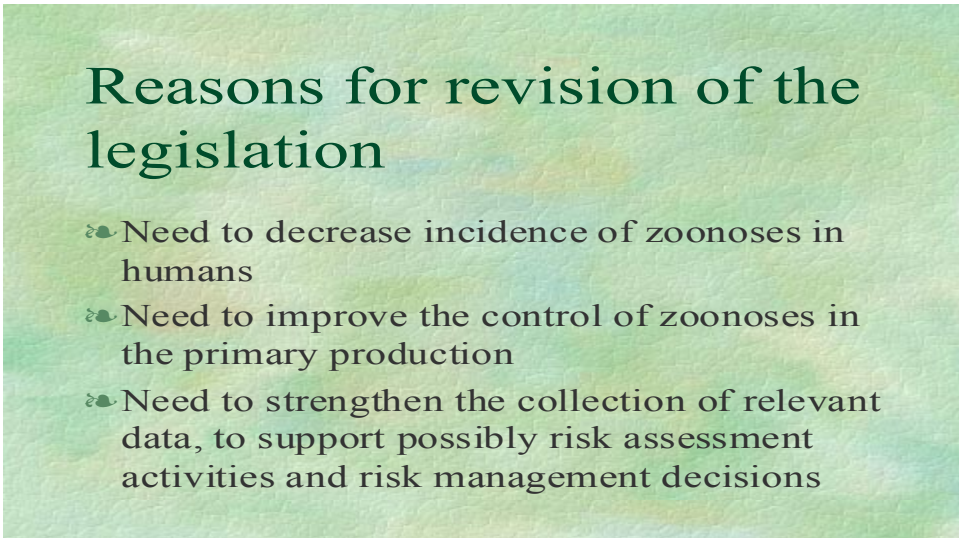

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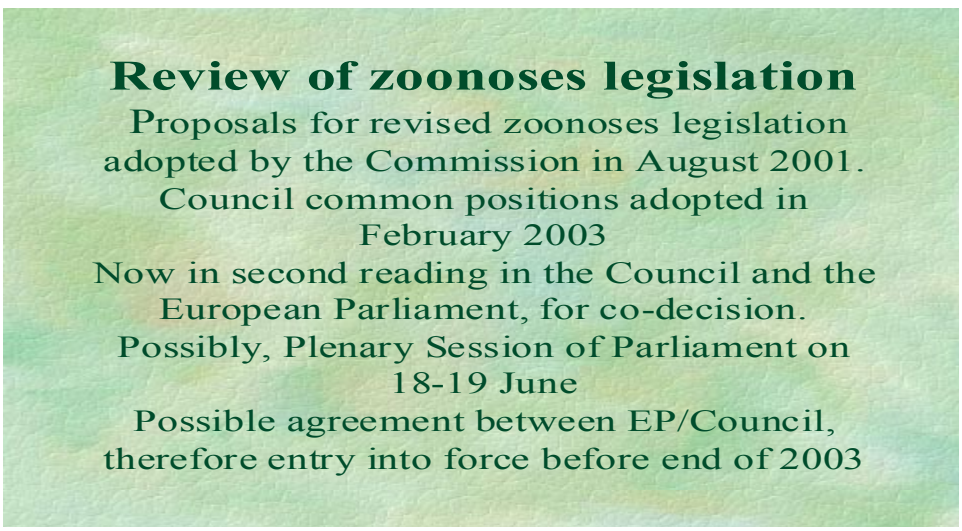

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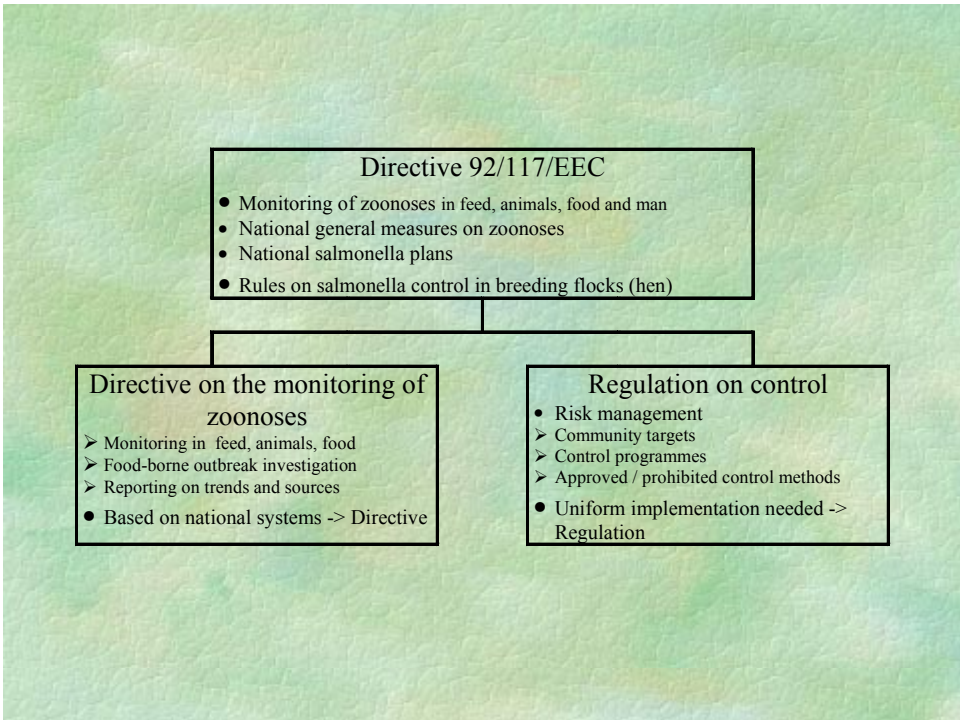
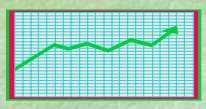


Slide 5

Slide 6

Proposed Directive on monitoring

- Co-operation between competent authorities in animal/feed/food/human health sectors in the MSs
- Data in humans collected through Communicable Diseases Network
- Coordinated monitoring programmes at Community level (e.g. pre-stage for control)



Slide 7

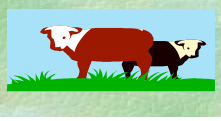
Proposed Directive on monitoring

- Food-borne outbreaks:
- obligation for food businesses to inform authorities about outbreaks
- competent authorities shall investigate the outbreaks
- annual reporting to EFSA and Commission

Slide 8

Proposed Directive on monitoring

- Monitoring of antimicrobial resistance:
  - in zoonotic agents (Salmonella, Campylobacter, and possibly other agents in the future)
  - in strains from animals (cattle, pigs, poultry) and food derived therefrom
  - complementary to monitoring in human isolates



Slide 9

Proposed Directive on monitoring

- MSs report annually to Commission; EFSA prepares the Community report
  - Deadlines: MS/end May (unchanged); EFSA/end November
  - Reports made available to public without delay (EP)
- The Community report could contain also data obtained from other sources (animal health, human communicable diseases)

Slide 10

Data in humans

- human data will be collected through the CDN
  - monitoring of sources and trends
  - verify effectiveness of control measures taken
  - risk assessments of zoonotic agents

Slide 11

Issues for implementation

- Need to prepare schemes and methods for harmonised monitoring of zoonotic agents, antimicrobial resistance along the food chain
- Rules for keeping isolates
- Rules on foodborne outbreak investigations
- Reflection on Community Reference Laboratories (in conjunction with draft Regulation on Official Feed and Food Controls and revision of microbiological criteria)

Slide 12

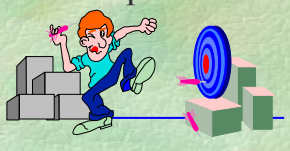
Proposed Regulation on control of salmonella and other foodborne zoonotic agents

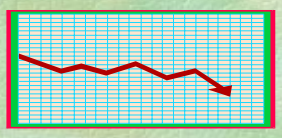
- Creates a framework for zoonoses control by setting targets for the reduction in prevalence of pathogens (salmonella), in animal populations essentially
- Control measures will be defined more closely by Commission Decisions

Slide 13

Proposed Regulation on control

- Pathogen reduction targets to be set
  - target is 'XX prevalence and/or XX % reduction in prevalence by year YY'
  - monitoring schemes to verify achievement of target (consider scheme for study, incl. method)
  - EFSA opinion needed





Slide 14

Proposed Regulation for control

- When targets established
  - MSs prepare a national control programme
    - methods for controlling decided by MS; certain control methods may be restricted/banned/approved by Commission decisions
    - responsibilities of food/feed businesses described
  - MSs' programmes approved by Commission
  - Food/feed businesses may have own programmes as part of national programme

Slide 15

Proposed Regulation on control of specified zoonoses

- Progressive approach:
  - Starting with salmonella with public health significance in poultry breeding flocks
  - Extending progressively to Salmonella with phs in layers, broilers, turkeys, and pigs
  - (possibility to include other zoonoses and other stages of food-chain)

Slide 16

Proposed Regulation on control of specified zoonoses

- Timetable:
  - Different targets set each year; national plans operational 18 months later
  - Poultry breeding flocks: target set 1 year after entry into force (EIF)
  - Layers: target set 2 years after EIF
  - Broilers: target set 3 years after EIF
  - Turkeys/slaughter pigs: 4 years after EIF
  - Breeding pigs: 5 years after EIF

Slide 17

Proposed Regulation on control of specified zoonoses

- Criteria to define salmonella serotypes with public health significance
- Cost/benefit analyses to be performed before proposing targets
- Transitional periods of three years for poultry to limit the targets to maximum 5 serotypes

Slide 18

Proposed Regulation for control of specified zoonoses

Minimum sampling requirements

- Zoonosis / zoonotic agent (salmonella with public health significance -phs)
- Animal species
- Sampling shall cover at least certain phases of production

Slide 19

Proposed Regulation for control

- Rules on trade in live animals and hatching eggs
  - after target established -> certification in intra-Community trade
  - MSs may require same guarantees as they apply themselves, for a transitional time period

Slide 20

Proposed Regulation for control

- Predefined specific measures:
  - Fowl breeding flocks infected with SE/ST: slaughter/heat treatment/destruction
  - Table eggs: have to originate from salmonella negative flocks (starting 6 years after EIF)
  - Poultry meat: criterion of absence of salmonella in 25g or industrial heat treatment salmonella (starting 7 years after EIF)

Slide 21

Proposed Directive on Monitoring

- Community financing possible for
  - Co-ordinated monitoring programmes
  - Community Reference Laboratories
  - New mandatory control measures (Commission report on financial arrangements due 3 years after entry into force)

Slide 22

Issues for implementation

- Prepare for setting of first target(s): breeders (and laying hens); need to
  - know before hand prevalence of serotypes in this/these animal populations and
  - organise sufficiently harmonised sampling/testing schemes
- Consultation EFSA on different issues (specific control methods, target setting)

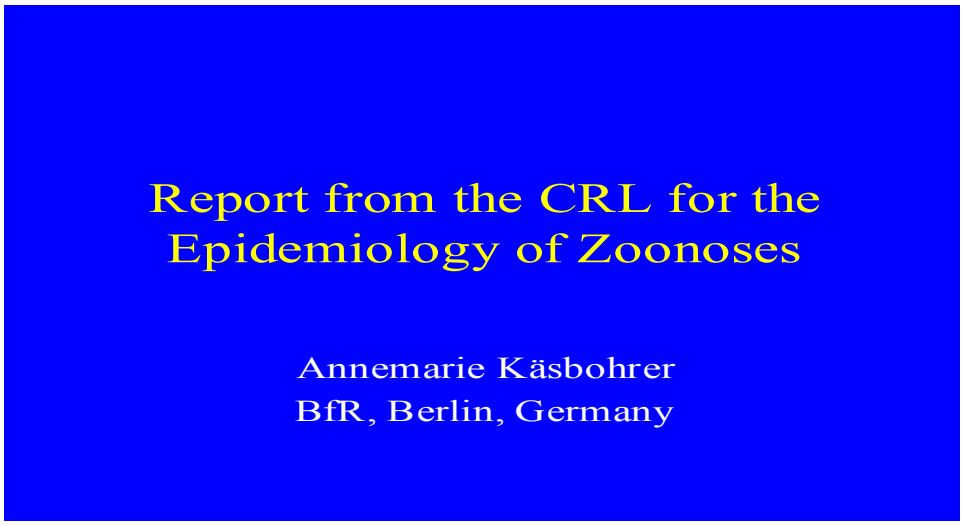
Slide 23

Laboratories

- CRLs/NRLs: to be appointed and tasks to be defined
- Requirement for accreditation 2 years after EIF
- Laboratories to take part in RTs organised by NRLs
- Testing: methods/ validated recommended by International standardization bodies as reference methods (possibility of alternative methods validated in accordance with internationally recognized rules)

Appendix 20. Slides of presentation 2.3

Slide 1



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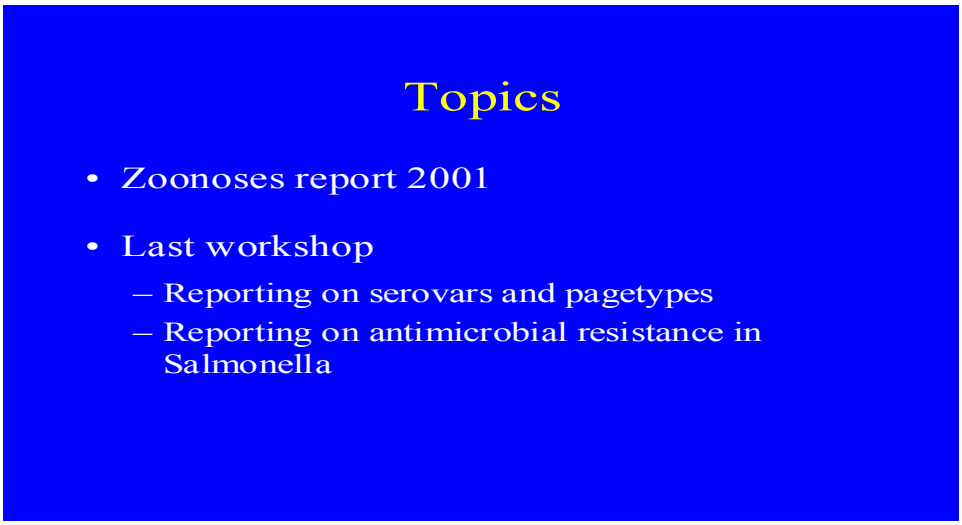
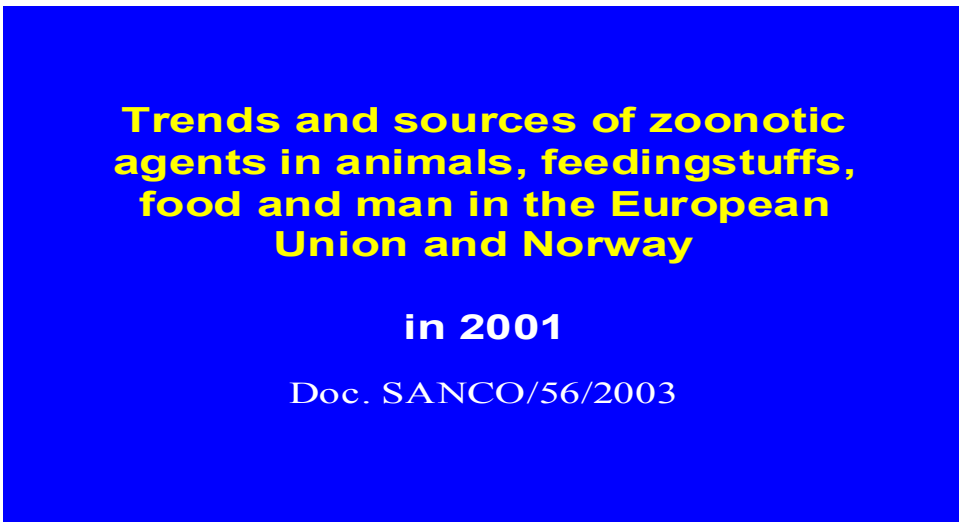


Slide 3



Slide 4

Salmonella - feed materials

- Animal derived
- Vegetable derived

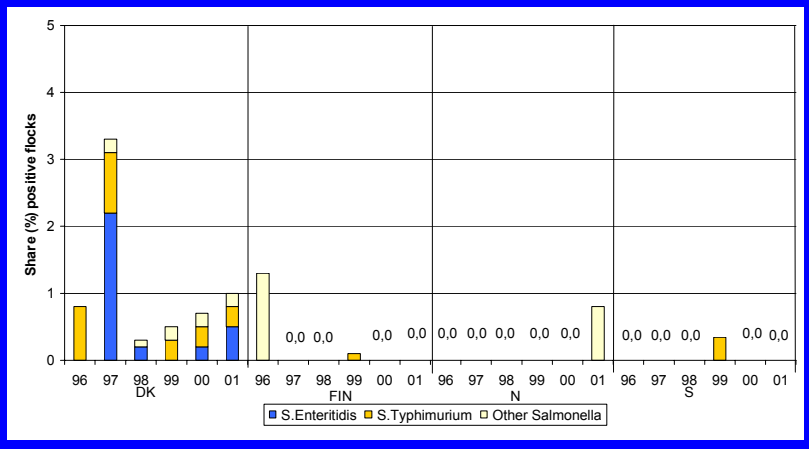
Salmonella could be detected in both categories

- Higher rates in some countries
- Fishmeal > meat and bone meal > other proteins
- Higher rates in some countries
- Oil seeds and products > cereals
- sunflower > soyabean > rapeseed

Mainly other than S.Enteritidis and S.Typhimurium

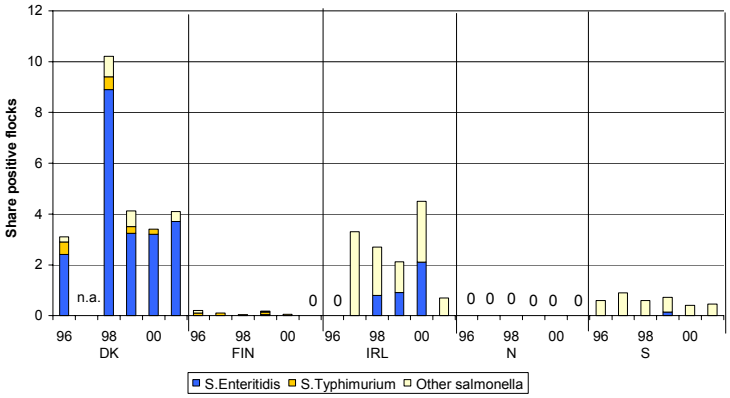
Slide 5

Approved control programme I Breeding flocks



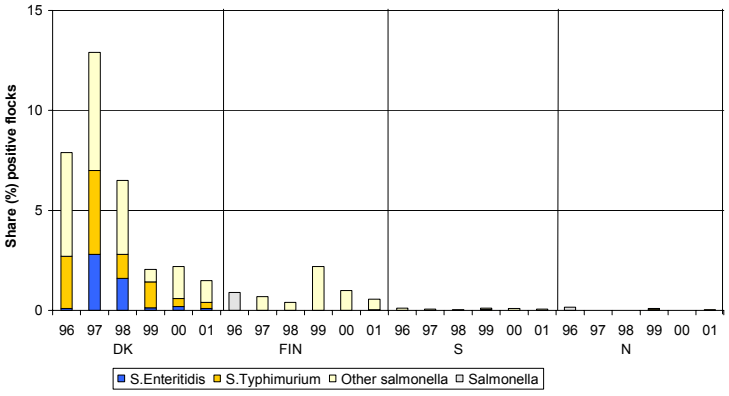
Slide 6

Approved control programme I Laying hens



Slide 7

Approved control programme I Broilers



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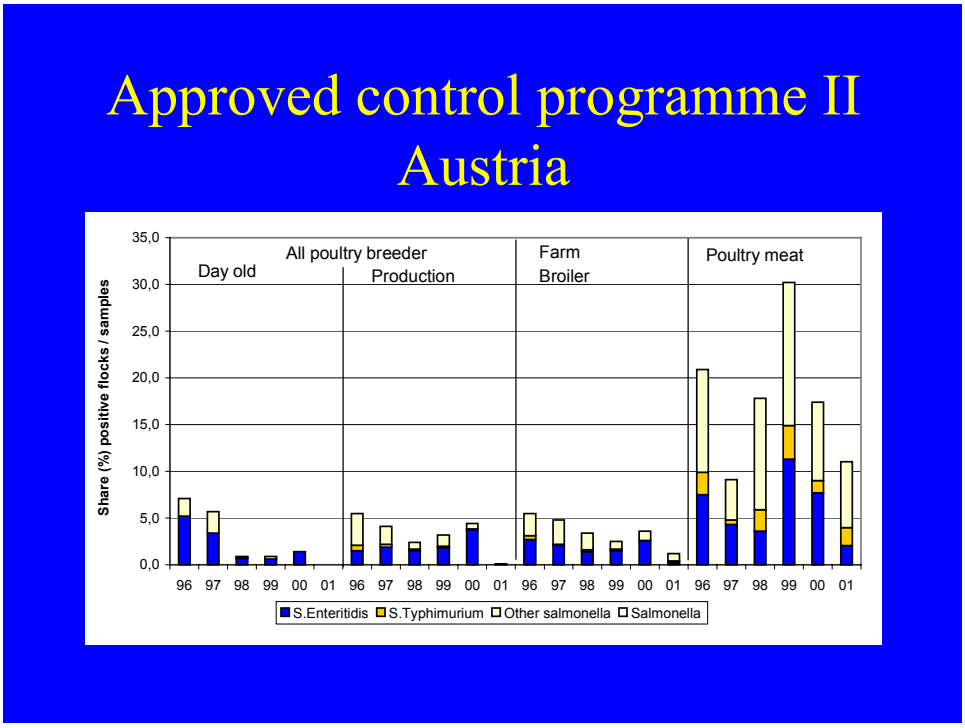

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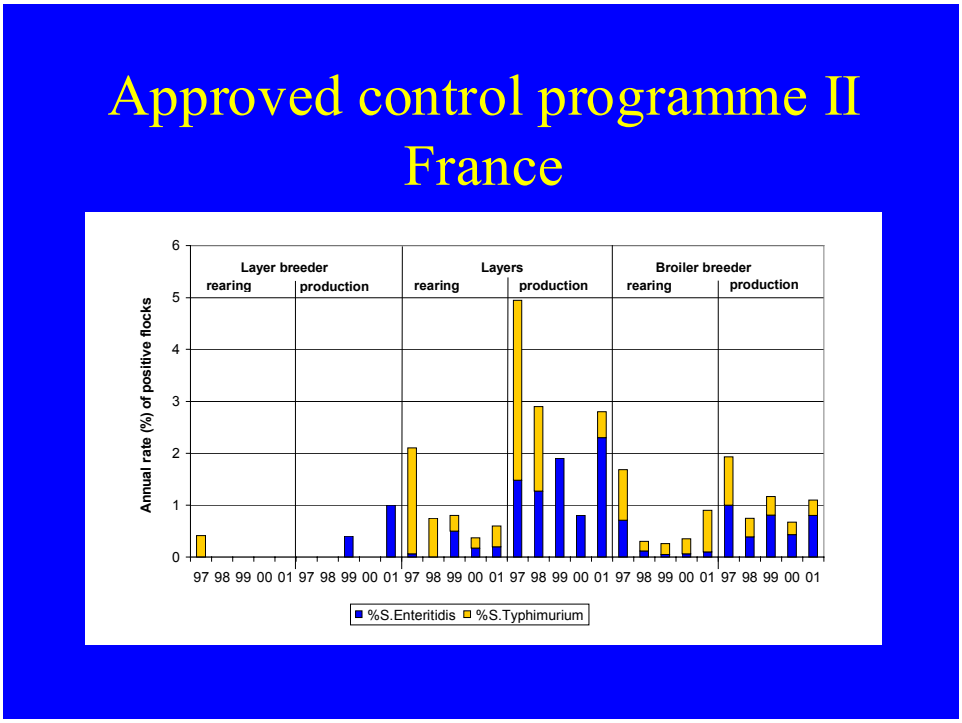
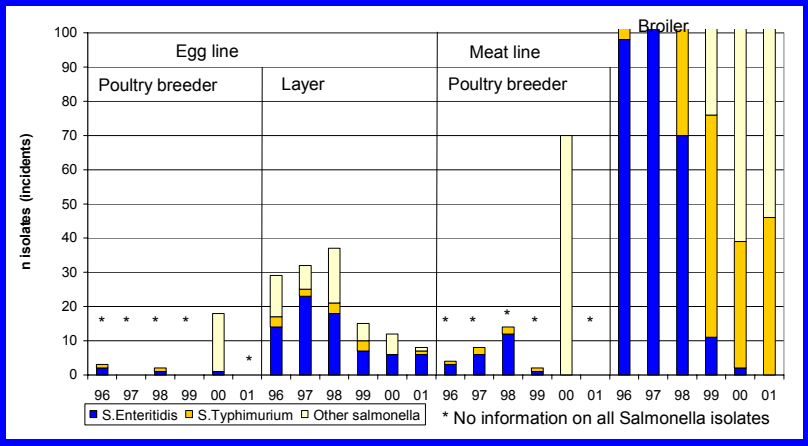


Slide 10

Monitoring programme acc Dir. Great Britain



Slide 11

Salmonella in eggs

- Denmark:
  - Danish Grade A eggs 0.06% positive
  - Imported Grade A and B 0.7% positive
- Germany:
  - 2001 0.60%
  - 2000 0.53%
- S.Enteritidis dominating

Slide 12

Salmonella in pigs

- Favourable situation
  - Sweden, Finland, Norway (0 - 0.12%)
- Varying rates in other countries - pigs
  - DK: 3.2% by meat juice monitoring
  - D: 7.3% by bacteriological methods

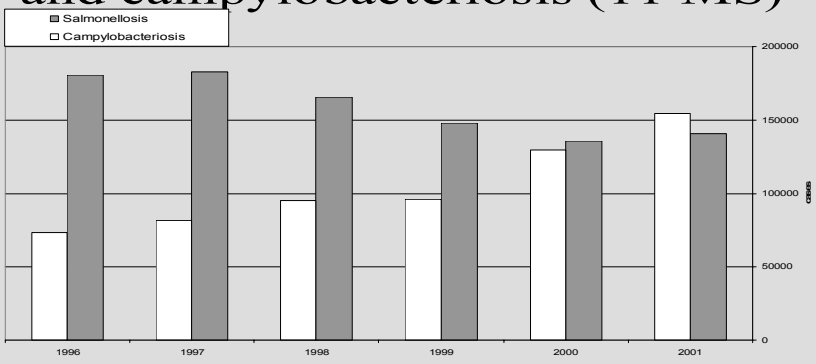
Slide 13

Salmonella in cattle

- Favourable situation
  - Sweden, Finland, Norway (0.03 - 0.31%)
- Varying rates in other countries
  - DK: 2.2% by bacteriological methods
  - NL: 1.0%
- Contamination rate of beef is lower compared to poultry meat and pork

Slide 14

Trend in human salmonellosis and campylobacteriosis (11 MS)



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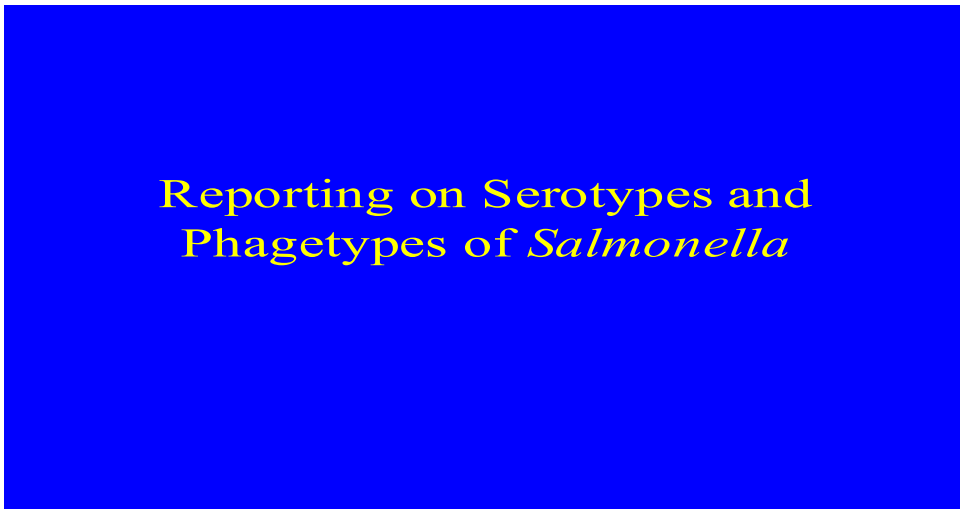

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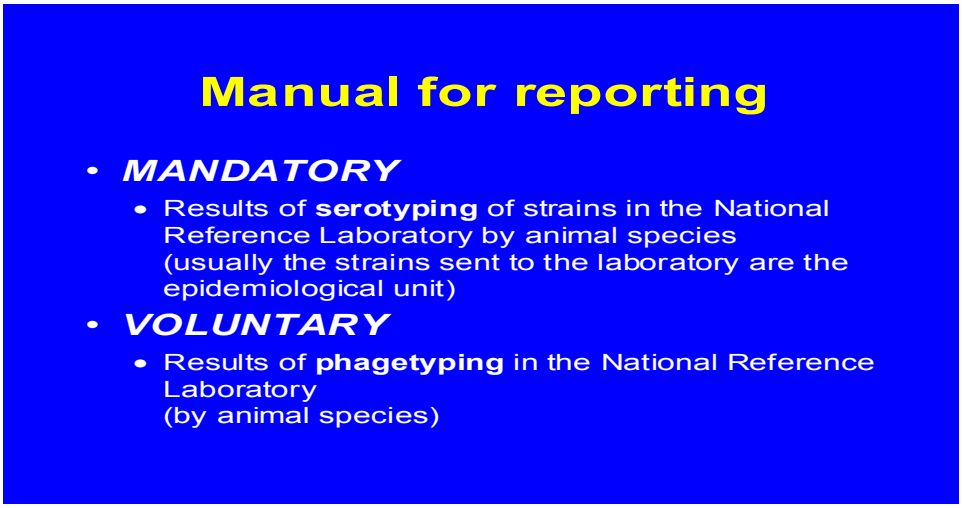


Slide 17

Slide 18

Categories (detailed)

- Poultry productine line
  - Layer breeder / layer / eggs
  - Broiler breeder / broilers / poultry (fowl meat)
- Poultry species
  - Broiler - Fowl meat
  - Turkey / turkey meat
  - Geese / geese meat
  - Ducks / duck meat

Slide 19

Sources of information

- Isolates available at the reference laboratory
- Isolates received in a monitoring programme / study

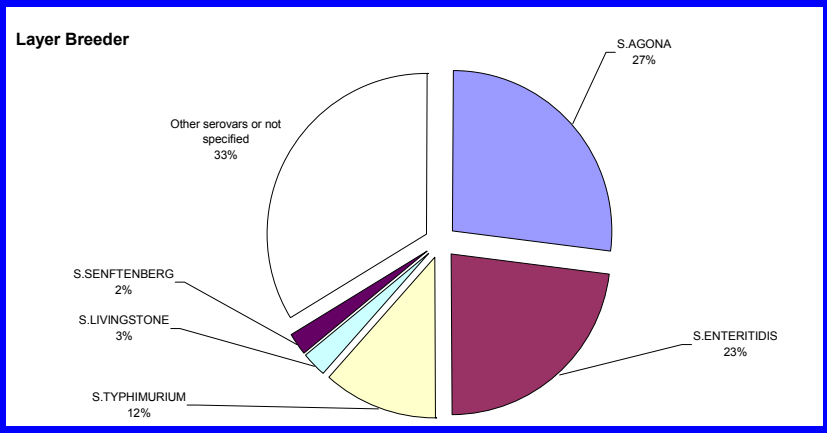
Slide 20

Presentation of the data

- Summarizing all countries and all Salmonella isolates where information on the servar was available
  - Ranking of top five
  - Frequency distribution
- Overall pattern by country
  - Ranking of top ten
  - Frequency distribution
- Weighted frequency distribution (ie each country same weight or by population size)

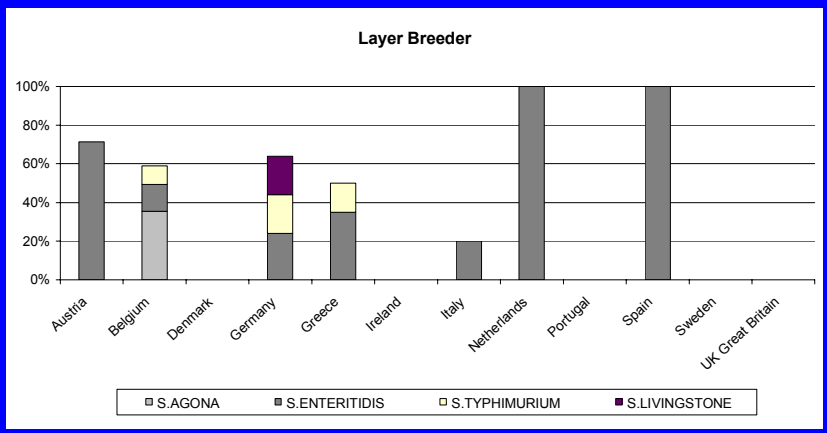
Slide 21

Five most frequent serovars - layer breeder



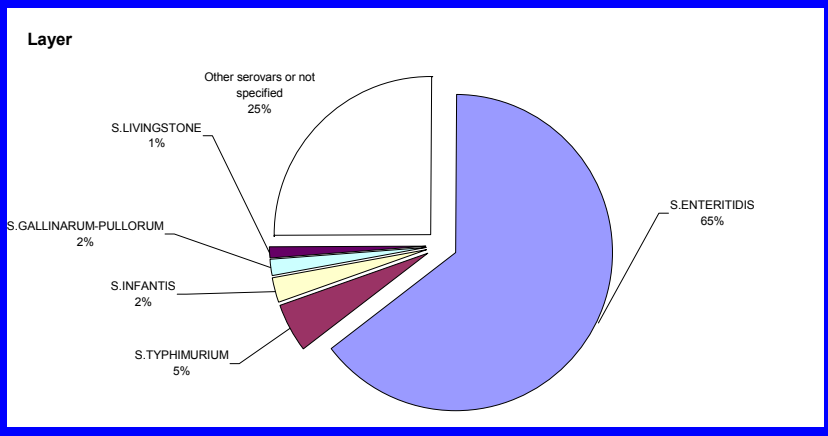
Slide 22

Five most frequent serovars - layer breeder



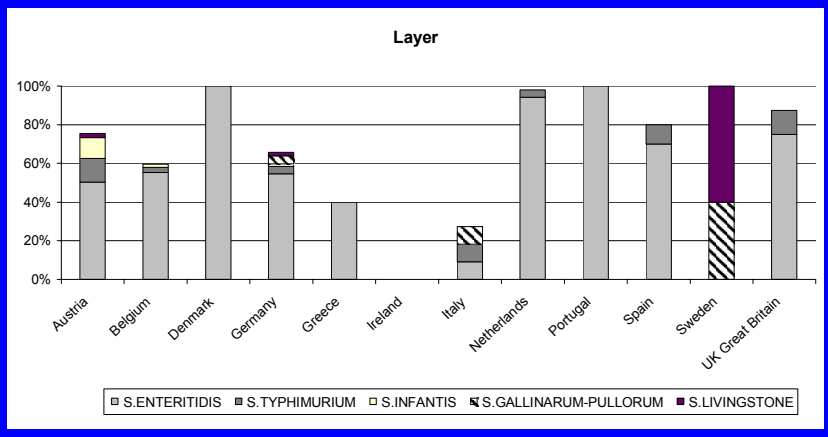
Slide 23

Five most frequent serovars - layers



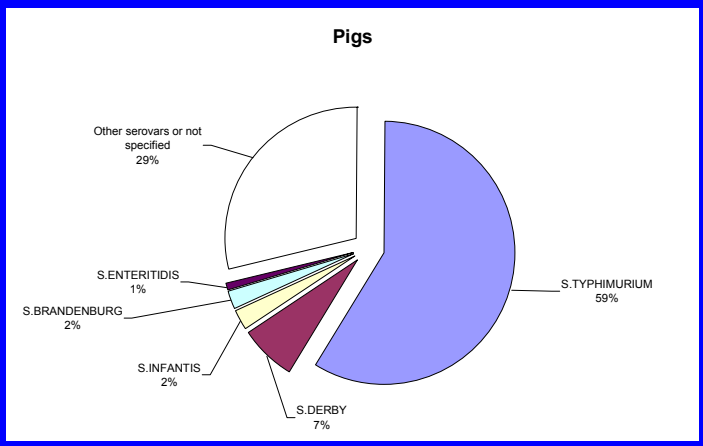
Slide 24

Five most frequent serovars - layers



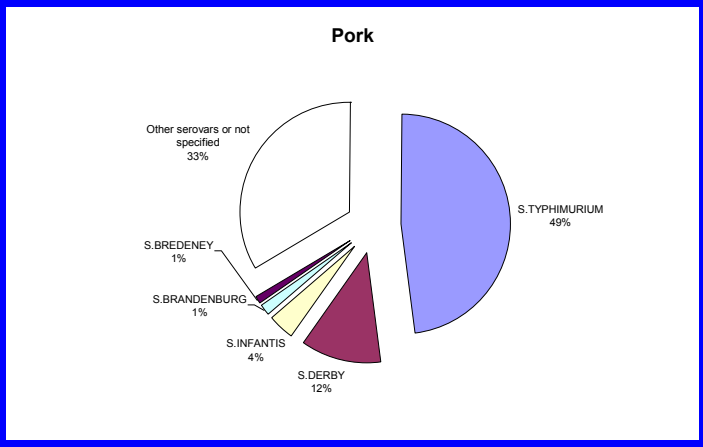
Slide 25

Five most frequent serovars - pigs



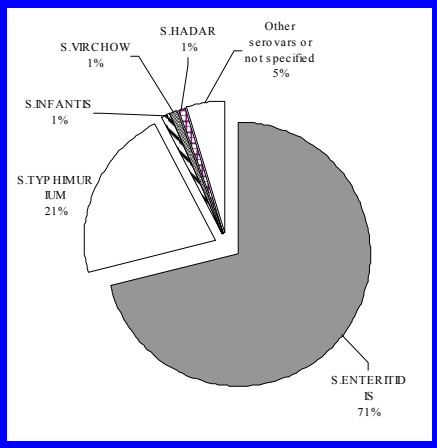
Slide 26

Five most frequent serovars - pork



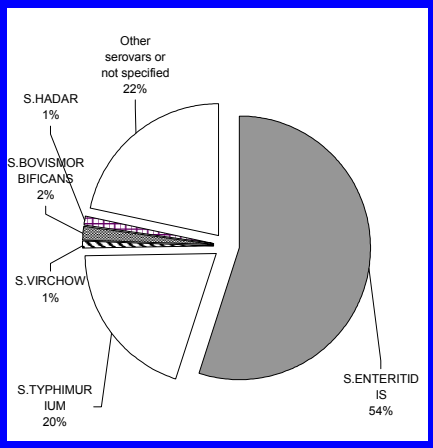
Slide 27

Main serovars in human salmonellosis (13 countries)



