

RIVM report 284500021/2002

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

Ploufragan (France), 28 May 2002

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(editors)

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Samenvatting

Op 28 mei 2002 is door het Communautair Referentie Laboratorium voor *Salmonella* (CRL-*Salmonella*) een workshop georganiseerd in Ploufragan, Frankrijk. Alle Nationale Referentie Laboratoria voor *Salmonella* (NRLs-*Salmonella*) van de EU lidstaten, met uitzondering van die van Griekenland en Noord-Ierland, waren vertegenwoordigd. In totaal waren er 34 deelnemers.

Het programma van de workshop bestond uit verschillende delen. Het eerste deel bestond uit een toelichting op de stand van zaken met betrekking tot de nieuwe zoonose richtlijn. Daarnaast werd verslag gedaan van de workshop van het CRL voor Epidemiologie van Zoönosen (gehouden najaar 2001) en was er een bijdrage over (nieuw) opkomende *Salmonella* typen in Nederland. Het tweede en derde deel van de workshop bestond uit bijdragen op het gebied van de bacteriologie, respectievelijk, typering. In deze delen werden o.a. reeds uitgevoerde ringonderzoeken besproken en werd bediscussieerd hoe de aankomende ringonderzoeken er uit zouden moeten zien. In het vierde en laatste deel werden enkele meer algemene onderzoeksbijdragen ondergebracht.

Summary

At 28 May 2002 a workshop was organised by the Community Reference Laboratory for *Salmonella* (CRL-*Salmonella*) in Ploufragan, France. All National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) of the EU Member States, with the exception of the Greek and the Northern-Ireland NRLs-*Salmonella*, were represented. In total there were 34 participants.

The workshop consisted of four parts. In the first part the proposed revision of the zoonosis legislation was presented. Also a report was given on the last workshop of the CRL for Epidemiology of Zoonosis (held in autumn 2001) and one on emerging *Salmonella* types in the Netherlands. The second and third part were devoted to bacteriology and typing, respectively. Results of ringtrials were evaluated and the set-up of upcoming ringtrials was discussed. The fourth, and last part, consisted of several more general research contributions.

1. Opening and introduction of participants

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

Opening

First of all I would like to sincerely welcome you all at this workshop. We are with many people, that is, at least 1 to 3 persons of each of 13 EU Member States (Greece and Northern Ireland are not represented).

A special word of welcome to Mr. Jean-Charles Cavitte as the representative of the Commission among us. Also a special word of welcome for Ms. Annemarie Kaësbohrer from the CRL-Epidemiology of Zoonoses in Berlin. I am also very pleased that this year Ms. Linda Ward from the Public Health Laboratory Service (Colindale, UK) is among us as for several years already we have co-operated in the collaborative typing studies.

During this day I will be your chairman as the head of CRL-*Salmonella*. With these words the workshop is opened!

Aims

What can we expect from this workshop (see Appendix 3 for the programme). The functions and duties of the CRL-*Salmonella* according to the zoonoses directive are:

1. Providing national laboratories with details of analytical methods and comparative testing;
2. Co-ordinating the application by national reference laboratories of the methods, referred to under the first mentioned point, in particular by organising comparative testing;
3. Co-ordinating research into new analytical methods and informing national laboratories of advances in this field;
4. Conducting initial and further training courses for the benefit of staff from national reference laboratories; and
5. Providing scientific and technical assistance to the Commission of the European Community.

The aims of the workshop are defined as to discuss:

- General issues of relevance for CRL-/NRLs-*Salmonella*, for example at 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.

Participating are (see Appendix 2): the representative of the EU Commission, a representative of the CRL-Epidemiology of Zoonoses, representatives of NRLs-*Salmonella* and representatives of CRL-*Salmonella*.

2. Review of the more general presentations

2.1 State of play on the proposed revision of the zoonoses legislation and developments for CRL and NRLs

Jean-Charles Cavitte, European Commission (See Appendix 4)

The revision of the legislation follows in particular from the perceived need to decrease the incidence of zoonoses in humans; to improve the control of zoonoses in the primary production ; to strengthen the collection of relevant data, to support possibly risk assessment activities and risk management decisions.

The proposals are currently in discussion in the Council and the European Parliament, for co-decision.

The Parliament adopted its position in first reading on 15.05.02. It largely endorsed the principles underpinning the Commission's proposals. Through most of its amendments, it improved the proposals from an editorial or technical point of view. As regards monitoring, the objective to collect comparable data throughout the food chain, including feed, not only on products of animal origin but also on plant products is strengthened. The connection and co-ordination between the "veterinary" and the human sectors were highlighted also. In this respect, it should be noted that the proposed Directive does not foresee collection of data in humans, which should be a task for the "(human) communicable diseases network" under Council and parliament Decision 2119/98/EC. The need to make information available to the public in a transparent manner, and in a faster timetable than proposed by the Commission, is further stressed. According to the Parliament's amendments, the Member States authorities would have to supply their national reports within 3 months instead of 5 months currently. The parliament asks for the monitoring of anti-microbial resistances to cover not only zoonotic agents but also other bacteriological agents of interest. The Commission supported most of the amendments of the Parliament but not the shortening of the deadline for Member States to report on their national situation. As regards the Regulation on control of *Salmonella* and other food-borne zoonotic agents, the Parliament tends to reinforce elements of the proposal, by amending or adding targets to reduce the prevalence of *Salmonella* in different animal populations and the related measures in flocks. In particular, it adds slaughter pigs, calves, other cattle and sheep and measures should systematically cover all *Salmonella* serotypes with "public health significance". A ban on intra-Community trade of animals and related products would be placed on Member States that would not have an approved programme. Also, additional guarantees in trade for zoonotic agents other than *Salmonella* are requested by the Parliament and a recital would be included in the Regulation stating that the use of antibiotics for growth promoting and preventative purposes should be prohibited at all events. The involvement of the feed business operators in the *Salmonella* control programmes is further

clarified. Strangely, the Parliament would introduce food of plant origin into this 'control' Regulation, but this should be tackled under the "food hygiene package". The Parliament demands also that the deadline for laboratories to be accredited according to international standards be reduced from January 2005 to January 2004. The Commission could accept a majority of amendments but however decided to reject a number of them for different reasons in particular the need for preliminary scientific advice. In particular the issue of sanctions and additional guarantees, the additional targets on cattle and sheep, the recital to prohibit use of antimicrobials, the shortening of deadlines for countries to report and laboratories to be accredited, were rejected.

In the Council, experts groups have taken place under the former Belgian presidency and the current Spanish presidency. There is not yet a common view on the proposals and the positions of the Member States seem to depend largely on their national situation in respect of *Salmonella*, in particular. The main issues of substance relate to: enlarged monitoring of antimicrobial resistance, scope and timetable for the implementation of the targets for the reduction of *Salmonella* prevalence and the criteria for defining serotypes with public health significance. A number of amendments required by the Parliament go along the same lines as the developments in the Council, but some amendments go further than what was discussed in the Council so far. One particular element introduced in the Directive during discussions in the Council was the requirement for operators to arrange for strains of zoonotic agents detected during own checks to be kept, so that further investigations can be made in particular in the light of the epidemiological situation in humans. A concern expressed by certain Member States in the Council relates to Community co-financing. These Member States call for Community support to the new control measures, in particular. The Commission will have to prepare revised proposals in the lights of the amendments of the Parliament that it has accepted and the Council will now have to look at the amendments required by the Parliament. From July, the Danish presidency will handle the file.

There will be a need for the Commission to prepare, together with the Member States and Community bodies involved, sampling and testing schemes to evaluate the prevalence of *Salmonella* in animal populations before setting *Salmonella* reduction targets and to monitor the achievement of the targets under the proposed Regulation. Similarly, schemes for harmonised and statistically based monitoring of zoonotic agents under the Directive will need to be drafted.

Developments as regards CRLs and NRLs:

According to the zoonoses proposals, CRLs shall to be appointed and their tasks defined and the NRLs should be appointed by the Member States authorities and certain of their tasks may be defined at Community level. However, the Commission is in the process of finalising a draft regulation on official feed and food control and the issue of the laboratories involved in official control (what the CRLs and NRLs are) will be tackled. A final decision has not been made yet whether the CRLs and NRLs on zoonoses should be covered by that latter legislation or be maintained under the zoonoses legislation.

As regards methods, the development of legislation goes towards requiring the use of international standards or methods validated against those standards according to international systems. In this context, it would be important to consider standardising the *Salmonella* detection in faeces or environmental samples.

On 15-16 January 2002, the Commission organised a first co-ordination meeting of CRLs involved in Veterinary Public Health. Various technical organisational and financial issues were discussed. As a result a decision was made to organise the yearly financing of CRLs in such a way that a decision on the aid be taken before the beginning of the year (meaning: decision made at the end of December of 2002 on the financial support for year 2003). Also, as regards ring trials, an improvement was made to support the cost of sending samples by a specific chapter instead of taking it from the overhead. For workshops, the intention is to repeal the maximum of 850 € by person for reimbursement of travel expenses, but to use the rules as for participants to Commission meetings. It is likely also that from 2003, the financing of workshops would be integrated into the yearly financial support of the CRL, as a specific envelope, instead of having a separate arrangement, which often create difficulties. Cooperation with non-member countries, follow-up of ring trials, accreditation of laboratories and development of websites were also among the issues discussed. A procedure has been drafted for the follow up required of under-performing laboratories; it was supplied to the CRLs (and Chief Veterinary Officers) together with the minutes of the meeting. A web-page including the details, tasks and yearly work-programmes of the CRLs has recently been created on the Commission website.

Discussion:

DAVIES: Which are the criteria for selecting public health significant serotypes (PHS-serotypes) ?

CAVITTE: Selection of a limited number of PHS-serotypes will not be easy. Different factors, such as the population targetted, the invasiveness of a serotype, its relevance to human cases and the severity of its infection, have to be taken into account.

DAVIES: What does this mean for *S. Dublin* in cattle?

CAVITTE: This is an example of a serotype for which we should take into account the relevance to human cases as well as the severity of the disease it can cause.

VANDEGIESSEN: Will the list of PHS-serotypes be different per Member State?

CAVITTE: No, there will be one list including the serotypes that are most prevalent in the different Member States.

2.2 Report from the CRL for the Epidemiology of Zoonoses

Annemarie Käsbohrer, CRL-Epidemiology, Berlin, Germany (See Appendix 5)

Some results from the last workshop, which was held in Berlin in October 2001, relevant for the representatives of the National Reference Laboratories were presented. Additionally, some findings of the new zoonoses report (Doc. SANCO/927/2002) which was just finished were shown. Finally, future requirements for the work were discussed.

During the workshop it was agreed to have tables summarising the monitoring and control strategies for *Salmonella* in poultry. This type of presentation makes obvious the variety of the schemes applied, even in poultry breeders. To achieve more comparable data, knowledge on the sensitivity of the overall strategy, taking into account the individual type of sample, the combination of different samples and the frequency of sampling is highly desirable.

On the basis of the new reporting system for antibiotic resistance, agreed on in 2000, data were reported by 14 countries in 2001. Additional recommendations for the reporting were accepted. From the NRLs information should be given on the share of the tested isolates in relation to the number of all available strains in the laboratory. Additionally, human data and information on turkey should be given separately. Preferably quantitative data should be collected such as diameter of inhibition or the dilution point of the MIC. Data should be given, if available, separately for all *Salmonella* strains together, *S. Typhimurium* (overall picture), *S. Typhimurium* DT104, *S. Typhimurium* non - DT104, *S. Enteritidis* and all other serovars together.

In the report on trends and sources of zoonotic agents in animals, feedingstuffs, food and man in the European Union and Norway in 2000, due to differences in the monitoring scheme applied, the current situation is given separately for four different groups of countries, i.e. those countries running an approved control programme for several years now, those which have started that since 1999 or 2000, the countries, which apply a monitoring scheme based on the sampling procedures in the Zoonoses Directive and those countries, which run other sampling schemes.

In the future, more information is needed on the sensitivity of the method applied in the monitoring programmes. Therefore, the representatives of the NRL *Salmonella* were requested to provide data on the sensitivity of the methods taking into account the complex schedules applied. As regards the antibiotic resistance testing, participants were encouraged to provide the information as discussed. Additionally, the results of serotyping and phage typing should be provided by the main animal species and food categories.

Discussion:

HELMUTH: Currently, three activities are going on in parallel, i.e. Enternet, the Communicable Disease Network (CDN) and the CRL-Epidemiology. Is the Commission aware of this and how will the Commission deal with it in future?

CAVITTE: In fact, Enternet is a project linked to the CDN-system. At present, data on human cases are collected both by Enternet and the CRL-Salmonella. In the future, under the new regulations, these data collection systems will be complementary: human data will be collected by the CDN, whereas data on the occurrence of zoonotic agents in the food chain will be collected according to the new Zoonoses Directive.

HENKEN: For risk analyses it is important that human data and data obtained from the food chain will be linked.

CAVITTE: The linkage of these data will be ensured by the EFSA, to which both the human data and the food chain data have to be reported.

2.3 Emerging Salmonella types in the Netherlands

Wilfrid van Pelt, National Institute for Public Health and the Environment, Bilthoven, the Netherlands (See Appendix 6)

The Dutch National Reference Laboratory is the reference laboratory for *Salmonella* spp. isolated from both humans and from (farm) (exotic) animals, foods and the environment. Background data concerning these isolates have been recorded since 1984. As *Salmonella* types are in general host-specific the data have been used for a rather straightforward computation to establish which fraction of cases of human Salmonellosis can be attributed to which category of farm animals. The data are also used in algorithms for automated detection of outbreaks of laboratory-confirmed infections in humans and for algorithms that provide indicators for emerging infections or developments of resistance to antibiotics in animal husbandry that may pose a threat to human health. Two recent examples of such emerging *Salmonella* types are discussed below.