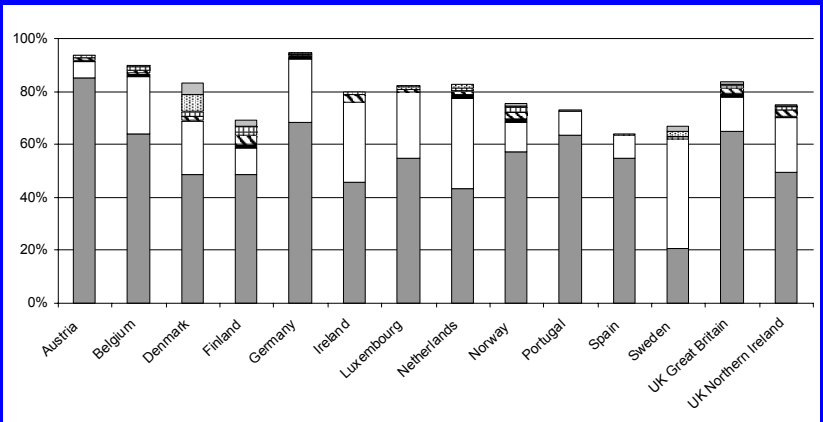
Slide 28

Main serovars in human salmonellosis (13 countries) - weighted by country



Slide 29

Main serovars in human salmonellosis

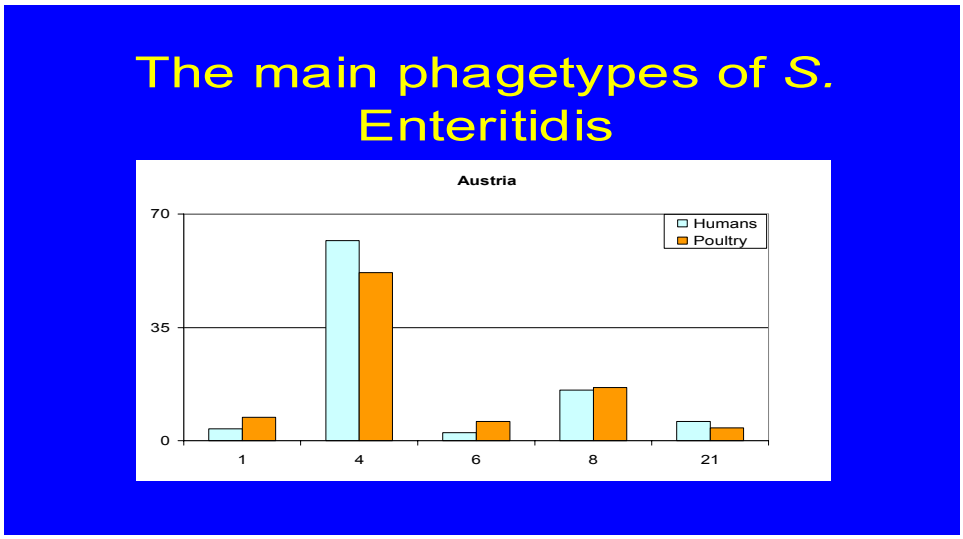


Slide 30

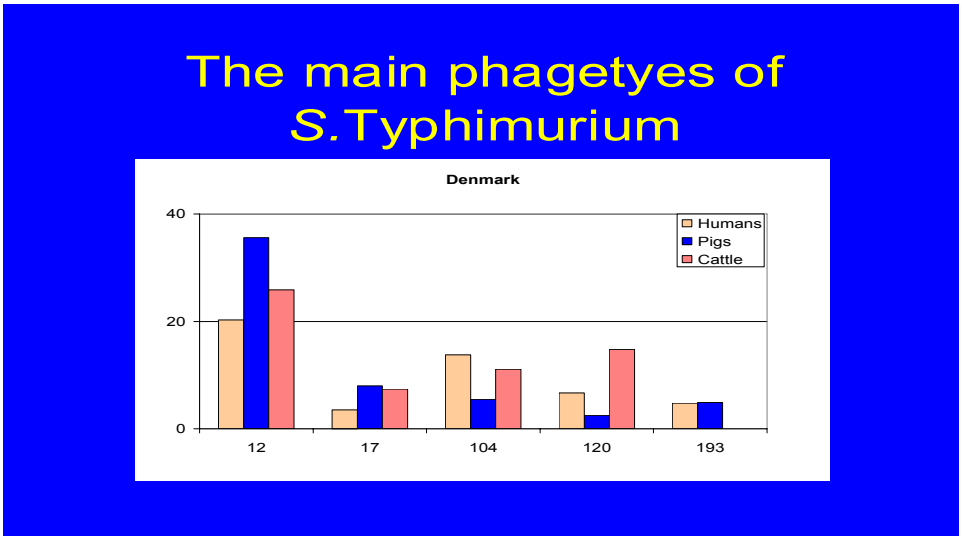
Data on phagetyes of *S.Enteritidis* and *S.Typhimurium*

in 2001

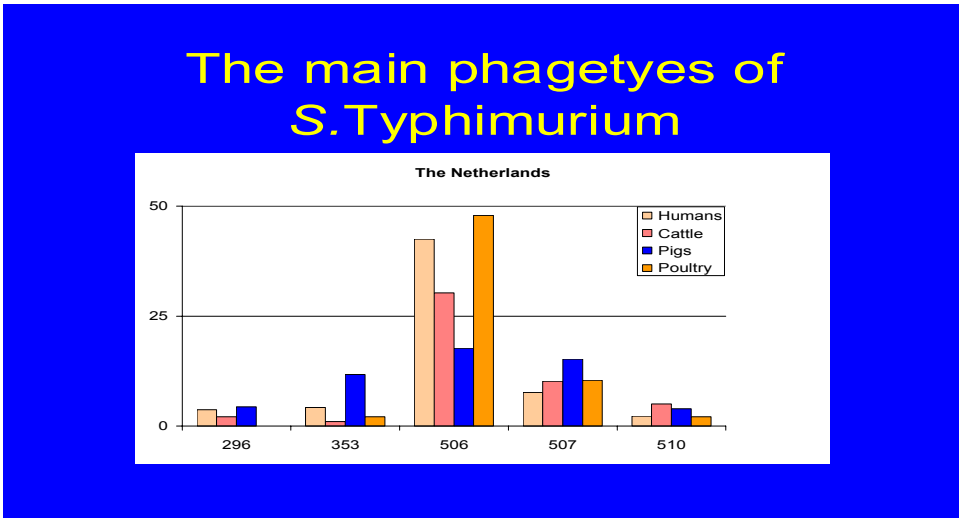
Slide 31



Slide 32



Slide 33



Slide 34

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

Slide 35

Data basis

Most countries keep on the instructions given in the manual

- Mandatory
  - Serotypes
- 14 countries supplied the serotype distribution in different species
- 1 country is able to specify the source of the data

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

Slide 36

Antibiotic resistance in Salmonella Experiences with the reporting system - update 2001

Slide 37

Antibiotic resistance testing

- **Reporting countries**
  - 2000: 14 reports gaps: EL, L
  - 2001: 16 reports

Slide 38

Antibiotic resistance testing

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

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

Slide 39

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

Slide 40

Antibiotic resistance testing

- **Monitoring frame**
  - at least 60 isolates of each serotype per animal species

	cattle	pigs	poultry
Salmonella spp:	5	8	7
S.Enteritidis:	-	-	5
S.Typhimurium:	2	6	2

Slide 41

Antibiotic resistance testing

- **Monitoring frame**
  - isolates should be selected in **randomized way** among isolates at NRLs
  - clustering **is to be avoided**
  - information about whether isolates derive from **active** or **passive** surveillance
  - as close to the level of **primary production** as possible

Slide 42

Antibiotic resistance testing 2001

Antimicrobials in test panel	Countries
– Tetracycline (TE)	16
– Chloramphenicol (C) or Florfenicol (FFN)	16 (15/8)
– Ampicillin (AMP)	14
– 3rd generation cephalosporin (CEF)	14
– Ciprofloxacin or enrofloxacin (CIP)	16
– Nalidixic acid (NA)	16
– Sulfonamide/TMP (SXT)	12 (12/9/16)
– Streptomycin (S)	14
– Gentamycin / Neomycin / Kanamycin	15/10/7 (13)

Slide 43

Antibiotic resistance testing 2001

	Countries	
• Antimicrobials in test panel		
– 3rd generation cephalosporin, (CEF)	14	
• Cefotaxime	9	
• Ceftiufur	5	
• Ceftazidime	1	
– Sulfonamide/TMP (SXT)	12	(12/9/16)
• SXT + Sulfonamide	8	
• SXT + Trimethoprim	5	
• SXT + SU or TMP	9	

Slide 44

Antibiotic resistance testing

- Reporting the methods used
  - test method :
    - Agar diffusion 11 countries
    - Agar / Broth dilution 7 countries
  - testing standard used :
    - NCCLS 11
    - BASC/ DIN /CASFM 1 / 1 / 1
    - Provider 2 countries

Slide 45

Antibiotic resistance testing

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

Slide 46

Antimicrobial resistance - Salmonella

- Salmonella
  - Tetracyclin: Resistance common
  - Ampicillin, streptomycin, sulfonamides: Resistance often detected
  - Nalidixic acid, enrofloxacin: detected
- S. Enteritidis
  - very low rates
- S. Typhimurium
  - high resistance rates

Slide 47

Results of the workshop - Salmonella

- Quantitative data
  - new table to include MIC values / zone diameters
- Decision on the antimicrobials to be tested
  - chloramphicol and florfenicol
  - more than one cephalosporin
  - one fluorochinolone
  - aminoglycosides: streptomycin / gentamycin / neomycin or kanamycin
- Reporting of multiresistance

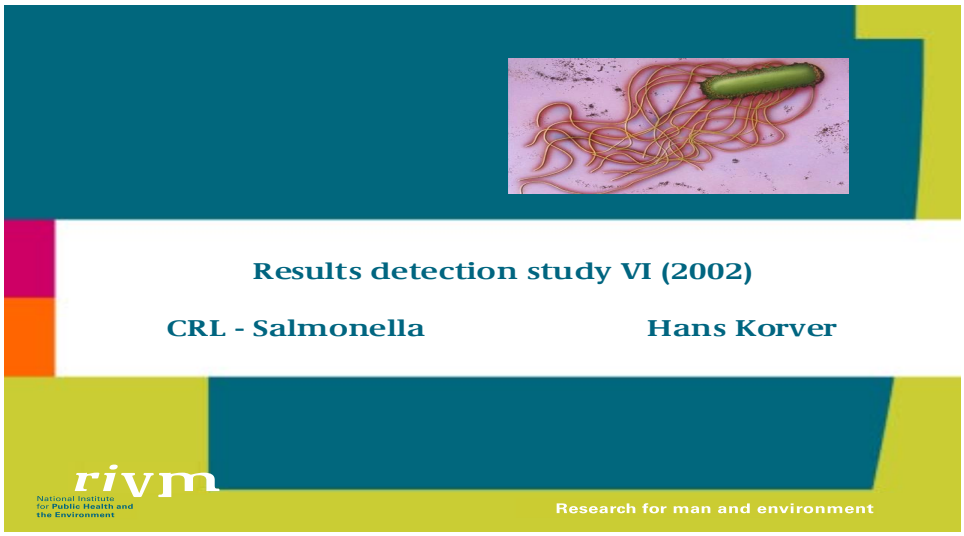
Slide 48

Results of the workshop

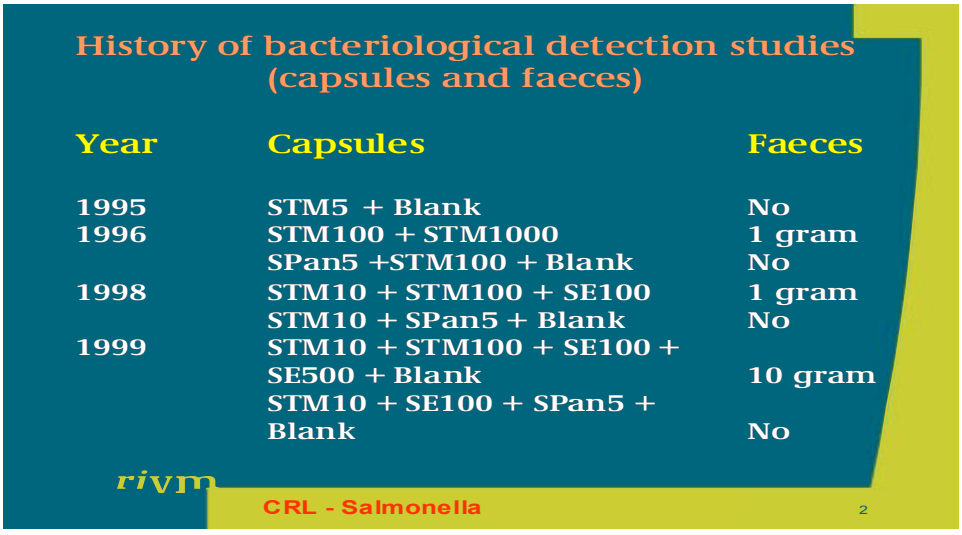
- Campylobacter
  - *C.cejuni* and *C.coli* separate
  - primary production; at least poultry (C.j.) and pigs (C.c.)
  - human isolates
  - 60 isolates per *Campylobacter* species and animal source
  - Quantitative data
  - Antimicrobials to be tested
    - erythromycin , ciprofloxacin , nalidixic acid tetracycline , ampicillin , gentamicin

Appendix 21. Slides of presentation 2.4

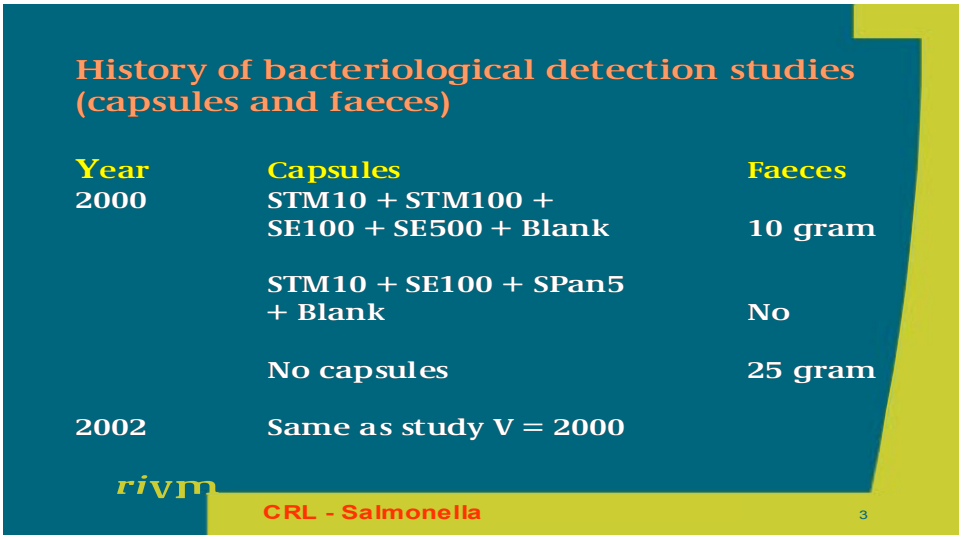
Slide 1



Slide 2



Slide 3



Slide 4

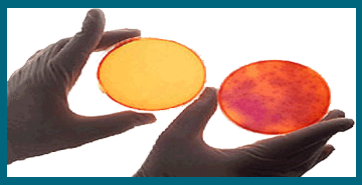
History of bacteriological detection studies (media)

Year	Sel.enrichment	Plating-out
1995	RV + SC	BGA + own
1996	RV + SC + own	BGA + own
1998	RV and own	BGA + own
1999	RV or RVS + MSRV+ own	BGA + own
2000	Same 1999	BGA + XLD

rivm CRL - Salmonella 4

Slide 5

Media for study 2002



rivm CRL - Salmonella 5

Slide 6

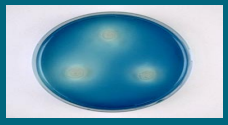
RV

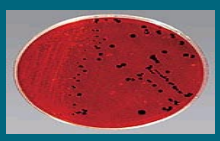
BGA

MKTTn

XLD

MSRV





rivm CRL - Salmonella 6

Slide 7

Overview content and number of capsules

Capsules	Control capsules (n = 10) No faeces added	Test samples (n=25) with 10 g *Salmonella*- negative faeces	Test samples (n=20) with 25 g *Salmonella*- positive faeces
S. Panama 5	2	---	---
S. Enteritidis 100	3	5	---
S. Enteritidis 500	---	5	---
S. Typhimurium 10	3	5	---
S. Typhimurium 100	---	5	---
Blank	2	5	---
No capsules		---	20

rivm CRL - Salmonella 7

Slide 8

Level of contamination and homogeneity of SE and STM capsules

	Test batch (n=10)		Final batch (n=25)	
	Mean cfp per capsule	Homogeneity (T_2 / (I-1)	Mean cfp per capsule	Homogeneity (T_2 / (I-1)
STM 10	10	0,49	11	0,75
STM 100	150	0,92	139	0,90
SE 100	111	0,73	92	1,28
SE 500	487	0,40	389	0,77

rivm CRL - Salmonella 8

Slide 9

Incubation time

Medium	ISO or SOP	Time
BPW	16-20 hrs	4 labs > 20 h < 23 h
RVS	21-27 hrs	3 labs < 21 h > 17 h
MKTTn	21-27 hrs	5 labs < 21 h > 18 h 1 lab > 27 h < 28 h
MSRV	21-27 hrs	6 labs < 21 h > 18 h

rivm CRL - Salmonella 9

Slide 10

Incubation temperature

Medium	ISO or SOP	Temperature
BPW	37 ± 1 °C	2 labs < 36 > 32.5
RVS	41.5 ± 1 °C	2 labs < 40.5 > 39.1 4 labs > 42.5 < 43.0
MKTTn	37 ± 1 °C	3 labs < 36 > 34 2 labs 41.2 - 42.8
MSRV	41.5 ± 1 °C	3 labs < 40.5 > 37 2 labs > 42.5 < 43

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CRL - Salmonella 10

Slide 11

MKTTn: ISO 6579 or otherwise ?

Lab.code	Manufacturer	pH
ISO 6579		8.2 ± 0.2
1	Home made	7.0
11/16	Home made ISO	8.2 / 8.2
12/14	Biokar	7.8 / 7.3
2	Biolife	?
3/13	Biorad	? / 8
4	Difco	8.1
5/6/10/15/17	Oxoid	8.0/8.0/8.0/7.9/?
9	Merck	7.4
7	Scharlau	8.3

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CRL - Salmonella 11

Slide 12

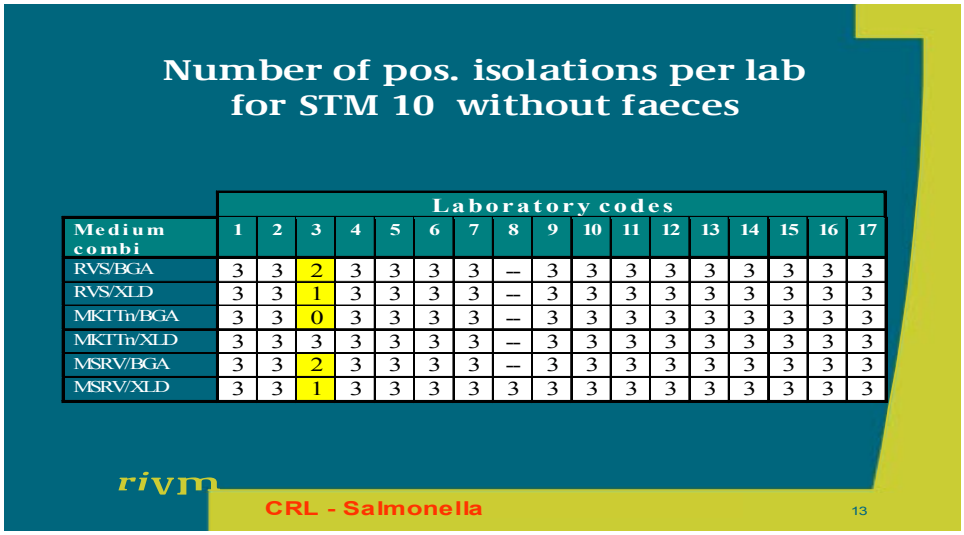
Number of pos. isolations per lab for S.Panama 5 without faeces

	Laboratory codes																
Medium combination	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
RVS/BGA	0	2	2	2	2	2	2	--	2	2	2	2	2	2	2	2	2
RVS/XLD	2	2	0	2	2	2	2	--	2	2	2	2	2	2	2	2	2
MKTTn/BGA	0	2	1	2	2	2	2	--	2	2	2	2	2	2	2	2	2
MKTTn/XLD	2	2	1	2	2	2	2	--	2	2	1	2	2	2	2	2	2
MSRV/BGA	2	2	2	2	2	2	2	--	2	2	2	2	2	2	2	2	2
MSRV/XLD	2	2	0	2	2	2	2	1	2	2	2	2	2	2	2	2	2

rivm

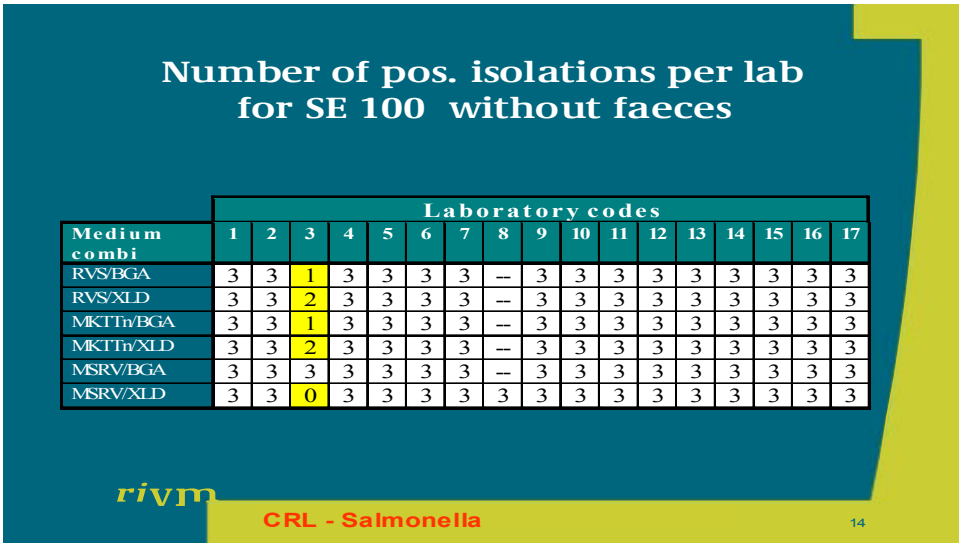
CRL - Salmonella 12

Slide 13



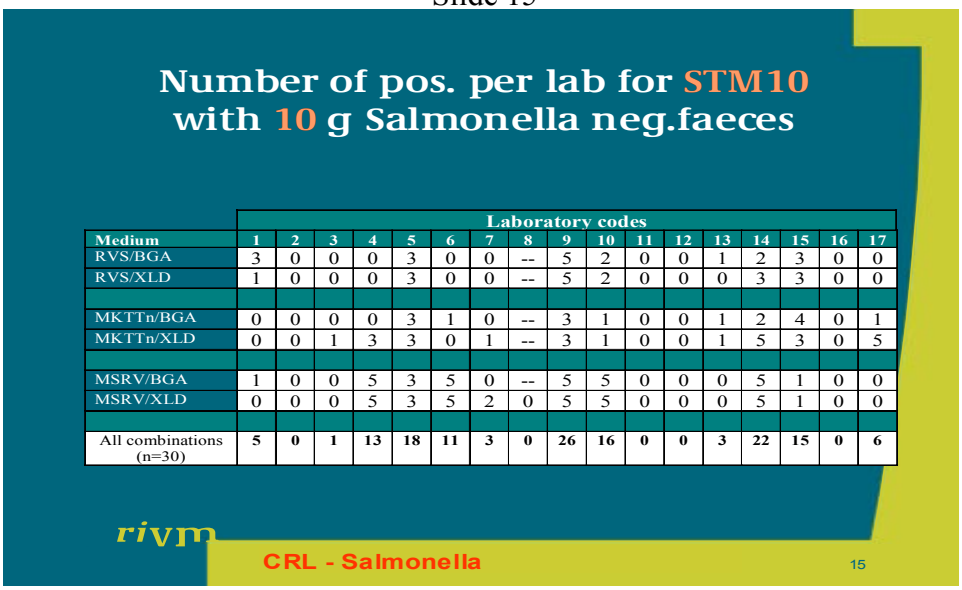
Medium combi	Laboratory codes 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
RVS/BGA	3	3	2	3	3	3	3	–	3	3	3	3	3	3	3	3	3
RVS/XLD	3	3	1	3	3	3	3	–	3	3	3	3	3	3	3	3	3
MKTTn/BGA	3	3	0	3	3	3	3	–	3	3	3	3	3	3	3	3	3
MKTTn/XLD	3	3	3	3	3	3	3	–	3	3	3	3	3	3	3	3	3
MSRV/BGA	3	3	2	3	3	3	3	–	3	3	3	3	3	3	3	3	3
MSRV/XLD	3	3	1	3	3	3	3	3	3	3	3	3	3	3	3	3	3

Slide 14



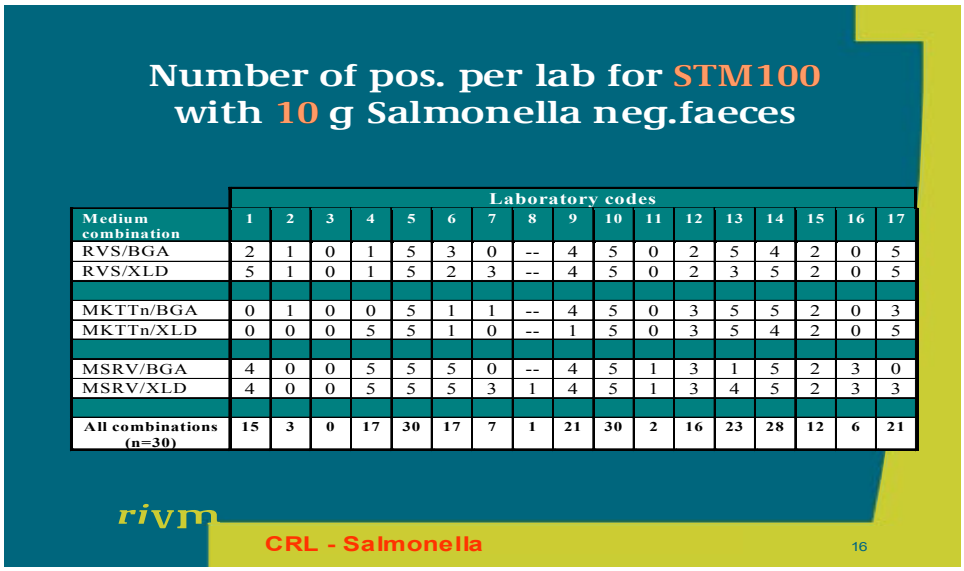
Medium combi	Laboratory codes 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
RVS/BGA	3	3	1	3	3	3	3	–	3	3	3	3	3	3	3	3	3
RVS/XLD	3	3	2	3	3	3	3	–	3	3	3	3	3	3	3	3	3
MKTTn/BGA	3	3	1	3	3	3	3	–	3	3	3	3	3	3	3	3	3
MKTTn/XLD	3	3	2	3	3	3	3	–	3	3	3	3	3	3	3	3	3
MSRV/BGA	3	3	3	3	3	3	3	–	3	3	3	3	3	3	3	3	3
MSRV/XLD	3	3	0	3	3	3	3	3	3	3	3	3	3	3	3	3	3

Slide 15



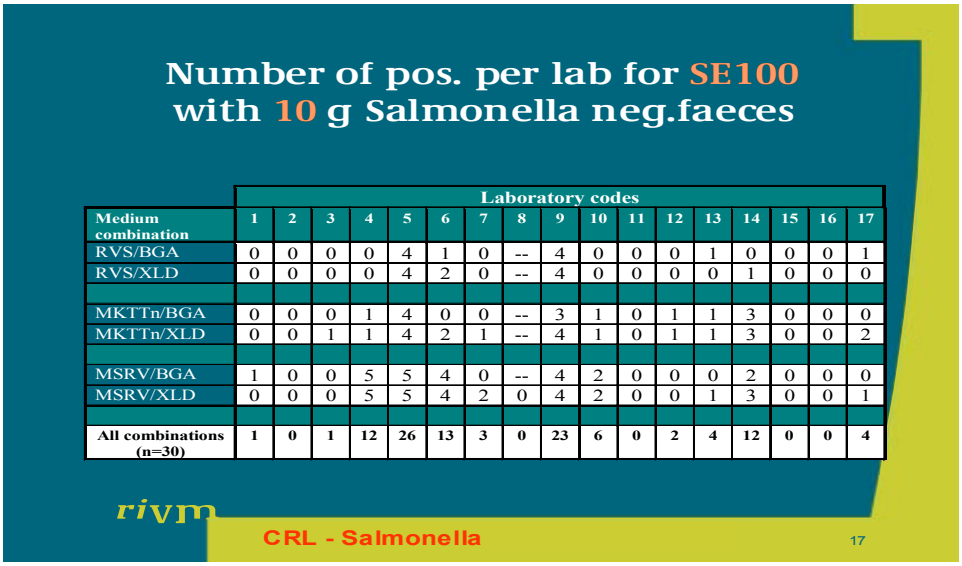
Medium	Laboratory codes 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
RVS/BGA	3	0	0	0	3	0	0	--	5	2	0	0	1	2	3	0	0
RVS/XLD	1	0	0	0	3	0	0	--	5	2	0	0	0	3	3	0	0
MKTTn/BGA	0	0	0	0	3	1	0	--	3	1	0	0	1	2	4	0	1
MKTTn/XLD	0	0	1	3	3	0	1	--	3	1	0	0	1	5	3	0	5
MSRV/BGA	1	0	0	5	3	5	0	--	5	5	0	0	0	5	1	0	0
MSRV/XLD	0	0	0	5	3	5	2	0	5	5	0	0	0	5	1	0	0
All combinations (n=30)	**5**	**0**	**1**	**13**	**18**	**11**	**3**	**0**	**26**	**16**	**0**	**0**	**3**	**22**	**15**	**0**	**6**

Slide 16



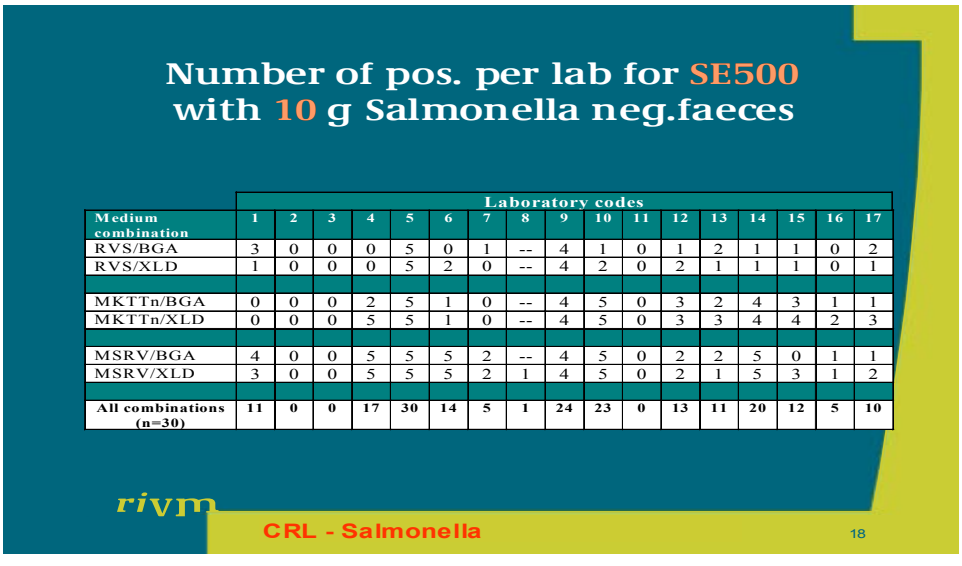
Medium combination	Laboratory codes 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
RVS/BGA	2	1	0	1	5	3	0	--	4	5	0	2	5	4	2	0	5
RVS/XLD	5	1	0	1	5	2	3	--	4	5	0	2	3	5	2	0	5
MKTTn/BGA	0	1	0	0	5	1	1	--	4	5	0	3	5	5	2	0	3
MKTTn/XLD	0	0	0	5	5	1	0	--	1	5	0	3	5	4	2	0	5
MSRV/BGA	4	0	0	5	5	5	0	--	4	5	1	3	1	5	2	3	0
MSRV/XLD	4	0	0	5	5	5	3	1	4	5	1	3	4	5	2	3	3
All combinations (n=30)	**15**	**3**	**0**	**17**	**30**	**17**	**7**	**1**	**21**	**30**	**2**	**16**	**23**	**28**	**12**	**6**	**21**

Slide 17



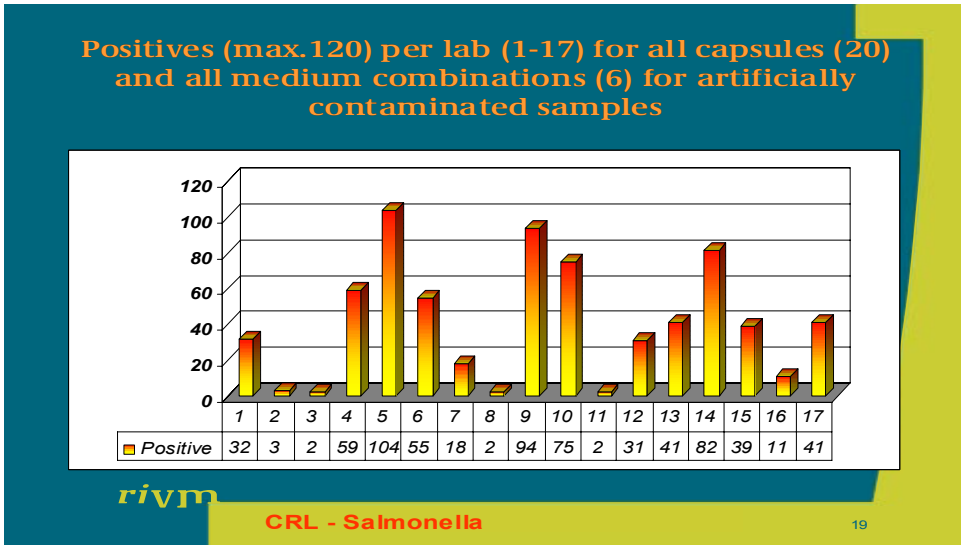
Medium combination	Laboratory codes 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
RVS/BGA	0	0	0	0	4	1	0	--	4	0	0	0	1	0	0	0	1
RVS/XLD	0	0	0	0	4	2	0	--	4	0	0	0	0	1	0	0	0
MKTTn/BGA	0	0	0	1	4	0	0	--	3	1	0	1	1	3	0	0	0
MKTTn/XLD	0	0	1	1	4	2	1	--	4	1	0	1	1	3	0	0	2
MSRV/BGA	1	0	0	5	5	4	0	--	4	2	0	0	0	2	0	0	0
MSRV/XLD	0	0	0	5	5	4	2	0	4	2	0	0	1	3	0	0	1
All combinations (n=30)	**1**	**0**	**1**	**12**	**26**	**13**	**3**	**0**	**23**	**6**	**0**	**2**	**4**	**12**	**0**	**0**	**4**

Slide 18

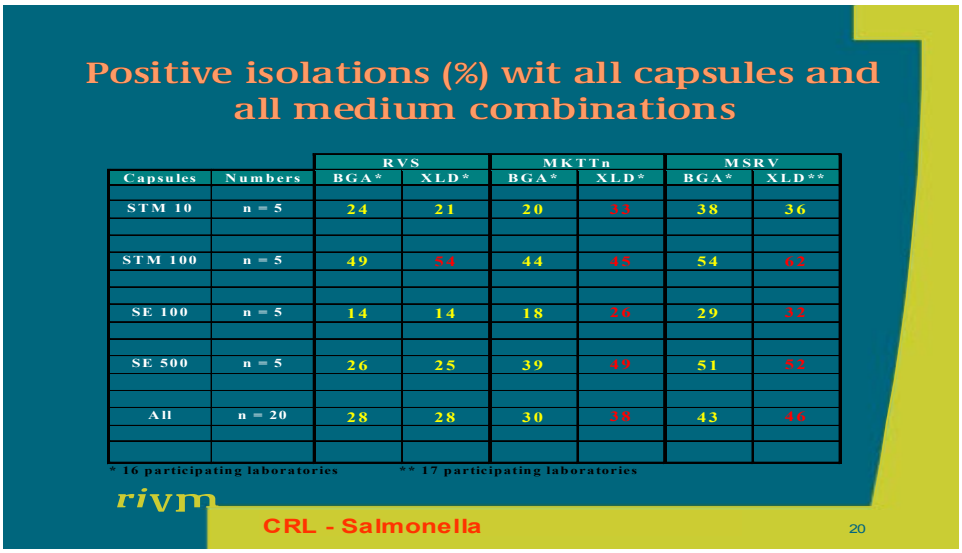


Medium combination	Laboratory codes 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
RVS/BGA	3	0	0	0	5	0	1	--	4	1	0	1	2	1	1	0	2
RVS/XLD	1	0	0	0	5	2	0	--	4	2	0	2	1	1	1	0	1
MKTTn/BGA	0	0	0	2	5	1	0	--	4	5	0	3	2	4	3	1	1
MKTTn/XLD	0	0	0	5	5	1	0	--	4	5	0	3	3	4	4	2	3
MSRV/BGA	4	0	0	5	5	5	2	--	4	5	0	2	2	5	0	1	1
MSRV/XLD	3	0	0	5	5	5	2	1	4	5	0	2	1	5	3	1	2
All combinations (n=30)	**11**	**0**	**0**	**17**	**30**	**14**	**5**	**1**	**24**	**23**	**0**	**13**	**11**	**20**	**12**	**5**	**10**

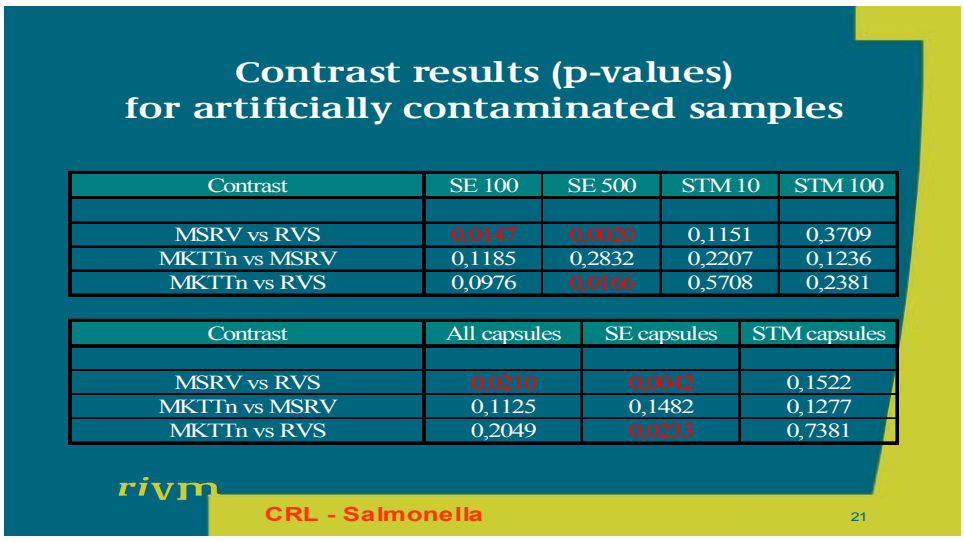
Slide 19



Slide 20



Slide 21



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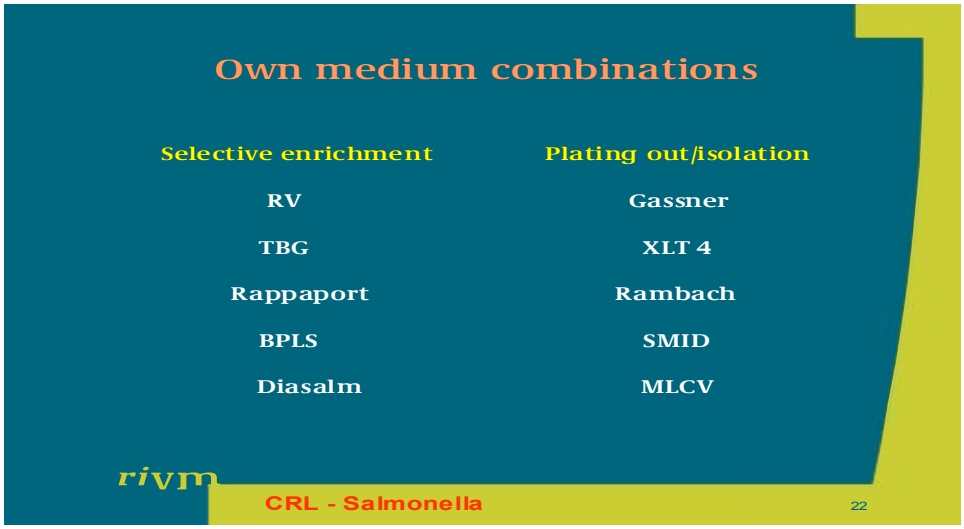

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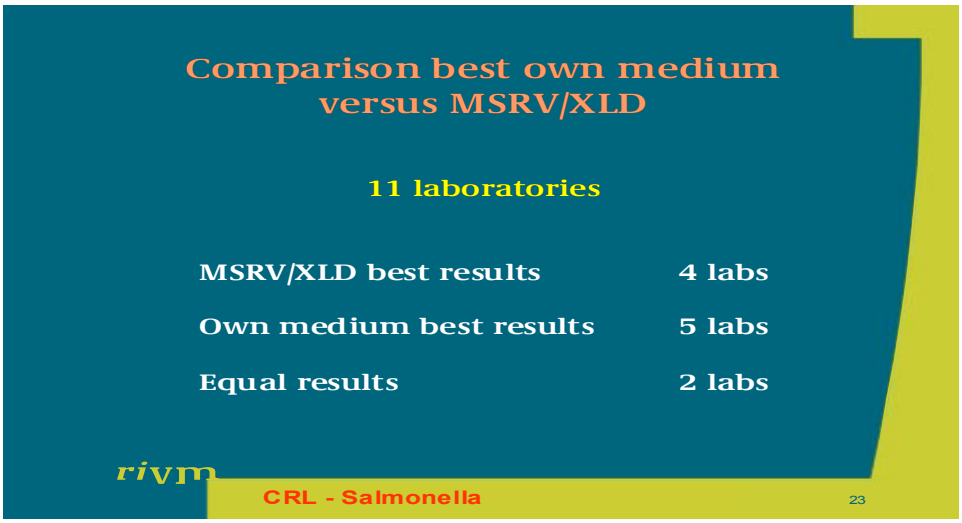

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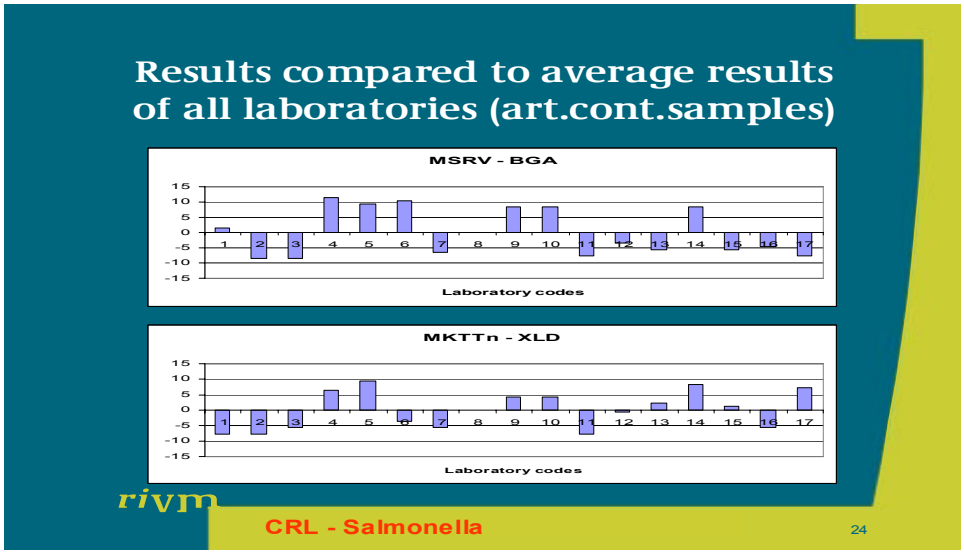
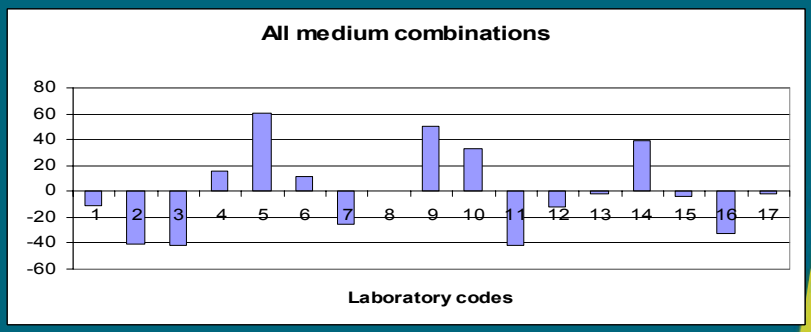


Slide 25

Results compared to average results of all laboratories (art.cont.samples)



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Result of faeces samples naturally contaminated with *Salmonella*

Medium combination	Laboratory codes 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
RVS/BGA	7	20	0	15	20	3	0	--	19	20	8	15	7	12	19	17	18
RVS/XLD	6	20	20	15	20	7	6	--	19	20	9	20	2	11	19	12	19
MKTTn/BGA	2	19	0	1	17	2	2	--	4	20	10	20	9	18	20	20	7
MKTTn/XLD	0	20	6	18	20	0	3	--	11	20	18	20	7	18	19	20	15
MSRV/BGA	5	4	1	20	19	20	0	--	19	20	14	20	9	19	19	13	8
MSRV/XLD	1	20	17	20	19	20	5	20	19	20	14	20	6	19	19	16	17

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Overall results all medium combinations for naturally contaminated samples

Capsules		RVS BGA*	RVS XLD*	MKTTn BGA*	MKTTn XLD*	MSRV BGA*	MSRV XLD**
None	Positives	200	225	171	215	210	272
	%	63	70	53	67	66	85

* 16 participating laboratories ** 17 participating laboratories

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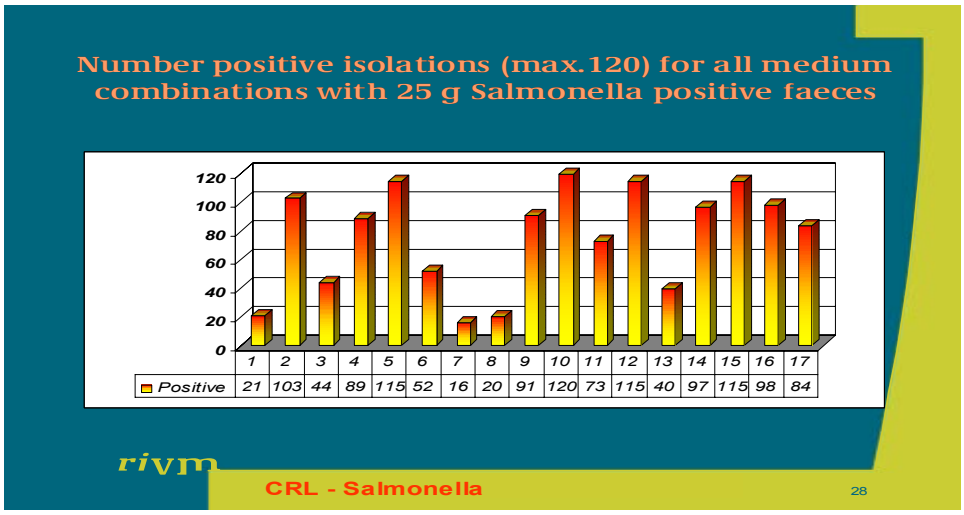
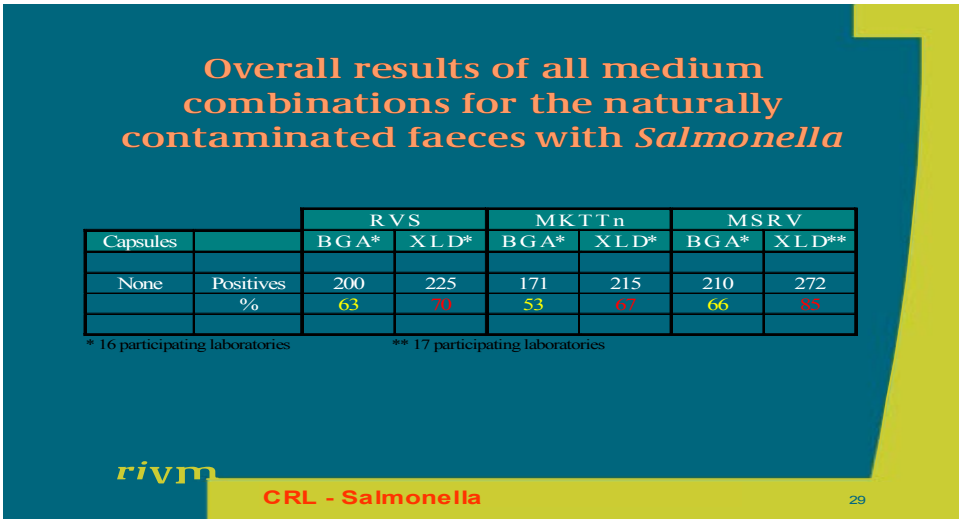


Slide 29



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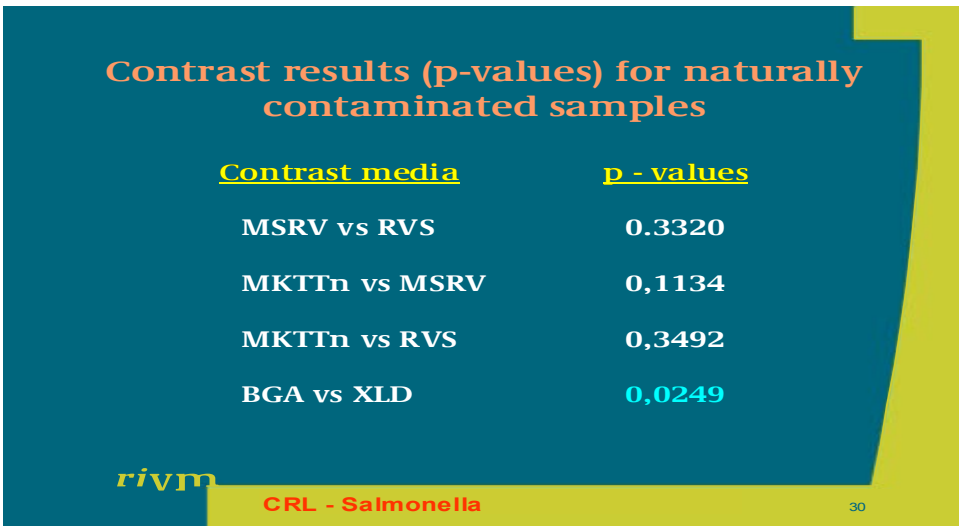

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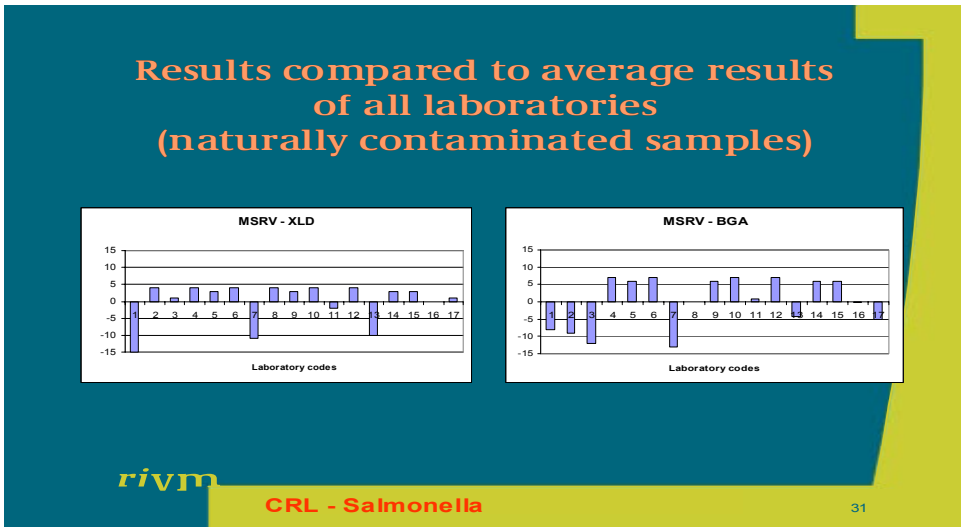
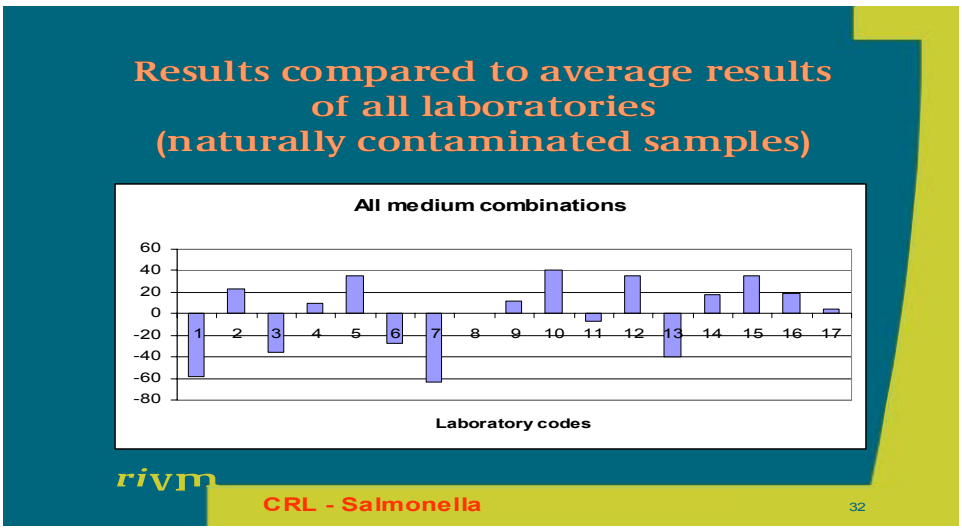


Slide 32



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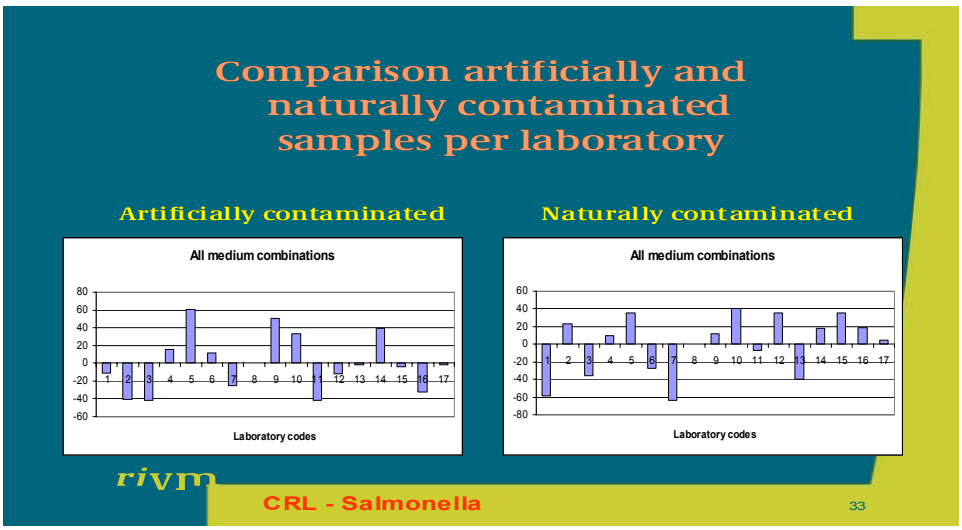

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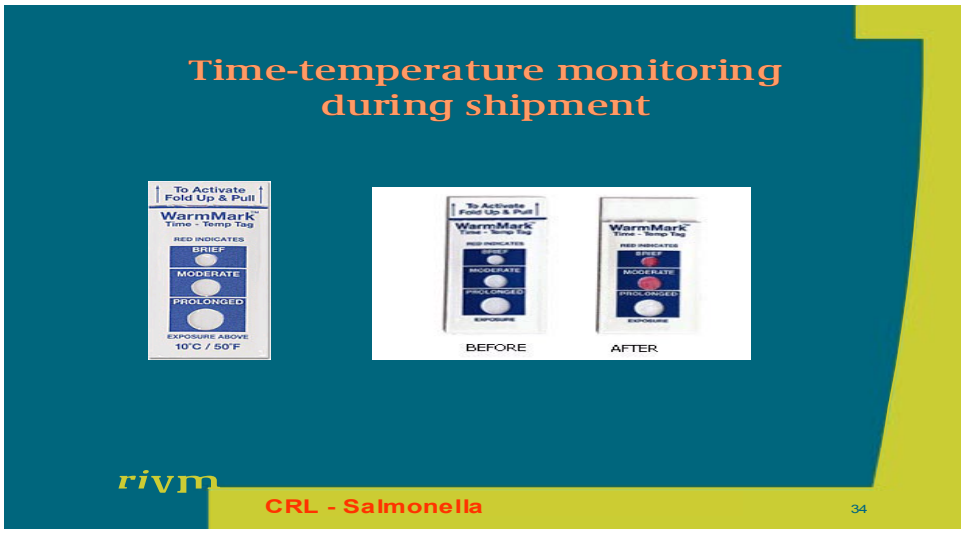

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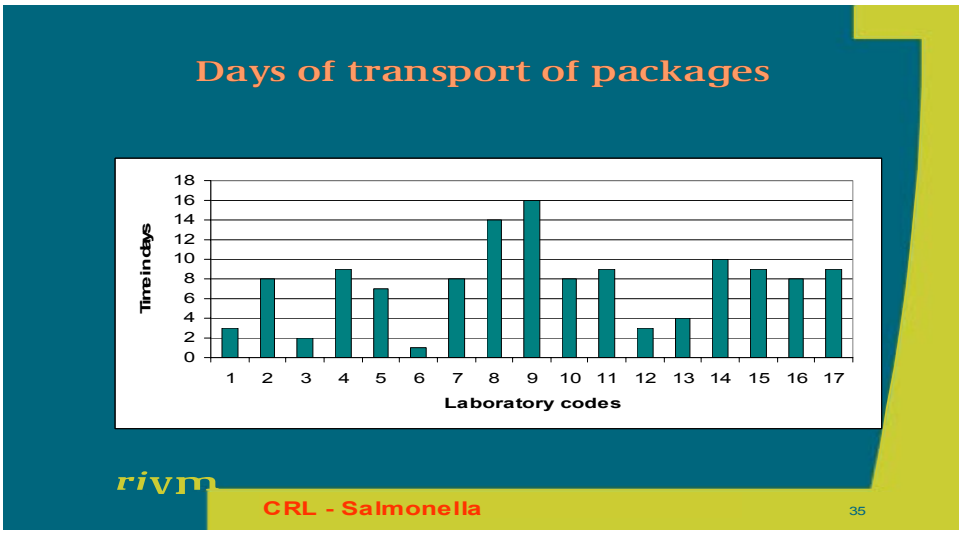

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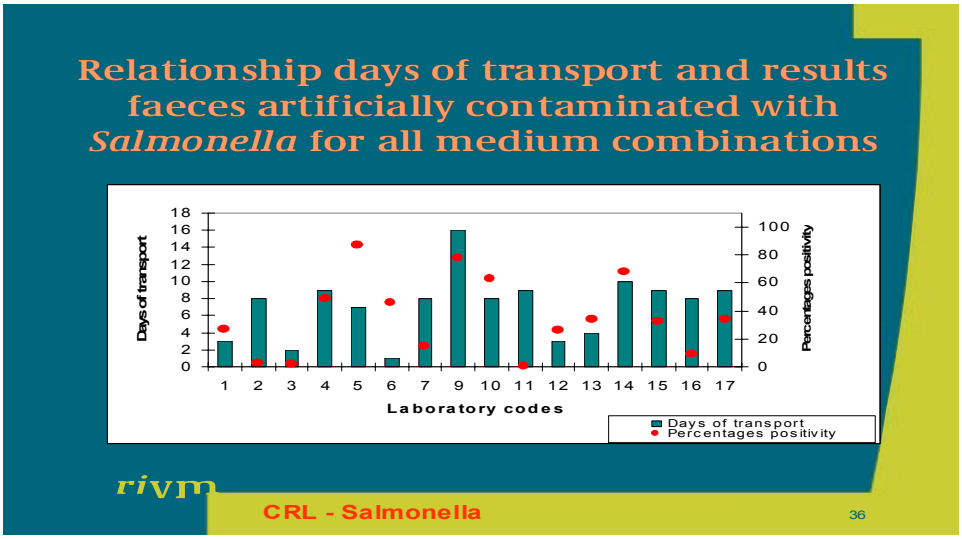

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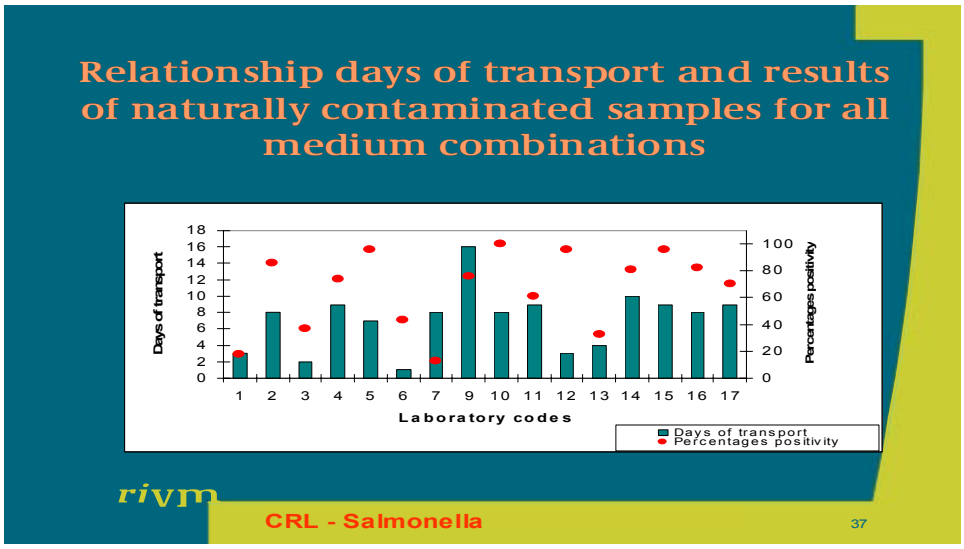

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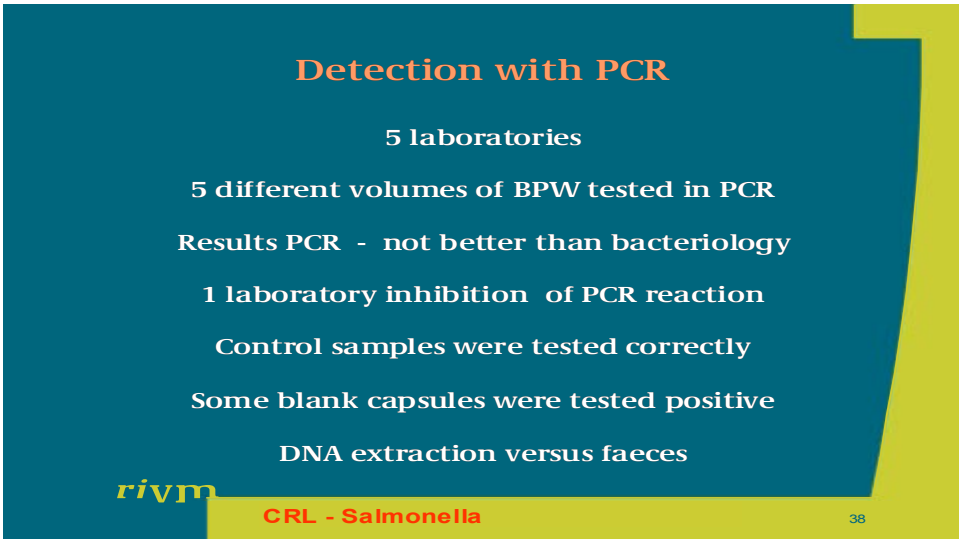
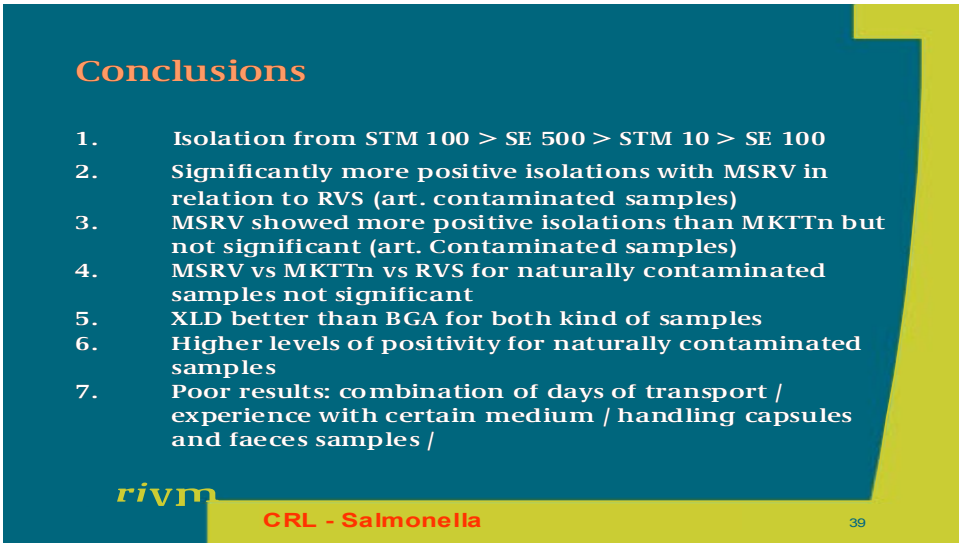
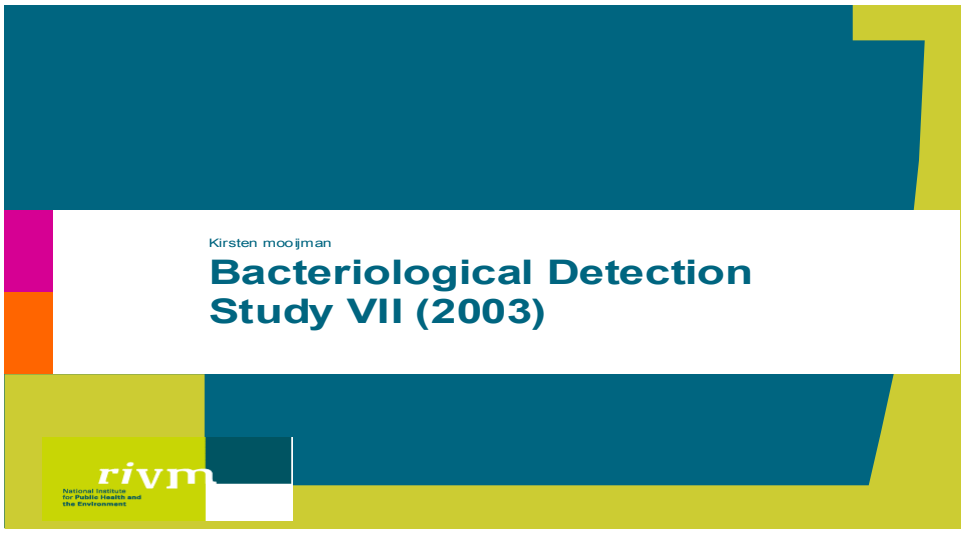