Human laboratory confirmed infections with penta-resistant *Salmonella* Typhimurium DT104 doubled in 2001 compared to former years. The fraction DT104 runs now in line with the emerging of this pathogen in pigs and cattle, the most important animal reservoirs for DT104 in The Netherlands. Together with Ireland the Dutch fraction of DT104 (18% of all isolates) in humans is now the highest in Europe. Dutch laboratory surveillance data on *Salmonella* in humans, farm animals and foods indicates that consumption of food contaminated with DT104 may have a higher risk to result in an infection. A comparative epidemiological study of patients with a *Salmonella* infection in March, April and May 2001 supports the hypothesis that the clinical course of DT104 infections is more severe than infections with other *S.*Typhimurium infections or a *S.*Enteritidis infection. Hence, additional an epidemiological study of DT104 is of importance. Purchase of manure seems to be an important factor in the spread of DT104 among farm animals in The Netherlands. More research is of importance about the contamination of manure and as to how to minimise this riskfactor.

In The Netherlands *S. Paratyphi B* variation Java increased in poultry from less than 2% of all isolates before 1996 up to 40% in 2001. This development in poultry runs in parallel with that in Germany and appears not to occur in other European countries. A German study shows that in the late nineties it concerns isolates of only one multiresistant clone of Java (in The Netherlands as well) whilst isolates before the mid-nineties were genetically much more heterogeneous and sensitive to antibiotics. Although the exposition of humans to contaminated poultry meat is relatively high, human patients with a Java infection are rare. Treatment of poultry flocks with quinolones was about 13% in 2000-2001. Resistance to flumequin of Java increased from 3% between 1996-1999 to 20% between 2000-2002 whilst that of other serotypes in poultry remained about 7%. Java is also fast becoming less sensitive to ciprofloxacin which is the antibiotic of first choice in serious cases of salmonellosis in humans. The ministries of public health, agriculture and the production boards, with their research institutes, together with the poultrymeat production chain integrations have recently decided to work together in order to determine the public health importance of the Java epidemic in poultry and finding measures for effective control in the poultry industry.

Discussion:

WARD: In the UK, *S. Paratyphi B* var. Java is also increasing, both in poultry and in humans. In the past year, we had eight human cases. The strains were multi-drug resistant.

HELMUTH: This is an example of how the list of public health significant serotypes may change. In all probability, selective pressure from the environment, e.g. by exposure to certain substances added to drinking water for animals, will select for resistant clones of bacteria. If this happens to *Salmonella*, the result may have a severe impact to public health.

IMBERECHTS: In Belgium in 2001 three strains of this serotype were isolated.

BAGGESEN: This serotype has not been found in Denmark so far. In which type of poultry does it occur in The Netherlands?

VANPELT: In broilers.

3. Review of the presentations on bacteriology

3.1 Activities of the Dutch National Reference Laboratory for *Salmonella*

Arjen van de Giessen, NRL-*Salmonella*, Bilthoven, the Netherlands (See Appendix 7)

An overview was given of the activities of the Dutch National Reference Laboratory for *Salmonella*. More specifically, the role of the NRL *Salmonella* in the Dutch national *Salmonella* monitoring and control programme in the poultry sector was elucidated. This control programme was implemented in 1997 by the Dutch Production Boards for Livestock, Meat and Eggs under supervision of the Dutch National Authority based on the regulations laid down in the EU Zoonoses Directive. In order to co-ordinate the application of analytical methods in this programme, a national steering group was formed including representatives from the Production Boards, the National Authority and the NRL *Salmonella* as well as *Salmonella* experts from other national institutes. It was concluded that the ISO-method for detection of *Salmonella* in foods and feed (ISO 6579) is not appropriate for examination of faecal samples. Therefore, comparative studies were conducted both at the NRL *Salmonella* and the Animal Health Service leading to the establishment of a branch method for bacteriological detection of *Salmonella* in various poultry samples. This branch method is a modification of the above mentioned ISO method including MSRV as the selective enrichment medium. Furthermore, criteria were defined for participation of diagnostic laboratories in the control programme including approval by the National Authority, accreditation of the branch method and the obligatory participation in ring trials organized by the NRL *Salmonella*. For this, collaborative studies on the detection of *Salmonella* in chicken faeces are organized by the NRL *Salmonella* twice a year using reference capsules containing various levels of *Salmonella* contamination. In the latest ring trial 23 laboratories participated including 17 labs from The Netherlands, 5 from Belgium and 1 from Germany. A repeated failure of a laboratory to meet the criteria for the results of the trials set by the Production Boards will have consequences for its participation in the programme. Recently, alternative methods for bacteriological detection of *Salmonella* have been allowed after validation studies had indicated that these alternative methods offer equivalent results to those obtained by the branch method. The approved alternative methods include the PROBELIATM PCR-method for *Salmonella* detection in poultry faeces, fluff and neck skins and the VIDAS-SLM-method for *Salmonella* detection in fluff. As for the typing of *Salmonella* isolates, identification of SE and STM and of the main *Salmonella* serogroups is performed at a few diagnostic laboratories, while a selection of isolates is sent to the NRL *Salmonella* for sero- and phagetyping and antibiotic resistance testing. In the near future, the NRL *Salmonella* will support a limited number of diagnostic laboratories to implement a limited typing scheme for

identification of specific serotypes covering about 90% (top 10 ranking types) of the *Salmonella* isolates from poultry. For this, the NRL *Salmonella* will provide training courses on serotyping and organize collaborative studies on serotyping.

Discussion:

CAVITTE: Which method is used by the participating laboratories for the testing of *Campylobacter* in ring trials ?

VANDEGIESSEN: There is a branch method for testing in which the method to be used is described.

BAGGESEN: Is it possible for the participating NRLs to buy capsules for their national collaborative trials for standardisation, to reduce the costs and to make the outcome more reliable ?

HENKEN: This is very difficult because the CRL (RIVM) is not producing these capsules commercially. Therefore, each country may have to look for other possibilities. We should also discuss this with the commission. The major problem will be the funding.

CAVITTE: Which international rules did you use for the development of the alternative methods in the long term ?

VANDEGIESSEN: The draft ISO in limited form.

MOOIJMAN: The Joint Research Center (geel, Belgium) has 35 reference materials but they are very expensive. She does not think that they will fund non-certified reference materials.

IMBERECHTS: Suggests to send the method for making these reference materials to the NRLs so that they will know how to do this.

HENKEN: The need for reference materials is getting more wide-spread. The situation is difficult. Should this kind of work fall under the umbrella of the NRLs ?

VANDEGIESSEN: There are also Belgian and German laboratories participating in the Dutch ring trials so the need for reference materials is definitely there.

3.2 Specificity using ISO 6579:2002

Marylène Bohnert, NRL-*Salmonella* France (See Appendix 8)

Specificity data were generated for *Salmonella* and non-*Salmonella* strains using the ISO 6579: 2002 enrichment protocol with isolation agars used by the participants in the EC project SMT4 – CT 96 2098 collaborative study. Plate Count Agar (PCA) enumeration of all *Salmonella* strains was performed after pre-enrichment and after selective enrichment to compare growth levels achieved. In general, after overnight incubation in Buffered Pepton Water (BPW), and after incubation in Mueller Kauffmann Tetra Thionate plus novobiocin (MKTTn) or Rappaport Vassiliadis Soy (RVS), most *Salmonella* levels were approximately

10⁸ cfu/ml (included *S. Paratyphi* B). The growth levels of few *Salmonella* strains (included *S. Virchow*, *S. Dublin*), were lower in RVS and/or MKTTn.

Following selective enrichment in either MKTTn or RVS, the *Salmonella* strains were spread plated onto different selective agars. These selective agar counts were compared against those on PCA plated from the same broth tube and the appearance of colonies were described. The majority of strains produced similar levels on all selective agars, except XLT4, compared to PCA. A higher incidence of decreased growth was seen on XLT4.

One strain of *S. Paratyphi* C and 8 strains of *S. Typhi* survived enrichment in selective broths, but the growth levels were lower than others *Salmonella*.

For the non-*Salmonella* strains tested, growth levels in RVS and/or MKTTn appears to be equal or minor. The colony morphology of surviving micro-organisms are different than typical *Salmonella* for the selective agars evaluated, except some colonies onto BGAgar.

Discussion:

VANDEGIESSEN: Which second medium besides XLD would you suggest ?

BOHNERT: She would suggest Bismuth Sulphite for lactose positive *Salmonella*, but she says that it is very hard to properly interpret the results if you do not have enough experience with this medium.

3.3 Revision of ISO 6579 and working group on horizontal methods

Kirsten Mooijman, CRL-*Salmonella*, The Netherlands (See Appendix 9)

ISO 6579

In the last few years, ISO 6579 (Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.) has been revised. At present the revised ISO is in its final stage. Voting on the Final Draft International Standard (FDIS) was recently closed and was in favour of the revised version. This will result in publication of the final ISO version probably by the end of 2002.

Although the ISO describes a horizontal method for *Salmonella* detection it ‘may not be appropriate in every detail for certain products’.

The main differences with the former version of this ISO are:

Table 3.3.1 Comparison ISO 6579: 1993 and 2002

ISO 6579: 1993	ISO 6579:2002
Pre-enrichment: BPW, (37 ± 1) °C, (18 ± 2) h	Pre-enrichment: (no change) BPW, (37 ± 1) °C, (18 ± 2) h
Selective enrichment: Rappaport-Vassiliadis magnesium chloride-malachite green medium (RV); 42 °C, 24 h Selenite/Cystine medium; 35 °C or 37 °C, 2 x 24h	Selective enrichment: Rappaport-Vassiliadis medium with Soya (RVS); (41.5 ± 1) °C, (24 ± 3) h Muller-Kauffmann tetrathionate-novobiocin broth (MKTn); (37 ± 1) °C, (24 ± 3) h
Plating-out: Phenol red/brilliant green agar; 35 °C or 37 °C, 2 x 24 h 2. Agar of choice	Plating-out: Xylose lysine deoxycholate agar (XLD); (37 ± 1) °C, (24 ± 3) h Agar of choice
Biochemical and serological confirmation	Biochemical and serological confirmation (no change)

Working group

In 2001 a European (ad hoc) Working group was set up named ‘Salmonella-criteria for evaluation’. This Working group was requested to advise the European Commission on criteria to be applied in the evaluation of new methods for Salmonella detection, with the aim to determine whether they are equivalent to the authorised standard methods. The Working group has met three times in 2001 and evaluated several international procedures on validation and comparison of methods. Results of the discussions were summarised in a draft report and will be finalised in the ‘Scientific Committee on Veterinary Measures relating to Public Health’. After agreement of the Scientific Committee the opinion will be sent to the European Commission.

The following recommendation is made in the report:

‘It is desirable that the validation of the alternative method follows an official procedure. The Committee would favour the procedure currently presented collectively by the CEN and the ISO (prEN ISO/FDIS 16140: ‘Microbiology of food and animal feeding stuffs - Protocol for the validation of alternative method’) once adopted’.

Discussion:

MOOIJMAN: If you have any additional information about what should be in the ISO, please make sure that the people who make the ISO get this. This ISO is mainly for food and animal feeding stuff, so not for animal faeces.

BAGGESEN: Do you have the exact components of the tetrathiocyanate ?

MOOIJMAN: I will send you a copy.

HELMUTH: Is there an ISO standard for testing in human material ?

HENKEN: I am not aware of such a standard.

3.4 Quantification of *Salmonella* spp. using a miniturisation of MSRV enrichment medium: mini-MSRV

Philippe Fravallo, Yann Hascoët, Magali Le Fellici, Stephane Queguiner, Julie Petton, Gilles Salvat, NRL-*Salmonella*, Ploufragan, France (See Appendix 10)

Introduction

Salmonella enterica, is considered in most industrialized country as a major cause of foodborne diseases. Integrative epidemiological investigations from “ farm to fork ” are able to give a qualitative evaluation of the respective role of each production step from the primary production to the consumer plate in the occurrence of human salmonellosis. Even if Salmonellosis is most of the time a consequence of the ingestion of large amounts of microorganisms due to successive accumulation of mistakes, the infection primarily originates from the small quantities of *Salmonella* that may occur in the first step of the production. Numeration of *Salmonella* at the early stages of the contamination is of interest for many purposes.

At the primary production steps, numeration of *Salmonella* is a tool to evaluate the efficiency of corrective and preventive measures that may be applied and to quantify their respective efficiency.

At the retail level, it allows a quantitative risk assessment by defining precisely the starting point of the contamination of a retail product, and combined with a monte Carlo simulation, provides an estimation of the risk taking into account the mistakes that may occur during food conservation and preparation by the consumer.

In case of foodborne diseases, quantification of *Salmonella* on a control meal would allow evaluation of the dose response curve.

Unfortunately, routine quantification of *Salmonella* is not possible by direct plating of a sample suspension on a petri dish, and as a consequence, most probable number (MPN) techniques are the only ones to be used for such a purpose. Classical MPN techniques are unreliable and time and money consuming. Published miniaturized MPN methods (2) are more efficient and precise but are still fastidious. As a consequence, this paper try to present a new MPN method, able to analyse simultaneously a lot of samples at a lower cost. This technique is based on the ability of the MSRV medium to select and enriched motile *Salmonella* that may be further isolated on chromogenic agars such as Rambach. The proposed method has been evaluated with 3 different purposes, i.e. to determine:

- Contamination of live pigs;
- Contamination of the environment of live pigs;

- Neck skin contamination of turkey carcasses.

Materials and methods

Mini-MSRV: Samples were diluted 10 fold with peptone buffered water and were homogenised 1 min with a stomacher like for a classical *Salmonella* detection procedure. Two and a half ml of this suspension was distributed in each vial (3) of the first row of a cell culture plate containing a total of 12 vials (3X4). Half a milliliter of each vial from the first row was transferred into 2 ml of PBW in the second row to obtain the first 1/5 dilution. Two additional successive dilutions were done in the same way, all with a microtiter pipette of 500 µl. This pre-enrichment plate was incubated for 16 to 20h at 37°C. The initial suspension in the stomacher bag was also incubated for a classical *Salmonella* detection (16 to 20 h pre-enrichment in PBW at 37°C, enrichment 24 to 48h at 41.5°C on MSRV and streaking of characteristic migrations on Rambach agar incubated 24h at 37°C; identification and serotyping of characteristic colonies).