Slide 39

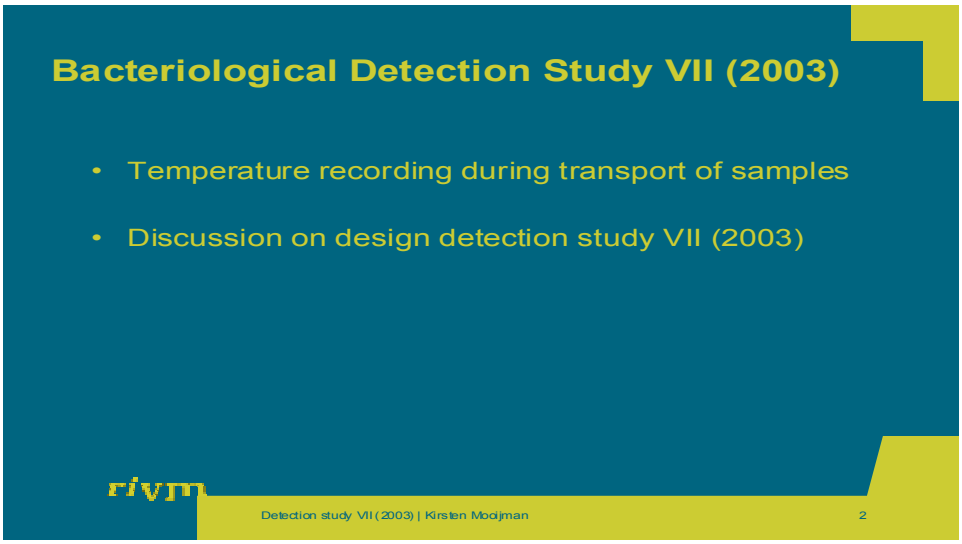


Appendix 22. Slides of presentation 2.5

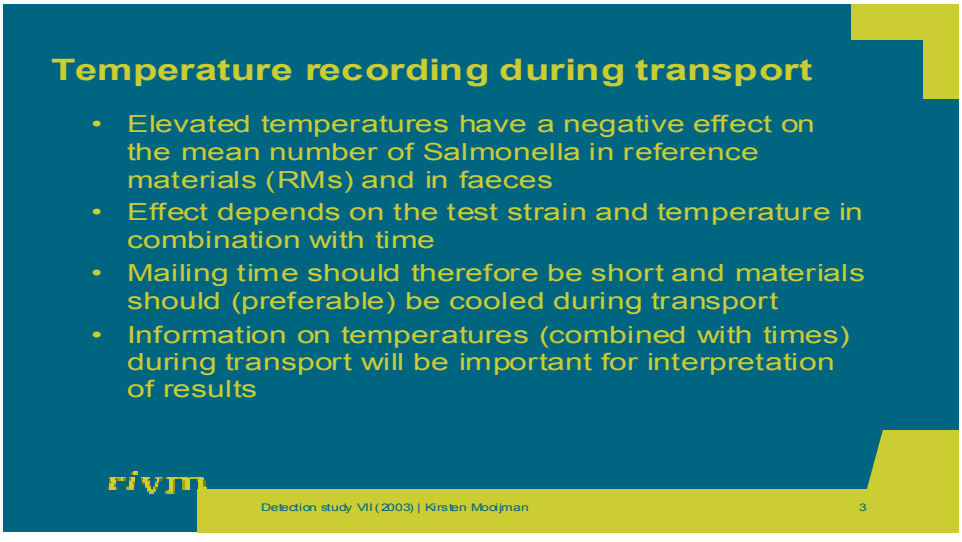
Slide 1



Slide 2

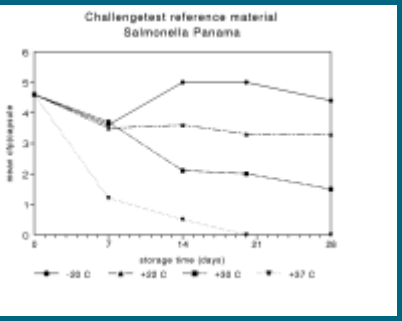


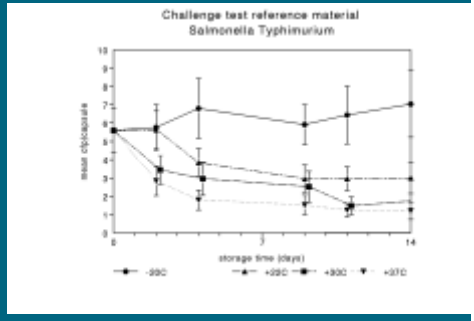
Slide 3



Slide 4

Effect of temperature on Salmonella in RMs





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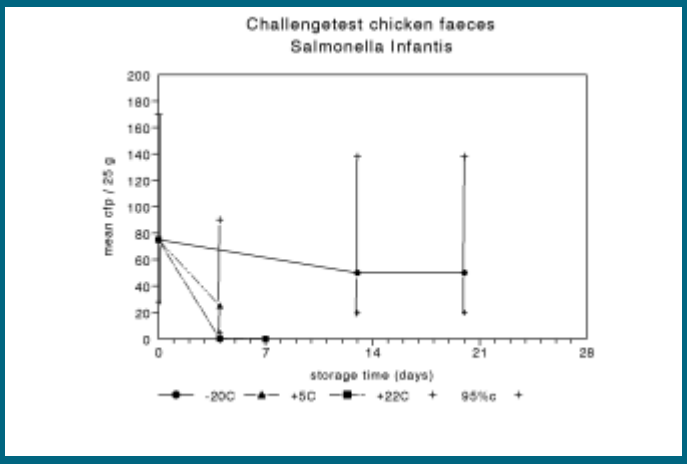
Slide 5

Effect of temperature on Salmonella in faeces



rivm

Slide 6

Temperature recording during transport

- Up to now we used 'Warm-Mark Time-Temperature Tags'
- Tags give an indication whether the temperature has exceeded the 'Tag-temperature' (e.G. 10 °C)
- Tags do not give much info on how long the temperature was above the 'Tag-temperature'

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Slide 7

Temperature recording during transport

- Preferable more exact info on temperature combined with times
- E.g. by using 'Tomprobes'
- Tomprobes will only be of use if receiving lab immediately returns Tomprobe to CRL

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Slide 8

Proposal for design of bact detection study VII (2003)

- ca November 2003;
- same no. and type of samples as study VI, but less methods

Samples:

- 5 STM10 + 10 g faeces (Salm-)
- 5 STM100 + 10 g faeces (Salm-)
- 5 SE100 + 10 g faeces (Salm-)
- 5 SE500 + 10 g faeces (Salm-)
- 5 Blank + 10 g faeces (Salm-)
- 3 STM10 no faeces
- 3 SE100 no faeces
- 2 SPan5 no faeces
- 2 Blank no faeces
- 20 x 25 g Salmonella+ faeces

Methods:

- 'Reference' method:
  - pre-enrichment: BPW
  - selective enrichment: MSRV
  - Plating-out: BGA & XLD
- 'Own' method:
  - Preferable the (one) method routinely used

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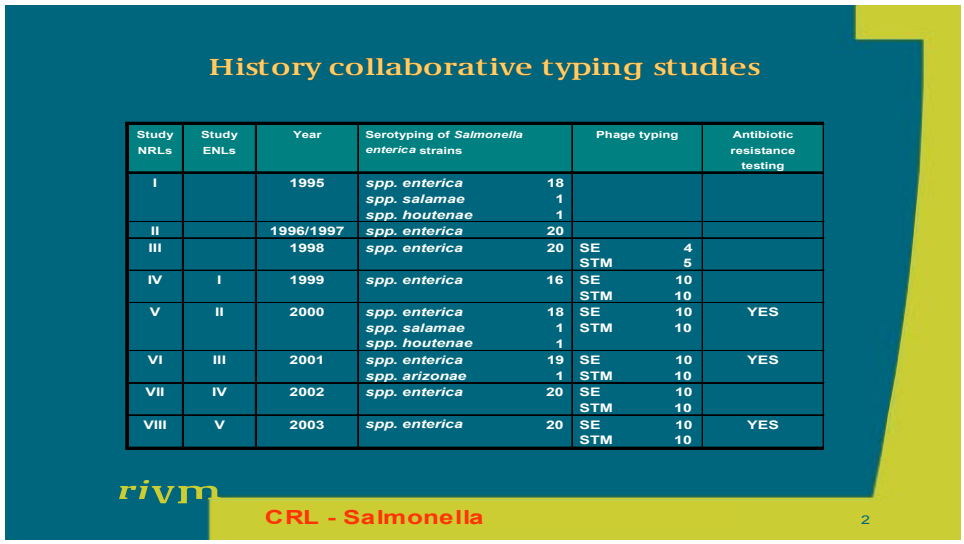
Appendix 23. Slides of presentation 2.6

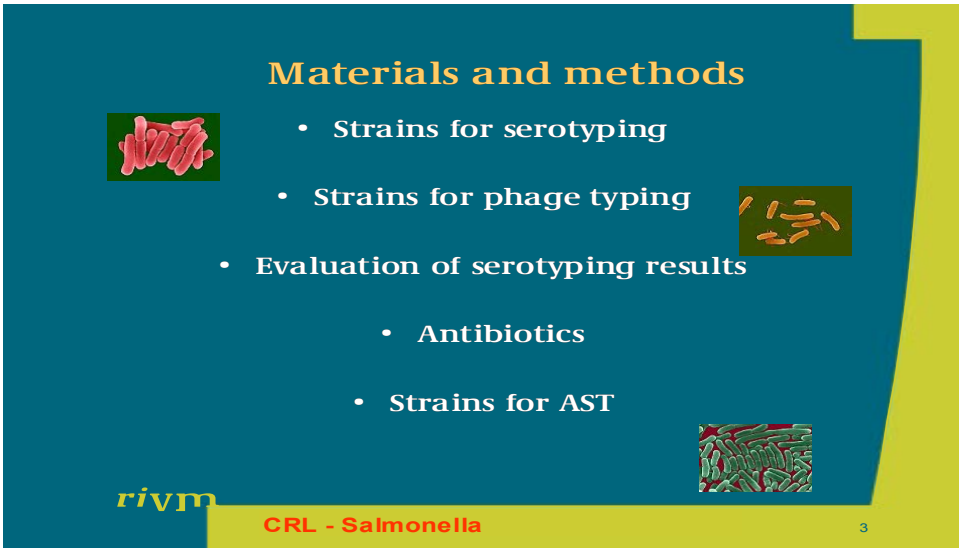
Slide 1

Slide 2

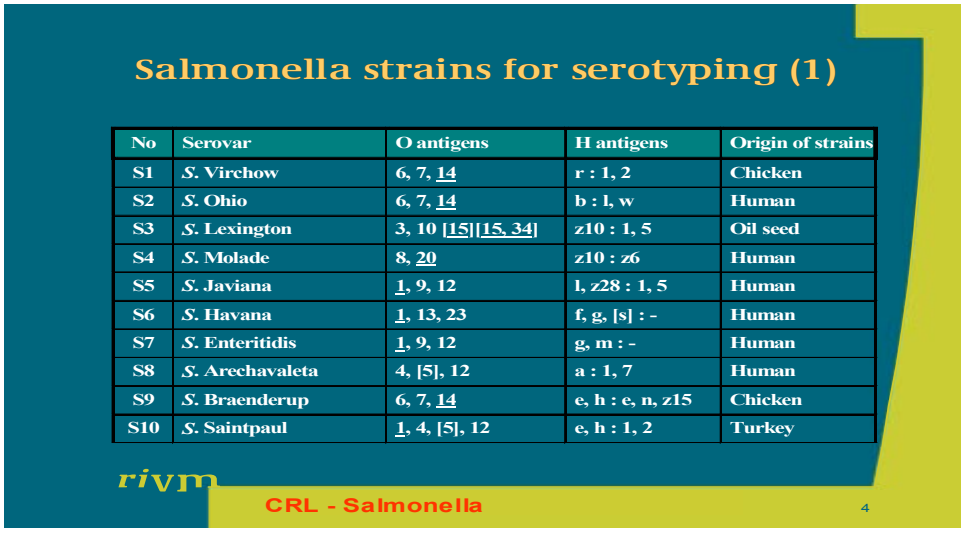


Study NRLs	Study ENLs	Year	Serotyping of *Salmonella enterica* strains		Phage typing		Antibiotic resistance testing
I		1995	*spp. enterica* *spp. salamae* *spp. houtenae*	18 1 1			
II		1996/1997	*spp. enterica*	20			
III		1998	*spp. enterica*	20	SE STM	4 5	
IV	I	1999	*spp. enterica*	16	SE STM	10 10	
V	II	2000	*spp. enterica* *spp. salamae* *spp. houtenae*	18 1 1	SE STM	10 10	YES
VI	III	2001	*spp. enterica* *spp. arizonae*	19 1	SE STM	10 10	YES
VII	IV	2002	*spp. enterica*	20	SE STM	10 10	
VIII	V	2003	*spp. enterica*	20	SE STM	10 10	YES

Slide 3

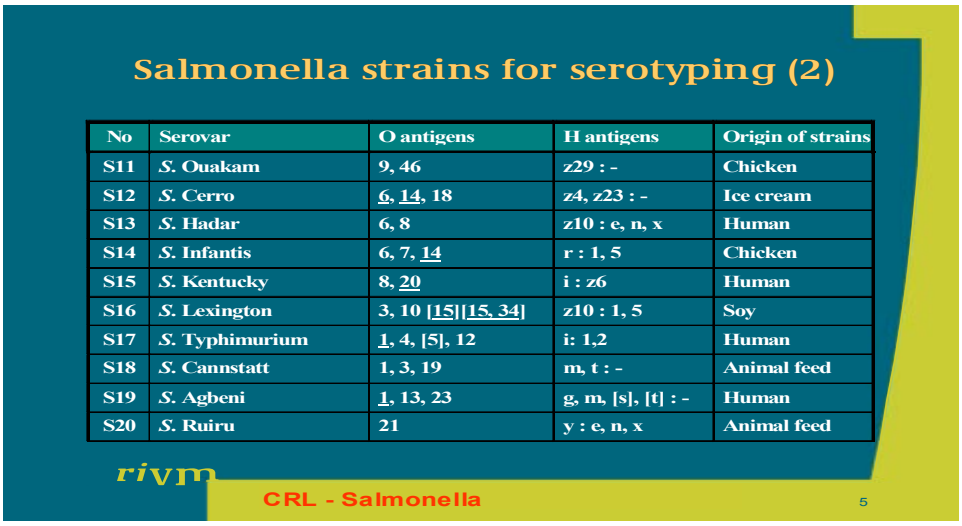


Slide 4



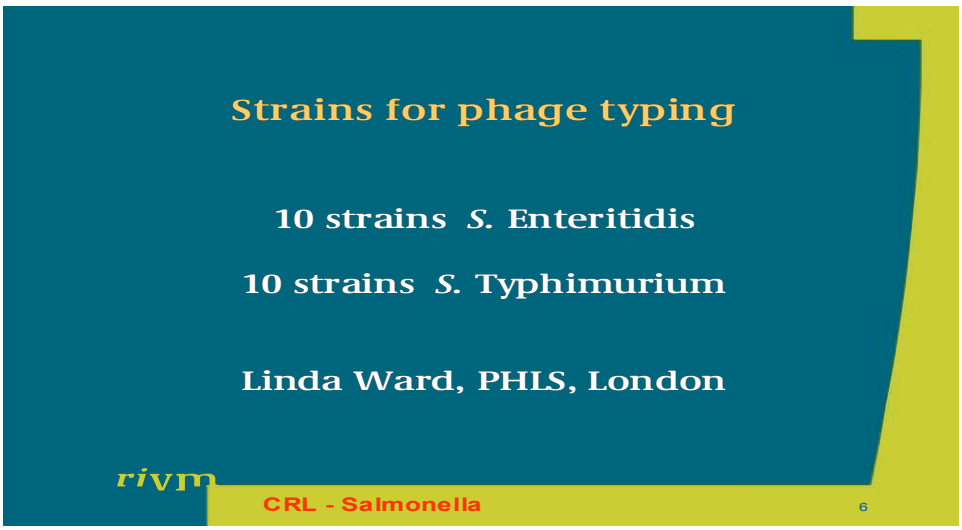
No	Serovar	O antigens	H antigens	Origin of strains
S1	*S.* Virchow	6, 7, 14	r : 1, 2	Chicken
S2	*S.* Ohio	6, 7, 14	b : l, w	Human
S3	*S.* Lexington	3, 10 [15][15, 34]	z10 : 1, 5	Oil seed
S4	*S.* Molade	8, 20	z10 : z6	Human
S5	*S.* Javiana	1, 9, 12	l, z28 : 1, 5	Human
S6	*S.* Havana	1, 13, 23	f, g, [s] : -	Human
S7	*S.* Enteritidis	1, 9, 12	g, m : -	Human
S8	*S.* Arechavaleta	4, [5], 12	a : 1, 7	Human
S9	*S.* Braenderup	6, 7, 14	e, h : e, n, z15	Chicken
S10	*S.* Saintpaul	1, 4, [5], 12	e, h : 1, 2	Turkey

Slide 5



No	Serovar	O antigens	H antigens	Origin of strains
S11	*S.* Ouakam	9, 46	z29 : -	Chicken
S12	*S.* Cerro	6, 14, 18	z4, z23 : -	Ice cream
S13	*S.* Hadar	6, 8	z10 : e, n, x	Human
S14	*S.* Infantis	6, 7, 14	r : 1, 5	Chicken
S15	*S.* Kentucky	8, 20	i : z6	Human
S16	*S.* Lexington	3, 10 [15][15, 34]	z10 : 1, 5	Soy
S17	*S.* Typhimurium	1, 4, [5], 12	i: 1,2	Human
S18	*S.* Cannstatt	1, 3, 19	m, t : -	Animal feed
S19	*S.* Agbeni	1, 13, 23	g, m, [s], [t] : -	Human
S20	*S.* Ruiru	21	y : e, n, x	Animal feed

Slide 6



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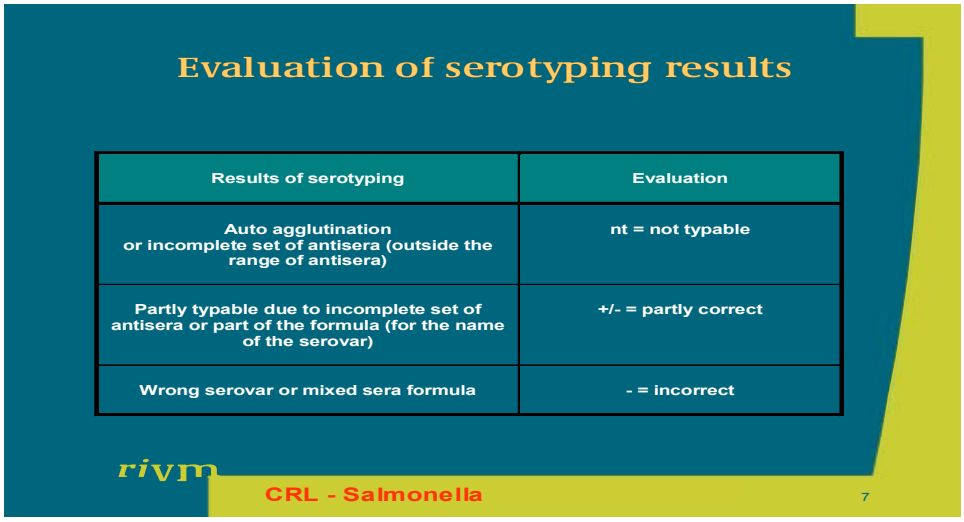

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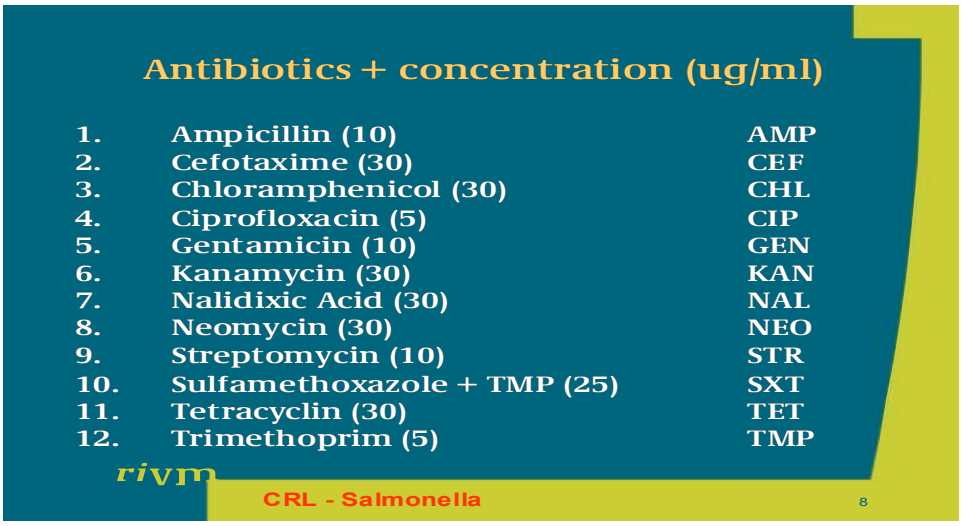

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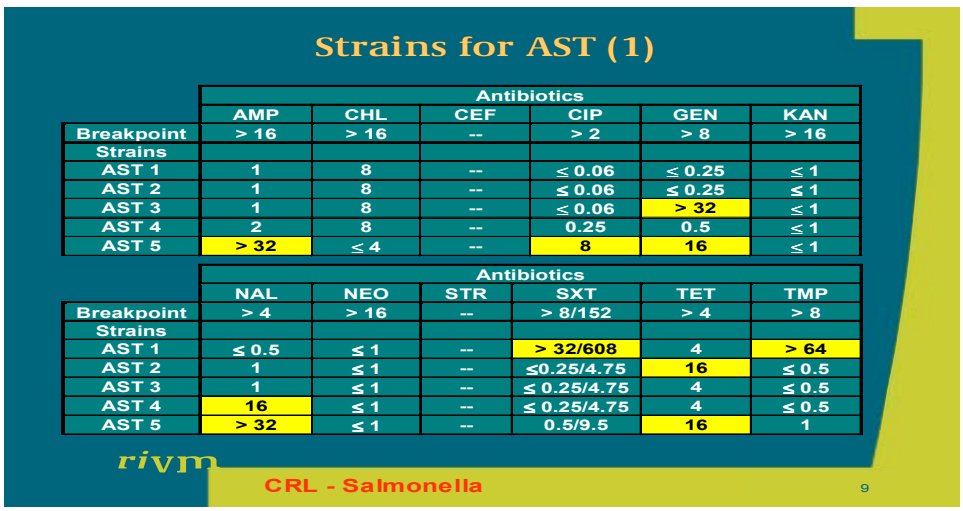

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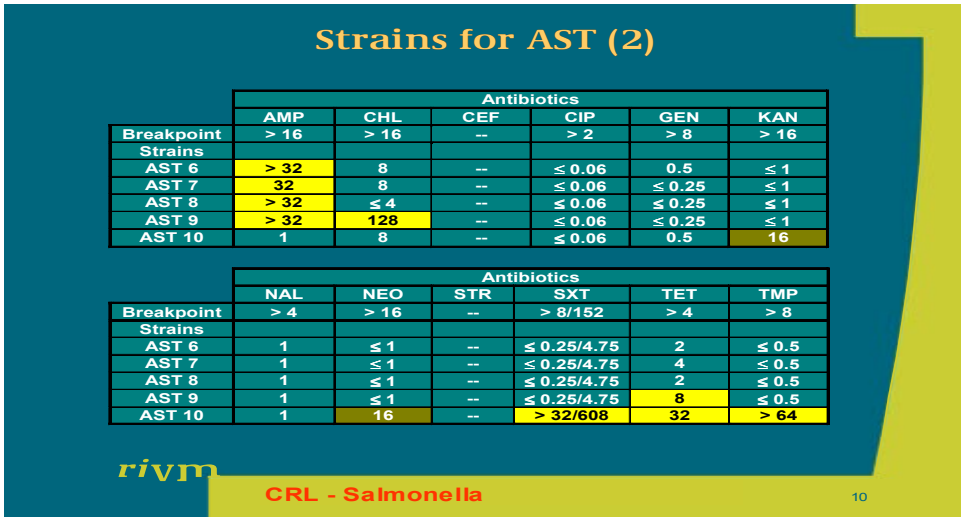
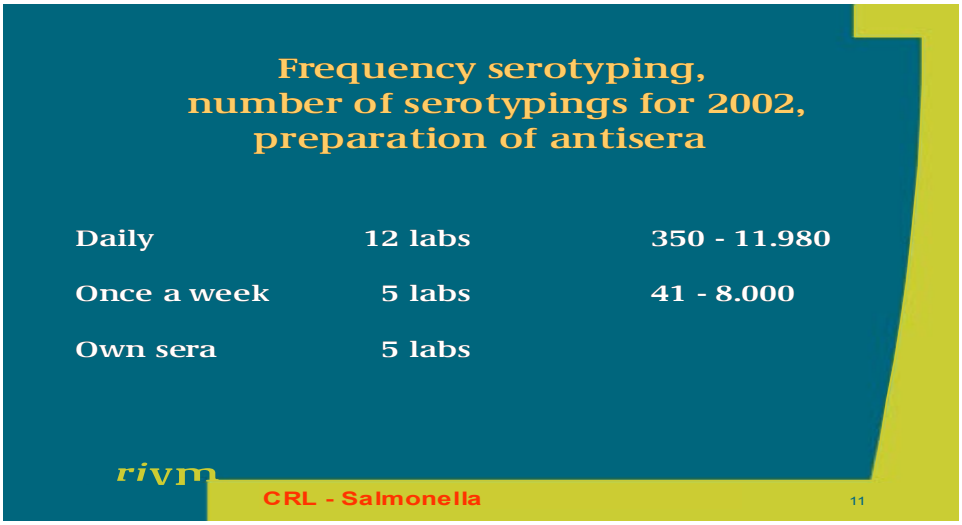


Slide 11



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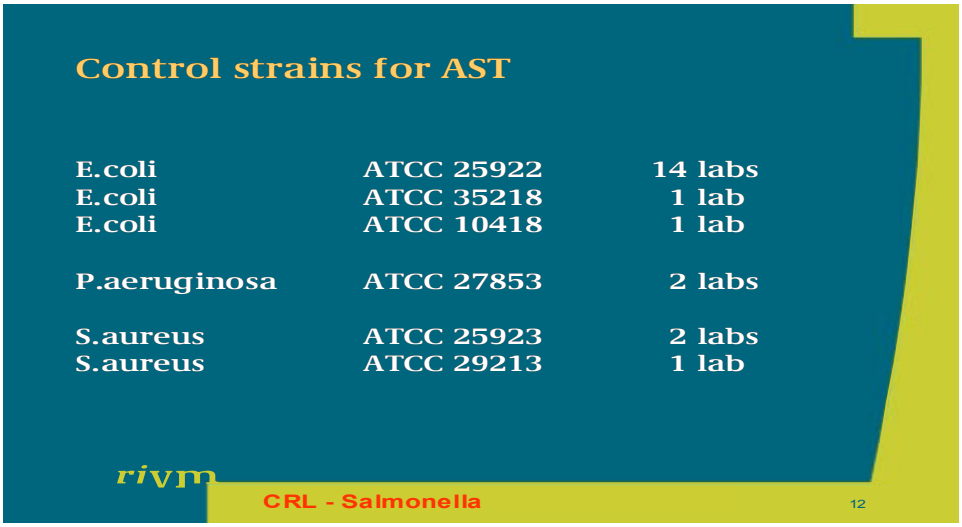

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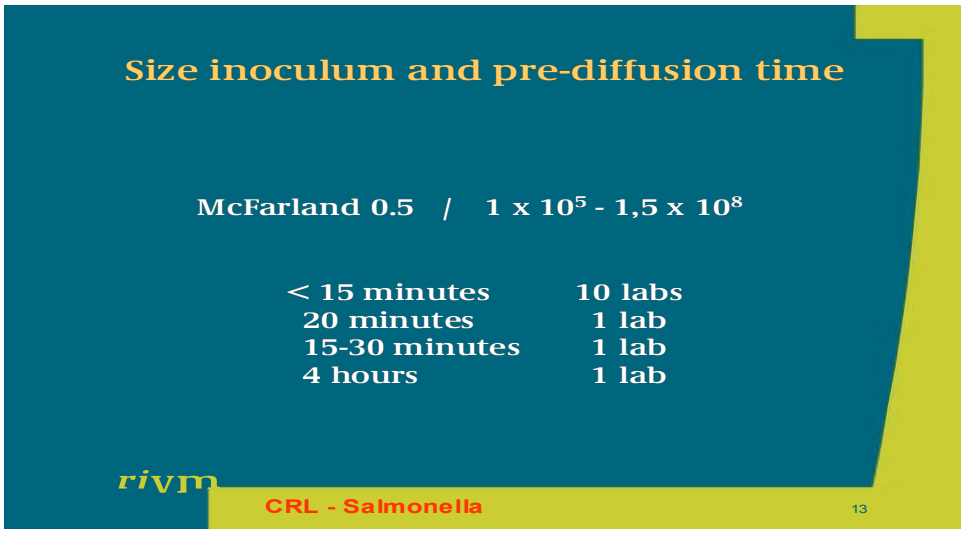

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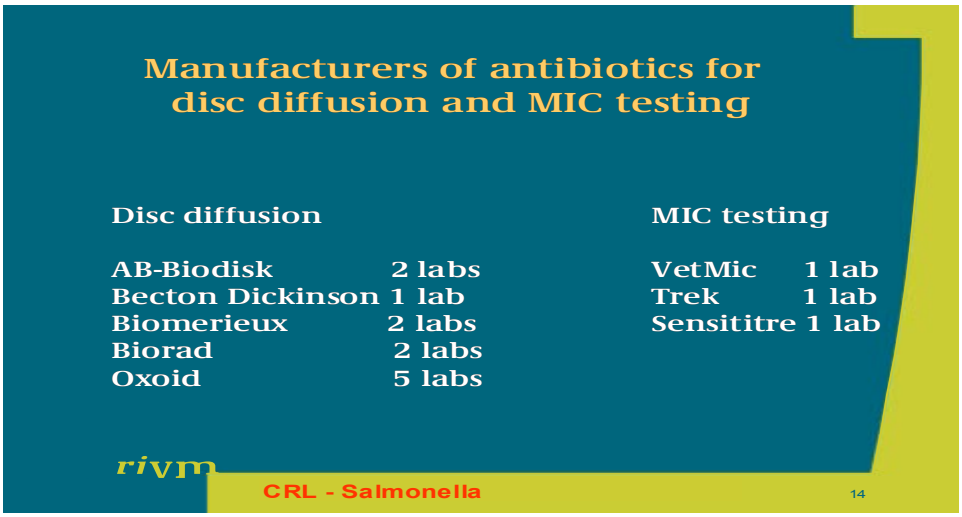

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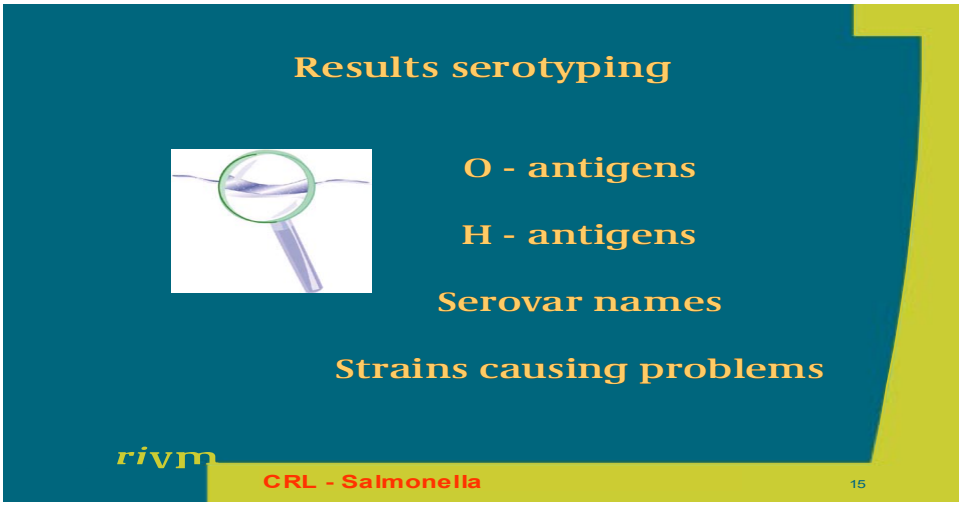

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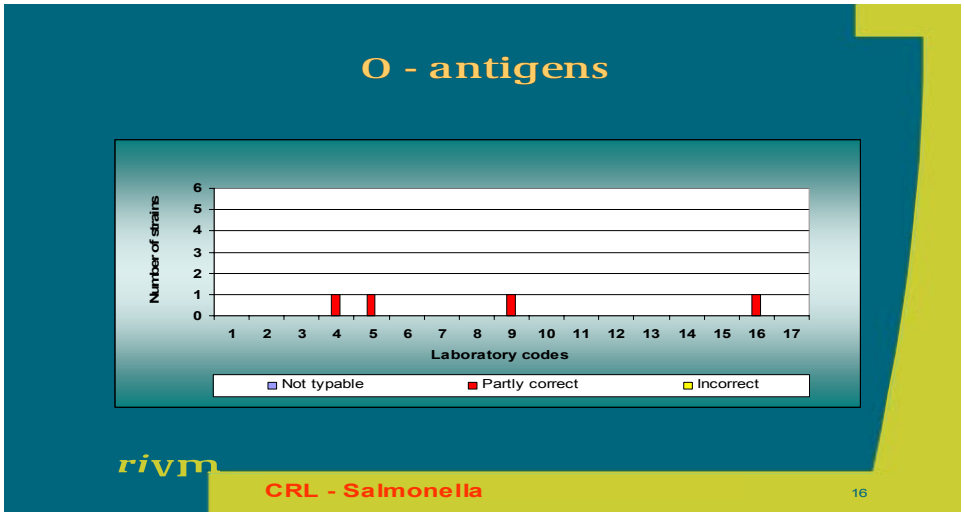

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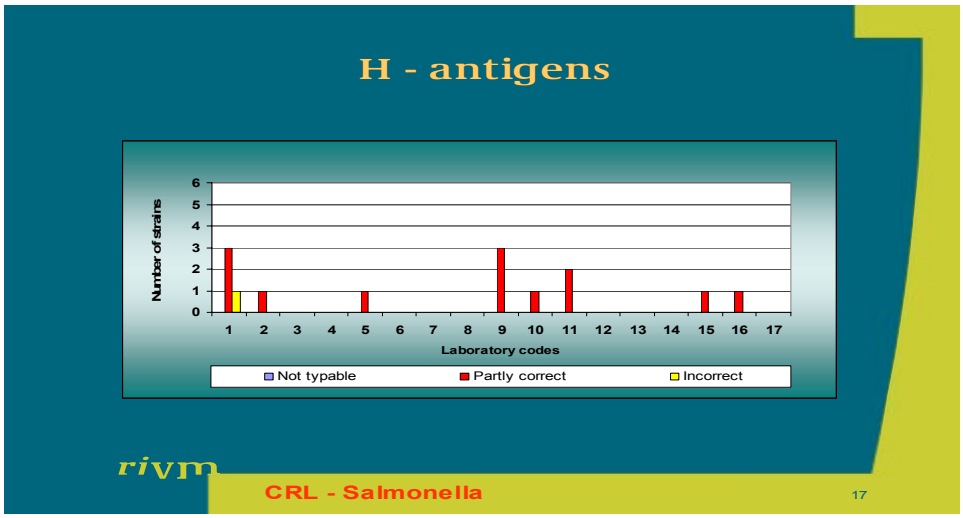
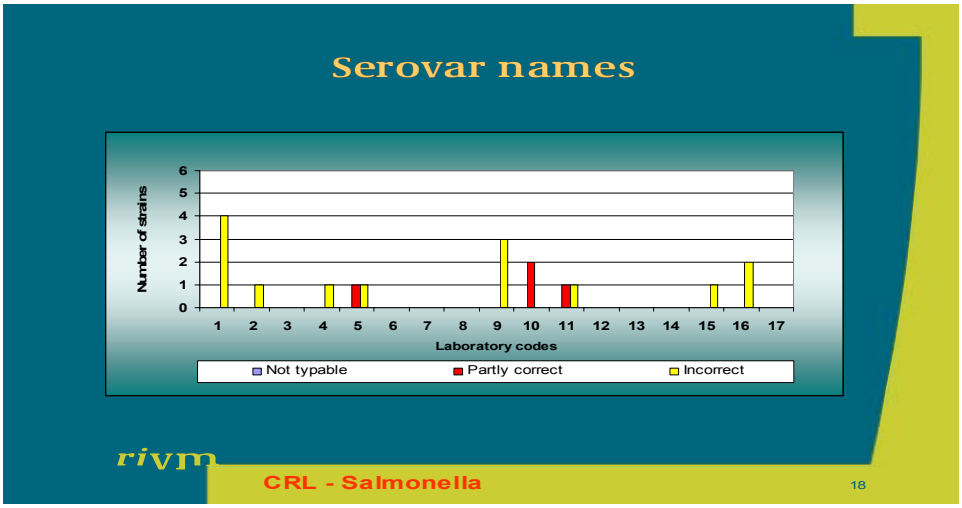


Slide 18



Slide 19

Strains causing problems (1)

Strain		O antigen detected				H antigen detected				Name serovar			
No.	Serotype	+	nt	+/-	-	+	nt	+/-	-	+	nt	+/-	-
3	S. Lexington	20	0	0	0	19	0	1	0	19	0	1	0
4	S. Molade	20	0	0	0	19	0	1	0	19	0	0	1
5	S. Javiana	20	0	0	0	19	0	1	0	19	0	1	0
7	S. Enteritidis	19	0	1	0	20	0	0	0	19	0	0	1
8	S. Arechavaleta	20	0	0	0	19	0	1	0	19	0	0	1
9	S. Braenderup	20	0	0	0	19	0	1	0	19	0	0	1
10	S. Saintpaul	20	0	0	0	19	0	1	0	19	0	0	1
11	S. Ouakam	20	0	0	0	20	0	0	0	19	0	1	0
12	S. Cerro	17	0	3	0	20	0	0	0	17	0	1	2
13	S. Hadar	20	0	0	0	18	0	1	1	18	0	0	2
18	S. Cannstatt	20	0	0	0	15	0	5	0	15	0	0	5

rivm CRL - Salmonella 19

Slide 20

Strains causing problems (2)

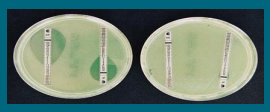
Serovar	O-antigens	H-antigens	Problem
S. Cerro	6, 14, 18	z_4 , $z_{2\ 3}$	O-antigen 18 not detected by 3 labs
S.Cannstatt	1, 3, 19	m, t	H-antigens g, m and t detected by 5 labs

rivm CRL - Salmonella 20

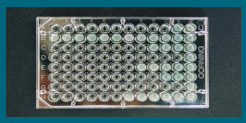
Slide 21

Antimicrobial susceptibility testing (AST)

Disc diffusion



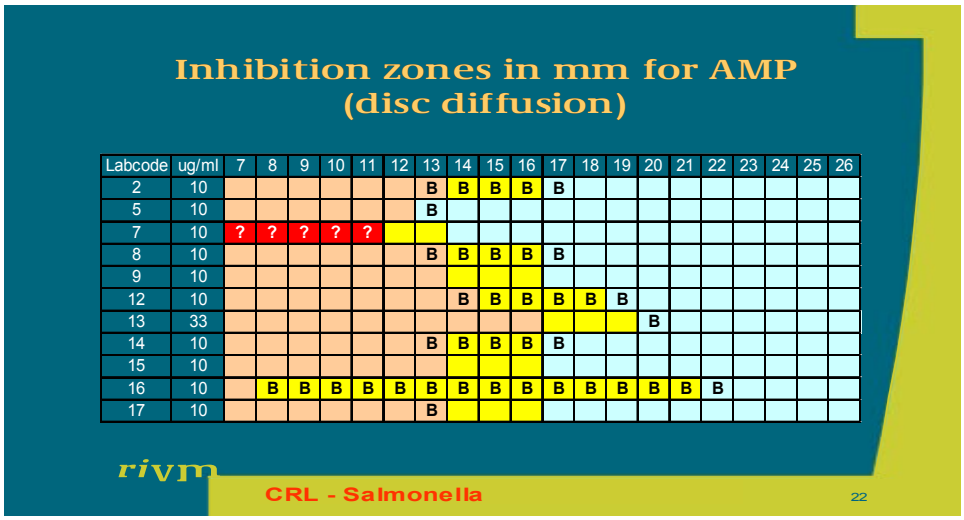
E-test



MIC (+ Breakpoint MIC)

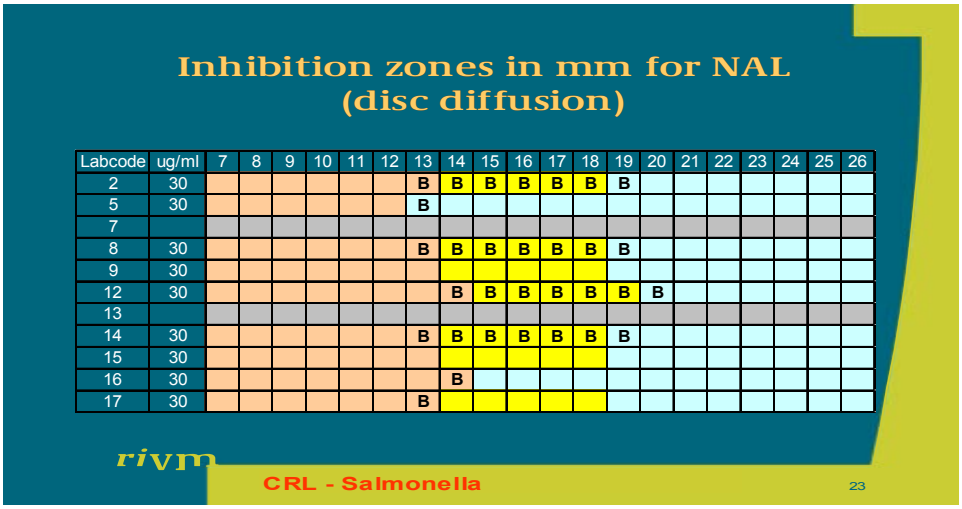
rivm CRL - Salmonella 21

Slide 22



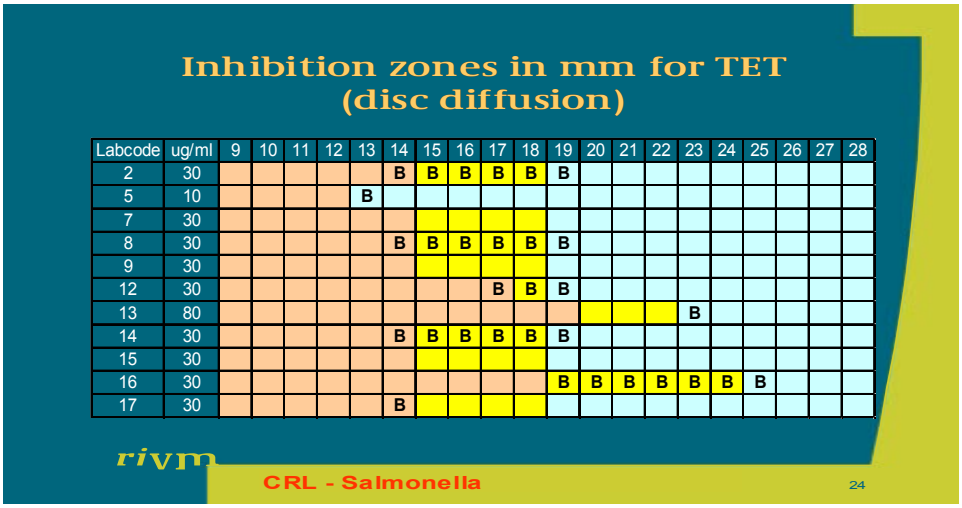
Labcode	ug/ml	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
2	10							B	B	B	B	B									
5	10							B													
7	10	?	?	?	?	?															
8	10							B	B	B	B	B									
9	10																				
12	10								B	B	B	B	B	B							
13	33														B						
14	10							B	B	B	B	B									
15	10																				
16	10		B	B	B	B	B	B	B	B	B	B	B	B	B	B	B				
17	10							B													

Slide 23



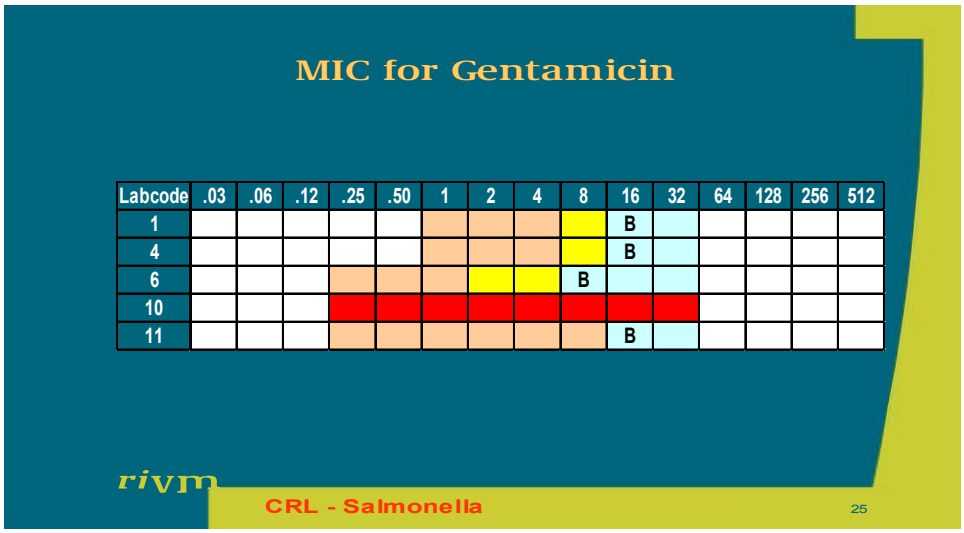
Labcode	ug/ml	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
2	30							B	B	B	B	B	B	B							
5	30							B													
7																					
8	30							B	B	B	B	B	B	B							
9	30																				
12	30								B	B	B	B	B	B	B						
13																					
14	30							B	B	B	B	B	B	B							
15	30																				
16	30								B												
17	30							B													

Slide 24

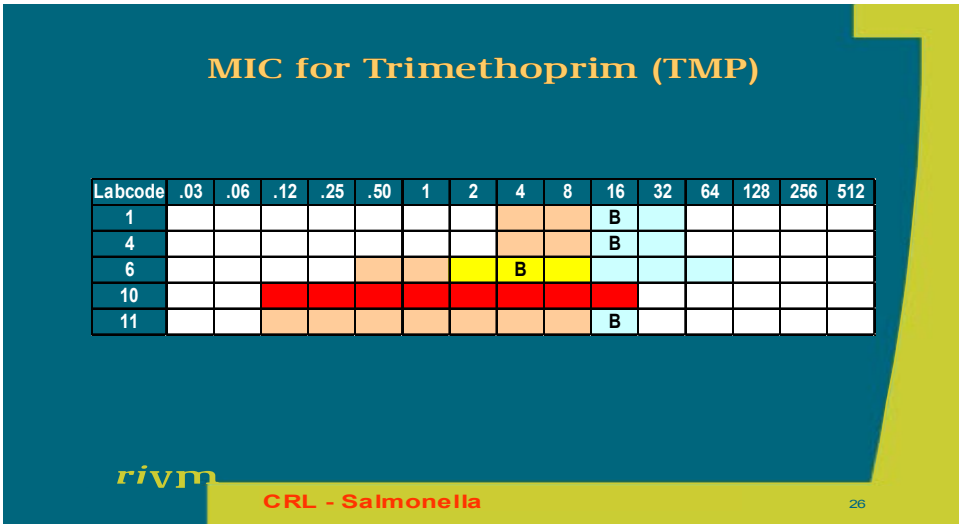


Labcode	ug/ml	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
2	30						B	B	B	B	B	B									
5	10					B															
7	30																				
8	30						B	B	B	B	B	B									
9	30																				
12	30									B	B	B									
13	80															B					
14	30						B	B	B	B	B	B									
15	30																				
16	30											B	B	B	B	B	B	B			
17	30						B														

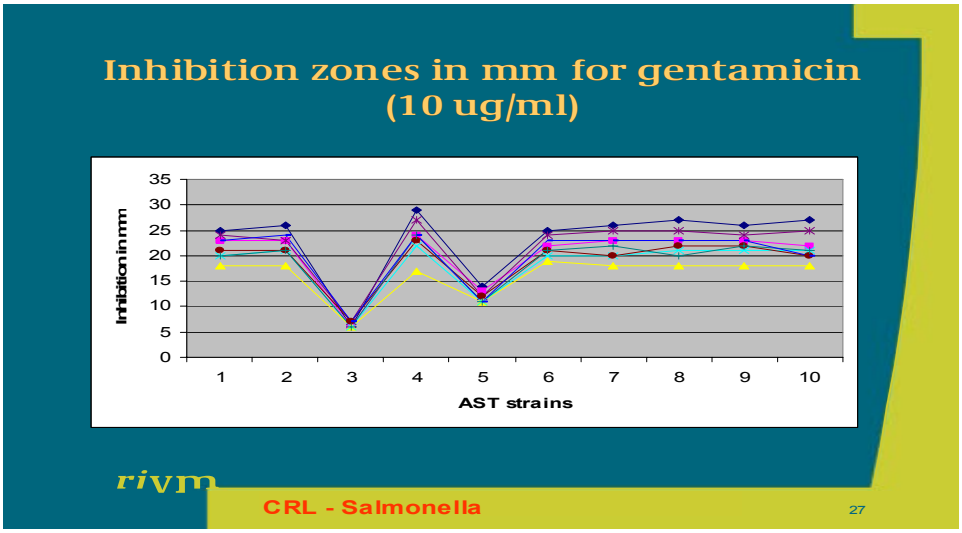
Slide 25



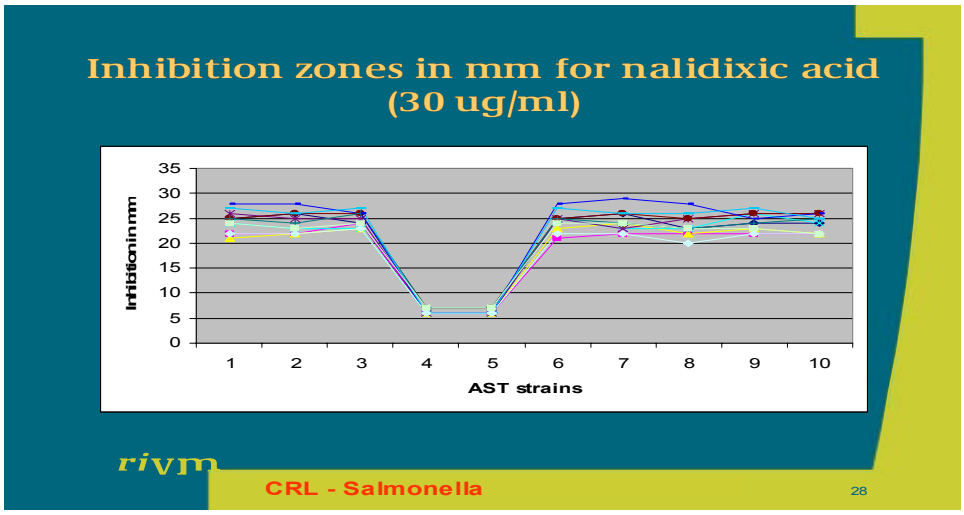
Slide 26



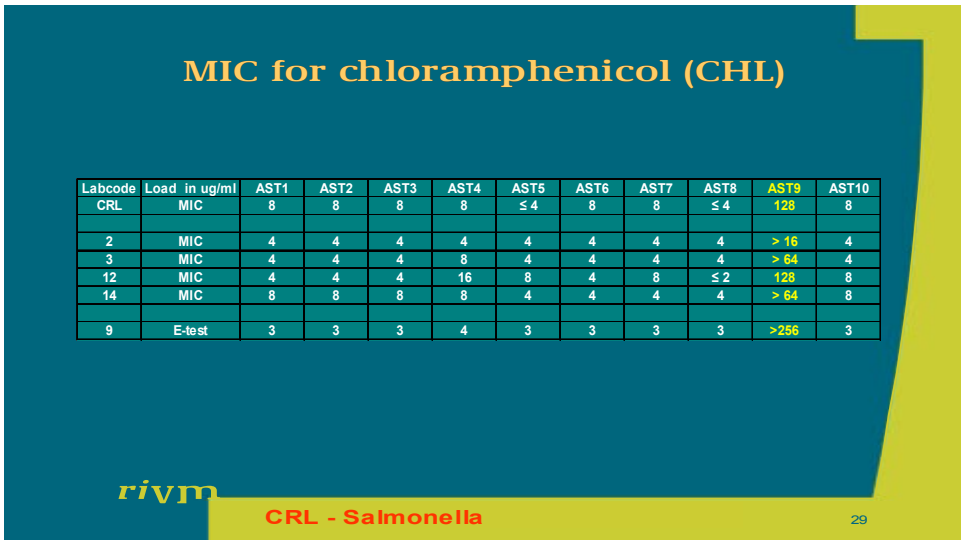
Slide 27



Slide 28

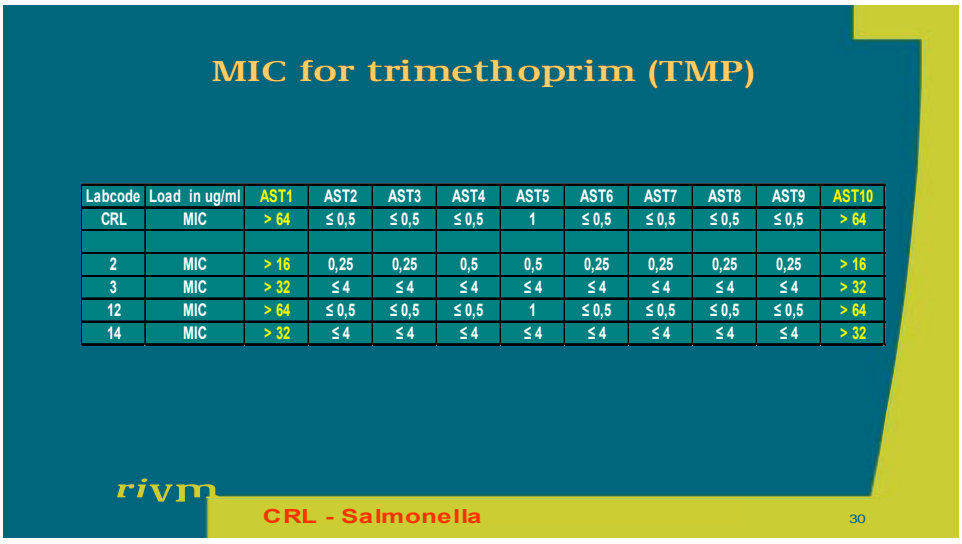


Slide 29



Labcode	Load in ug/ml	AST1	AST2	AST3	AST4	AST5	AST6	AST7	AST8	AST9	AST10
CRL	MIC	8	8	8	8	≤ 4	8	8	≤ 4	128	8
2	MIC	4	4	4	4	4	4	4	4	> 16	4
3	MIC	4	4	4	8	4	4	4	4	> 64	4
12	MIC	4	4	4	16	8	4	8	≤ 2	128	8
14	MIC	8	8	8	8	4	4	4	4	> 64	8
9	E-test	3	3	3	4	3	3	3	3	>256	3

Slide 30



Labcode	Load in ug/ml	AST1	AST2	AST3	AST4	AST5	AST6	AST7	AST8	AST9	AST10
CRL	MIC	> 64	≤ 0,5	≤ 0,5	≤ 0,5	1	≤ 0,5	≤ 0,5	≤ 0,5	≤ 0,5	> 64
2	MIC	> 16	0,25	0,25	0,5	0,5	0,25	0,25	0,25	0,25	> 16
3	MIC	> 32	≤ 4	≤ 4	≤ 4	≤ 4	≤ 4	≤ 4	≤ 4	≤ 4	> 32
12	MIC	> 64	≤ 0,5	≤ 0,5	≤ 0,5	1	≤ 0,5	≤ 0,5	≤ 0,5	≤ 0,5	> 64
14	MIC	> 32	≤ 4	≤ 4	≤ 4	≤ 4	≤ 4	≤ 4	≤ 4	≤ 4	> 32

Slide 31

Achievements in % correctness (serotyping)

	2001	2002	2003
O-antigens	94	98	99
H-antigens	94	94	96
Serovar names	90	92	95

rivm CRL - Salmonella 31

Slide 32

Achievements in % correctness (phage typing)

	2001	2002	2003
*S.*Enteritidis	84	81	90
*S.*Typhimurium	92	87	97

rivm CRL - Salmonella 32

Slide 33

Conclusions

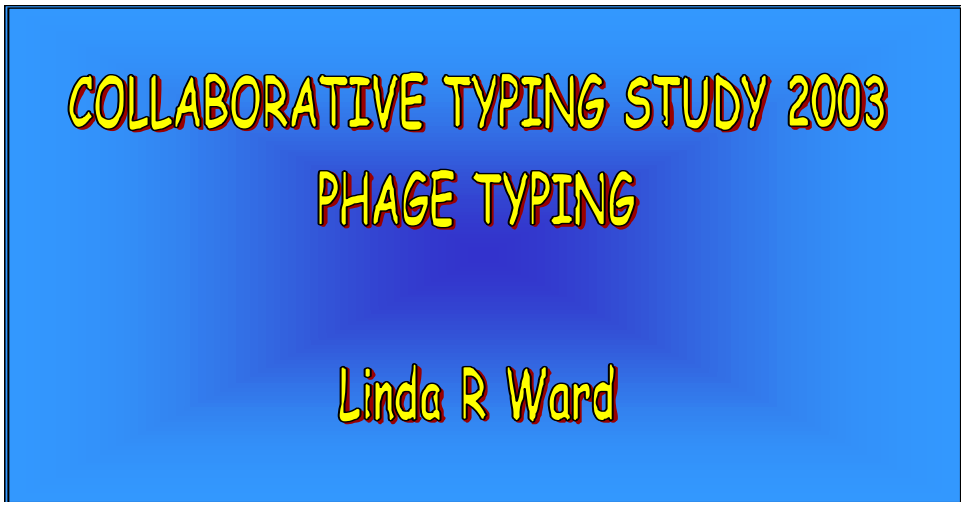
Serotyping: Better results than in previous years

Antimicrobial susceptibility testing:
Disc method / E - test / MIC test
Interpretation of results
Other antibiotics
Other strains / more strains ?

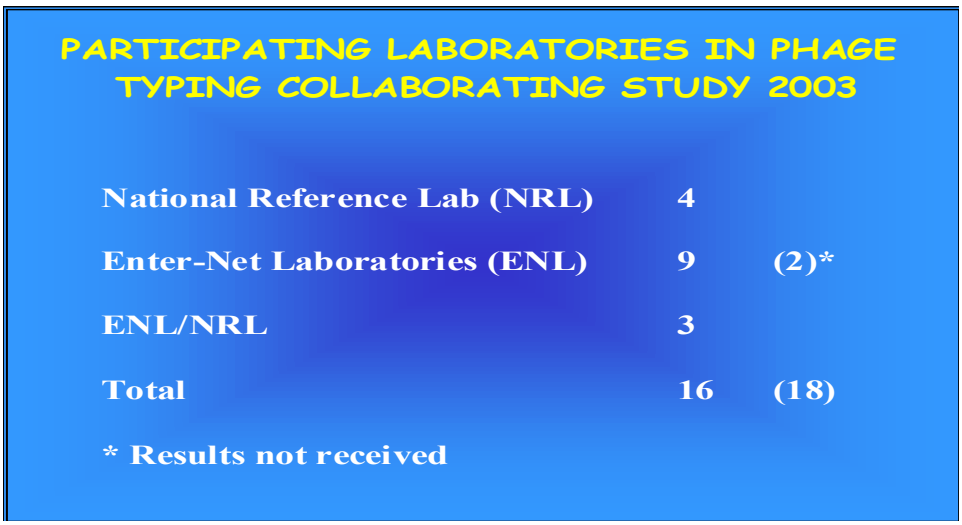
rivm CRL - Salmonella 33

Appendix 24. Slides of presentation 2.7

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	7	12	14	15
E1	4	4	4	4	4	4	4	4
E2	6a	6a	6a	6a	6a	6a	6a	6a
E3	8	8	8	8	8	8	8	8
E4	5c	5b	5	5c	5b/c	6	5c	5c
E5	14b	14b	14b	14b	14b	14b	14b	14b
E6	3	3	3	3	3	3	3	3
E7	1	1	1	1	1	1	1	1
E8	13a	13a	13a	13a	13a	13a	13a	13a
E9	21	21	21	21	21	21	21	21
E10	33	9b	33	33	9b	33	33	9b

Slide 4

Results of Salmonella *Enteritidis phage typing by the ENLs*

		Phage type of each laboratory										
Strain	PT	A	B	C	E	F	H	J	K	P	T	Y
E1	4	4	4	4	4	4	4	4	4		4	
E2	6a	6a	6a	6a	6a	6a	6a	6a	6a		6a	
E3	8	8	8	8	8	8	8	8	8		8	
E4	5c	5b	5	5c	5c	5c	5c	5c	NST		5b	
E5	14b	14b	14b	14b	14b	14b	14b	14b	14b		14b	
E6	3	20a	3	3	3	3	3	3	3		3	
E7	1	1	1	1	1	1	1	1	1		1	
E8	13a	28	13a	13a	13a	13a	13a	13a	13a		13a	
E9	21	21	21	21	22	21	21	21	21		21	
E10	33	9b	33	33	33	33	33	33	33		9b	

Slide 5

Strain E 4 *Salmonella* Enteritidis

		Phages 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
PHLS	5c	-	scl	+	scl	scl	scl	+	scl	ol	ol	+	scl	scl	-	-	-
3	5b	-	scl	-5	scl	± ±	±	±	ol	scl	ol	+l	scl	± ±	-	-	-
4	5	++	scl	++	± ±	scl	+++	<Scl	scl	scl	scl	+	cl	+++	± ±	-	+
12	6	-	scl	-	scl	-	scl	l	ol	ol	ol	-	-	+	-	-	-
A	5b	-	scl	+	scl	++	++	++	scl	++	scl	+	+++	+++	-	-	-
K	NST	+	cl	++	ol	+++	scl	+++ scl	ol	ol	ol	+++ scl	scL	scl	+		
T	5b	-	scL	+++	scl	+++	++ +	+	ol	ol	scl	+	ol	++ +	-	-	-
B	5	±	ol	++	scl	scl	++	++	ol	ol	ol	++	cl	scl	-	-	-

Slide 6

Strain E 10 *Salmonella* Enteritidis

		Phages 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
PHLS	33	-	-	-	-	-	-	-	-	-	-	-	-	-	cl	-	-
3	9b	-	-	+ +	-	< cl	-	-	-	-	-	-	< cl	-	< cl	-	-
7	9b	-	-	+ +	-	< scl	-	-	-	-	-	-	scl	-	cl	-	-
15	9b	-	-	scl	-	cl	-	-	-	-	-	-	cl	-	cl.	-	-
A	9b	+ + +	-	+ + +	-	scl	-	-	-	-	-	-	scl	-	scl	-	-
T	9b	-	-	cl	-	scl	-	-	-	-	-	-	cl	-	cl	-	-

Slide 7

Results of Salmonella *Typhimurium phage typing by the NRLs*

		Phage type of each laboratory						
Strain	PT	3	4	6	7	12	14	15
M11	4	4	4	4	4	Nt	4	4
M12	104L	104L	104L	104L	104	Nt	104	104L
M13	37	37	37	36	37	Nt	37	37
M14	193	193	193	193	193	Nt	193	193
M15	160	160	160	160	160	Nt	160	160
M16	141	141	141	141	141	Nt	RDNC	141
M17	2	2	2	2	2	Nt	2	2
M18	8	8	8	8	8	Nt	8	8
M19	40	40	40	40	40	Nt	40	40
M20	U302	U302	U302	U302	U302	Nt	U302	U302

Slide 8

Results of Salmonella *Typhimurium phage typing by the ENLs*

		Phage type of each laboratory										
Strain	PT	A	B	C	E	F	H	J	K	P	T	Y
M11	4	4	4	4	4	4	4	4	4		4	
M12	104L	104L	104L	104L	104	104H	104	104L	104		104	
M13	37	36	37	37	37	36	37	36	37		37	
M14	193	193	193	193	193	193	193	193	193		193	
M15	160	160	160	160	160	160	160	160	160		160	
M16	141	141	141	52A	141	141	141	4*	NST*		141	
M17	2	2	2	2	2	2	2	2	2		2	
M18	8	8	8	8	8	8	8	8	NST*		8	
M19	40	40	40	40	40	40	40	40	40		120	
M20	U302	U302	U302	U302	U302	U302	U302	U302	U302		U302	

* Correct Readings

Slide 9

Strain M 13 (A) *Salmonella* Typhimurium

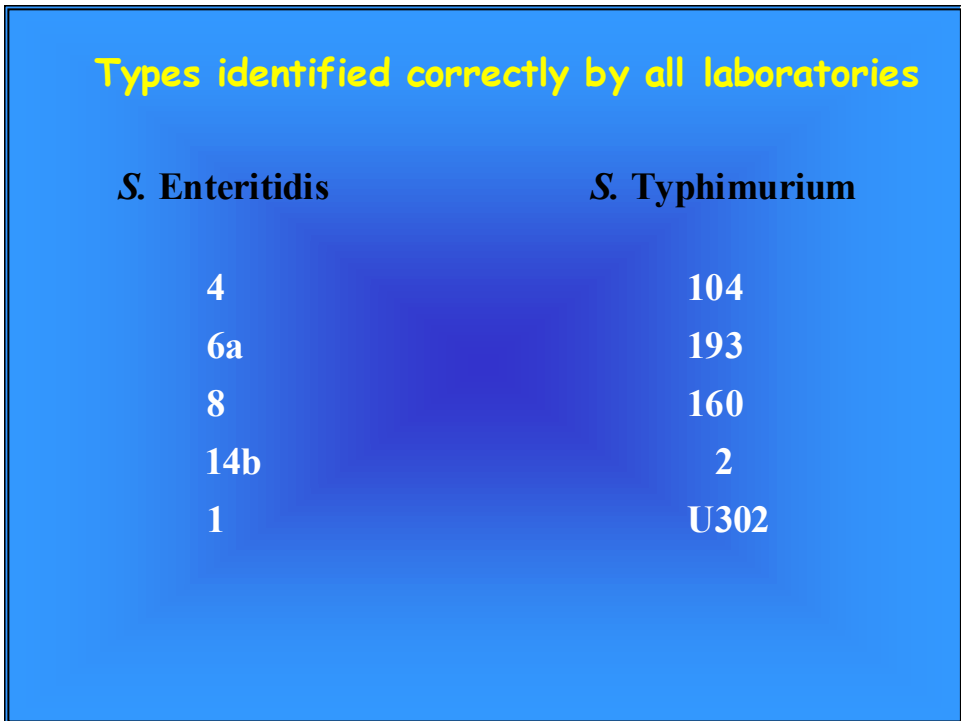
		Phages at Routine Test Dilution (*S.* Typhimurium)														
Lab code	Phage type	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16
PHLS	37	cl	scl	cl	cl	cl	cl	cl	cl	cl	cl	-	cl	cl	cl	cl
6	36	cl	scl	cl	cl	cl	cl	cl	cl	cl	cl	5n	ol	cl	cl	cl
A	36	ol	scl	ol	scl	scl	scl	ol	scl	scl	scl	++	scl	ol	ol	ol
J	36	cl	<cl	cl	cl	cl	cl	cl	cl	<cl	cl	<<	cl	cl	cl	cl

Slide 10

Strain M 16 (A) *Salmonella* Typhimurium

Lab code	Phage type	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	19
PHLS	141	-	-	-	+++	+++	±	-	-	cl	++	±	+	-	ol	ol	-	-	scl
14	RDNC	-	-	-	±	-	-	-	-	scl	++	±	±	-	<ol	scl	-	-	-
C	52A	-	-	-	scl	scl	-	-	-	cl	?	-	-	-	scl	scl	-	-	scl
J	4	-	-	-	scl	+++	+++	-	-	cl	cl	+	+	-	scl	scl	-	-	+++
K	NST	-	-	-	++	++	-	-	-	scl	+++	+	+	-	ol	ol	-	-	++

Slide 11



Slide 12

Summary *Salmonella* Enteritidis phage typing

% Correct	NRL	ENL	Total (%)
100	2	4	6 (38)
90	3	3	6 (38)
80	2	1	3 (19)
60	-	1	1 (6)
	7	8	16

Slide 13

Summary *Salmonella* Typhimurium phage typing

% Correct	NRL	ENL	Total (%)
100	4	4	8 (53)
90	2	3	5 (33)
80	-	2	2 (13)
	6	9	15

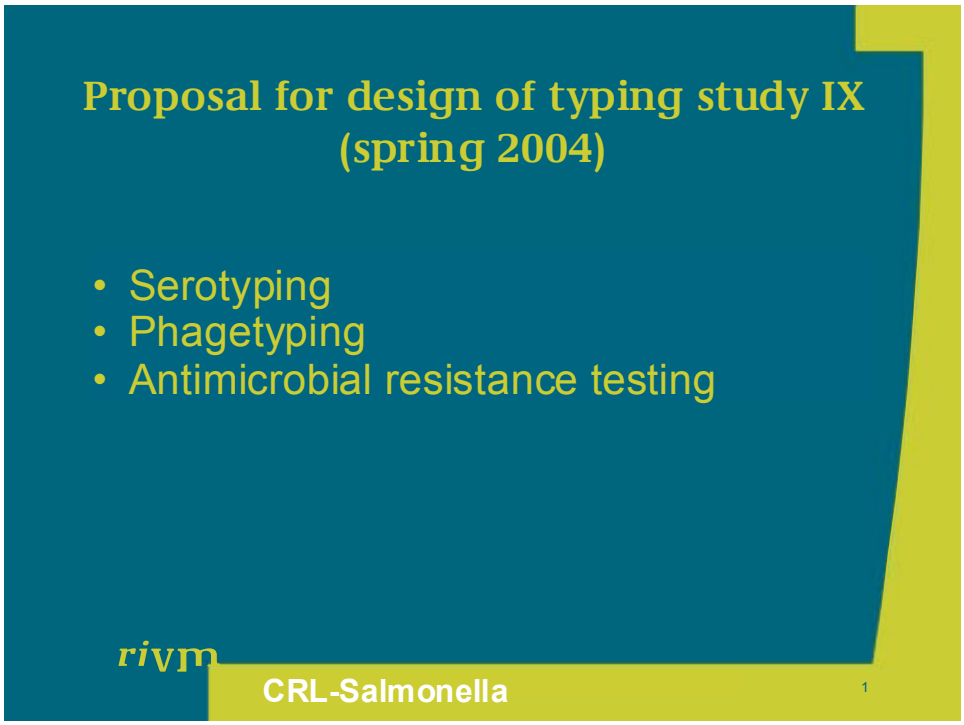
Slide 14

Summary Phage Typing Collaboration Study 2003

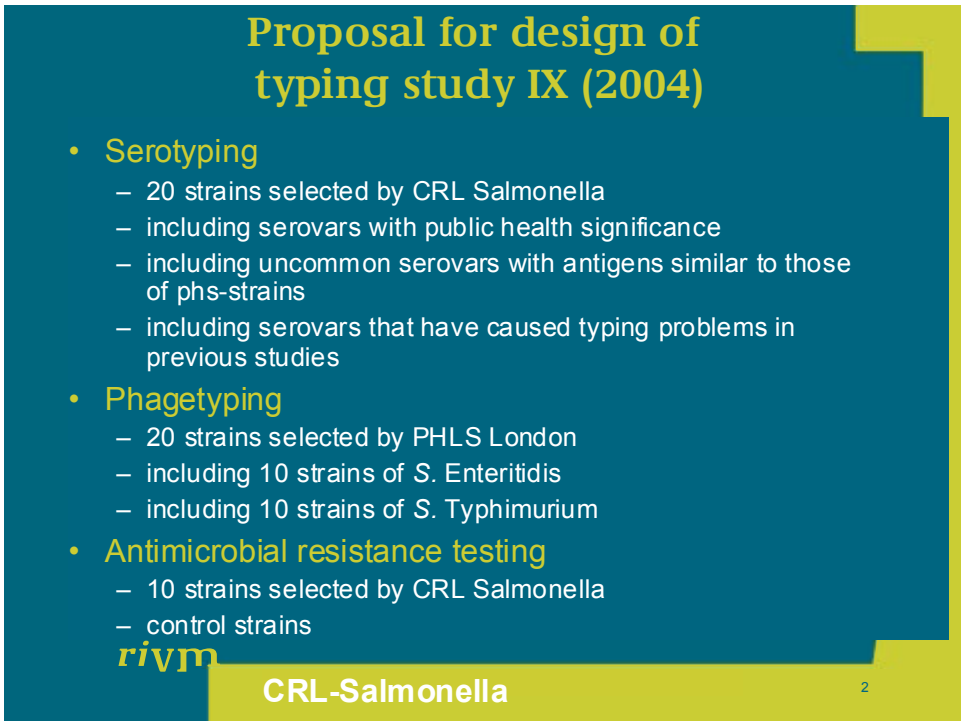
% Correct	NRL	ENL	Total (%)
100%	0	2	2 (13)
95%	5	3	8 (50)
90%	2	1	3 (19)
85%	-	2	2 (13)
75%	-	1	1 (6)
	7	9	16