The pre-enriched 12 vials plate was then agitated 3 to 5 min at 500 rpm on an orbital agitator (IKA 130, labo moderne, France), and then 20 microliter of each vial was replicated with a microtiter pipette on a second 12 vials plate containing 2 ml of MSRV in each vial. The plate was then incubated 24 to 48h at 41.5°C. Characteristic migrations were confirmed by streaking each of them on Rambach agar and identification and serotyping of characteristic colonies. The MPN characteristic number is obtain by counting the number of positive vials in the 4 dilutions 3 repetitions system used in this experiment. The MPN characteristic number was converted in a number of Salmonella/g of the initial sample by using a dedicated software developed and provided freely by the Institut Universitaire de Technologie of Quimper (France) and based on De Man MPN Tables.

Applications to various samples

In order to evaluate the reliability of the technique for the quantification of *Salmonella*, 25 g of pig feces was inoculated with serial dilutions of a suspension of *Salmonella* Typhimurium (c.a. 0.8 to 80 /g).

Then the method was tested on several samples of:

- (i) 25 g of conventional pig feces sampled at an abattoir,
- (ii) large swabs done on 300cm² of lairage ground cleaned and disinfected in a pig abattoir
- (iii) 10g of turkey neck skin sampled at an abattoir and issued from a flock tested positive during rearing.

Results

Table 3.4.1 Salmonella Typhimurium numeration in artificially contaminated pig feces

	Number of S.T.	CI	Number of S.T.	CI	Number of S.T.	C.I.
Inoculum (CFU/g)	0.8		8		80	
Results	1.2	0.2-8.6	9.3	3-27	77	24-241
mini-	1.2	0.2-8.6	4.9	1.5-16	192	60-611
MSRV	1.2	0.2-8.6	17	5.4-53	133	53-431
(CFU/g)	1.2	0.2-8.6	4.9	1.5-16	53	17-160
	1.2	0.2-8.6	3.9	1.1-14	88	27-281
	2.5	0.6-11	17	5.4-53	53	17-160
	2.5	0.6-11	33	10-102	192	60-611
	0	0	53	17-160	133	41-431
Mean mini- MSRV	1.4	0.3-8.1	18	5.6-55	115	36-366

CI : Confidence Interval of the MPN value as calculated by the software

Results provided by the MPN technique generally overestimated the real number of inoculated *Salmonella* Typhimurium, but remained close to the inoculum. Only one sample inoculated with the lowest dose (0.8/g) was not detected by the mini MSRV MPN technique. Both classical detection and mini MSRV MPN numeration technique were applied to 224 samples of pig feces issued from contaminated farms. Mini MSRV numeration technique showed to be less sensitive than classical detection as 43 samples were detected by the *Salmonella* detection procedure while only 18 samples were found positive with the MPN technique. Despite that, mini MSRV MPN technique is of great interest as it could provide an estimation of the distribution of *Salmonella* numbers in pig feces. Results of this distribution were mentioned in table 2.

Table 3.4.2 Distribution of the pig population regarding their Salmonella enterica contamination in feces

<i>Salmonella</i> numeration	Number of samples
< 1 cfu/25g	181
1 cfu/25g < <i>Salmonella</i> < 3.5 cfu/g	25
3.5 /g	1
6 /g	4
33 /g	4
179 /g	3
>890 /g	6
Total	224

The same comparison was done with 192 swabs (large tissues) of 300cm² of lairage zone in a pig abattoir. Twenty seven results were found positive with both classical detection and mini MSRV MPN numeration (sensitivity 100%). Nearly all samples gave results below 1cfu/cm²; In only one sample a result of 4 cfu/cm² was found .

One hundred and twenty two samples of 10 g of turkey neck skin originating from a positive flock were analysed with both techniques. Fourteen samples were detected positive by the routine method while 9 were positive for the numeration. Results were always less than 10 salmonella's /g of neck skin (see table 3) confirming other results on poultry samples.

Table 3.4.3 Distribution of the Salmonella enterica contamination in turkey neck skin

<i>Salmonella</i> numeration	Number of samples
<i>Salmonella</i> <0.1 cfu/g	113
0.1 cfu/g < <i>Salmonella</i> <1 cfu/g	4
1 cfu/g < <i>Salmonella</i> <10 cfu/g	5

Discussion

The mini-MSRV MPN technique presented in this paper is not a detection method. As the amount of primary dilution analysed is less than those of the routine method, the sensitivity of the method is lower. One of the limiting parameters of the method is the way to confirm positive wells. In this paper, the only method used to confirm the migration of positive wells is streaking on Rambach agar plates. This method is time consuming and difficult to automatize. Other confirmation strategies such as sero-enrichment (2) or multiplex PCR (3) may be used to simplify this step. At least, the results given by the method are not precise, as a great confidence interval due to the MPN technique itself is given. For instance when an average amount of 8.3 *Salmonella*/g is given by the method, the confidence interval is 3.1-21.9. The result provided by the mini-MSRV MPN technique is an estimation and is to be considered like that.

Nevertheless, the mini-MSRV MPN technique has its own positive aspects as it allows a quantification of *Salmonella* which is useful for risk assessment purposes.

In the example given in this work, quantification of the amount of *Salmonella* excreted by healthy carrier pig is of great interest in order to take measures at the farm level or to delay their slaughtering to the end of the day to prevent massive cross contamination at the abattoir. In the same extend, the evaluation of cleaning and disinfection measures in the lairage area at the abattoir is a way to quantify the risk of contamination among live pigs just before slaughtering. In our example, the contamination at this stage appears to be very low (nearly all the positive samples were contaminated by less than 1 *Salmonella*/cm²).

The quantification study done on turkey neck skin issued from flocks identified as positive at the farm level, proves that even when birds were positive before their slaughtering, if the abattoir applies GMP and HACCP, the amount of *Salmonella* numerated on the carcasses are

very low. This result would not justify the use of disinfectants on carcasses in order to lower the risk of Salmonellosis.

Considering those three examples, the importance of *Salmonella* quantitation for risk assessment purposes is proved.

The method described in this study can be used as a routine technique and can be more efficient than a streaking method as the competing flora is inhibited by MSRV medium. During retrospective inquiries after foodborne diseases where control meals are available, this technique would allow determination of the dose to which the humans were exposed and in combination with dose-response data this would be very useful for quantitative risk assessment.

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Identification by a multiplex PCR-based assay of Salmonella typhimurium and Salmonella enteritidis strains from environmental swabs of poultry houses. Lett Appl Microbiol. 1999 Jul;29(1):1-6.

Discussion

HENKEN: Can this method play a role in prevention ?

SALVAT: It can also be used to evaluate the remaining contamination after treatment of poultry. The question is: is it interesting for PHLs or not ?

HELMUTH: Can you also use microtiter plates ?

SALVAT: The method would be more time consuming and you can not use MSRV with too small holes because you would not be able to see the migration. However you could use microtiter plates with enriched media.

3.5 Discussion on Bacteriological collaborative study VI

André Henken, Director CRL-*Salmonella*, the Netherlands (See Appendix 11)

The sixth bacteriological collaborative study will (in principle) have the same set-up as study IV and V. However, now the revised ISO 6579 is almost final, some small changes will be introduced.

It was agreed that the following methods will be used in the sixth study:

- Pre-enrichment in Buffered Peptone Water: BPW (same as study V);
- Selective enrichment in:
 1. Rappaport-Vassiliadis medium with soya: RVS (was RV in study V);
 2. Modified semi-solid Rappaport Vassiliadis medium: MSRV (same as study V);
 3. Mueller-Kauffmann tetrathionate-novobiocin broth: MKTTn (extra in comparison to study V).
- Plating-out on:
 1. Xylose lysine deoxycholate agar: XLD (same as study V);
 2. Brilliant Green agar: BGA (same as study V).
- Biochemical confirmation, urea, TSI and LDC (same as study V).

The number of capsules to be used in the sixth study will remain the same as the number used in study V. Also the amount of faeces to be added to the capsules (10 g) will remain the same. It was preferred that also naturally contaminated samples would be distributed for analyses. The sixth study will most probably be organised in October 2002.

Discussion:

HENKEN: Study VI will have the same features as studies 4 and 5 to be able to compare the results over time.

BAGGESEN: Suggestion - Use new ISO as well as old ISO next to each other.

VANDEGIESSEN: Why should MKTT be used in the ring trial ?

BAGGESEN: This offers an important opportunity and evaluation for the ring trial.

HENKEN: Should the proposal for the next bacteriological ring trial include the new ISO ?

PARTICIPANTS: Yes.

4. Review of the presentations on typing

4.1 Results sixth (2001) typing study

Hans Korver, CRL-*Salmonella*, the Netherlands (See Appendix 12)

The sixth collaborative typing study for *Salmonella* was organised by the CRL-*Salmonella* in collaboration with the PHLS, London. Seventeen National Reference Laboratories (NRLs) and 15 EnterNet Laboratories (ENLs) participated in the study. In total, 19 strains of the species *Salmonella enterica* subspecies *enterica* and one strain of the species *Salmonella enterica* subspecies *arizonae* were selected for serotyping and antimicrobial susceptibility testing, while 10 strains of *Salmonella* Typhimurium (STM) and 10 strains of *Salmonella* Enteritidis (SE) were selected for phage typing.

In general, problems with the serotyping of the O-antigens were of minor importance. Most problems occurred with the typing of the second phase of the H-antigens.

The overall results of the phage typing were good. The majority of laboratories again achieved over 90% correct identifications.

Some laboratories used a quantitative method (six in total) like the MIC and all other laboratories tested the susceptibility with an agar disc diffusion test. Both methods gave comparable results.

Susceptibility testing with a variety of antibiotics revealed data which show a certain standardisation in the technique is required for comparison between laboratories. The type of the antibiotic as well as the number of antibiotics should be standardised.

The results of the sero- and phage typing and the antimicrobial susceptibility testing are partly shown in the appendix. The achievements of the sero- and phagotyping results for NRLs and ENLs are shown in the tables below. All results will be presented in report to be published in the summer of 2002.

Table 4.1.1 Achievements in % correctness by the NRLs

Labcodes	O-antigens n=20	H-antigens n=20	Serovar names n=20	SE Phage n=10	STM Phage n=10
1	100	100	100	90	100
2	85	85	85		
3	100	100	100	90	100
4	90	95	90		
5	95	95	95		
6	100	100	100	100	100
7	90	100	90		
8	90	80	65		
9	100	90	95	90	90
10	85	90	80		
11	100	100	100	90	
12	100	100	100		
13	80	90	70	60	60
14	100	90	90		
15	100	95	95		
16	100	100	100	70	100
17	85	95	80		

Table 4.1.2 Achievements in % correctness by the ENLs

Labcodes	O-antigens n=20	H-antigens n=20	Serovar names n=20	SE Phage n=10	STM Phage n=10
A	100	95	95	90	90
B	100	100	100	90	100
C	100	100	100	100	100
D	100	90	90		
E	100	100	95	100	90
F	95	80	70	80	90
H	100	100	100	100	100
J	100	100	100	80	100
K	100	100	100	90	100
L	100	95	95		
P	100	100	100	90	40
R	100	100	100		
S				70	80
T	100	95	95		
V				100	90
W	95	100	95	70	60

4.2 Preliminary results seventh (2002) typing study

Hans Korver, CRL-*Salmonella*, the Netherlands (See Appendix 13)

This presentation was divided over two subjects. At first the preliminary serotyping results of the NRLs were discussed and secondly the problems around the antimicrobial susceptibility testing.

Seventeen NRLs for *Salmonella* participated in the study. The typing results of one laboratory did not arrive in time to include these in the preliminary results. The results of the ENLs were not available at the moment of the workshop. In total, 20 strains of the species *Salmonella enterica* subspecies *enterica* were selected for serotyping by CRL-*Salmonella*.

Table 4.2.1 Evaluation of correct serotyping results

O-antigens	No. of labs		H-antigens	No. of labs		Serovar names	No. of labs
20	12		20	6		20	6
19	3		19	5		19	5
18	0		18	3		18	3
17	0		17	0		17	0
16	0		16	2		16	0
15	1		15	0		15	1
14	0		14	0		14	1

Some laboratories had problems in serotyping strains 6, 10, 11 and 16 respectively belonging to serovars *S. Paratyphi B* var Java, *S. Paratyphi B* var Java, *S. Vinohrady* and *S. Oranienburg*.

In the past two collaborative serotyping studies (V and VI) antimicrobial susceptibility testing was included. Due to the amount of antibiotics (total >50) used in these two studies and the two methods (MIC and disc diffusion) a need was felt for standardisation and harmonisation. The amount of antibiotics to be tested in a next study will be decreased to twelve. These twelve antibiotics are: ampicillin, chloramphenicol, ciprofloxacin, florfenicol, gentamicin, kanamycin, nalidixic acid, neomycin, streptomycin, tetracyclin, trimethoprim and sulfamethoxazole/trimethoprim (see also newsletter of June 2002). In one of the upcoming newsletters CRL-*Salmonella*

want to include a questionnaire in which questions about the following subjects will be asked: method, antibiotic load, inhibition zones in mm, maximum number of antibiotics per plate, tickness of medium, incubation time and temperature, inoculum size and measurement of the

inoculum size. Furthermore insight is needed in the relationships of MIC-breakpoints per antibiotic/country and the diameter of the inhibition zones (disc diffusion) to notations resistant/intermediate/sensitive.

Discussion

GUERRA: An antibiotic belonging to one of the cefalosporine should also be included in the list, this is recommended by the NCCLS.

RICCI: It is quite difficult to find the discs for chloramphenicol, that is the reason it is not used very often in Italy. Also quality control should be included in the questionnaire.

CAVITTE: Is there a possibility to make the determination of the H-antigens easier ?