Appendix 25. Slides of presentation 2.8

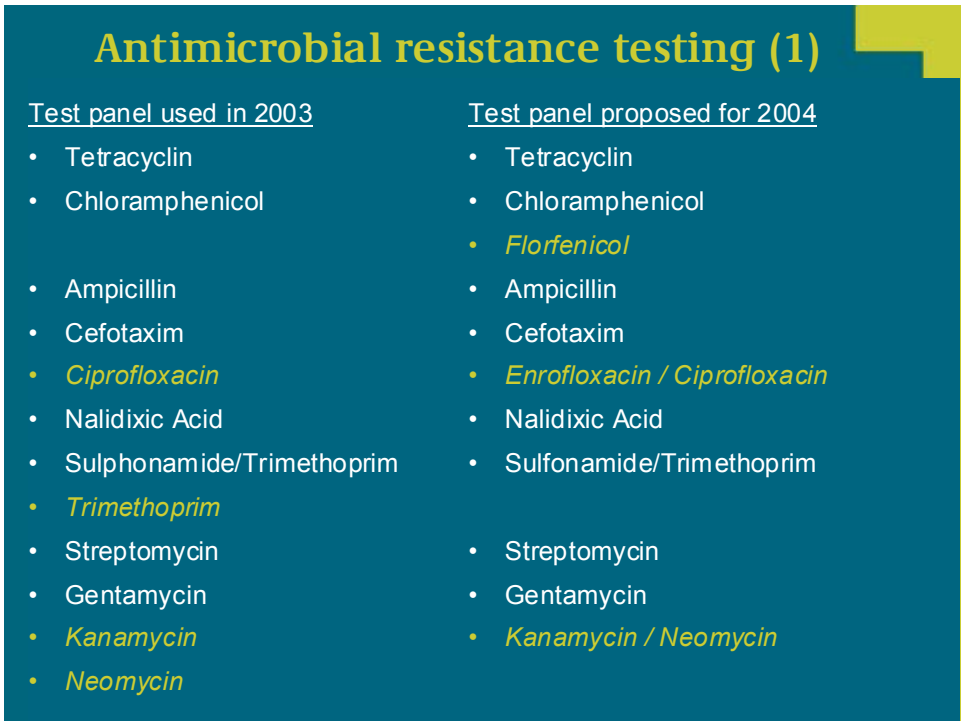
Slide 1



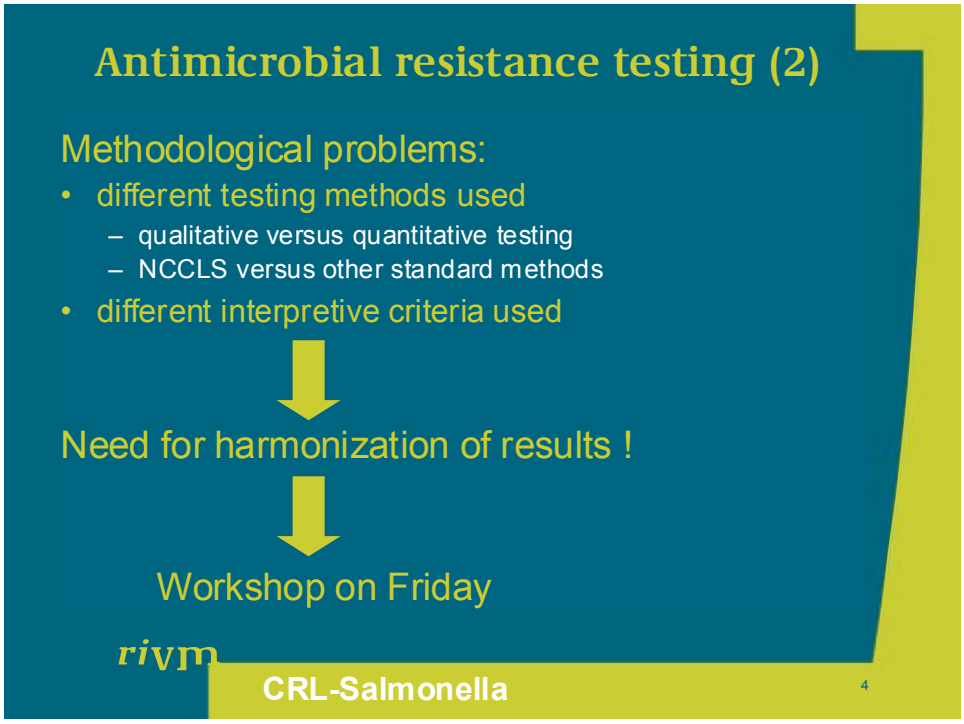
Slide 2



Slide 3

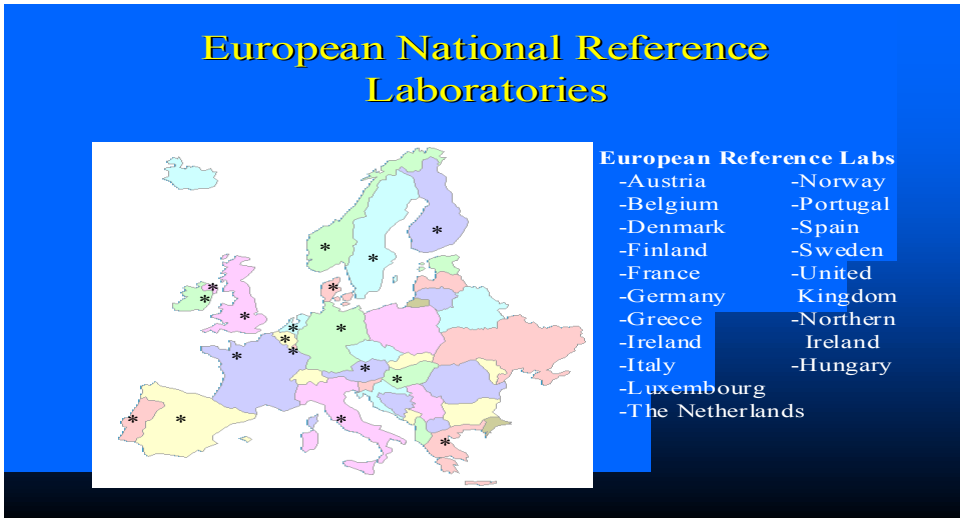


Slide 4



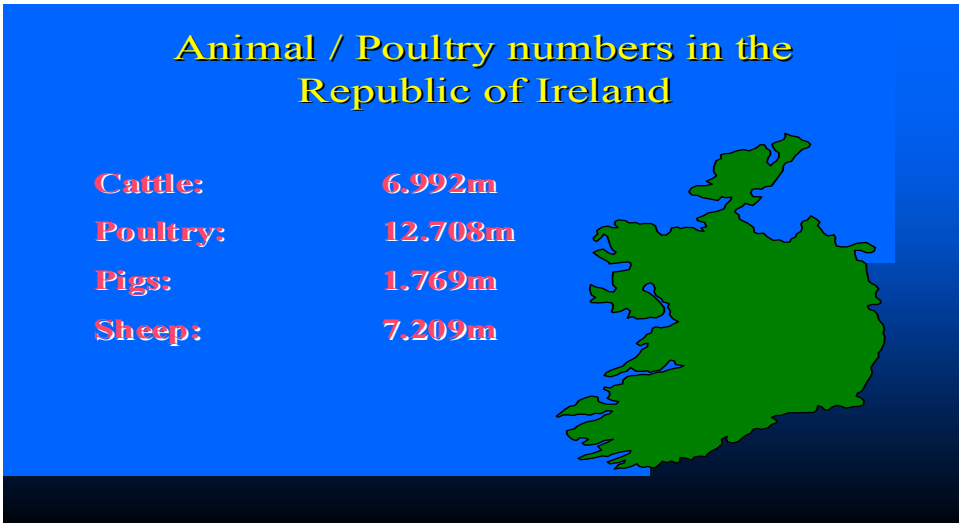
Appendix 26. Slides of presentation 2.9

Slide 1



Slide 2

Slide 3



Slide 4

CVRL the NRL for Salmonella in the ROI under the zoonoses directive 92/117/EEC

- Duties of the NRL, include:
  - Providing advice on various analytical methods
  - Examining official samples
  - Approving and monitoring private laboratories
  - Providing typing & antibiotic resistance testing
  - Organising comparative testing & improve standardisation of methods
  - Participating in CRL-collaborative studies

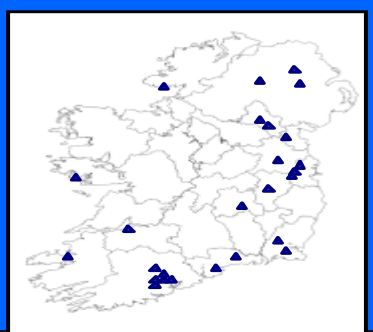
Slide 5

DAF Approved Laboratories-April 2003

- Advanced Micro Services
- Aire Laboratories
- Anser Laboratories
- Aqua Lab
- Biosearch
- City Biologic
- Complete Laboratory Solutions
- Consult-Us Laboratories
- Dairygold Pathogen Lab
- Envirolab
- Food Safety Lab, Cork Co. Co.
- Foodtech Consultants
- Foodtech Laboratories
- Independent Micro Lab
- Irish Equine Centre
- Microchem Laboratories
- Microlab
- Mid-Antrim Laboratory Services
- Monaghan Veterinary Lab
- National Food Centre, Dublin
- National Food Centre, Limerick
- Oldcastle Laboratories
- Q-Lab
- Ryland Research
- Slaney Foods
- Southern Scientific Labs

Slide 6

DAF Approved Private Labs

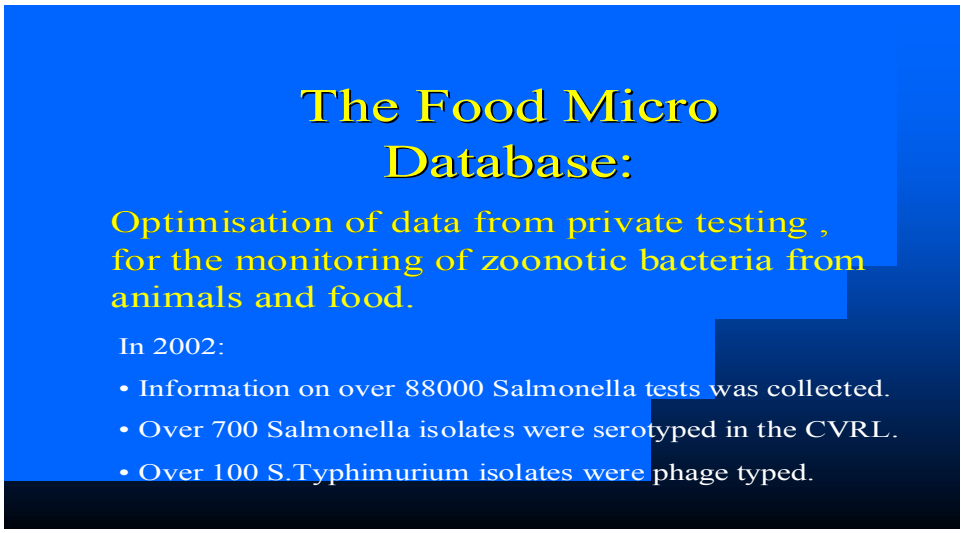


Currently there are 26 private labs approved by DAF.

-23 in ROI

-3 in NI, (testing samples from ROI)

Slide 7



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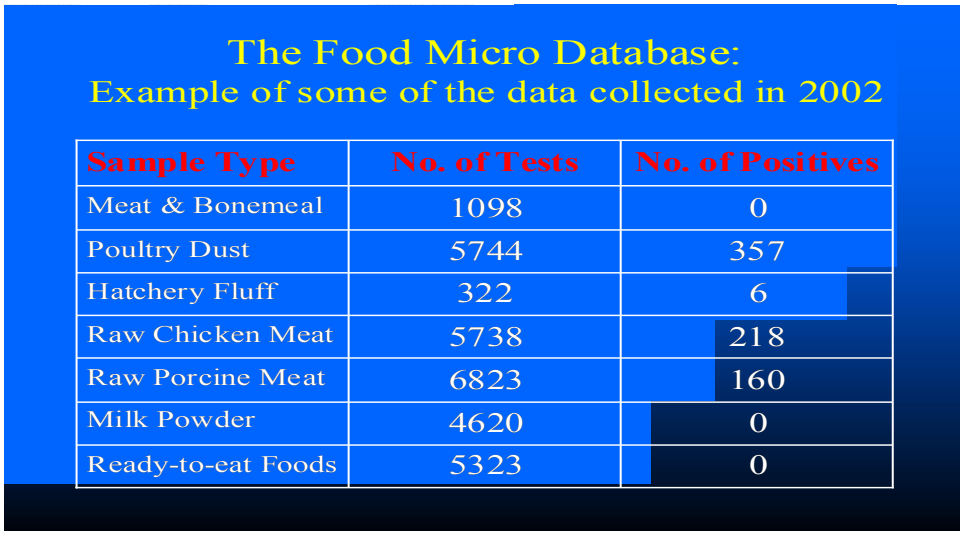

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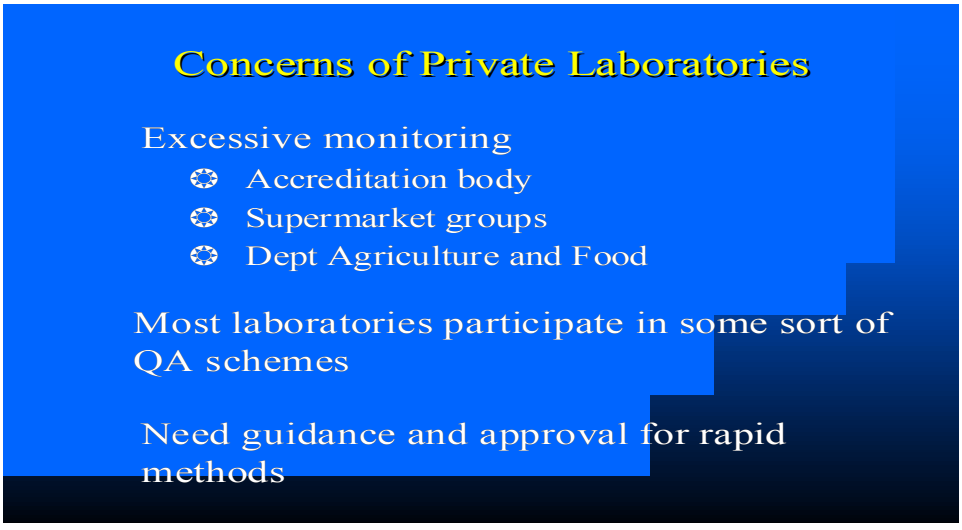

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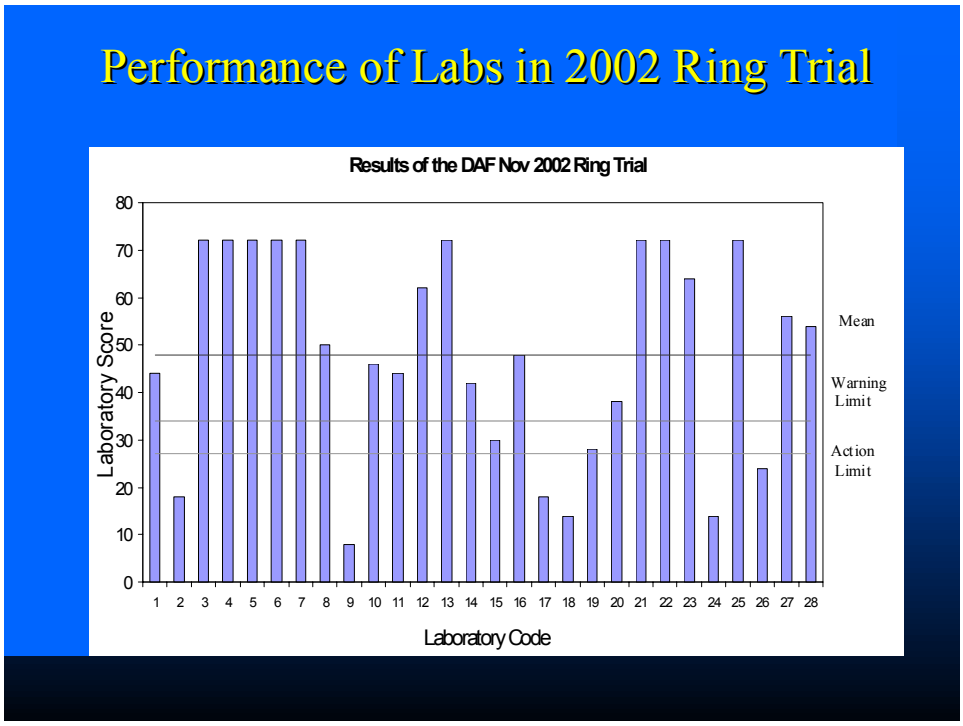
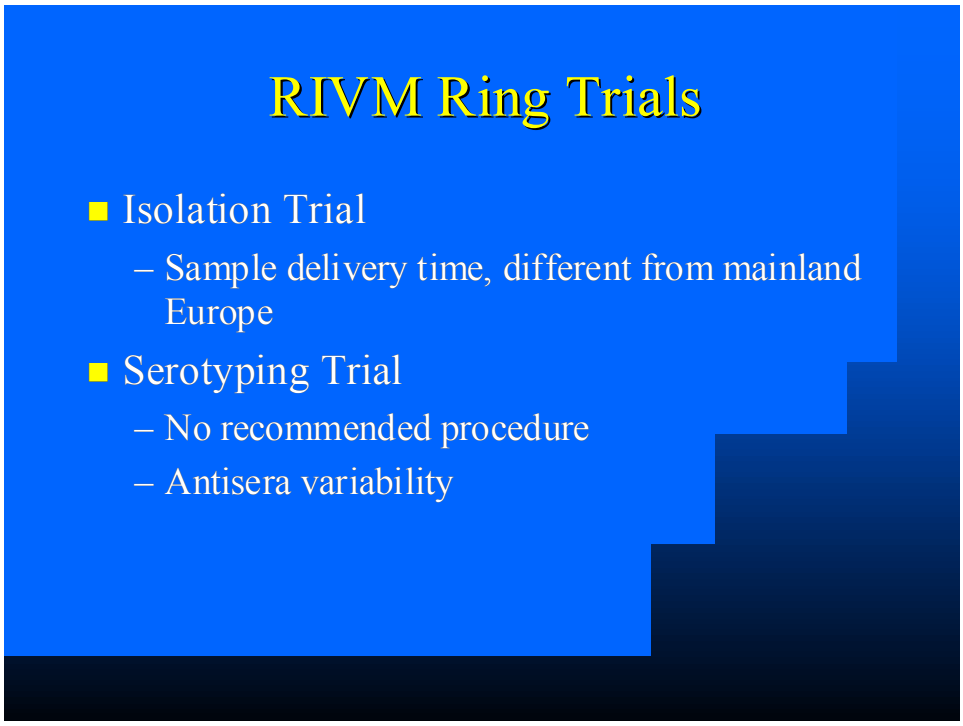


Slide 11

Slide 12

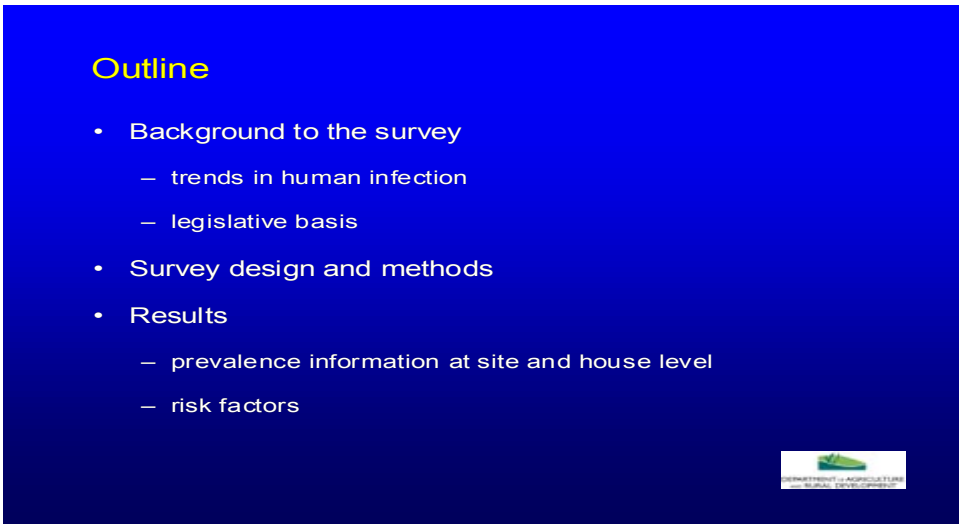


Slide 13

Appendix 27. Slides of presentation 2.10

Slide 1

Slide 2



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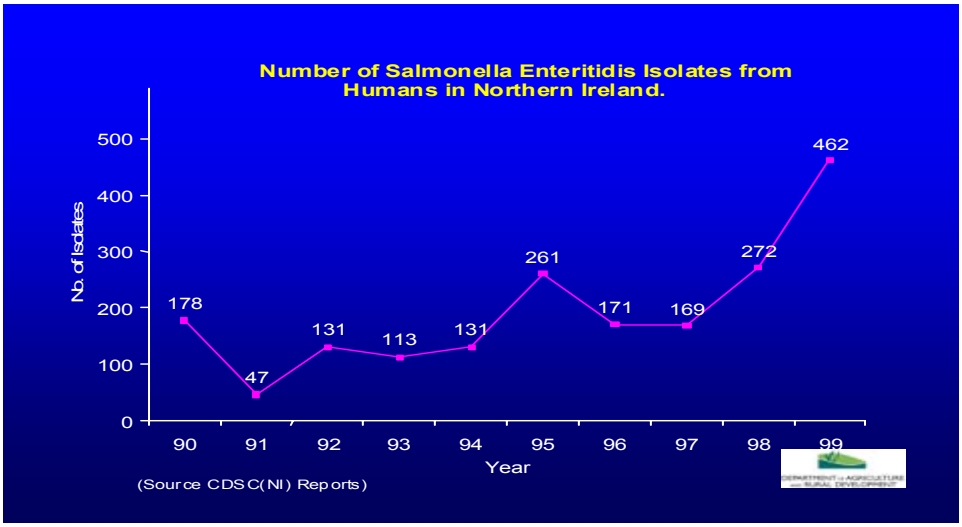

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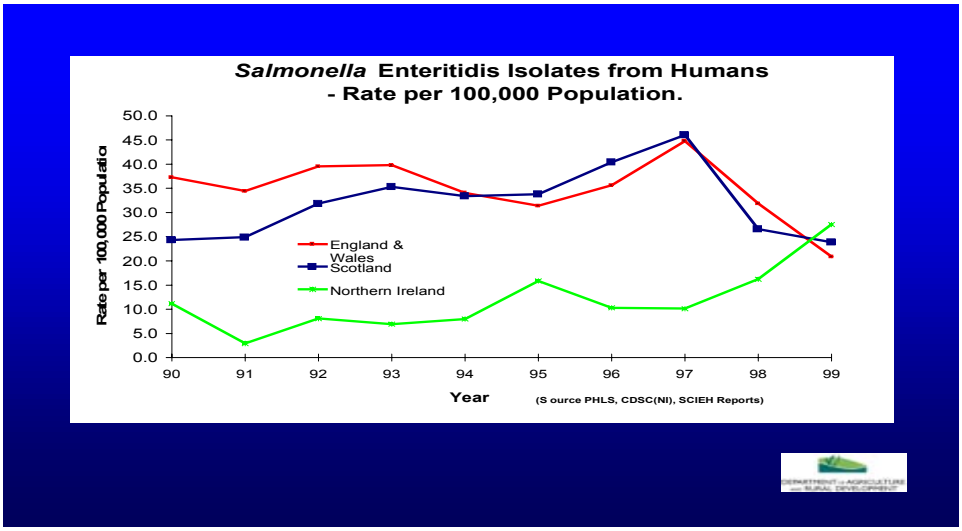


Slide 5

Legislative Basis for Salmonella Testing

- Legislation requiring testing of poultry flocks was first introduced in GB in 1989 & in NI in 1990
  - applied to both breeding flocks and commercial egg layers
- Requirement to test commercial egg layers later revoked following the 1993 ACMSF report on salmonella in eggs
- Since 1993, testing of commercial flocks has been voluntary and DARD data limited to laboratory notifications
- The Zoonoses Order (NI) 1991 does however have general powers to investigate where there is suspicion of infection

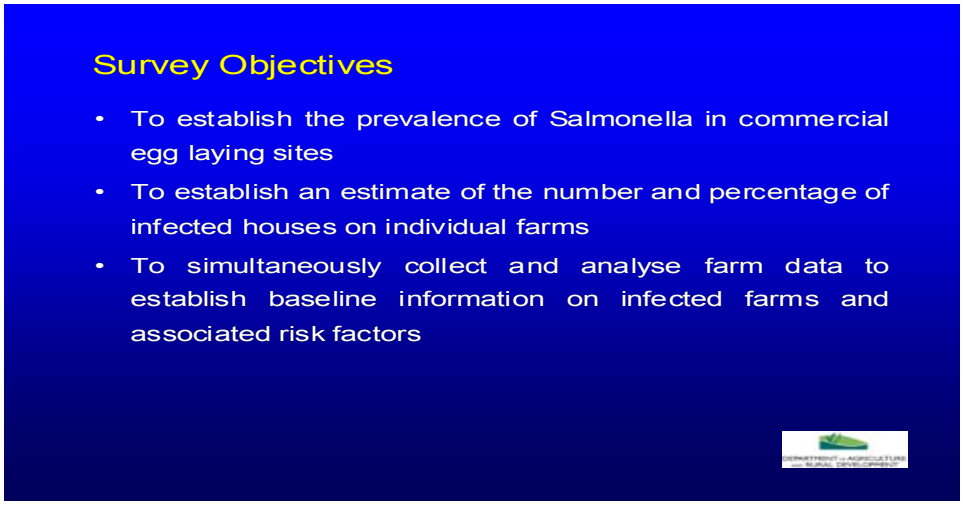
Slide 6

NI Egg Laying Industry

- Commercial layers*
  - Total flock size ~2.3 million (~6% of the UK total)
  - 1253 farms with egg laying birds
  - 129 have >1,000 birds and account for >99% of the population
  - 1124 with <1,000 birds and account for < 1%
- Rearing pullets*
  - Total estimated ~0.8 million
  - 38 have >1,000 birds and account for >99% of the population
- Free range / perchery barn systems account for ~ 11% of birds

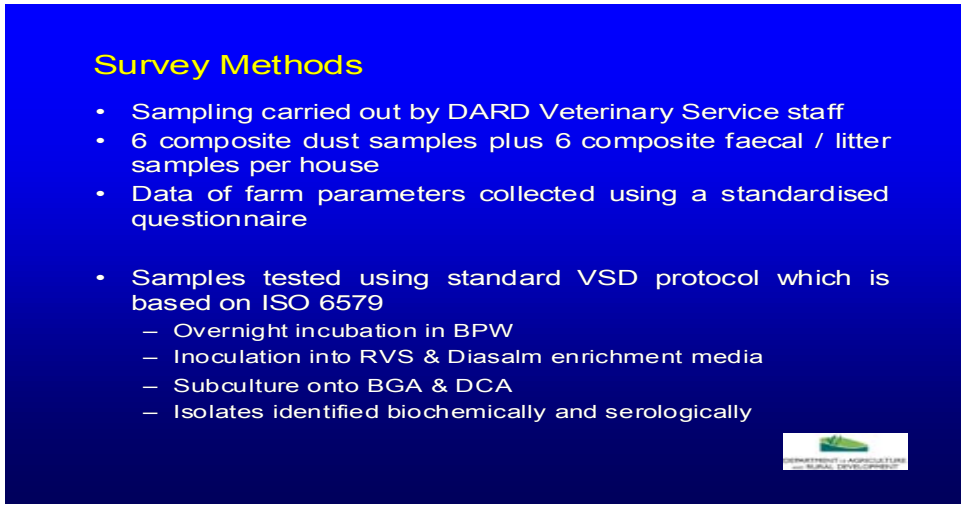
*Source DARD June 2000 census data

Slide 7



Slide 8

Slide 9



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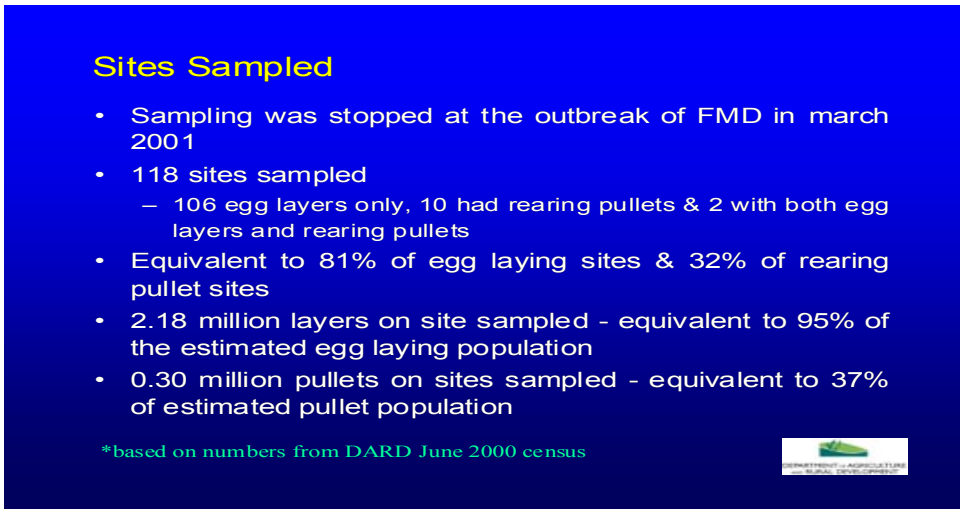

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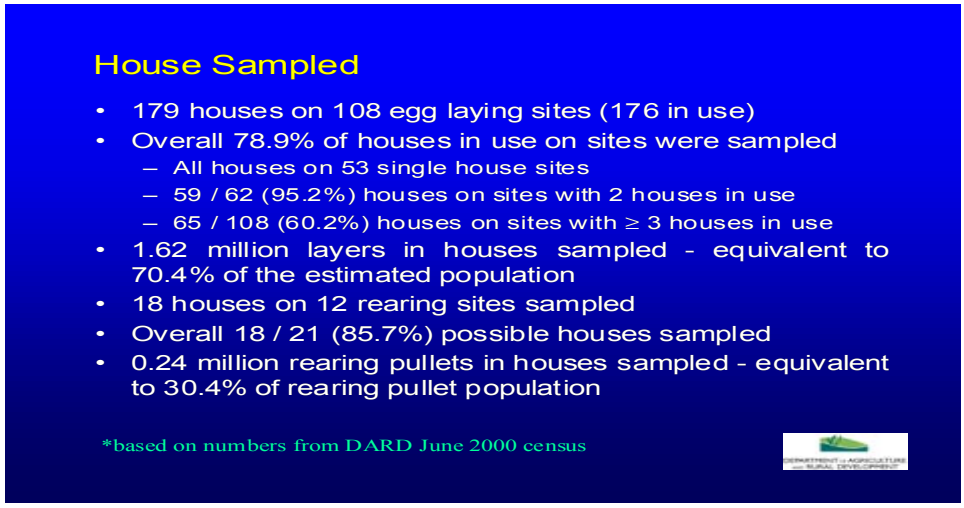

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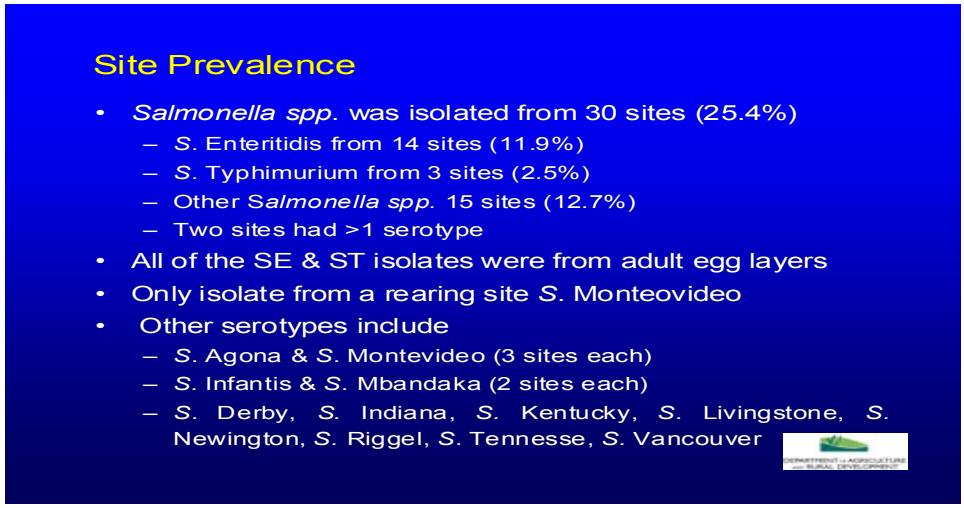

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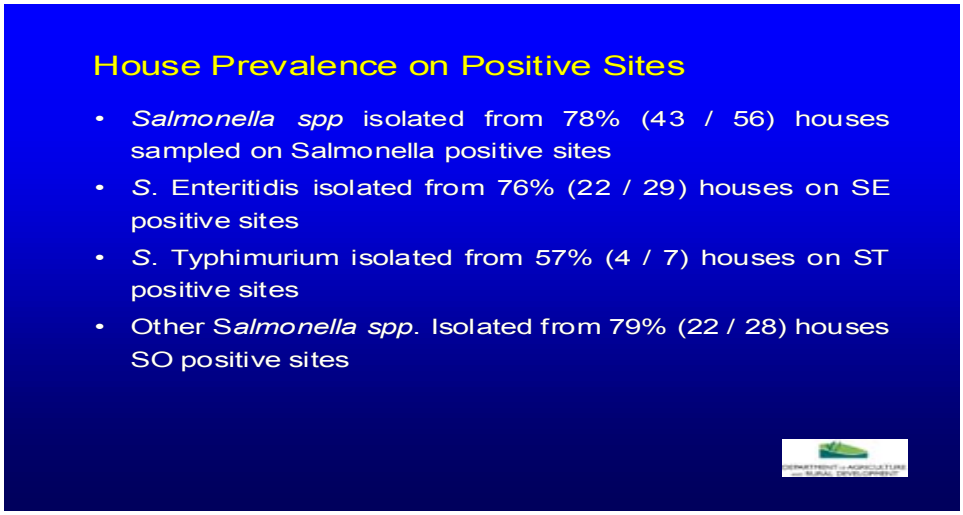

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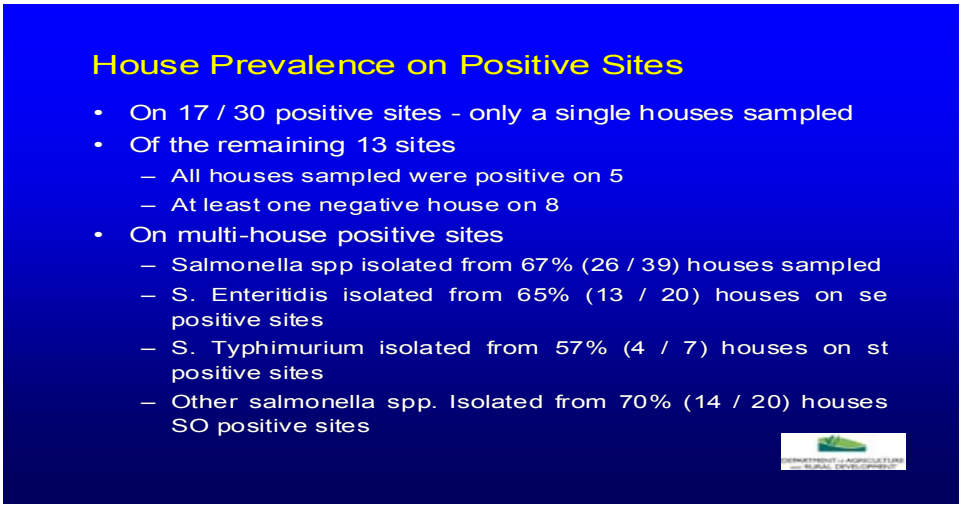

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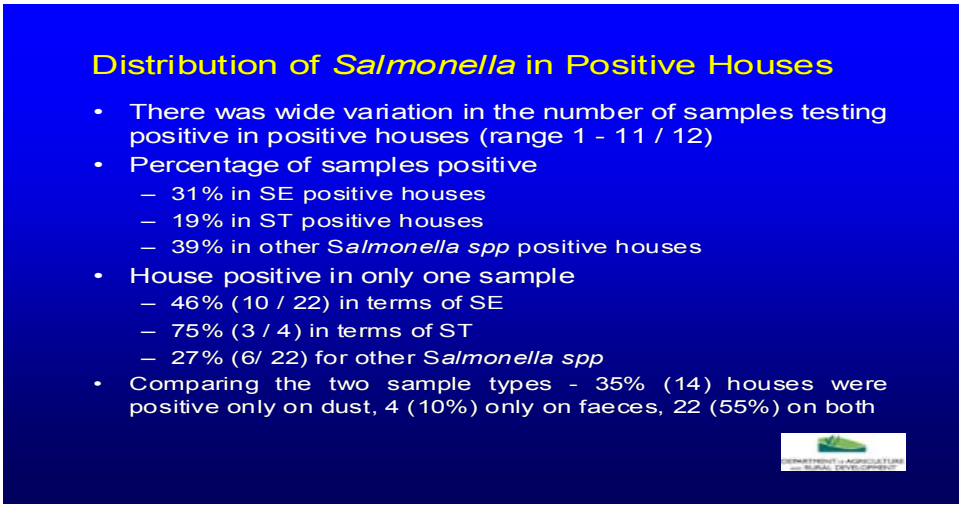


Slide 16

Univariate Analysis for *S. Enteritidis*

Factor		OR	95% CI	P-value
Houses in use	1	1.00	-	-
	2	0.27	0.03 – 2.44	0.203
	≥ 3	3.92	1.07 – 13.90	0.040
Layers on site	<10K	1.00	-	-
	10K – <20K	2.42	0.49 – 11.88	0.416
	≥20K	8.17	1.66 – 40.30	0.005
Feed – home-mixer		12.00	1.62 – 88.78	0.017
Rodent control by contractor		0.20	0.05 – 0.73	0.015
VSD History of SE on site		8.89	2.17 – 36.49	0.002
Vaccination (Not vacc)		2.12	0.63 – 7.19	0.330
Free - range		0.17	0.02 – 1.43	0.101

Slide 17

Vaccination in Previously Positive Sites

Vaccination Status	SE Status		
	Positive	Negative	Total
No	3	1	4
Yes	2	6	8
	5	7	12