WARD: There is a problem with commercially produced antisera, the factor 1 and 2 are not absorbed and therefore cross-reactions are possible. The PHLS in London will be testing antisera.

4.3 Salmonella Phage Typing

Linda R. Ward, Laboratory of Enteric Pathogens, Public Health Laboratory Service, London, United Kingdom (See Appendix 14)

Phage typing has over many years proved to be a very successful method of strain differentiation for the common *Salmonella* serovars. This technique has been invaluable as an epidemiological tool particularly in tracing and proving the source of many outbreaks.

There are currently ten salmonella typing schemes in routine reference use in the Laboratory of Enteric Pathogens (LEP) of the Public Health Laboratory Service in England and Wales; *Salmonella* Typhi, Paratyphi A, Paratyphi B / Java, Enteritidis, Typhimurium, Hadar, Virchow, Thompson, Pullorum and Agona. In addition, schemes for Newport, Kedougou and Bredeney have been developed.

The schemes for *Salmonella* Typhi, Paratyphi A and Paratyphi B are the most important in terms of disease whereas those for *S. Typhimurium* and Enteritidis are more important in terms of the number of human isolates in England and Wales. All the schemes continue to evolve with new types being recognised and new phages being isolated or adapted to type or differentiate previously untypable strains.

The phage-typing scheme for *S. Enteritidis* was first developed in the late 1950s and in 1960 had six typing phages that recognised nine types. Today the scheme contains 16 typing phages and defines in excess of 80 types. As the scheme develops the established phage

types can be further differentiated with the addition of the new typing phages, for example PT4 and PT1 have been split into PT4 and PT4b and PT1 and PT1b due to the addition of phage 16.

The use of the Enteritidis scheme has proved invaluable in following the emergence of PT4 in England and Wales. Numbers of human isolations rose from less than 400 in 1981 to 17,603 in the peak year of 1993. There has been a subsequent decline from 1997 (15218 isolates) to 5001 and 5036 in the years 2000 and 2001, respectively.

With day-to-day surveillance, new types are detected quickly and can be added to the scheme. *S. Enteritidis* PT44 was first defined in 1997 and is a type associated with travel to the Canary Islands. PT5c made a major impact in 2001 in England and Wales with 326 human infections, 28% with known foreign travel particularly to Tenerife and there were three outbreaks confirmed to be associated with egg consumption in England.

The most complex salmonella phage-typing scheme is the one for *S. Typhimurium*. The current scheme has been derived from that of Felix in 1943 followed by Callow in 1959 and Anderson et al. in 1977. Today's scheme contains 38 typing phages and recognises over 280 types. It has been very valuable in many large 'epidemics', particularly in the past decade following the emergence and increase of the multi-resistant strains of DT104 both in the UK and other countries worldwide. DT104 is also changing into new types; for example the provisional phage types U302 with multi-resistance and U310, which can carry multi-resistance identical to U302 but is more commonly resistant to tetracyclines alone. This strain has been linked to pigs.

In recent years a multi-resistant strain of *S. Paratyphi* B variant Java has been found in chickens and chicken products. To easily identify this strain, the LEP is currently developing a new typing phage from one of the *S. Paratyphi* B / Java typing phages.

Salmonella phage typing with its large historical data base continues to play a major role in salmonella epidemiology. Changes in the numbers of particular phage types and in the types themselves, together with the emergence of new types can be rapidly monitored worldwide.

Discussion

KORVER: In the last typing study we had some difficulties with type 104 in relation to type 104L. Please could you explain this ?

WARD: 104H(igh) is a parent of 104. 104L(ow) is multiresistant. Some people call 104 High and Low just 104, but others call them 104 High and 104 Low.

GUERRA: Is there a relationship between dt 120 and dt 104 ?

WARD: dt 120 is only resistant to phage 18. The resistant dt 120 is derived from 104 by blocking reactions to phage 12 + 13.

HELMUTH: If you would sequence them they would be unrelated, but phenotypically they are related.

WARD: From 104 Low we can make a 104b Low by addition of a phage.

HELMUTH: We can detect 104s but some behave like for instance *Salmonella* Dublin.

WARD: The phage typing scheme is very big and complicated and strains are evolving all the time.

4.4 Discussion on typing study 8 (2003)

Andre Henken, Director CRL-*Salmonella*, the Netherlands (See Appendix 15)

Discussion

The following was agreed upon:

The same number of serotypes will be used in the 8th typing study.

We will continue with the same system that we used before, i.e. using the most important types from a public health perspective and those that may be easily confounded with those important ones.

It is agreed to wait taking decisions how to proceed on antimicrobial testing until the questionnaires returned and evaluated.

5. Review of the presentations on miscellaneous subjects

5.1 Overview of research on *Salmonella* in pigmeat production at VLA

Rob Davies, NRL-*Salmonella*, Addlestone, United Kingdom (See Appendix 16)

Intensive faecal and environmental sampling was carried out on over 30 pig farms and this resulted in over 99 visits and culture of nearly 21,000 samples. Sensitive *Salmonella* culture techniques were used and all isolates were serotyped and, when appropriate, phage typed. Selected isolates were tested by disc diffusion technique for resistance to 16 antimicrobials and examined by plasmid profile analysis. Interventions such as improved disinfection and rodent control, feed acidification, vaccination and reduced breeding stock replacement were examined by carrying out additional sampling visits. An analytical questionnaire, in combination with *Salmonella* testing was applied to all rearing and finishing farms in an integrated company. Studies of contamination in the slaughter process were carried out in a series of visits to two abattoirs in which intestinal contents and carcass swabs, as well as a selection of other tissues, were taken. An ELISA test which was comparable with the Danish Meat juice ELISA was developed and evaluated on samples from experimentally infected pigs and from pig herds and abattoirs. The range of the test was extended by adding additional antigens and applying the test to alternative tissues. The survival of *S. Typhimurium* DT104 on a range of building and bedding materials was evaluated quantitatively over a 31 month period and a surface faecal contamination model was developed to compare commonly used agricultural disinfectants.

Multiple resistant *S. Typhimurium* appeared to survive well in pig herds for at least two years but then begin to reduce on some of the farms. In most cases the greatest level of contamination was in groups of finishing pigs, especially in continuously occupied or poorly disinfected houses. Breeding stock was less likely to be involved except where cleansing and disinfection or rodent control in farrowing rooms was poor or there was a high turnover in replacement breeding stock. Rodents, wild birds and farm cats were commonly involved when the level of environmental contamination was high but these were not considered to be primary vectors. They did however cause contamination of nearby stores holding grain for onward sale. Sampling in disinfected houses showed current methods to be inadequate in most cases and recontamination of pens by mouse droppings after disinfection was common. A wide range of *Salmonella* serotypes and phage types were found on most of the farms. In some cases these changed over time, suggesting introduction of new strains was the

predominant factor rather than persistence. This observation was supported by the finding of *Salmonella* in faeces samples from replacement stock on several occasions. Persistence of particular strains was also identified on some farms however.

Interventions such as vaccination, feed acidification and acidified liquid feed were not successful but a high level of *Salmonella* control was achieved in batch finishing systems by dry cleaning and disinfection with 2% formalin solution. In many cases this success was compromised by restocking the disinfected unit with pigs which were already carrying *Salmonella*. On 3 farms which were either closed or ceased buying in breeding stock during the study *S.Typhimurium* disappeared. It is thought that this is likely to be due to the development of herd immunity, which is difficult to achieve on larger farms with a high breeding stock replacement rate.

The survey of 161 farms in an integrated company identified 70% of farms as currently infected with *Salmonella*, with nursery farms more heavily infected than finishing farms. The risk of *S.Typhimurium* infection also increased with increasing herd size and with longer established premises, suggesting both persistence and newly introduced *Salmonella* infection was involved.

The *Salmonella* ELISA developed at VLA was shown to be an effective means of identifying highly infected farms but it failed to identify all individual pigs excreting *Salmonella* at slaughter.

The abattoir studies demonstrated that virtually all surface contamination of pigs could be eliminated by scalding at 62°C or more, followed by singeing but then evisceration and carcass splitting resulted in up to 20% of carcasses being contaminated on a day when highly infected herds were slaughtered. There was also evidence of cross-contamination of subsequent batches of salmonella-free pigs slaughtered but little carry-over of contamination from day-to-day.

Salmonella Typhimurium DT104 survived for at least 31 months on building materials and for at least 3 months in soil on an outdoor pig unit. Only formaldehyde solution was effective in decontaminating surfaces contaminated with DT104 in a thin faecal layer.

The work on pig farms, together with the results of the first National Abattoir Survey, has shown that there is a considerable problem with multiple antimicrobial resistant *Salmonella*, particularly *S.Typhimurium*, in the UK pig herd. A large part of the industry, particularly the primary breeding sector, has adopted biosecurity measures to limit the dissemination of major porcine pathogens. *Salmonella*, being largely subclinical and in the absence of any statutory control measures is unaffected by these so specific control measures are required. The work carried out in this project has shown that the organism is normally widespread and persistent on infected farms. Many farms are continuously occupied so there is no opportunity to eliminate *Salmonella* from the site. Similarly, long-term carriage of *Salmonella* in adult stock replacements occurs and may be a source of infection which is difficult to control. The study has also shown that standards of cleaning and disinfection and pest control on many pig farms are insufficient to eliminate contamination from animal housing so that infection is

perpetuated by a mixture of environmental contamination and subclinical infection in carrier pigs and wildlife vectors.

Attempts made to reduce *Salmonella* by use of fermented or acidified feed, depopulation and vaccination were largely unsuccessful as were ad-hoc attempts to upgrade hygiene. More systematic hygiene improvements did have a beneficial effect but were not sustained because of the cost of the increased labour and chemical disinfectant requirement. Formaldehyde and phenolic disinfectants were the most effective under field and model faecal contamination conditions.

Studies carried out in the abattoir showed that although the majority of pig carcasses were contaminated after killing/bleeding the scald and singe process was effective so careful evisceration techniques can limit subsequent contamination. It is therefore worth investing more effort at this point. Molecular genetic studies of isolates of *S. Typhimurium* DT104 from farms showed a greater diversity in plasmid profile type than in pulsed field gel electrophoresis type, possibly as a result of greater mobility of plasmids in a situation where selective pressure is high due to frequent and antimicrobial therapy. The results of this project helped in the design of a Code of Practice and more detailed guidance document for prevention and control of *Salmonella* in pigs and contributed to the design of the MLC/BPEX National *Salmonella* Monitoring and Control Initiative.

The longitudinal studies element of this project is being continued as part of project OZ0316, which will also incorporate a nationwide prevalence survey, analytical study and intervention studies. Other work in progress involves development of an ELISA test protocol for use on bulked meat juice and assessment of the effect of different feeding and housing systems on *Salmonella* infection.

Discussion

KORVER: May a 1% formaldehyde solution still be used ?

DAVIES: Yes, as a precaution.

5.2 Antimicrobial Resistance in *Salmonella* and *E. coli* from abattoir surveys and passive surveillance

Rob Davies, NRL-*Salmonella*, Addlestone, United Kingdom (See Appendix 17)

Antimicrobial resistance was detected in both cattle-derived salmonella isolates but not in the isolate from sheep. One of the *S. Typhimurium* isolates from cattle was DT 12 which demonstrated multiple antimicrobial resistance (to tetracycline, ampicillin, chloramphenicol, streptomycin and compound sulphonamide). The other cattle isolate, *S. Typhimurium* PT193,

was resistant to furazolidone. In 1999, 9 isolates of *S.Typhimurium* DT 12 were tested for antimicrobial susceptibility from incidents of clinical disease in cattle and of these, two isolates (22.2%) had an identical resistance pattern to the isolates recovered in the abattoir survey. There were 15 isolates of *S.Typhimurium* DT 193 recovered from incidents of clinical disease in cattle and of these only one was resistant to furazolidone, with an identical pattern to that recovered in the abattoir survey. *Salmonella Typhimurium* DT 104 was not recovered from cattle during the abattoir survey and although this organism has affected all types of cattle, dairy cows and calves have been most affected, rather than beef animals.

75.6% of salmonella isolates from pigs at slaughter were resistant to tetracyclines, 44.8% to sulphonamide, 28.1% to ampicillin, 27.1% to streptomycin, 24.3% to trimethoprim/sulphonamide, 17.9% to chloramphenicol, 7.4% to cefoperazone and 4.1% to nalidixic acid. Resistance to other antimicrobials in the panel was uncommon and no resistance to cefuroxime, amikacin, amoxycillin/clavulanic acid or colistin was identified. The widest spectrum of resistance was seen in *S.Typhimurium*, where 93.6% of isolates were resistant to tetracycline and 7.4% resistant to nalidixic acid. *S.Derby* showed 90.9% resistance to tetracycline, 18.8% to sulphonamide and 15.2% to trimethoprim/sulphonamide.

Amongst the other more commonly isolated serotypes resistance to tetracycline, sulphonamide and trimethoprim/sulphonamide also predominated. 77.4% of *S.Goldcoast* were fully sensitive as were 70% of *S.Panama* and 23.1% of *S.Kedougou*. 42.3% of *S.Kedougou* isolates showed resistance to 2 antimicrobials and these largely comprised various combinations of tetracycline, streptomycin, sulphonamide and trimethoprim/sulphonamide.

The frequency of antimicrobial resistance in all salmonella serotypes and *S.Typhimurium* was lower in the abattoir survey isolates than in Zoonoses Order isolates with the exception of a low average level of furazolidone resistance and apramycin resistance in *S.Typhimurium*. *S.Typhimurium* showed the highest frequency of multiple resistance with 20.2% resistant to 6 antimicrobials and 0.6% (2 isolates) resistant to 9 antimicrobials (Am, C, S, Su, T, Na, Tm, Cn, Apr). Nalidixic acid resistance was found in 30 isolates, most commonly *S.Typhimurium* DT193 (17.7% resistant). Resistance to nalidixic acid was also found in 7.5% of DT104, 6.7% of 104B and 4.8% of U302 isolates and two probable rough variants of *S.Goldcoast* were also resistant.

The most common serotypes of salmonella recovered in the pig abattoir survey were *S.Typhimurium* and *S.Derby* and sulphonamide and trimethoprim resistance were the commonest resistances observed in the porcine salmonella serotypes, probably reflecting relatively common exposure of the types of salmonella involved to these antimicrobials. This is illustrated by the finding that 70% of *S.Derby* isolates were resistant to tetracyclines and that 13.1% were resistant to tetracyclines and trimethoprim/ sulphonamides; 8.1% of *S.Derby* isolates were fully sensitive. The most common resistance in *S.Typhimurium* (26.4% of isolates) was also to tetracycline alone. In all a large number of resistance patterns were present, thus demonstrating the likely diversity of the strains involved.

Resistance levels detected in commensal *E.coli* recovered during the abattoir surveys of cattle and sheep were markedly lower than levels detected in *E.coli* from animals and bovine/ovine specimens recovered from all VLA regional laboratories in 1998/1999. This will in part reflect the trend to decreasing resistance generally observed in older animals but also the nature of the population sampled (healthy animals at slaughter as opposed to clinical disease outbreaks). There was very low overall resistance in the isolates recovered from cattle and sheep at slaughter, with no particular antimicrobial giving cause for concern. In total, 67 isolates of commensal *E.coli* demonstrated resistance; 45 were isolated from cattle. Of these, 44 (29 from cattle) were resistant to multiple antimicrobials, representing 3.4% and 1.7% of isolates recovered from cattle and sheep respectively. Resistance was most frequently demonstrated against tetracycline in both species.

Resistance levels detected in commensal and other *E. coli* recovered during the pig abattoir survey were similar or rather lower than levels detected in *E.coli* from pigs/porcine samples referred to VLA regional laboratories during 1998 and 1999. A total of 2,491 *E.coli* isolates were recovered from pigs during the abattoir survey and of these 195 (7.8%) were sensitive to all 16 of the antimicrobials tested. Between 1986 and 1991, 3,590 *E.coli* isolates were received at VLA Weybridge for serotyping from the Veterinary Investigation Service in England and Wales. During this period, 14.6% of isolates received were sensitive to all of the antimicrobials tested. Resistance to tetracyclines was detected in 58% of isolates as opposed to 78.4% of isolates in the current abattoir survey. Clinical isolates of *E.coli*/non-haemolytic coliforms from pigs of all ages referred to VLA regional laboratories in 1998 and 1999 showed levels of resistance to tetracyclines of between 74 and 86%. Neomycin resistance was 17% in the 1986-1991 survey, and was 21.6% in the current abattoir survey. Neomycin resistance in *E.coli*/coliform isolates from pigs received at VLA regional laboratories was between 17 and 20% in 1998 and 1999. The level of ampicillin resistance recorded during the abattoir survey was 25.3%, very similar to the figure of 25% ampicillin resistance recorded by Wray et al in the 1986-1991 survey. This compares with figures of 41-45% in *E.coli*/non-haemolytic coliforms recovered from pigs of all ages at VLA regional laboratories in 1998 and 1999. Furazolidone resistance levels were 3.7%; similar to the level of 3% recorded in 1986-1991 by Wray et al. Trimethoprim/ sulphonamide resistance was 30.9% in the current abattoir survey and 19% in the 1986-1991 survey by Wray et al. Levels in clinical isolates from pigs at VLA regional laboratories in 1998 and 1999 ranged between 44 and 53%. Chloramphenicol resistance levels were 7% in the 1986-1991 survey of Wray et al., though were 20.9% in the abattoir survey. Levels of resistance to Gentamicin and Apramycin were similar in the current abattoir survey and in the survey of Wray et al. in 1986-1991. Apramycin resistance varied between 7 and 17% in 1998 and 1999 in *E.coli*/coliforms from pigs of all ages recovered at VLA regional laboratories. Streptomycin resistance levels were 37.3% in the current abattoir survey and 47% in the 1986-1991 survey by Wray et al. 3% of *E.coli*/coliform isolates from pigs of all ages referred to VLA regional laboratories showed resistance to Enrofloxacin, whilst only 0.6% of *E.coli* isolates recovered during the abattoir surveys were resistant to Nalidixic acid.

Resistance to one or more antimicrobials was identified in 14 of 186 isolates (7.5%) of VTEC O157 recovered from cattle. Of the 14 resistant isolates, 13 (92.9%) were resistant to tetracycline and sulphonamide compounds and this pattern of resistance to sulphonamides and tetracyclines has been reported to occur frequently in animal isolates. Multiple resistance was not detected.

The 7 *E.coli* O157 isolates recovered from pigs that were EAE positive and VT2 positive were sensitive to all of the antimicrobials in the panel of 16 tested. The 70 isolates of O157 recovered from sheep were also fully-sensitive.

Discussion

No questions

5.3 The Food Micro Database: Optimisation of data from private testing, for monitoring of *Salmonella* spp. in the Republic of Ireland

Karen McGillicuddy, NRL-*Salmonella*, Dublin, Ireland (See Appendix 18)

Salmonellosis accounts for over 600 cases of gastro-enteritis per year in the Republic of Ireland (ROI), making it the second most common cause of bacterial food poisoning in ROI. A control programme for salmonellae in poultry has been in operation here since 1988. Under this programme a two-tier sampling system was developed; official samples are taken by the Department of Agriculture Food and Rural Development (DAFRD) and tested at the Central Veterinary Research Laboratory (CVRL) and private samples collected by the industry are examined in commercial laboratories. Only laboratories approved by DAFRD are authorised to undertake these tests. One of the conditions for approval by DAFRD is that a report of all salmonella tests must be submitted to the CVRL on a monthly basis. Since 1996 the level of reporting has expanded to include the results of salmonella tests from animal feed and food for human consumption. This information is now being collated in a new database (The Food Micro Database) developed in 2001, and supported by the Food Safety Promotion Board (FSPB). Currently there are 21 laboratories contributing to this database, 19 from the ROI and 2 from Northern Ireland, which test samples from ROI customers. The approved laboratories in ROI also submit any *Salmonella* isolates they recover to the CVRL for further identification. In 2001 data on over 52000 submissions were

recorded; approximately 75% (38000) of these tests were results from food samples. Preliminary analysis of the data indicates that *Salmonella* are most frequently isolated from raw poultry and raw pork. The Food Micro Database stores information on the range of samples being tested for salmonellae and the identity of any positives isolated. This information should contribute significantly to the monitoring of *Salmonella* spp. in the ROI.

Discussion

CAVITTE: ISO 43 may be used for the organisation of ring trials. How are strains stored ?

MCGILLICUDDY: Frozen at the moment.

5.4 DNA fingerprinting for food-related salmonellosis – a European collaboration

Tansy Peters, E.J. Threlfall, I.S.T Fisher, O.N. Gill, London, United Kingdom - on behalf of the Salm-Gene Project Group (See Appendix 19)

Presented by Reiner Helmuth NRL-*Salmonella*, Berlin, Germany

Objectives

Much salmonellosis prevention and control depends on early outbreak recognition through a suitable surveillance system. The value of phenotypic typing methods as surveillance tools is well established and DNA fingerprinting is used as an adjunct in outbreak investigations in which enhanced strain discrimination is needed. Our study, which currently involves ten national reference laboratories within Europe, investigates the potential for improvement in the current surveillance system when DNA fingerprinting is used to routinely sub-type *salmonella* isolates. The participating centres include Austria, Denmark, England, Finland, Germany, Italy, The Netherlands, Scotland and Spain with France acting as an advisor on software compatibility.

The aim was to develop standard laboratory operating procedures for pulsed-field gel electrophoresis (PFGE) and for computer recognition of the results, and to develop and assess other DNA-based methods for the subtyping of salmonella bacteria.

Methods

Initially each participating centre received a set of 16 *S. enterica* strains to be used for quality assurance of the methods. Each laboratory then selected 500 strains of *S. enterica* representing currently defined serotypes of epidemiological importance within their country. An agreed protocol for rapid PFGE was used which involved proteinase K lysis of cells, a

series of washes at 50°C followed by digestion with *Xba*I. Gel images were exchanged in tag image file format (tiff files) for comparison between centres. Results: We have harmonised a method for PFGE that gives reproducible results and is currently being applied in selected European reference laboratories. By using defined parameters for electrophoresis the gel images produced were comparable between each centre despite slight variations in DNA preparation. Electronic recording and transmission of data between laboratories has enabled the formation of an international database of gel profiles.

Conclusions

For the control of *Salmonella* precise identification of the organism is an essential prerequisite. We used a harmonised PFGE protocol that takes into account some of the differences between different European centres. While standardisation of DNA preparation and digestion were not considered to be essential, standardisation of the parameters for electrophoresis was considered to be an absolute requirement. We are compiling a searchable database of DNA fingerprint information that will allow us to describe the range and incidence of sub-types for the major salmonella serotypes within Europe. The use of internationally agreed methods for DNA fingerprinting allows countries in the EU to rapidly compare subtypes of salmonella organisms responsible for international food-related outbreaks.

Discussion

No questions

6. Closing remarks

André Henken, CRL-*Salmonella*, the Netherlands

General discussion

The workplan for 2002 and 2003 has been discussed.

In the autumn of 2002 a sixth bacteriological collaborative study will be organised. This study will in principle have the same set-up as study IV and V. However, the revised ISO 6579 is almost final and therefore some small changes will be introduced.

It was agreed that the following methods will be used in the sixth study:

Pre-enrichment in Buffered Peptone Water: BPW (same as study V);

Selective enrichment in:

- Rappaport-Vassiliadis medium with soya: RVS (was RV in study V);
- Modified semi-solid Rappaport Vassiliadis medium: MSRV (same as study V);
- Mueller-Kauffmann tetrathionate-novobiocin broth: MKTTn (extra to study V).

Plating-out on:

- Xylose lysine deoxycholate agar: XLD (same as study V);
- Brilliant Green agar: BGA (same as study V).

Biochemical confirmation:

- urea, TSI and LDC (same as study V).

The number of capsules to be used in the sixth study will remain the same as the number used in study V. Also the amount of faeces to be added to the capsules (10 g) will remain the same. It was preferred that also naturally contaminated samples would be distributed for analyses. The sixth study will most probably be organised in October 2002.

Next spring (2003) an 8th typing collaborative study will be organised including serotyping, phagotyping and antibiotic resistance typing.

Serotypes selected will be ones that are important in terms of public health or ones that are easily confounded with those important ones. Again phagotyping will be included using 10 *S. Enteritidis* strains and 10 *S. Typhimurium* strains from PHLS. Depending on the outcome of a questionnaire on antibiotic resistance typing among the NRLs-*Salmonella* (to be included in the next newsletter) the typing of antibiotic resistance will be prescribed in the next study (e.g., antibiotics and methods used).

In the autumn of 2003 the 7th bacteriological collaborative study will be organised, the details of which will be discussed at the next workshop to be held in May 2003 in Bilthoven.

Each quarter the newsletter will be published. The CRL-Salmonella will be available for questions, for instance in case of follow-up to studies organised.

Evaluation of the workshop

If we go back to the objectives of the workshop, did we succeed? At the workshop a presentation was given of the new draft zoonosis directive, several reports of other workshops and meetings were given, results of collaborative studies of the CRL-Salmonella were presented and discussed, several research activities in Member States were reported and the plan of the CRL-Salmonella for the second half of 2002 and for 2003 was discussed. However, still hardly any national collaborative study was presented. This is at least partly due to the absence of suitable reference materials for such studies. The NRLs-Salmonella preferably would like to use the same kind of reference materials (capsules) the CRL-Salmonella is using, but these capsules, containing *S. Typhimurium* and *S. Enteritidis*, are not commercially available. The RIVM has the knowledge and experience to support production of capsules, but the EU Commission does not consider research and production of reference materials a task of a CRL. At the January meeting of all CRLs in the veterinary field in Brussels a similar need was expressed by other CRLs. It was suggested to make contact with the Joint Research Centre in Geel to discuss this issue. The contact person of the Commission promised to facilitate this discussion. In the meantime the CRL-Salmonella will inventarise possibilities of alternative solutions.

So, except for reports on the national collaborative activities, the objectives of the workshop were met.