OR 9.00; P=0.22

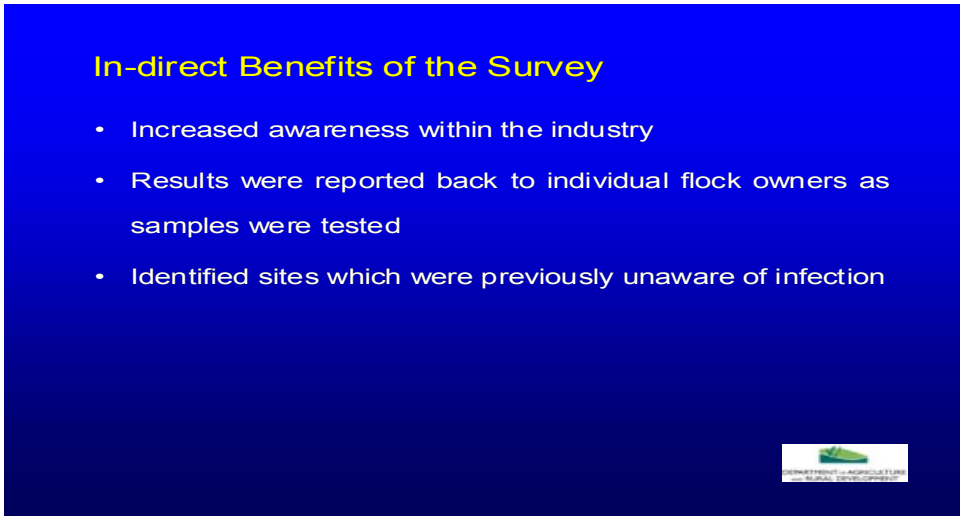
One site with mixed vaccination status excluded

Slide 18

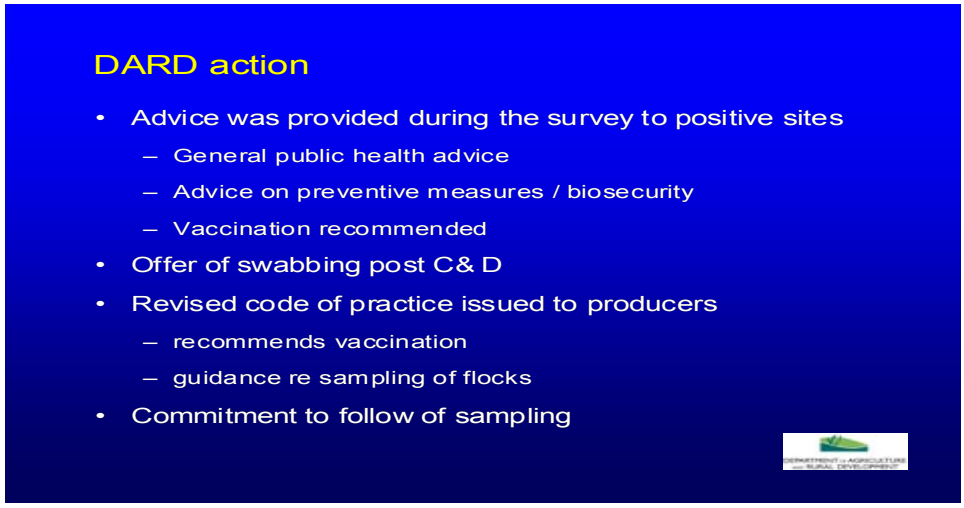
Univariate Analysis for Other *Salmonella Spp.*

Factor		OR	95% CI	P-value
Layers on site	<10K	1.00	-	-
	10K – <20K	2.42	0.49 – 11.88	0.416
	≥20K	8.17	1.66 – 40.30	0.005
VSD History of SO on site		12.00	1.62 – 88.78	0.017
Vaccination (Not vacc)		1.29	0.34 – 4.89	0.735

Slide 19



Slide 20



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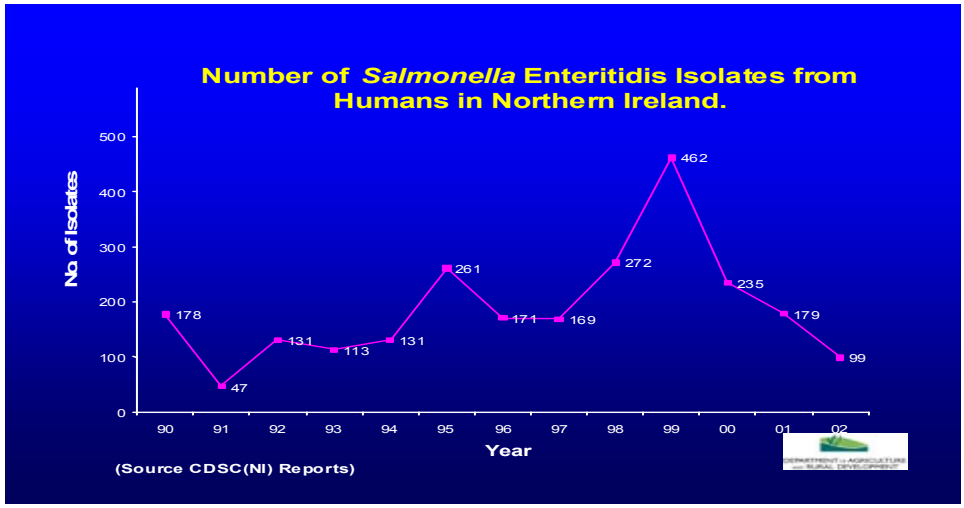

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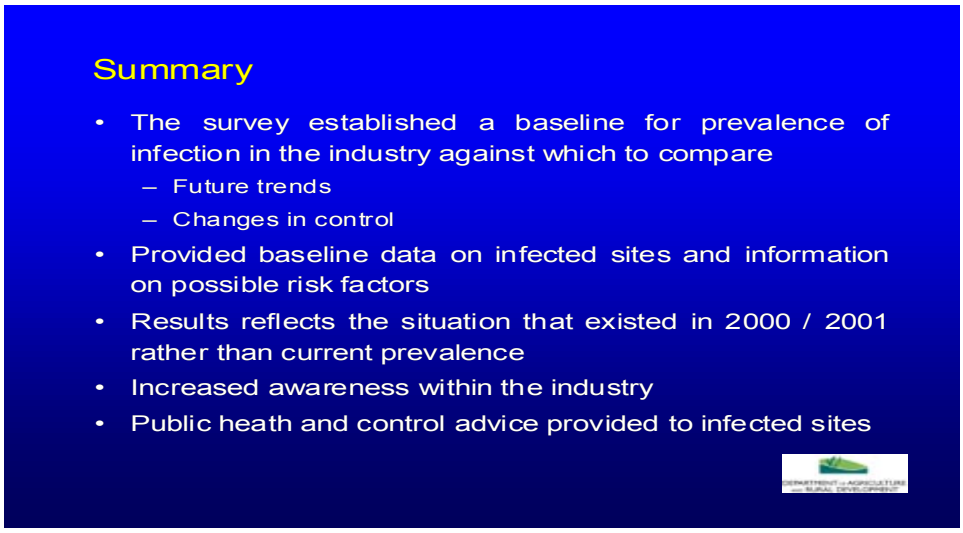
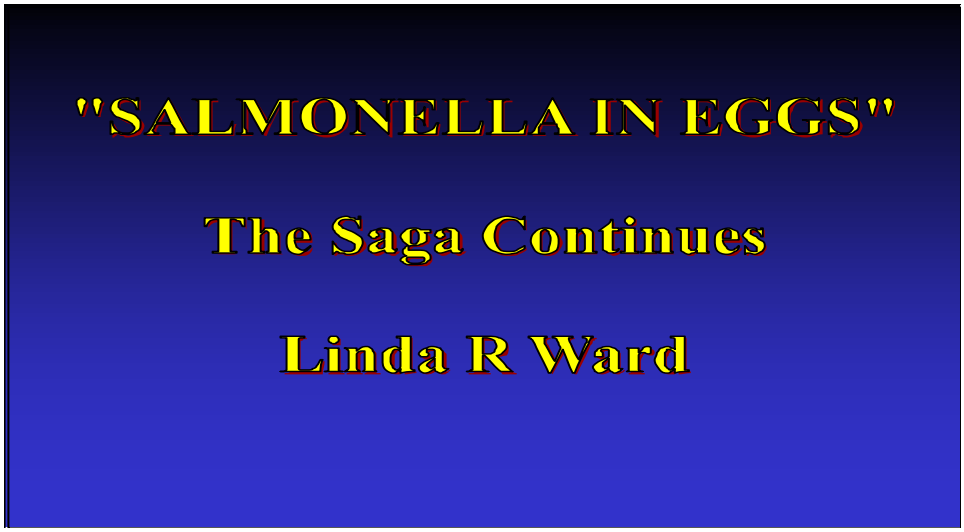


Slide 23

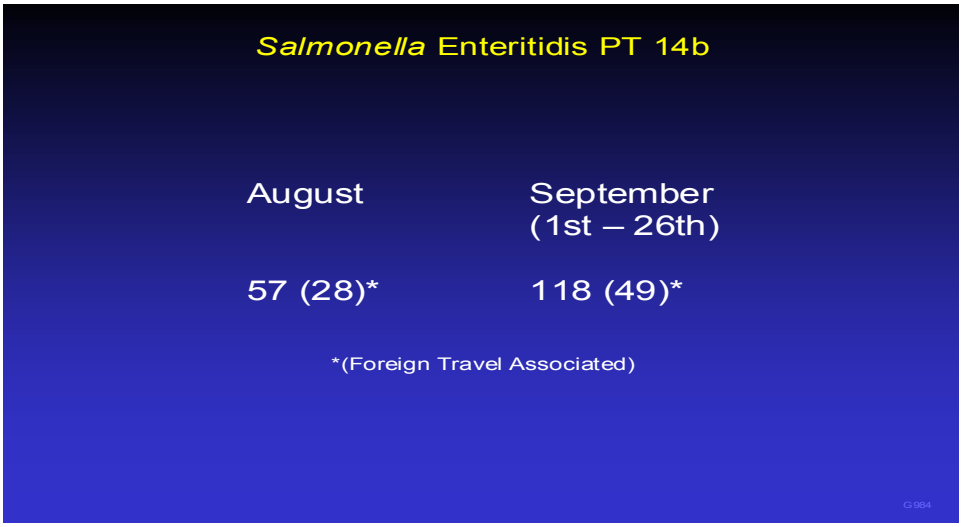
Appendix 28. Slides of presentation 2.11

Slide 1



Slide 2

Slide 3



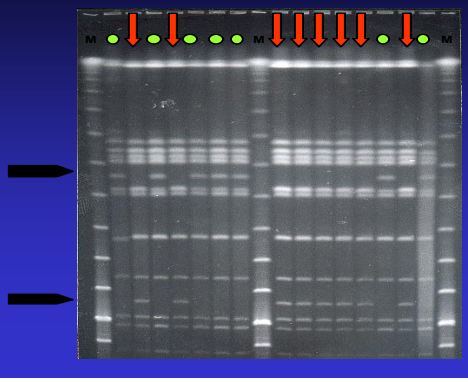
Slide 4

Salmonella Enteritidis PT 14b
26th September – 22nd October 2002

Country	Gas	Total
Greece	Anaerogenic	17
Portugal)		1
Belgium)	Aerogenic	1
Italy)		1
Other)		4
England	Aerogenic	>217

Slide 5

Salmonella Enteritidis PT14b Sept/Oct 2002
DNA Fingerprints



M Control
Anaerogenic PT14b
Outbreak PT14b differs by two bands

Slide 6

Salmonella Enteritidis PT 14b
National Outbreak

Location	Source		PT egg isolates
NW Cheshire	Bakery)))	Spanish eggs	PT6a
Basingstoke	Bakery)))		
Ormskirk	Chinese restaurant	? Eggs	?
Southwark	Primary school	Eggs	?

Slide 7

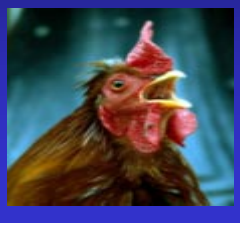
Salmonella Enteritidis PT 6a

Outbreak	Source	Eggs
London Hospitals	Eggs	PT 6a ($NxCp_L$) PT 14b - National OB PT 13a - Increase PT 6 - S.West OB PT 58 - ?

Slide 8

Egg Samples (Public Health Investigation)
October 2002-January 2003

Batches	Number of eggs	Number of pools	Number positive	% positive
29+	3960+	651	35	5.3%



Communicable Dis. Rep. CDR Wkly 2003 9th January

Slide 9

Egg Samples (Public Health Investigation)
October 2002-January 2003

Country of origin	Number of pooled samples	Number positive	% positive
Spain	449	23	5.1
UK (not Lion Quality)	74	1	1.3
UK Lion Quality	8	0	0
? Country not known	40	11	27.5
USA	60	0	0
Total		35	5.3

Communicable Dis. Rep. CDR Wkly 2003 9th January

Slide 10

Salmonella Egg Isolates October 2002-January 2003

Serotype	Country of origin	Positive pooled samples	Laboratory
S. Enteritidis	Spain) UK) Imported)	25	(Chelmsford (Leicester (London (Southampton
S. Altona	?	1	Chelmsford
S. Cerro	?	1	London
S. Infantis	?	5	Chelmsford
S. Livingstone	?	1	London
S. Ohio		2	Chelmsford

G984

Slide 11

Salmonella Enteritidis - Egg related Human England & Wales 2001-2002

Phage type	R-type	2001	2002
1	$NxCp_L$	892	1123
1c	Amp^R	10	20
4		4847	3575
5c		352	141
6		869	774
6a	Amp^R	294	464
6a	$NxCp_L$	22	187
6d	Amp^R	9	56
12	$NxCp_L$	4	20
13a		65	77
14b		375	661
14b	$NxCp_L$	5	18
58		-	2

LEP data

G984

Slide 12

Salmonella Enteritidis - Egg related Human England & Wales 2001-2002

Phage type	R-type	2001	2002	% Rise
1	$NxCp_L$	892	1123	21
6a	Amp^R	294	464	37
6a	$NxCp_L$	22	187	88
6d	Amp^R	9	56	84
12	$NxCp_L$	4	20	80
14b		375	661	43
14b	$NxCp_L$	5	18	73

LEP data

G984

Slide 13

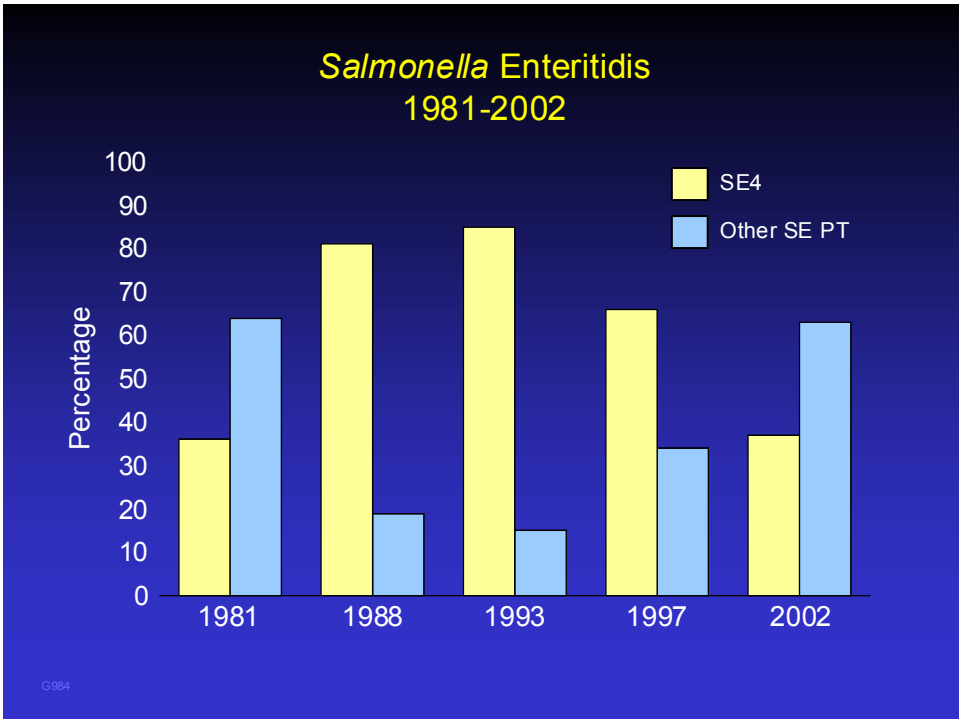
Selected *Salmonella* Enteritidis 'Outbreaks' 2002

Outbreak	PT	R-type	'Source'
Sandwich bar	1	$NxCp_L$	Homemade mayonnaise
Hospital	6a	$NxCp_L$	Spanish eggs*
Hospital	1	$NxCp_L$	Spanish eggs?, chicken
General	14b	-	Spanish eggs*
School	14b	-	Spanish eggs*
Chinese restaurant	14b	-	Spanish eggs*

*Human isolates and egg isolates are indistinguishable by plasmid profile and PFGE analysis

G984

Slide 14



Slide 15

Slide 16

Appendix 29. Slides of presentation 2.12

Slide 1

Evaluation of Pooled Serum and 'Meat Juice' in a *Salmonella* ELISA for Pig Herds

Rob Davies, Peter Heath, Sue Coxon & Robin Sayers

Veterinary Laboratories Agency, Weybridge, UK

Data derived from research funded by Meat and Livestock Commission & Defra

Grateful thanks to staff at VLA Bury St Edmunds and VLA Weybridge, MLC samplers, Farms and abattoirs

MJE SLIDE 1

Slide 2

Background

- *Salmonella* found in 23% UK pigs at slaughter
- Multiple resistant *S.*Typhimurium DT104 complex predominant
- New resistant *S.*Typhimurium DTs emerging
- Relative risk of *Salmonella* pig meat to humans in UK unknown
- Danish *Salmonella* Control Policy
- Cost of structured surveillance high in economically depressed UK pig industry

MJE SLIDE 2

Slide 3

Sampling

- 20 Commercial Pig Finishing Farms (*Salmonella* history unknown)
- 420 Pigs - one batch per farm
- Farm: visited day before slaughter - gauze swab swept through dunging area in pens to be slaughtered
- At slaughter:
  - pigs tagged - blood collected
  - guts tagged - caecum ligated and collected
  - carcase swabbed (USDA/Danish method) at meat inspection point
  - diaphragm muscle collected

MJE SLIDE 3

Slide 4

Testing

- Farm faeces samples (25g), caecal samples (25g) and carcase swabs cultured same day
  - BPW (18hr/37°C) --- Diassalm (24/48h/41.5°C) --- Rambach (24hr, 37°C)
- Serum and diaphragm samples frozen -20°C --- tested as batch
- Serum/meat juice from each farm tested as individuals, pools 5, pools 10, pools 20
- Guildhay ELISA kit - manufacturer's instructions
- *Salmonella* isolates fully serotyped and phagetyped
- Statistics : Herd level - Statistica (Statsoft, Inc)

MJE J SLIDE 4

Slide 5

Table 1: **Blood Sample Serology for Pigs from 20 Farms: Mean Results by Farm**

	Individual sera (IS)					Pooled Sera							
						5's		10's		20's		IS	SP 20's
Ref	OD	SP	N	N+ve	% +ve	OD	SP	OD	SP	OD	SP	% cat.	OD cat.
P0134	0.12	0.12	22	3	13.6	0.12	0.17	0.12	0.15	0.12	0.17	<=17%	0.1-0.4
P0135	0.18	0.21	22	5	22.7	0.19	0.20	0.20	0.22	0.18	0.18	18-50%	0.1-0.4
P0182	0.09	0.10	22	1	4.5	0.09	0.08	0.11	0.17	0.08	0.05	<=17%	<0.1
P0183	0.12	0.26	21	7	33.3	0.13	0.26	0.14	0.30	0.18	0.47	18-50%	>0.4
P0189	0.12	0.11	22	4	18.2	0.15	0.25	0.13	0.19	0.16	0.28	18-50	0.1-0.4
P0193	0.08	0.02	19	0	0.0	0.09	0.08	0.10	0.08	0.07	0.02	<=17%	<0.1
P0217	0.40	0.53	22	15	68.2	0.40	0.56	0.47	0.66	0.39	0.55	>50%	>0.4
P0276	0.23	0.37	22	9	40.9	0.23	0.66	0.28	0.86	0.32	1.05	18-50%	>0.4
P0277	0.22	0.33	22	8	36.4	0.22	0.63	0.21	0.59	0.23	0.68	18-50%	>0.4
P0306	0.09	0.03	22	1	4.5	0.07	0.02	0.09	0.08	0.10	0.09	<=17%	<0.1
P0320	0.26	0.36	20	10	50.0	0.28	0.33	0.30	0.37	0.35	0.44	18-50%	>0.4
P0328	0.08	0.03	22	0	0.0	0.08	0.05	0.09	0.07	0.09	0.08	<=17%	<0.1
P0329	0.08	0.03	22	0	0.0	0.08	0.05	0.08	0.05	0.09	0.07	<=17%	<0.1
P0353	0.21	0.26	20	6	30.0	0.19	0.21	0.21	0.24	0.19	0.20	18-50%	0.1-0.4
P0382	0.15	0.15	20	2	10.0	0.13	0.11	0.14	0.12	0.15	0.14	<=17%	0.1-0.4
P0399	0.20	0.23	22	7	31.8	0.18	0.45	0.18	0.47	0.19	0.50	18-50%	>0.4
P0400	0.18	0.19	22	6	27.3	0.21	0.59	0.18	0.47	0.18	0.46	18-50%	>0.4
P0466	0.48	0.72	22	12	54.5	0.82	1.18	0.83	1.19	0.96	1.39	>50%	>0.4
P0498	0.21	0.26	22	4	18.2	0.29	0.36	0.21	0.23	0.22	0.26	18-50%	0.1-0.4
P0516	0.15	0.23	22	9	40.9	0.16	0.39	0.17	0.14	0.22	0.32	18-50%	0.1-0.4
Overall Mean Result	0.18	0.23	430	109	25.3	0.21	0.33	0.21	0.33	0.22	0.37	NA	NA

MJE SLIDE 5

Slide 6

Table 2: **Meat Juice Serology Results for Pigs from 20 Farms: Mean results by farm**

	Neat juice					5's		10's		20's		
Ref	OD	SP	N	N+ve	%+ve	OD	SP	OD	SP	OD	SP	SP cat.
P0134	0.18	0.26	21	7	33.3	0.27	0.25	0.28	0.26	0.08	0.00	<0.1
P0135	0.24	0.28	21	6	28.6	0.28	0.32	0.30	0.34	0.30	0.35	0.1-0.4
P0182	0.07	0.03	22	0	0.0	0.09	0.01	0.08	0.00	0.11	0.03	<0.1
P0183	0.18	0.13	20	5	25.0	0.28	0.19	0.19	0.11	0.17	0.09	<0.1
P0189	0.37	0.38	21	10	47.6	0.53	0.43	0.73	0.62	0.76	0.64	>0.4
P0193	0.18	0.23	19	7	36.8	0.23	0.20	0.23	0.19	0.22	0.18	0.1-0.4
P0217	0.48	0.70	22	16	72.7	0.58	0.76	0.60	0.80	0.57	0.75	>0.4
P0276	0.55	0.69	22	16	72.7	0.99	0.88	0.92	0.81	0.83	0.73	>0.4
P0277	0.62	0.79	22	12	54.5	0.95	0.84	1.04	0.93	0.92	0.81	>0.4
P0306	0.31	0.48	22	9	40.9	0.44	0.46	0.51	0.55	0.32	0.30	0.1-0.4
P0320	0.32	0.65	20	12	60.0	0.44	0.67	0.42	0.64	0.42	0.64	>0.4
P0328	0.19	0.16	22	5	22.7	0.15	0.20	0.14	0.18	0.17	0.24	0.1-0.4
P0329	0.10	0.05	22	0	0.0	0.09	0.08	0.09	0.07	0.08	0.06	<0.1
P0353	0.26	0.50	20	13	65.0	0.29	0.40	0.30	0.42	0.29	0.40	0.1-0.4
P0382	0.19	0.22	19	4	21.1	0.19	0.23	0.18	0.20	0.17	0.19	0.1-0.4
P0399	0.63	0.51	21	14	66.7	0.65	0.55	0.71	0.62	0.74	0.64	>0.4
P0400	0.70	0.57	22	16	72.7	0.65	0.55	0.63	0.54	0.82	0.72	>0.4
P0466	0.64	0.87	19	12	63.2	1.01	1.68	1.12	1.89	1.14	1.92	>0.4
P0498	0.27	0.32	22	8	36.4	0.26	0.36	0.31	0.39	0.30	0.35	0.1-0.4
P0516	0.51	0.41	22	10	45.5	0.63	0.53	0.64	0.55	0.72	0.62	>0.4
Overall Mean Results	0.35	0.41	421	182	43.2	0.45	0.48	0.47	0.51	0.46	0.48	NA

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Table 3: **Bacteriological Results from Carcase Swabs, Caeca and Bulked Pen Faeces for Pigs from 20 Farms**

Ref	Carcase swabs N	N+ve	Typh. N+ve	%+ve	Typh. %+ve	Caecum N	N+ve	Typh. N+ve	N+ve	Typh. %+ve	%+ve	Faeces N	Typh. N+ve	N+ve	Typh. %+ve
P0134	22	2	2	9.1	9.1	21	11	8	52.4	38.1		19	14	9	73.7	47.4[c,b,m,j]
P0135	21	0	0	0.0	0.0	21	4	4	19.0	19.0		19	7	7	36.8	36.8[e]
P0182	22	0	0	0.0	0.0	22	0	0	0.0	0.0		20	6	0	30.0	0.0[g]
P0183	21	0	0	0.0	0.0	21	3	3	14.3	14.3		20	2	2	10.0	10.0[f,o]
P0189	20	0	0	0.0	0.0	20	3	2	15.0	10.0		15	1	1	6.7	6.7[f,b,j,e]
P0193	19	0	0	0.0	0.0	19	8	0	42.1	0.0		20	4	0	20.0	0.0[j]
P0217	22	1	1	4.5	4.5	22	7	3	31.8	13.6		15	8	2	53.3	13.3[n,e]
P0276	22	0	0	0.0	0.0	20	8	8	40.0	40.0		20	9	9	45.0	45.0[e,b]
P0277	22	1	1	4.5	4.5	22	4	4	18.2	18.2		20	5	5	25.0	25.0[f]
P0306	20	0	0	0.0	0.0	20	13	13	65.0	65.0		20	5	5	25.0	25.0[e,a]
P0320	20	0	0	0.0	0.0	20	6	6	30.0	30.0		19	12	11	63.2	57.9[e,a,n]
P0328	22	0	0	0.0	0.0	22	0	0	0.0	0.0		17	2	2	11.8	11.8[a]
P0329	22	0	0	0.0	0.0	22	0	0	0.0	0.0		20	0	0	0.0	0.0
P0353	20	0	0	0.0	0.0	20	2	2	10.0	10.0		20	15	15	75.0	75.0[e,p]
P0382	19	0	0	0.0	0.0	19	0	0	0.0	0.0		18	1	1	5.6	5.6[e]
P0399	22	0	0	0.0	0.0	22	3	3	13.6	13.6		15	0	0	0.0	0.0[d]
P0400	22	0	0	0.0	0.0	22	9	8	40.9	36.4		17	7	5	41.2	29.4[b,h]
P0466	19	1	1	5.3	5.3	19	7	2	36.8	10.5		16	11	3	68.8	18.8[b,d,j,k]
P0498	22	2	2	9.1	9.1	22	11	5	50.0	22.7		19	10	8	52.6	42.1[c,b,j,n]
P0516	22	0	0	0.0	0.0	22	3	3	13.6	13.6		20	2	2	10.0	10.0[e]
Overall Mean Results	422	7	7	1.7	1.7	420	102	73	24.3	17.4		369	121	87	32.8	23.6

Key: Superscripts: *Salmonella* types found in batch at slaughter or on farm: [a] Typh.104; [b]193; [c]208; [d]U302; [e]U308A; [f]U310; [g]Agona; [h]Enteritidis; [j]Derby; [k]Indiana; [m]Newport; [n]Reading; [p] Typh.Untypable (Typh.= *S.*Typhimurium)

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Table 4: Correlations of serum/juice with carcase/caecum/faeces

Variable	Correlations (Marked correlations are significant at p < .05000) Carcase %+ve	Carcase Typh.%+ve	Caecum %+ve	Caecum Typh.%+ve	Faeces %+ve	Faeces Typh.%+ve
Neat serum	0.20	0.20	0.14	0.15	0.44	0.29
SOD 5's	0.43	0.43	0.25	-0.01	**0.53**	0.16
SSP 5's	0.34	0.34	0.25	0.12	0.39	0.13
SOD 10's	0.35	0.35	0.22	-0.01	**0.54**	0.15
SSP 10's	0.26	0.26	0.24	0.15	0.43	0.15
SOD 20's	0.32	0.32	0.21	0.02	**0.48**	0.14
SSP 20's	0.23	0.23	0.22	0.17	0.34	0.15
Neat juice	0.10	0.10	0.42	0.40	0.44	0.43
JOD 5's	0.14	0.14	0.31	0.34	0.20	0.13
JSP 5's	0.28	0.28	0.33	0.23	0.43	0.20
JOD 10's	0.18	0.18	0.31	0.31	0.18	0.09
JSP 10's	0.30	0.30	0.33	0.21	0.40	0.16
JOD 20's	0.07	0.07	0.19	0.18	0.12	0.02

neat = individual sample % positive

S = serum
J = 'meat juice'
OD = optical density
SP = sample/positive ratio

Correlations significant at p<0.05 are underlined

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Table 5: Correlations of serum/juice pooled results with % positive individual (neat) sera

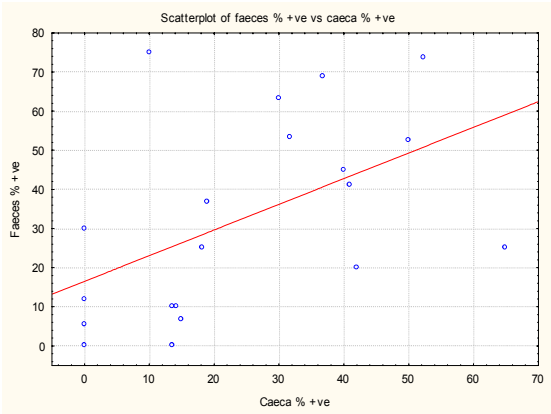
Variable	Correlations	
	Neat serum %+ve	Neat juice %+ve
SOD 5's	0.70	0.51
SSP 5's	0.77	0.69
SOD 10's	0.74	0.52
SSP 10's	0.76	0.67
SOD 20's	0.71	0.49
SSP 20's	0.75	0.64
JOD 5's	0.69	0.77
JSP 5's	0.74	0.71
JOD 10's	0.64	0.75
JSP 10's	0.70	0.68
JOD 20's	0.66	0.75
JSP 20's	0.70	0.68

S = serum
J = 'meat juice'
OD = optical density
SP = sample/positive ratio

MJE SLIDE 9

Slide 10

Figure 3: Correlation between % positive farm faeces and % positive caeca (C.C. 0.51)



MJE SLIDE 10

Slide 11

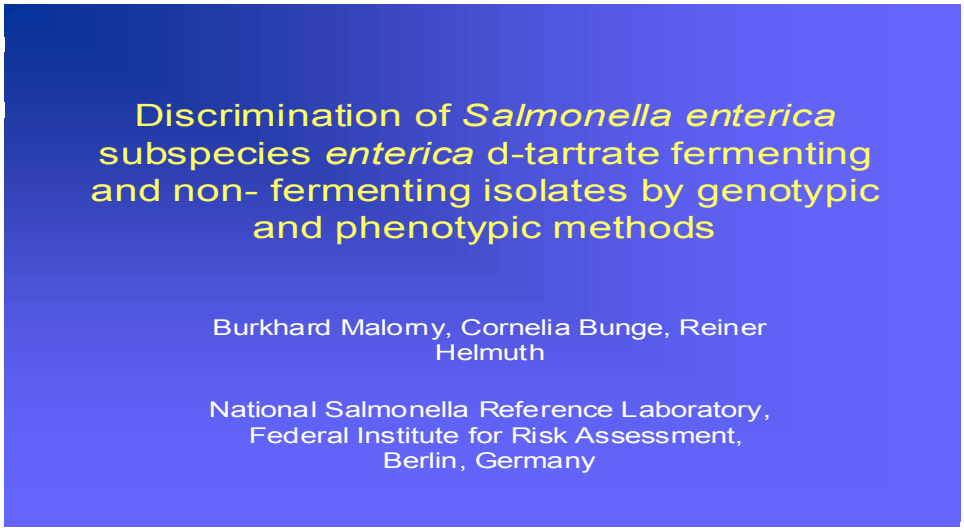
Conclusions

- *Salmonella* prevalence high in study farms
- *S.*Typhimurium predominant
- Poor correlation between serology and bacteriology
- Pen faeces correlated with caecal positives
- Pooled serum correlated with pen-positives
- Pooled serum or meat-juice mean OD or SP correlated with prevalence serological positives

MJE SLIDE 11

Appendix 30 Slides of presentation 2.13

Slide 1



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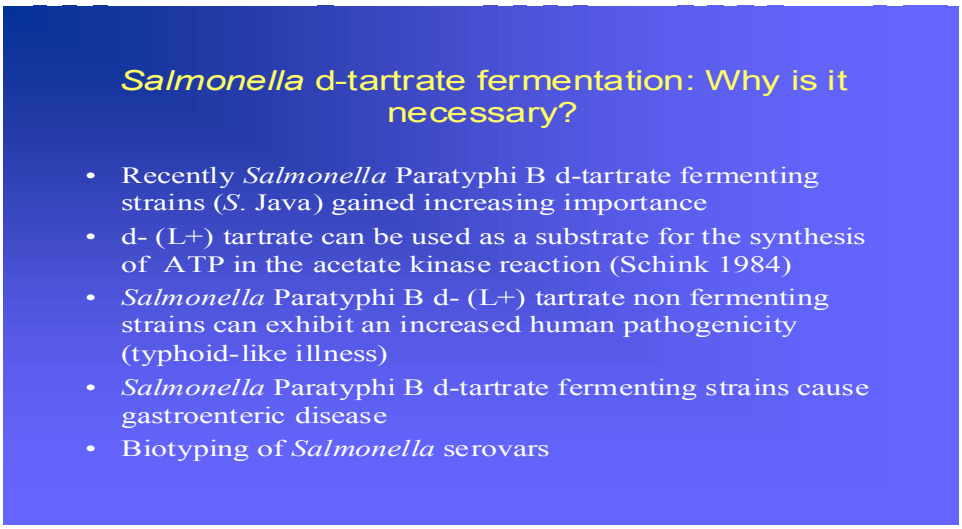
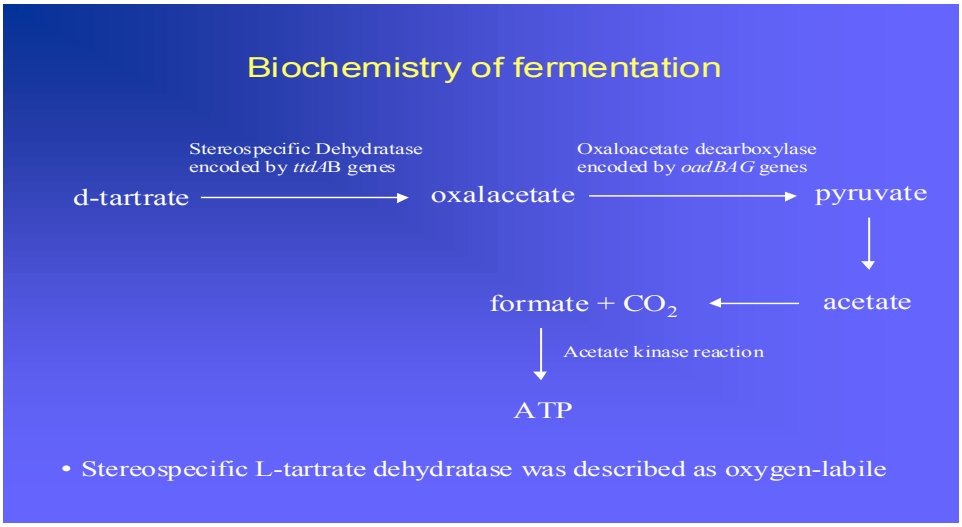


Slide 3



Slide 4

Lead acetate test

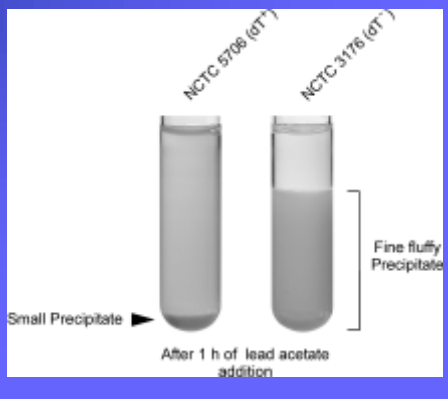
- Current WHO method for determination of d-tartrate fermenting according Alfredsson et al. (1972)

Conditions: static, aerated conditions with prolonged incubation times (3 and 6 days).