Actual closure of the workshop

The participants from the Member States 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 is acknowledged for their support also in financial terms to make this workshop possible. It is very much appreciated that we were allowed to have this meeting in Ploufragen in combination with the International Symposium Salmonella and Salmonellosis. The participants consider the workshop a necessary element in their annual programme. The CRL-Salmonella team is acknowledged for their work of the previous year, especially as it was a difficult year because 'old' staff members left. Luckily, new staff members could be hired and these are wished much success in their new job. The workshop organising team is thanked for their work which was related mostly to the content of workshop as such, because our French hosts did a great job by taking care of the logistics of the workshop. I therefore would like to express my sincere gratitude to our hosts, especially Ms. Genevieve Clement, for efforts and support to make 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. J. Sixma
06	Veterinary Public Health Inspector	drs. H. Verburg
07	Board of Directors RIVM	H.A.P.M. Pont
08	Director Sector Public Health	prof. dr. ir. D. Kromhout
09	Head of Microbiological Laboratory for Health Protection and Director CRL- <i>Salmonella</i>	dr. ir. A.M. Henken
10	Head of Laboratory for Analytical Residue Research	prof. dr. W.R. Stephany
11-44	Participants of the workshop	
45-47	Authors/Editors	
48	Dutch National Library for Publications and Bibliography	
49	SBC/Communication	
50	Registration agency for Scientific Reports	
51	Library RIVM	
52-65	Sales department of RIVM Reports	
66-70	Spare copies	

Appendix 2 Participants

National Reference Laboratories for *Salmonella*

Austria

Christian Berghold
Christian Kornschöber

Belgium

Hein Imberechts

Denmark

Dorte Lau Baggesen
Jens Chr. Jørgensen
Gitte Sørensen

Finland

Tuula Johansson
Sinikka Pelkonen

France

Gilles Salvat
Marylène Bohnert

Germany

Reiner Helmuth
Beatriz Guerra Roman
Christina Dorn

Ireland

John Egan
Bernard Bradshaw
Karen McGillicuddy

Italy

Antonia Ricci
Denis Vio

Luxembourg

Joseph Schon

The Netherlands

Arjen van de Giessen
Edda van Raamsdonk

Portugal

Alice Amado
Maria Do Rosario Vieira

Spain

Consuelo Rubio Montejano
Cristina de Frutos Escobar

Sweden

Ingrid Hansson
Anna Aspan

United Kingdom

Robert Davies
Linda Ward

CRL-*Salmonella*

André Henken
Hans Korver
Kirsten Mooijman
Wilfrid van Pelt

CRL-Epidemiology of Zoonoses

Annemarie Käsbohrer

Commission

Jean-Charles Cavitte

Appendix 3 Programme of the workshop

Monday 27 May

20.30 - 21.30 Social get together, bar hotel

Tuesday 28 May

appr. 8.00 Departure from hotel to place of workshop

8.30 - 8.45 Opening and introduction of participants
(André Henken)

8.45 - 9.30 State of play on the proposed revision of the zoonoses legislation and
developments for CRL and NRLs
(Jean-Charles Cavitte)

9.30 - 10.00 Report of the CRL-Epidemiology of Zoonoses
(Annemarie Kaesbohrer)

10.00 - 10.30 Emerging *Salmonella* types in the Netherlands
(Wilfrid van Pelt)

10.30 - 11.00 Coffee/tea

11.00 - 11.20 Dutch National Reference Laboratory for *Salmonella*
(Arjen van de Giessen)

11.20 - 11.40 Specificity using ISO 6579
(Marylène Bohnert)

11.40 - 11.55 Revision of ISO 6579 and working group on horizontal methods
(Kirsten Mooijman)

11.55 - 12.15 Quantification of *Salmonella* spp. Using a miniturisation of MSRV
enrichment medium: mini-MSRV
(Gilles Salvat)

12.15 - 12.30 Discussion on bacteriological collaborative study VI
(André Henken)

12.30 - 14.00	Lunch (during lunch: forms, copies of tickets, etc) (CRL- <i>Salmonella</i> team)
14.00 - 14.20	Test results of <i>Salmonella</i> typing by NRLs and ENLs – Collaborative study VI – 2001 (Hans Korver)
14.20 - 14.40	Preliminary results collaborative typing study VII – 2002 (Hans Korver)
14.40 - 15.00	A presentation on phage typing (Linda Ward)
15.00 - 15.15	Discussion on collaborative typing study VIII – 2003 (André Henken)
15.15 - 15.45	Coffee/tea
15.45 - 16.05	Epidemiological studies of multiresistant <i>Salmonella</i> Typhimurium in pigs (OZ0134) (Rob Davies)
16.05 - 16.25	Veterinary surveillance for antimicrobial resistance in <i>Salmonella</i> and <i>E.coli</i> (Rob Davies)
16.25 - 16.45	The Food Micro Database: optimisation of data from private testing, for monitoring of <i>Salmonella</i> spp. in the Republic of Ireland (Karen McGillicuddy)
16.45 - 17.05	The Salm Gene project (Reiner Helmuth)
17.05 - 17.30	Closing remarks (André Henken)
17.30 - 18.00	Last opportunity to provide the CRL- <i>Salmonella</i> team with your documents necessary for reimbursement of travel and subsistence costs (CRL- <i>Salmonella</i> team)
18.00 -	Departure to hotel
20.00 -	Dinner


Appendix 4 Slides of presentation 2.1

Slide 1

Revision of the Zoonoses legislation

Principle:
Safe food from healthy animals

J-Ch Cavitte, DG SANCO, Biological risks unit



Slide 2

Reasons for revision of the legislation

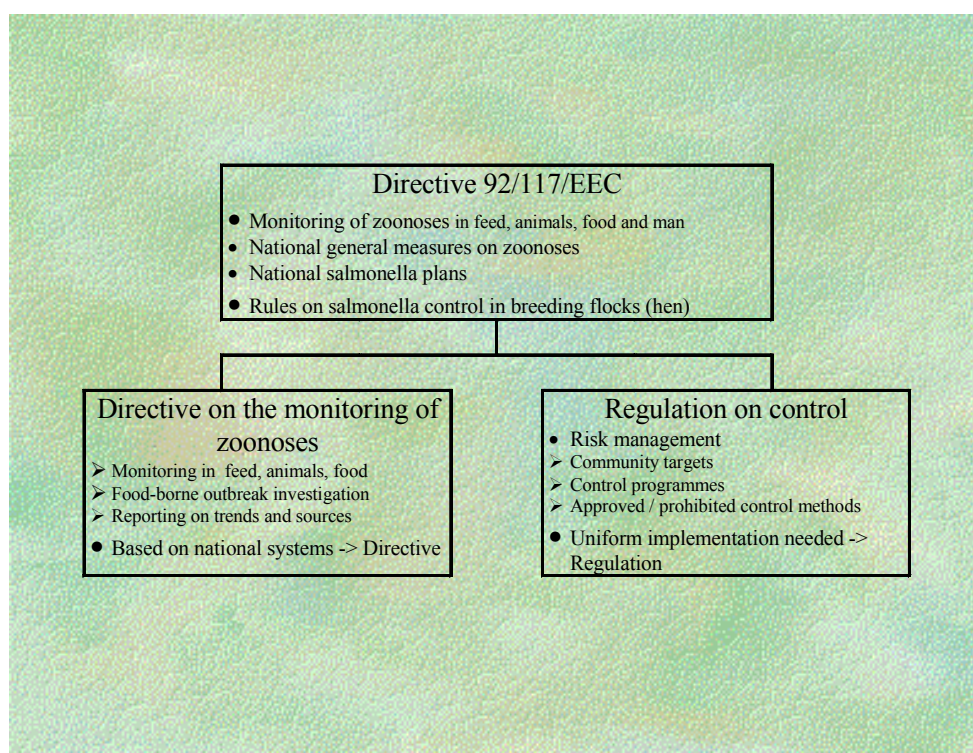
- Need to decrease incidence of zoonoses in humans
- Need to improve the control of zoonoses in the primary production
- Need to strengthen the collection of relevant data, to support possibly risk assessment activities and risk management decisions

Slide 3

Review of zoonoses legislation

The proposals for revised zoonoses legislation were adopted by the Commission in August 2001
Now in discussion in the Council and the European Parliament, for co-decision.
EP adopted its position in first reading on 15.05.02

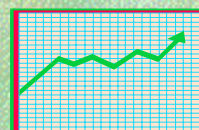
Slide 4



Slide 5

Proposed Directive on monitoring

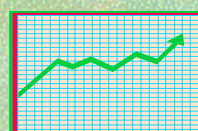
- Surveillance throughout the food chain
 - EP: food of animal and plant origin
- Monitoring based on the systems already in place in the MSs, but option to harmonise
 - EP; +/- C: stress on aim for comparable data
- Need to consider schemes and methods



Slide 6

Proposed Directive on monitoring

- Co-operation between competent authorities in animal/food/human health sectors in the MSs
 - EP: also animal feed and other authorities
- Coordinated monitoring programmes at Community level (e.g. pre-stage for control)



Slide 7

Proposed Directive on monitoring

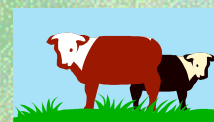
- Food-borne outbreaks:
- obligation for food businesses to inform authorities about outbreaks
- competent authorities shall investigate the outbreaks
- annual reporting to EFSA and Commission
- Council: keeping of strains by operators



Slide 8

Proposed Directive on monitoring

- Monitoring of antimicrobial resistance:
- in zoonotic agents (Salmonella, Campylobacter)
- in strains from animals (cattle, pigs, poultry)
- complementary to monitoring in human isolates
- C: isolates not only from animals but food derived therefrom
- EP; +/- C: and other bacteriological agents



Slide 9

Proposed Directive on monitoring

- MSs report annually to EFSA and the Commission; EFSA prepares the Community report
 - Shortening of deadline to produce national (EP: 5 to 3 months) and EU reports (EP 9 to 6; C 9 to 7), which should be 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

EP amendments

Comitology:

- decisions through SCFCAH, and where appropriate CDN
- systematic prior consultation of EFSA before amending annexes or taking measures (C: more flexible wording); valid also for Regulation on control
- certain elements moved from annexes into articles (also C); valid also for Regulation on control

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
 - EP: products of plant origin (food/feed)
- Control measures will be defined more closely by Commission Decisions



Slide 13

Proposed Regulation on control of specified zoonoses

Progressive approach:

- Starting with salmonella with phs in poultry breeding flocks
- Extending to Salmonella with phs (except layers: SE-ST) in all poultry flocks, turkeys and breeding pigs
- possibility to include other zoonoses and other stages of food-chain



Slide 14

Proposed Regulation on control of specified zoonoses

EP-C:

- EP (some delegations in C) : all salmonella with phs in layers
- EP: breeding pigs and slaughter pigs (also a number of delegations in C)
- EP: also calves, other cattle and sheep
- C: some delegations in favour of SE-ST only
- C: which serotypes with phs?

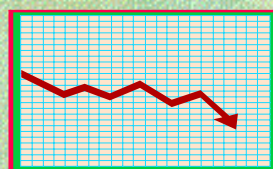


Slide 15

Proposed Regulation on control

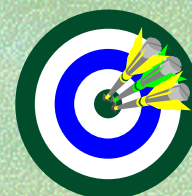
• Pathogen reduction targets to be set

- 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 16

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 17

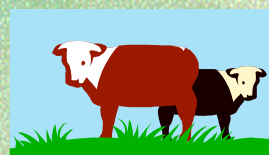
Proposed Regulation for control

Proposal in force	2003	2004	2005	2006	2007	2008
S. serotypes with P.H. importance; breeding flocks Gallus gallus		Target established	National control programmes Certification			
S. Enteritidis and S. Typhimurium ; laying hens			Target est.			
S. serotypes with P.H. importance; broilers				Target est.		
S. serotypes with P.H. importance; turkeys					Target est.	
S. serotypes with P.H. importance; breeding herds of pigs					Target est.	

Slide 18

Proposed Regulation for control

- C: different positions of delegations on timetable
- EP: no change to timetable for proposed targets, but additional timetable for calves (date as for broilers), and for slaughter pigs, other cattle and sheep (date as for breeding pigs)



Slide 19

Proposed Regulation for control of specified zoonoses



Minimum sampling

- Zoonosis / zoonotic agent (salmonella with public health significance -phs)
- Animal species
- (C: data)
- Sampling shall cover at least certain phases of production
- EP : testing on farm for pigs and for other livestock

Slide 20

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
- EP: ban on intra EC trade for MSs without approved programme (considered but very little support in in C); add guarantees not only for salmonella



Slide 21

Proposed Regulation for control

Predefined specific measures:

- Fowl breeding flocks infected with SE/ST: slaughter/heat treatment/destruction
 - EP: all salm with phs
- Table eggs: have to originate from salmonella negative flocks (starting from 1.1.2008)
 - EP: all salm with phs
- Poultry meat: criterion of absence of Salmonella in 25g or industrial heat treatment salmonella (starting from 1.1.2009)

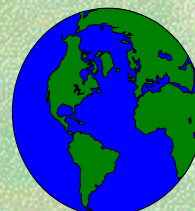


Slide 22

Proposed Regulation for control

Imports from third countries: equivalent conditions required

- national control programmes
- certification of live animals (and products)



Slide 23

Proposed Directive on Monitoring

- Community financing possible for
 - co-ordinated monitoring programmes
 - Community Reference Laboratories
 - the control measures financed currently, but no financing foreseen for new measures (pending the ongoing review of financial support for disease control in SANCO)
 - C: several MSs ask for additional EC co financing



Slide 24

And now?

- Intention from ESP presidency to go to the ministers for “political” discussion
- Need to look at the amendments from EP accepted by the Commission, and possibly come to a common position.
- Possibly 1 more working group under ESP presidency, then file for DK
- Unless C agrees on EP position, need to go for second reading.

Slide 25

Zoonoses proposals: laboratories

- Requirement for accreditation by Jan 2005
 - EP: 2004
- CRLs: to be appointed and tasks defined
 - Need to see with draft Regulation on official feed and food control
- NRLs: to be appointed and tasks may be defined
- Methods: international standards or validation
 - Sci opinion; Draft EN/ISO standard

Slide 26

Coordination meeting of CRLs VPH 15-16 January 2002

- Financing:
 - Yearly financing : earlier decision
 - Ring trials: cost of sending samples supported
 - Workshops: from next year, reimbursement of travel expenses similar to Commission meetings (economy class, but no threshold of 850€); probably integration into yearly financial aid
- Co-operation with third countries, in particular Candidate countries

Appendix 5 Slides of presentation 2.2

Slide 1

Report from the CRL for the Epidemiology of Zoonoses

Annemarie Käsbohrer
BgVV, Berlin, Germany

Slide 2

Topics

- Last workshop
 - Monitoring and control strategies for Salmonella
 - Reporting on antimicrobial resistance in Salmonella
- Zoonoses report 2000
- Future requirements

Slide 3

Monitoring and control strategies for Salmonella in poultry

Slide 4

Presentation of the data in the annual report

Tables summarizing the surveillance system

- Method
- Time and place of investigation
- Specimen collected
- Sample size and frequency

Slide 5

Schemes in poultry breeders

- Type of sample
 - Dead chicken, tissue samples
 - faecal samples, caecal droppings, faecal swabs
 - dust swabs
 - blood samples

Slide 6

Schemes in layers (table egg production)

- Type of sample
 - Egg samples
- Sample size
 - 24 - 60 ; 60 ; 60 - 90
- Frequencies
 - every 9 weeks; three times
 - 20 - 25 + 55 - 60 weeks
 - 24 + 40 + 55 weeks
 - Once max 9 weeks before delivery

Slide 7

Questions

- What is the sensitivity of the method ?
 - for the individual type of sample
 - in case of pooling of samples
 - for the combination of different samples
 - for the overall strategy involving different frequencies of sampling
 - for the overall strategy involving different time schedules

Slide 8

Antibiotic resistance

Experiences with the new reporting system

Slide 9

Antibiotic resistance testing

- **Monitoring frame**
 - 5 most important Salmonella serotypes
 - at least 60 isolates of each serotype per animal species
 - 3 main species of food animals (cattle, pigs, poultry)
 - further animal species may be included

Slide 10

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 11

Antibiotic resistance testing

- **Antimicrobials in test panel**
 - Tetracycline
 - Chloramphenicol
 - Florfenicol
 - Ampicillin
 - 3rd generation cephalosporin, eg. cefotaxim
 - Ciprofloxacin or enrofloxacin
 - Nalidixic acid
 - Sulfonamide/TMP (separate optional)
 - Streptomycin
 - Gentamycin
 - Neomycin
 - Kanamycin

Slide 12

Antibiotic resistance testing

- **Antimicrobials in test panel**

– Tetracycline	14
– Chloramphenicol	13
– Florfenicol	9
– Ampicillin	12
– 3 rd generation cephalosporin, eg. cefotaxim	14
– Ciprofloxacin or enrofloxacin	13
– Nalidixic acid	14
– Sulfonamide/TMP (separate optional)	9 (8/9)
– Streptomycin	11
– Gentamycin	12
– Neomycin	9
– Kanamycin	7

Slide 13

Antibiotic resistance testing

- **Reporting**

- should include a methods table:
 - test method
 - testing standard used
 - breakpoints used
 - does the lab use quality control strains
- Results should be reported as percent resistant among isolates tested;
- For each serotype to be reported separately

Slide 14

Antibiotic resistance testing

- **Methods**

- Agar diffusion: 9 countries
- Broth dilution: 5 countries

- **Standards**

- NCCLS
- Other: BSAC 1991, CASFM, DIN
- ??

Slide 15

Antibiotic resistance testing

- **Breakpoints**
 - According to NCCLS standard
 - No NCCLS standard fixed
 - Deviations from the NCCLS standard
 - Other methods with other breakpoints

Slide 16

Results of the working group - 1

- Share of the tested isolates in relation to the number of all available
- Additional categories
 - Human
 - Turkey
 - Other animal species together
- Attention to the sampling strategy for the isolates

Slide 17

Results of the working group - 2

- Preferably NCCLS protocol
- Otherwise to be specified:
 - Disk content for agar diffusion test
 - Breakpoints
- Preferably quantitative data
 - Diameter of inhibition or
 - Dilution point of the MIC

Slide 18

Results of the working group - 3

- Data should be given, if available, separately for
 - All *Salmonella* strains together
 - *S. Typhimurium* (overall picture)
 - *S. Typhimurium* DT104
 - *S. Typhimurium* non - DT104
 - *S. Enteritidis*
 - Other serotypes together

Slide 19

Trends and sources of zoonotic agents in animals, feedingstuffs, food and man in the European Union and Norway

in 2000

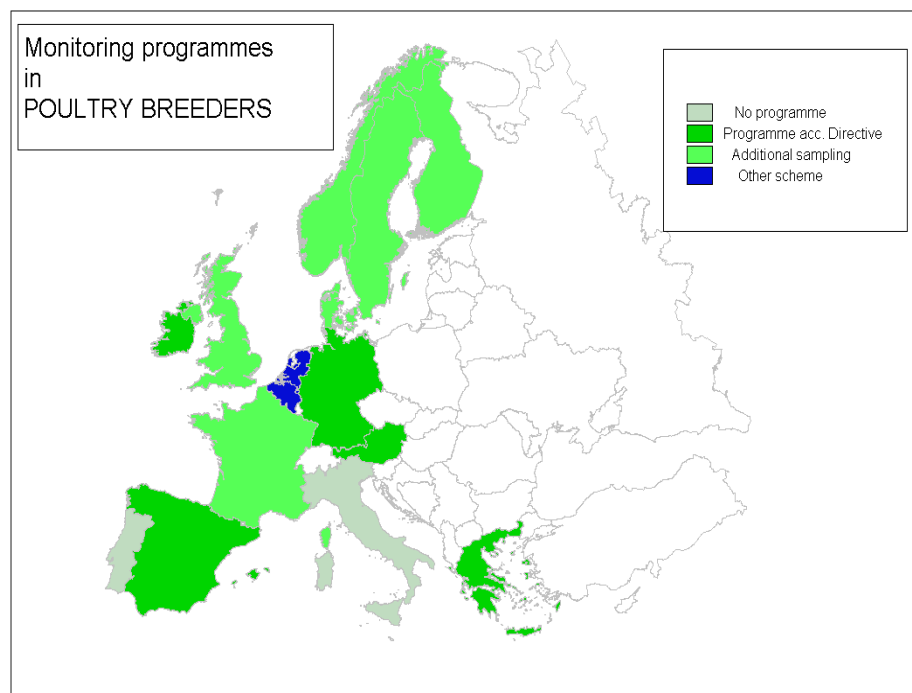
Doc. SANCO/927/2002

Slide 20

Salmonella - poultry breeders

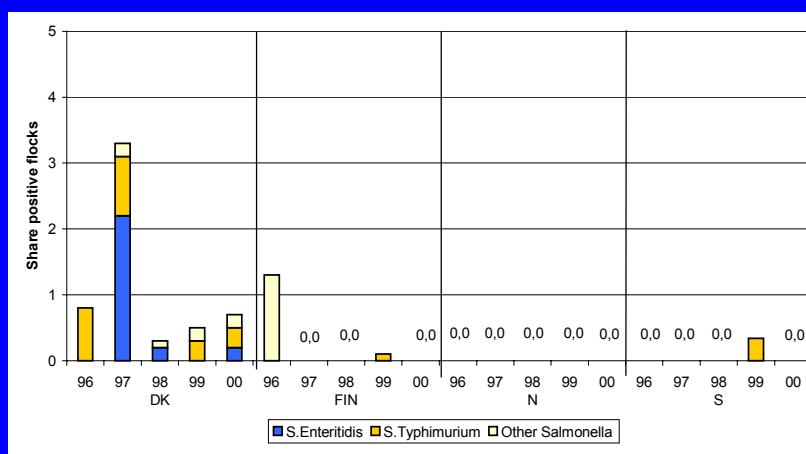
- Countries running an approved control programme for several years
 - DK, FIN, S, IRL, N
- Countries running an approved control programme since 1999 or 2000
 - A, F
- Countries, which apply a monitoring scheme based on the sampling procedures in the Zoonoses Directive
 - UK, D, E
- Countries, which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