Reference strains used:

NCTC 5706 (dT^+)

NCTC 3176 (dT^-)



Slide 5

Properties of lead-acetate protocols tested

Properties	Protocol 1 (current WHO method) (according Alfredsson et al.)	Protocol 2	Protocol 3
Broth	8 ml, pH 7.4 1% Difco Bacto-Peptone, 1% d-tartrate, 0.0023% bromothylmol blue	8 ml, pH 7.4 1% Difco Bacto-Peptone, 1% d-tartrate, 0.0023% bromothylmol blue	8 ml, pH 7.4 1% Difco Bacto-Peptone, 1% d-tartrate, 0.0023% bromothylmol blue
Inoculate	5 x 10^7 bacteria in 0.85% NaCl	5 x 10^7 bacteria in 0.85% NaCl	Loopful bacteria from plate
Incubation atmosphere	Air, 37°C	10% CO_2, 37°C	10% CO_2, 37°C
Incubation time	3 and 6 days	3 and 6 days	3 and 6 days

Slide 6

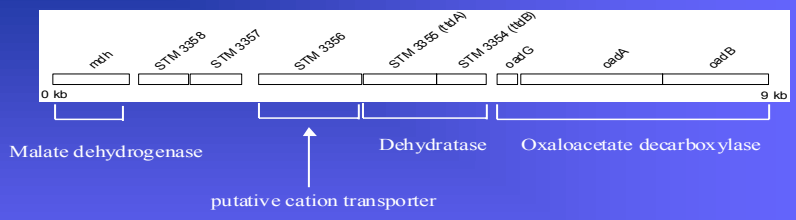
Results of the lead acetate protocols

Serovar	No. of strains tested	Country (no. of strains)	Year	No. of strains tested as d-tartrate positive (triplicates)					
				Lead acetate (Protocol 1)		Lead acetate (Protocol 2)		Lead acetate (Protocol 3)	
				3 d	6 d	3 d	6 d	3 d	6 d
Paratyphi B	81	Germany (63), England (3), Belgium (5), Australia (3), Austria (2), Unkown (2), France (2), Netherlands (1)	1961-2002	33	71	44	73	68	81
Paratyphi B	21	Germany (14), France (4,) Netherlands (2), Unkown (1)	1964-2002	0	0	0	0	0	0

- 88% accuracy between WHO protocol 1 and modified protocol 3 (after 6 days of incubation)

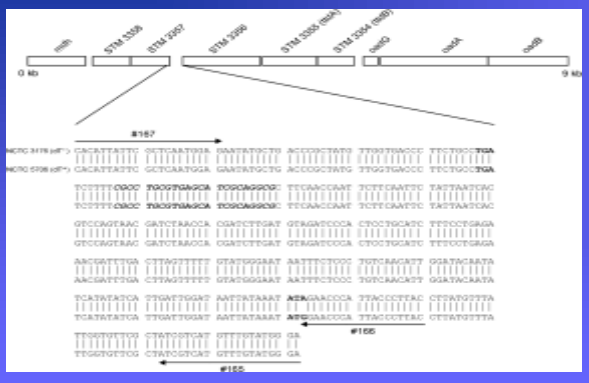
Slide 7

9 kb map derived from *S.* Typhimurium LT2



Slide 8

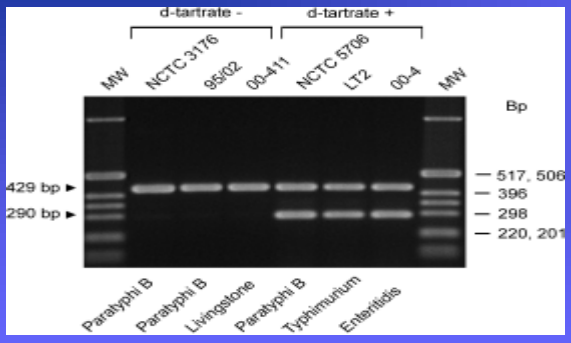
d-tartarte PCR assay: Primers and sequence



- PCR based on the presence or absence of the ATG start codon for gene STM 3356, encoding a putative cation transporter.
- d-tartrate + strains: ATG
  d-tartrate - strains: ATA
- 290 bp PCR product with primers #167-#166

Slide 9

PCR-based discrimination of dT⁻ and dT⁺ isolates



- 290 bp product: d-tartrate fermenting strain
- 429 bp product: pos. control for *Salmonella* specific DNA (ST11-ST15)

Slide 10

Comparison PCR- lead acetate tests

Serovar	No. of strains tested	Country (no. of strains)	Year	No. of strains tested as d-tartrate positive (triplicates)						
				PCR	Lead acetate (Protocol 1)		Lead acetate (Protocol 2)		Lead acetate (Protocol 3)	
					3 d	6 d	3 d	6 d	3 d	6 d
Paratyphi B	81	Germany (63), England (3), Belgium (5), Australia (3), Austria (2), Unkown (2), France (2), Netherlands (1)	1961-2002	81	33	71	44	73	68	81
Paratyphi B	21	Germany (14), France (4,) Netherlands (2), Unkown (1)	1964-2002	0	0	0	0	0	0	0

Slide 11

Summary

- An improved lead acetate protocol was developed using 5 to 10 times more bacterial cells for inoculation and incubation in the presence of 10% CO_2 for 6 days
- A PCR assay was developed which showed 100% accuracy with the modified lead acetate protocol
- Consequently, the PCR d-tartrate assay should be considered to be the method of choice for discrimination of d-tartrate fermenting and non-fermenting Salmonella strains in the future.

Slide 12

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Multicenter Validation of the Analytical Accuracy of *Salmonella* PCR: towards an International Standard

Burkhard Malorny,[1] Jeffrey Hoorfar,[2] Cornelia Bunge,[1] and Reiner Helmuth[1]*

Federal Institute for Health Protection of Consumers and Veterinary Medicine, D-12277 Berlin, Germany,[1] and Danish Veterinary Institute, DK-1790 Copenhagen, Denmark[2]

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As part of a major international project for the validation and standardization of PCR for detection of five major food-borne pathogens, four primer sets specific for *Salmonella* species were evaluated in-house for their analytical accuracy (selectivity and detection limit) in identifying 43 *Salmonella* spp. and 47 non-*Salmonella* strains. The most selective primer set was found to be 139-141 (K. Rahn, S. A. De Grandis, R. C. Clarke, S. A. McEwen, J. E. Galán, C. Ginocchio, R. Curtiss III, and C. L. Gyles, Mol. Cell. Probes 6:271-279, 1992), which targets the *invA* gene. An extended determination of selectivity by using 364 strains showed that the inclusivity was 99.6% and the exclusivity was 100% for the *invA* primer set. To indicate possible PCR inhibitors derived from the sample DNA, an internal amplification control (IAC), which was coamplified with the *invA* target gene, was constructed. In the presence of 300 DNA copies of the IAC, the detection probability for primer set 139-141 was found to be 100% when a cell suspension containing 10^4 CFU/ml was used as the template in the PCR (50 CFU per reaction). The primer set was further validated in an international collaborative study that included 16 participating laboratories. Analysis with 28 coded ("blind") DNA samples revealed an analytical accuracy of 98%. Thus, a simple PCR assay that is specific for *Salmonella* spp. and amplifies a chromosomal DNA fragment detected by gel electrophoresis was established through extensive validation and is proposed as an international standard. This study addresses the increasing demand of quality assurance laboratories for standard diagnostic methods and presents findings that can facilitate the international comparison and exchange of epidemiological data.

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Standardization of diagnostic PCR for the detection of foodborne pathogens

Burkhard Malorny[a,*], Panayotis T. Tassios[b], Peter Rådström[c], Nigel Cook[d], Martin Wagner[e], Jeffrey Hoorfar[f]

[a]*Federal Institute for Health Protection of Consumers and Veterinary Medicine, Diedersdorfer Weg 1, 12277 Berlin, Germany*
[b]*Department of Microbiology, Medical School, University of Athens, M. Asias 75, 11527 Athens, Greece*
[c]*Applied Microbiology, Center for Chemistry and Chemical Engineering, Lund Institute of Technology, Lund University, Lund, Sweden*
[d]*Central Science Laboratory, Sand Hutton, YO411LZ York, UK*
[e]*Institute for Milk Hygiene, Milk Technology and Food Science, Veterinärplatz 1, 1210 Vienna, Austria*
[f]*Danish Veterinary Institute, Bülowsvej 27, 1790 Copenhagen, Denmark*

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Abstract

In vitro amplification of nucleic acids using the polymerase chain reaction (PCR) has become, since its discovery in the 1980s, a powerful diagnostic tool for the analysis of microbial infections as well as for the analysis of microorganisms in food samples. However, despite its potential, PCR has neither gained wide acceptance in routine diagnostics nor been widely incorporated in standardized methods. Lack of validation and standard protocols, as well as variable quality of reagents and equipment, influence the efficient dissemination of PCR methodology from expert research laboratories to end-user laboratories. Moreover, the food industry understandably requires and expects officially approved standards. Recognizing this, in 1999, the European Commission approved the research project, FOOD-PCR (http://www.PCR.dk), which aims to validate and standardize the use of diagnostic PCR for the detection of pathogenic bacteria in foods. The present review focuses on the harmonization procedure and standardization criteria for detection of foodborne pathogens by PCR. The progress of standardization so far and future perspectives of diagnostic PCR are discussed.

Keywords: PCR; Harmonization and standardization; Detection; Foodborne pathogens

Int. J. Food Microbiol. (2003) 83 39-48

Appendix 31 Slides of presentation 2.14

Slide 1

Microbiological methods

- In the current EC legislation only one method laid down:
  the Salmonella methods used in the context of additional salmonella guarantees of Finland and Sweden (ISO 6579 and NMKL 71)

Slide 2

Proposed EC legislation

- The reference methods will be laid down (microbiol. criteria, control programmes)
- these are usually international standard methods (ISO, CEN) , if available
- preference given to horizontal methods in the food sector

Slide 3

Alternative methods

- Alternative microbiological methods can be used
  - if they are validated in accordance with internationally recognised rules, such as EN/ISO 16140 standard, (against the reference method), and
  - if they offer equivalent guarantees to the reference method

Slide 4

Antimicrobial resistance

- Collection of data foreseen in the proposed zoonoses Directive
- minimum surveillance: *salmonella* spp., *Campylobacter jejuni* and *coli* from cattle, pigs and poultry and in food of animal origin derived from them

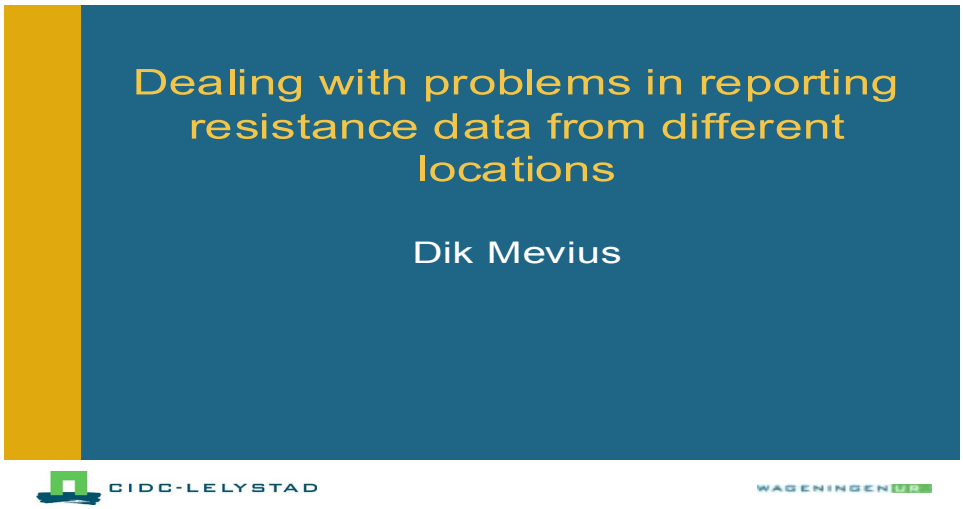
Slide 5

Antimicrobial resistance

- The national reports have to describe among other things:
  - antimicrobials included in testing
  - laboratory methods used for detection of resistance and identification of strains
- guidelines for sampling and testing provided by OIE and EARSS

Appendix 32 Slides of presentation 3.2

Slide 1



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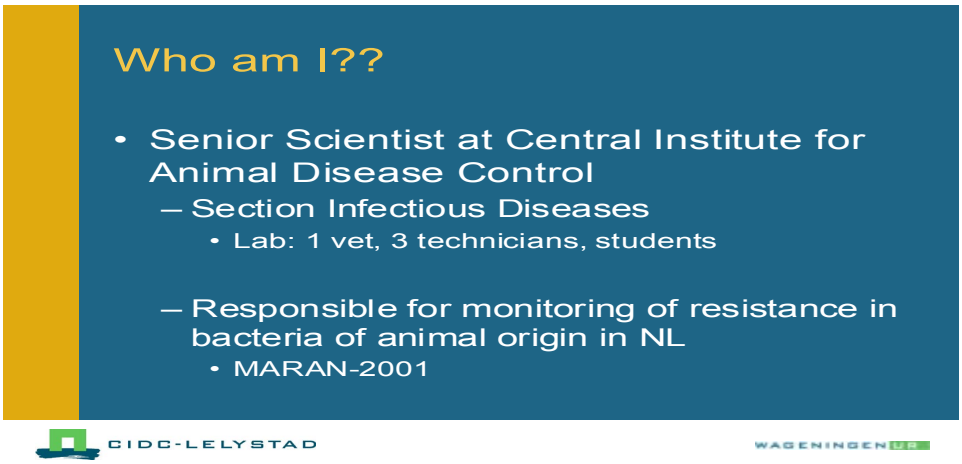

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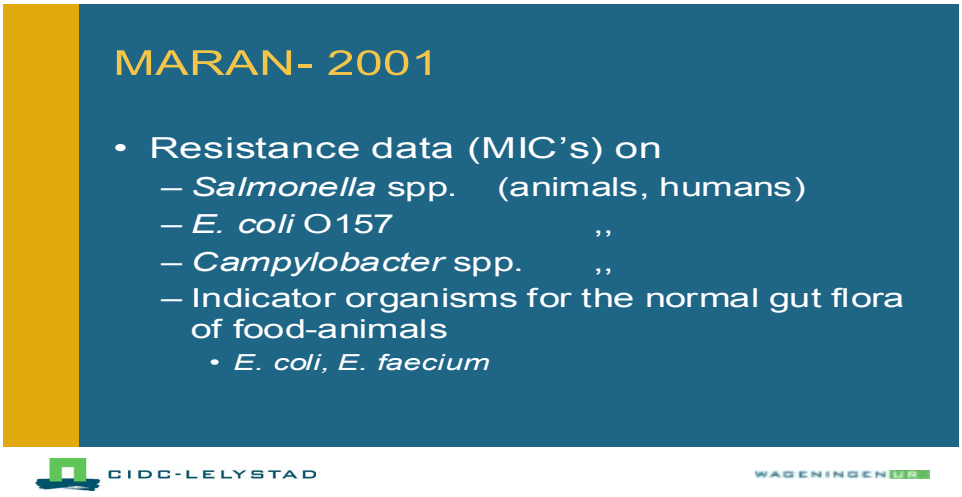
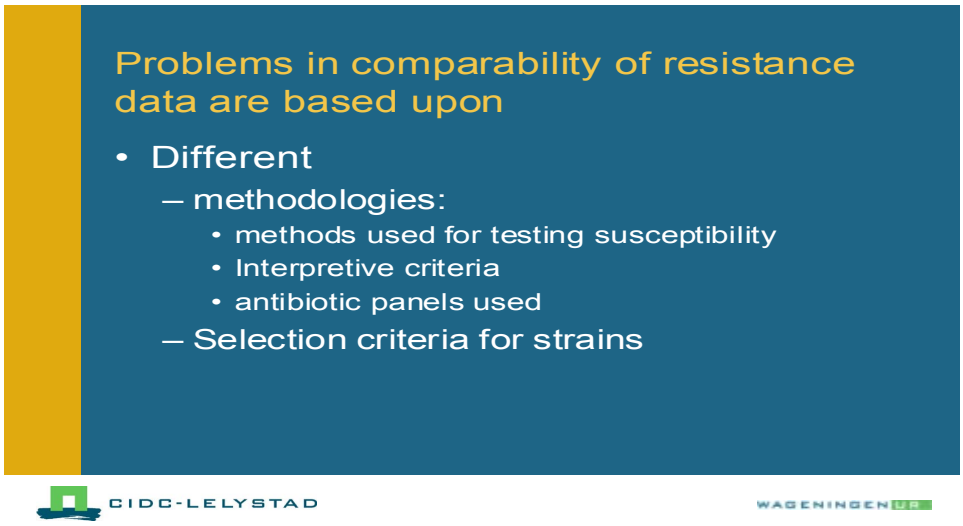
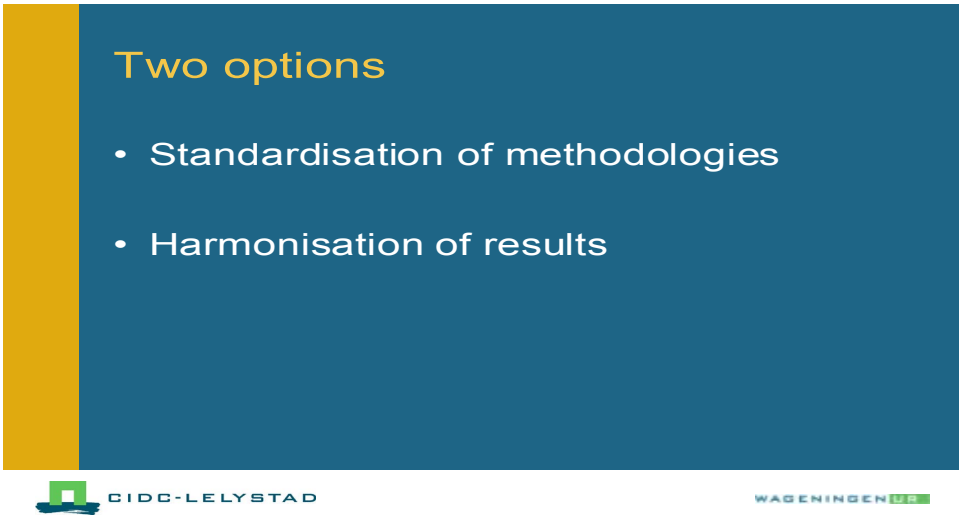


Slide 4



Slide 5



Slide 6

Slide 7

Harmonisation of results (I)

- Two options
  - Prescribe an Internal Quality Control System (IQAS) using ATCC strains (Surveillance Standard)
    - If results comply with criteria (e.g. NCCLS)
    - Then: data are comparable

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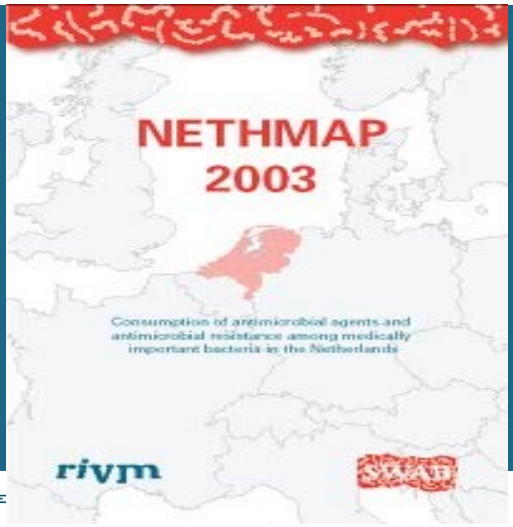
Slide 8



http://www.swab.nl

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Slide 9

Harmonisation of results (II)

- Two options
  - Organise an External Quality Assurance System (EQAS)
  - Essential conditions are
    - Reference laboratory
      - Reference strain collections identified en tested with reference methodologies
      - Sufficient expertise

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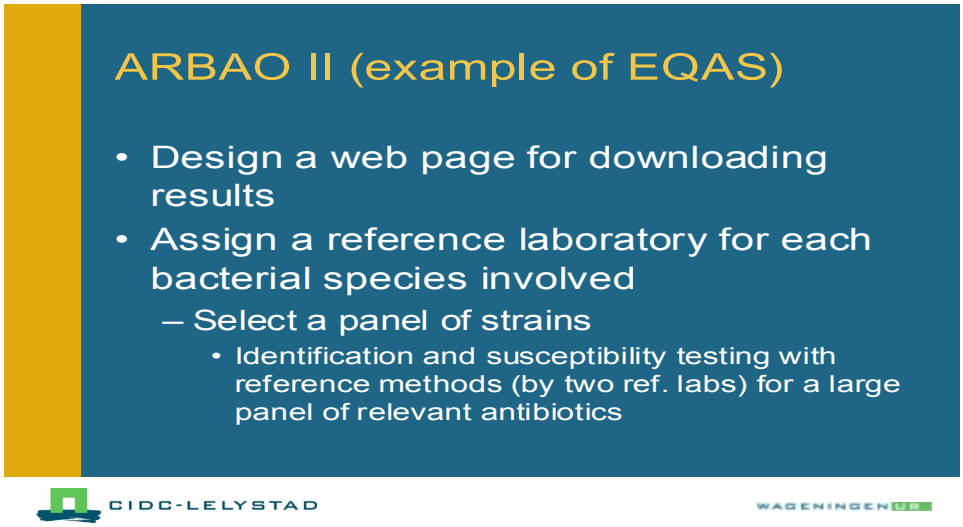
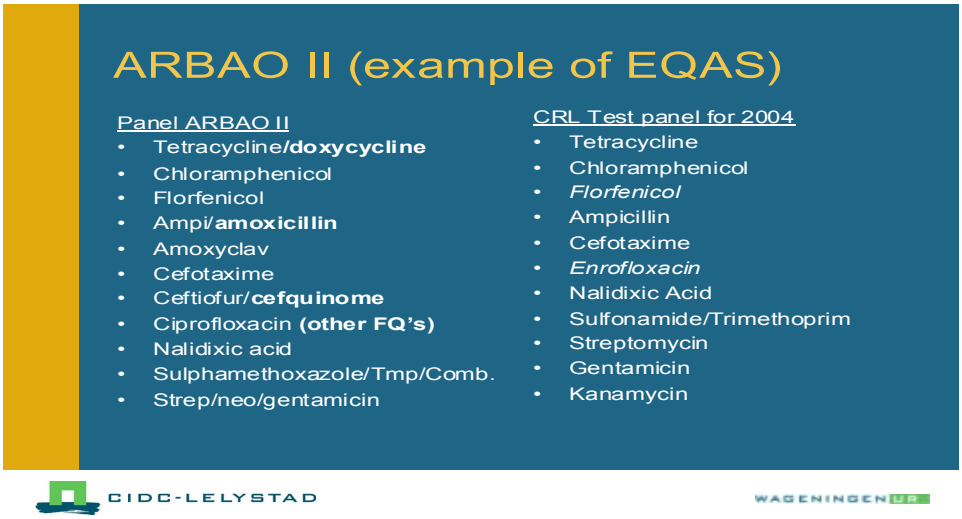
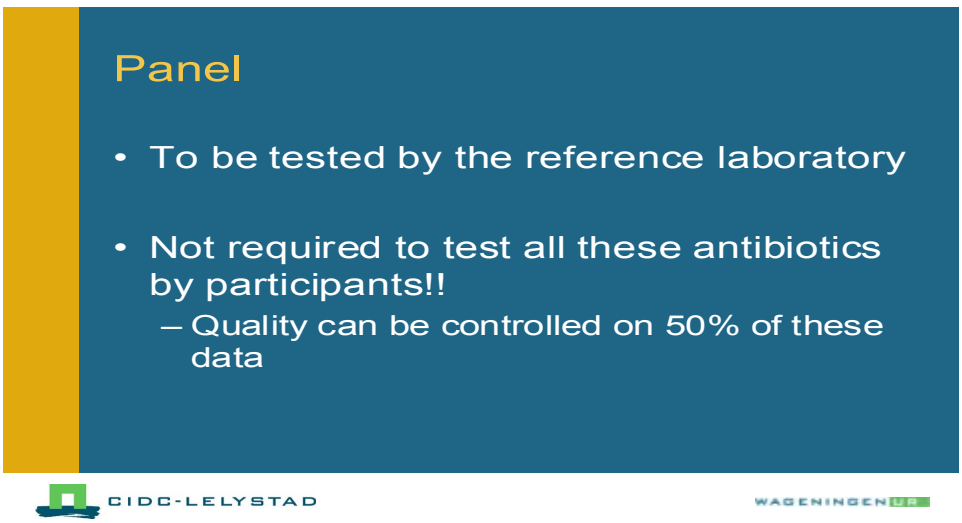
Slide 10



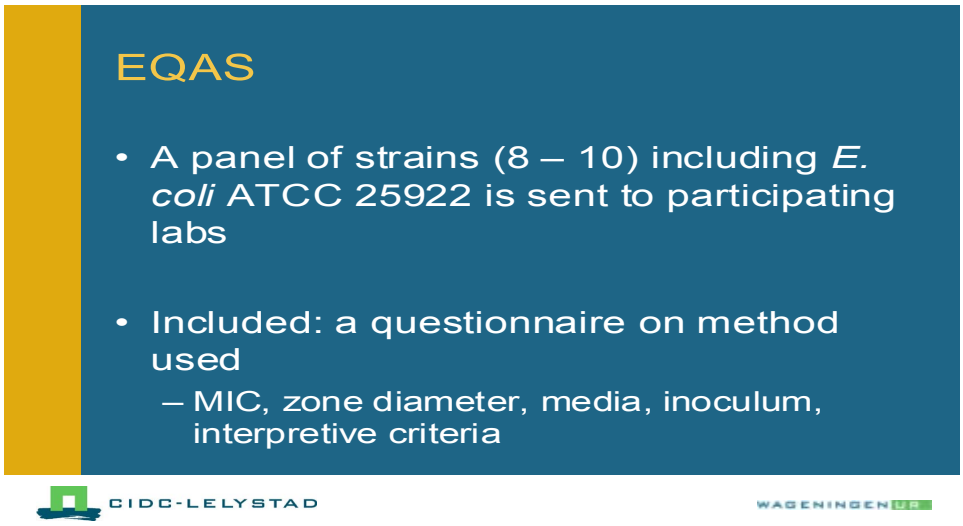
Slide 11



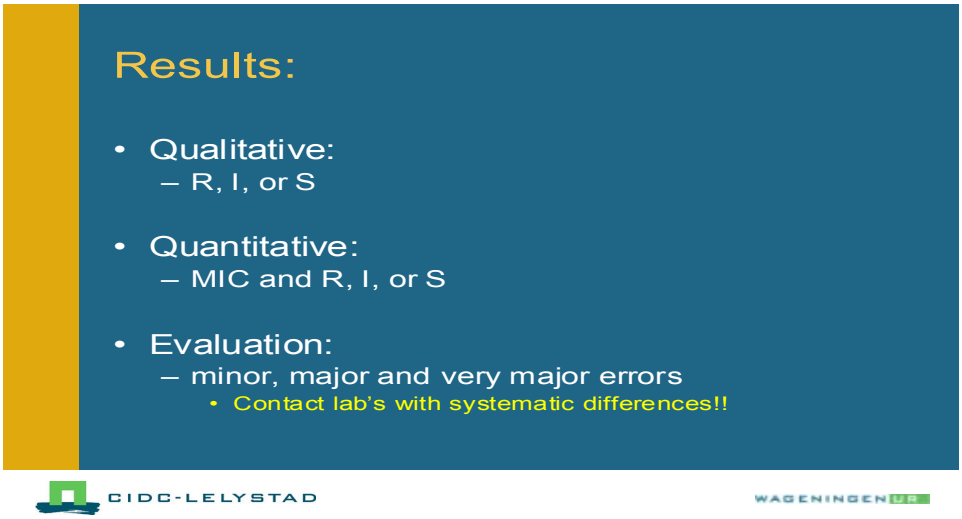
Slide 12



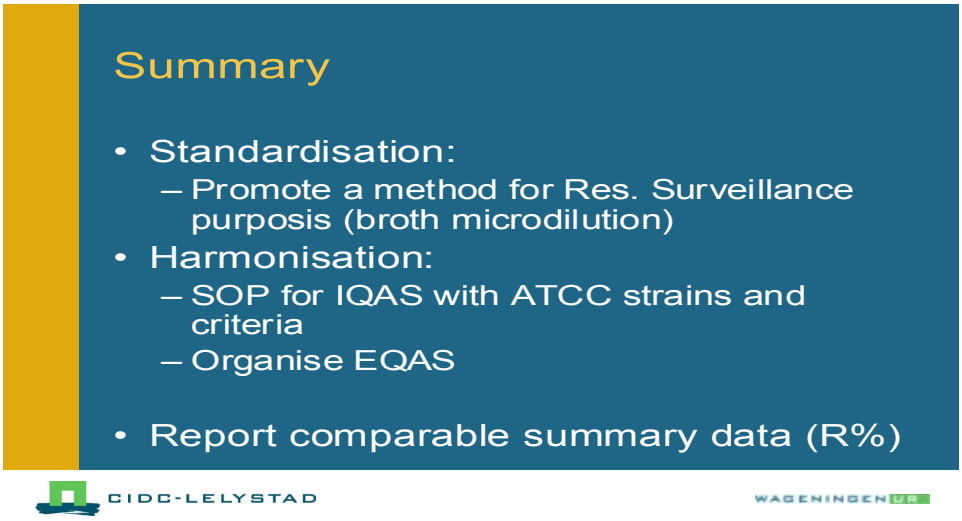
Slide 13



Slide 14



Slide 15



Appendix 33 Slides of presentation 3.3

Slide 1

Standardised method for the detection of Salmonella in poultry matrices,especially poultry faeces

Henk Stegeman, RIKILT, Wageningen, The Netherlands

Introduction
Dutch branch method
Validation studies for poultry faeces
Other validation studies
Recommendation / discussion

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Slide 2

Standards for Salmonella

Matrix	ISO	Selective enrichment	Isolation media
Food/feed	6579:1993	RV + SC	BGA + second
Food/feed	6579:2002	RVS + MKTTn	XLD + second
Milk products	6785:2001	RVS + SC	BGA + second
Water	6345:1995		BGA, XLD, BSA
Poultry faeces	-	-	-

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Slide 3

Control of Salmonella in Poultry chain

Dutch Production Boards for Livestock, Meat and Eggs (PVE) introduced in 1997 a branch method within the frame of *Action Plan Salmonella* for the control of Salmonella in the poultry chain.

Poultry chain: from hatchery to slaughterhouse

Matrices: down, faeces ,neck skins, meat.

The method is based on:
Modified Semi-Solid Rappaport-Vassiliadis (MSRV), developed by De Smedt et al.1986. J.Food.Prot..49.510-14

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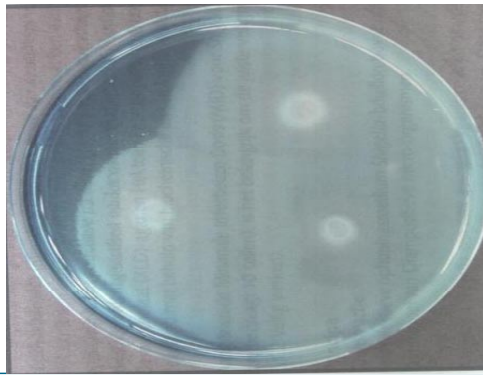
Slide 4

NL branch method for the detection of Salmonella

SAMPLE
↓
1:10 in BPW
for 16-18 h at 37 °C
↓
0,1 ml (3 drops)
on MSRV plate
↓
Incubation 2 x 24 h at 42 °C
↓
BGA
for 24 h at 37 °C
↓
Confirmation UA, TSI and LDC
or diagnostic kit such as API, Crystal



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Slide 5

Validation of Dutch method - Investigation 1

Dutch Animal Health Service
1998

Matrix: Poultry faeces
N= 2249 samples

Selective medium	Salmonella-positive samples		S. Enteritidis isolates	
	24 h	48 h	24 h	48 h
RV	47 %	62 %	2	4
RVS	62 %	69 %	4	3
MSRV	79 %	93 %	7	8
MSRV+ RV	90 %	94 %		8
MSRV+RVS	95 %	94 %		8

Hartman, 1999, Report 1, Dutch Animal Health Service, Deventer

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Slide 6

Validation of Dutch method - Investigation 2

Dutch Animal Health Service
1998

Matrix: Poultry faeces
N= 807 samples

Selective medium	Salmonella-positive samples		S. Enteritidis isolates	
	24 h	48 h	24 h	48 h
RV	74 %	83 %	3	3
RVS	39 %	39 %	2	1
SEL	39 %	69 %	0	0
MSRV	65 %	87 %	4	4
MSRV+ RV	87 %	91 %	4	
MSRV+RVS	70 %	87 %	4	
ISO 6579:1993	78 %	91 %	3	

Hartman.1999. Report 1. Dutch Animal Health Service, Deventer

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Slide 7

Validation of Dutch method - Investigation 3A

RIVM
Matrix: faecal samples from layer flocks and broiler flocks
N = 1022 and 892

Selective medium	Salmonella-positive samples			
	Poultry layer flocks		Poultry broiler flocks	
	24 h	48 h	24 h	48 h
RV	35 %	41 %	57 %	60 %
MSRV	89 %	92 %	92 %	93 %
RV + MSRV	91 %	95 %	96 %	98 %

Voogt et al. 2001. Letters in applied Microbiology 32:89 - 92

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Slide 8

Validation of Dutch method - Investigation 3B

RIVM

Matrix: faecal samples from layer flocks and broiler flocks

N = 1022 and 892

Selective medium	Number of serotypes		
	S.Enteritidis	S.Panama	S.Enterica (I), non-motile
RV	21	3	4
MSRV	90		

Voogt et al. 2001. Letters in applied Microbiology 32:89 - 92

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Slide 9

MSRV versus Probelia™ PCR

Validation study with 2 laboratoria based on EN - ISO 16140:2002
(*Protocol for the validation of alternative method*)

Lab.1 and lab.2

Matrix	N	P0	NO	NV	PV	Accuracy	Sensitivity	Specificity
Faeces	83	27	52	2	2	95 %	93 %	96 %
Neck skin	76	37	32	2	5	91 %	95 %	86 %
Down	65	22	39	0	4	94 %	100 %	91 %
Faeces	102	36	58	0	8	92 %	100 %	88 %
Neck skin	99	30	66	3	0	97 %	91 %	100 %
Down	84	28	48	0	8	90 %	100 %	92 %

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Slide 10

MSRV versus ISO 6579:1993 - Food and Feed

National Inspection Service for Livestock and Meat (RVV)

Matrix	Number of samples	Salmonella-positive samples (%) ISO 6579: 1993	MSRV 48h
Fish products	120	2 %	2 %
Animal feed	199	71 %	91 %
Meat	111	55 %	82 %
Egg/ Milk products	95	69 %	93 %

Van Velzen et al.1999. De Ware(n)-Chemicus 29: 140-143

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Slide 11

MSRV versus ISO 6579:1993 - Food

Inspectorate for Health Protection and Veterinary Public Health (KvW)

Matrix	Number of samples	Salmonella-positive samples (%) ISO 6579: 1993	MSRV 48h
Meat	66	90 %	100 %
Egg	64	92 %	96 %
Chicken	66	100 %	100 %
Milk products	60	92 %	96 %

Van der Zee et al. 2001. De Ware(n)-Chemicus 31: 129-140

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Slide 12

Recommendation / Discussion

- There is a need for a standardized method for the detection of Salmonella in poultry matrices, especially poultry faeces as a reference method in the new Zoonoses Directive.
- ISO subcommission Food and Feed Microbiology - Bangkok 2002 - Resolution Nº 209 - Detection of Salmonella in poultry faeces
  - SC 9 (Food Microbiology) will ask TC34 for the enlargement of SC09 scope to include microbiology in food production to deal with the analysis of samples coming from e.g. poultry faeces.
- MSRV (in combination with RV/RVS) is a more suitable method than ISO 6579 :1993 for poultry faeces (and food and feed samples).
- MSRV is not suitable for all Salmonella serotypes (e.g non-motile)
- For economical reasons it may be advisable to use only MSRV for the daily control of Salmonella in the poultry chain ?

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