Slide 21



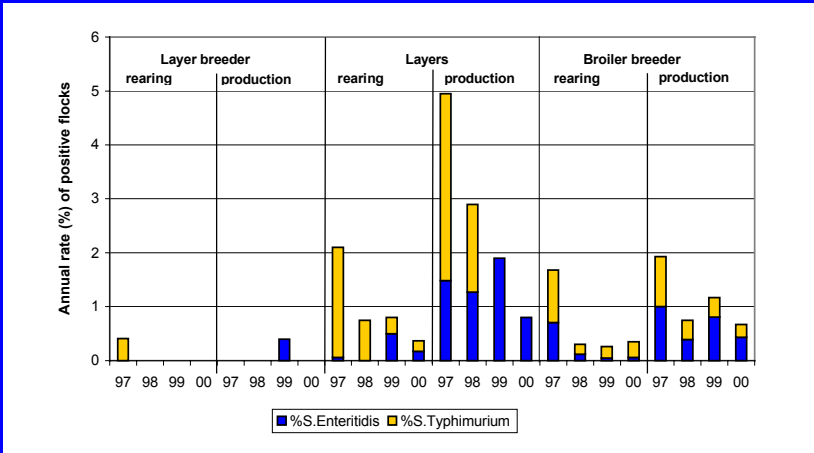
Slide 22

Approved control programme I



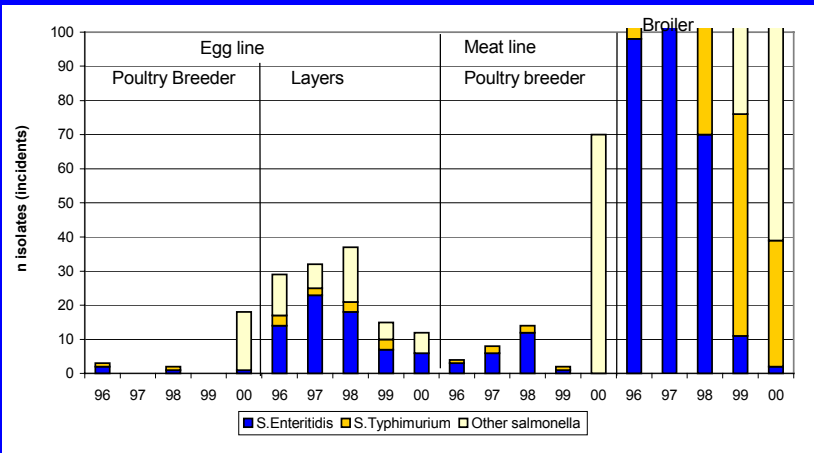
Slide 23

Approved control programme - France



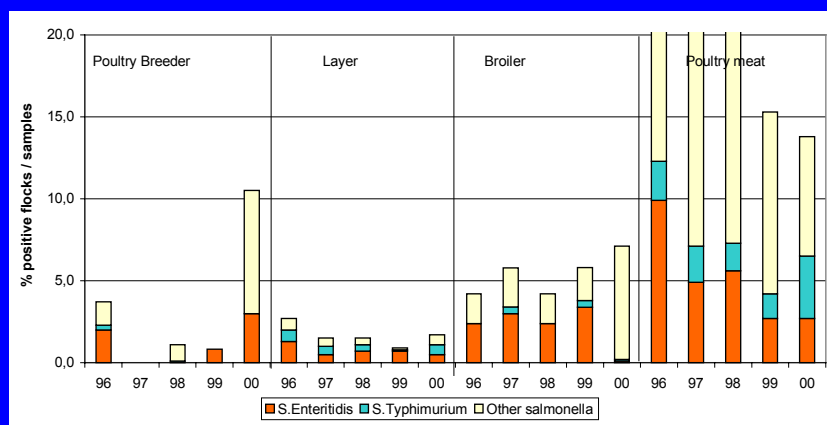
Slide 24

Monitoring programme acc Dir. - Great Britain



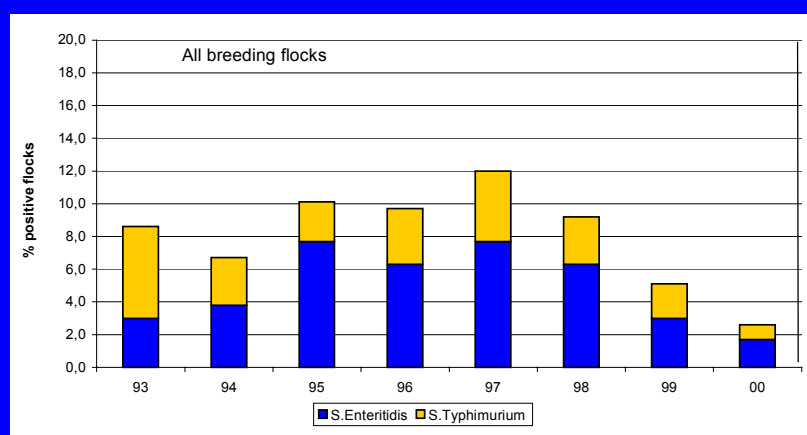
Slide 25

Monitoring programme acc Dir. - Germany



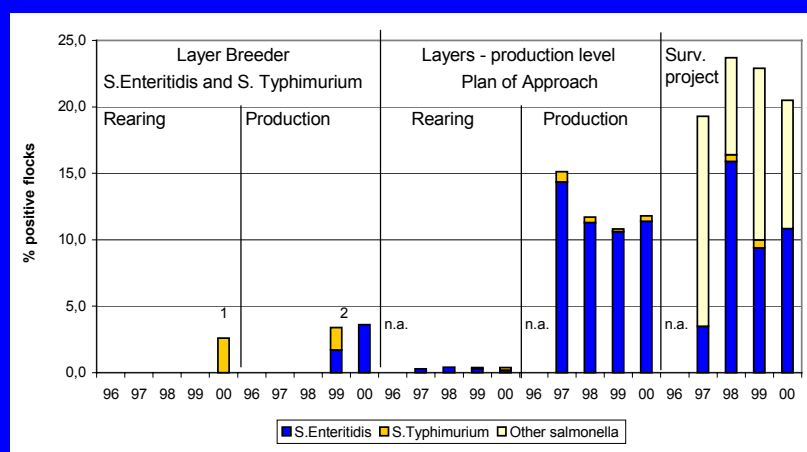
Slide 26

Other monitoring programme - Belgium



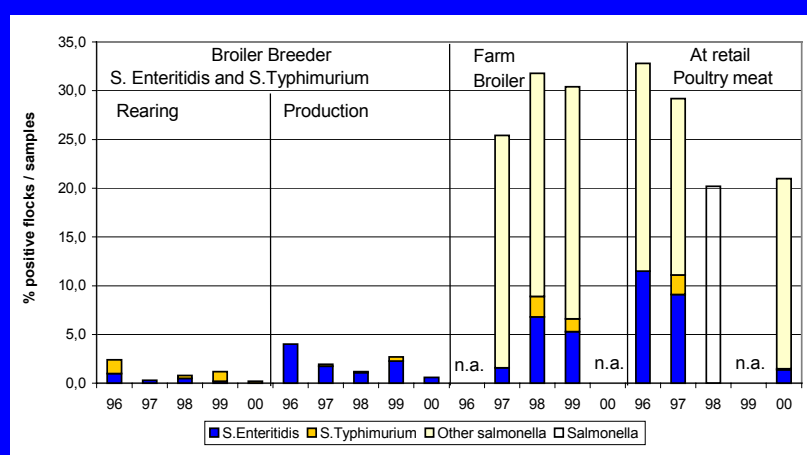
Slide 27

Other monitoring programme - The Netherlands

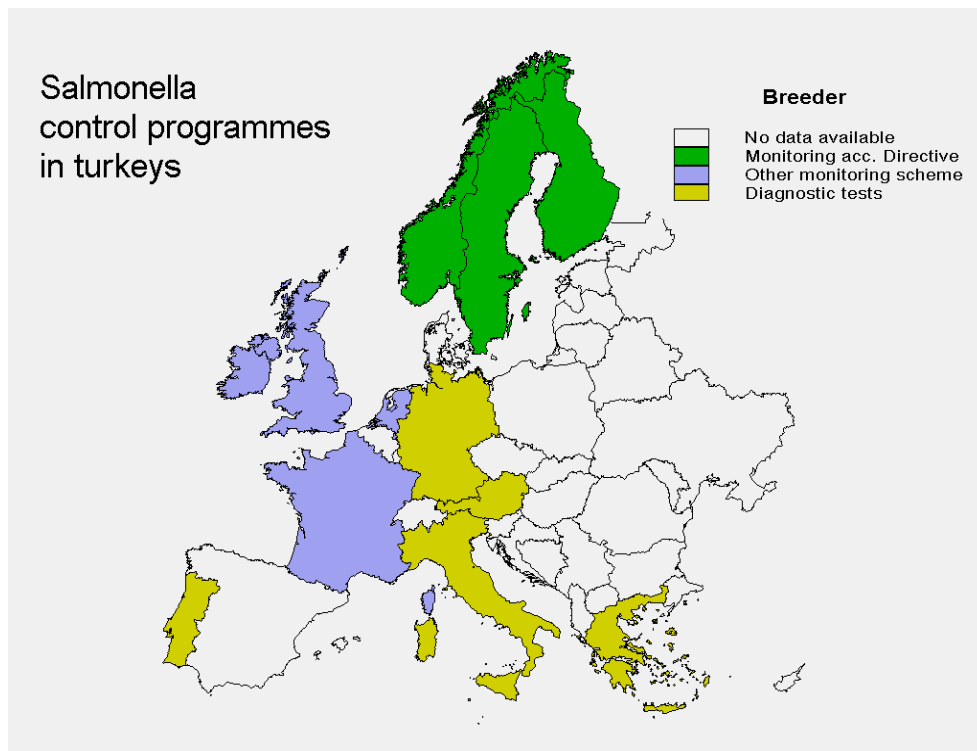


Slide 28

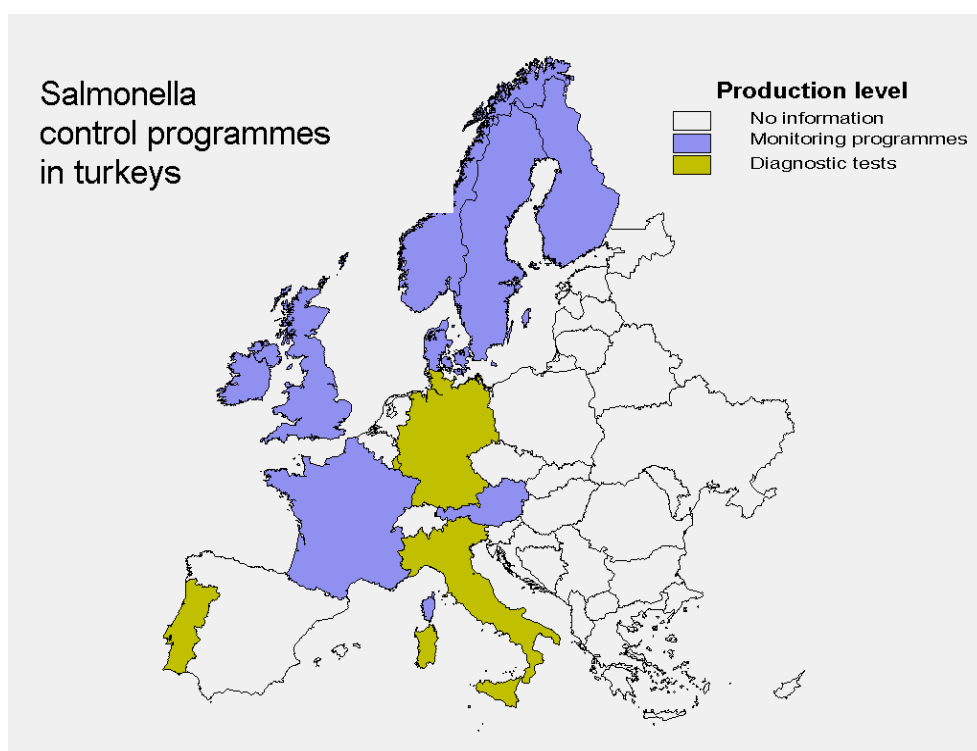
Other monitoring programme - The Netherlands



Slide 29



Slide 30



Slide 31

Salmonella in turkey

- Turkey breeders:
 - **No** positive turkey breeding flocks:
FIN, S, A, D, NL, N
- Production level:
 - Varying infection rates
 - S. Enteritidis, S. Typhimurium or others dominating
- Turkey meat:
 - Varying contamination rates

Slide 32

Most common serotypes in turkey - Austria

AUSTRIA	Turkey ¹	Humans
Serotype	%	rank
S.SAINTPAUL	31%	9
S.HEIDELBERG	19%	
S.READING	13%	
S. ENTERITIDIS	6%	1
S. INFANTIS	6%	4
S. MONTEVIDEO	6%	
S. AGONA	3%	10
S. AMSTERDAM	3%	
S. KENTUCKY	3%	
S. KOTTBUS	3%	

¹ Routine
sampling

Slide 33

Most common serotypes in turkey - Denmark

DENMARK	Turkey ²	Human
Serotype	%	rank
S.AGONA	25%	3
S.HEIDELBERG	25%	
S.MONTEVIDEO	16%	
S.RISSEN	11%	
S.INFANTIS	9%	9
S.DERBY	7%	
S.NEWPORT	2%	8
S.INDIANA	2%	
S.HAVANA	2%	
S.BRANDENBURG	2%	

² Compulsory
ante-mortem
examination

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Most common serotypes in turkey - England and Wales

England and Wales	Turkey ³	Human
Serotype	%	rank
S.DERBY	16%	
S.TYPHIMURIUM	12%	2
S.AGONA	12%	8
S.NEWPORT	9%	6
S.FISCHERKIEZ	7%	
S.ORION	6%	
S.MONTEVIDEO	6%	9
S.SENFTENBERG	5%	
S.KOTTBUS	4%	
S.INDIANA	4%	

³ Laboratory
reports under
Zoonoses Order

Slide 35

Salmonella in pigs and cattle

- Favourable situation
 - Sweden, Finland, Norway
- Varying rates in other countries
- Contamination rate of beef is lower compared to poultry meat and pork

Slide 36

Salmonella contamination

	Cattle	Pigs	Beef	Pork
D	<		<	
DK	<		<	
I	<		<	
L		>		>
N	<			>
P	<		<	

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Salmonella Typhimurium

	Cattle	Pigs	Beef	Pork
D	<	>	<	
DK		>	<	>
I	<		<	>
L	<			>
N		>	<	>
P			<	

Slide 38

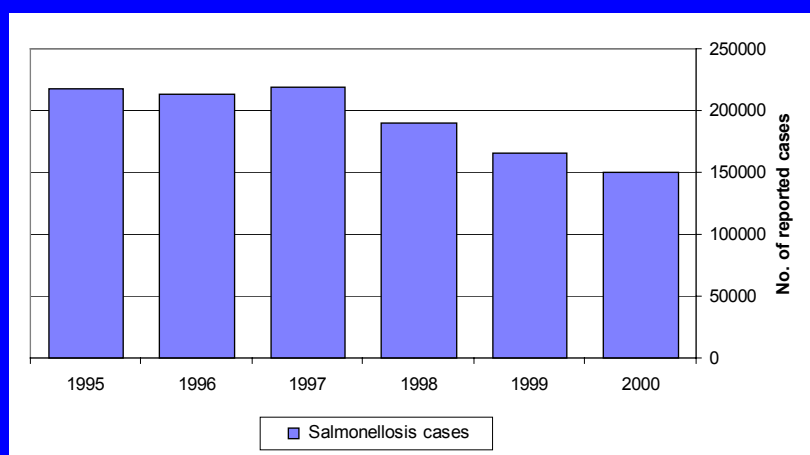
S. Typhimurium DT 104

Share of all S. Typhimurium isolates

DK	Pigs	3,4 %
	Pork	1,4 %
	Cattle	30,2 %
	Beef	0
NL	Cattle	52,3 %
	Pigs	25,4 %

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Trend in human salmonellosis



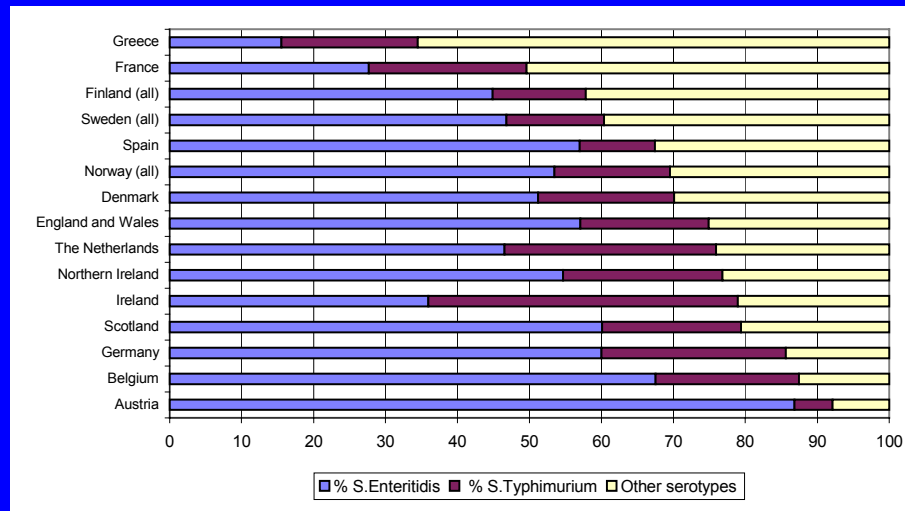
Slide 40

Main serotypes in human the EU, 2000

Serotype	%
<i>S. Enteritidis</i>	59,14
<i>S. Typhimurium</i>	13,03
<i>S. Hadar</i>	1,77
<i>S. Virchow</i>	1,36
<i>S. Infantis</i>	0,87
<i>S. Agona</i>	0,75
<i>S. Brandenburg</i>	0,68
<i>S. Newport</i>	0,53
<i>S. Blockley</i>	0,46
<i>S. Braenderup</i>	0,43

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Salmonellosis in man



Slide 42

Antimicrobial resistance - Salmonella

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

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Further requirements

- Sensitivity of the method applied
 - Please provide data on the sensitivity of the methods and schedules applied
- Antibiotic resistance testing
 - Please provide the information as discussed
- Serotyping / Phagotyping
 - Please provide the results of the testing by the main animal species and food categories

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Acknowledgement

- To all Member States and Norway contributing to the report
- To all scientists co-operating to make things more clear
- To the staff of the CRL-E
- To the Commission for funding the work of the CRL-E

Appendix 6 Slides of presentation 2.3

Slide 1

Ploufragan 28 mei 2002. CRL-meeting Wilfrid van Pelt

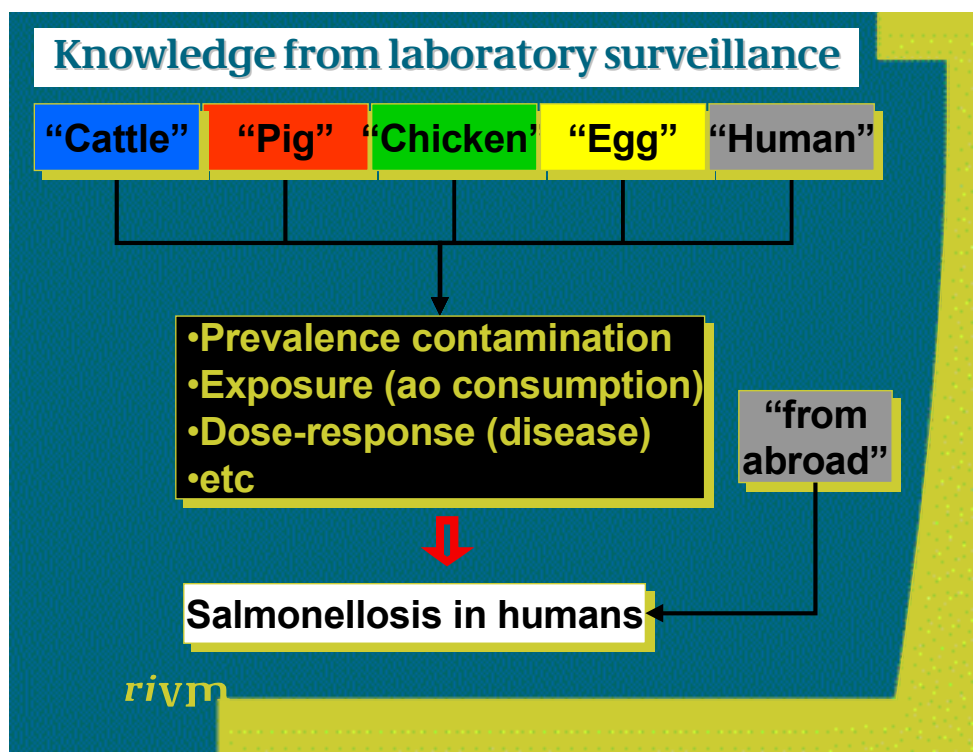
Emerging Salmonella types in the Netherlands

Thanks to: Matty de Wit, Yvonne van Duynhoven, Winette van den Brandhof, Arjen van de Giessen, and many others

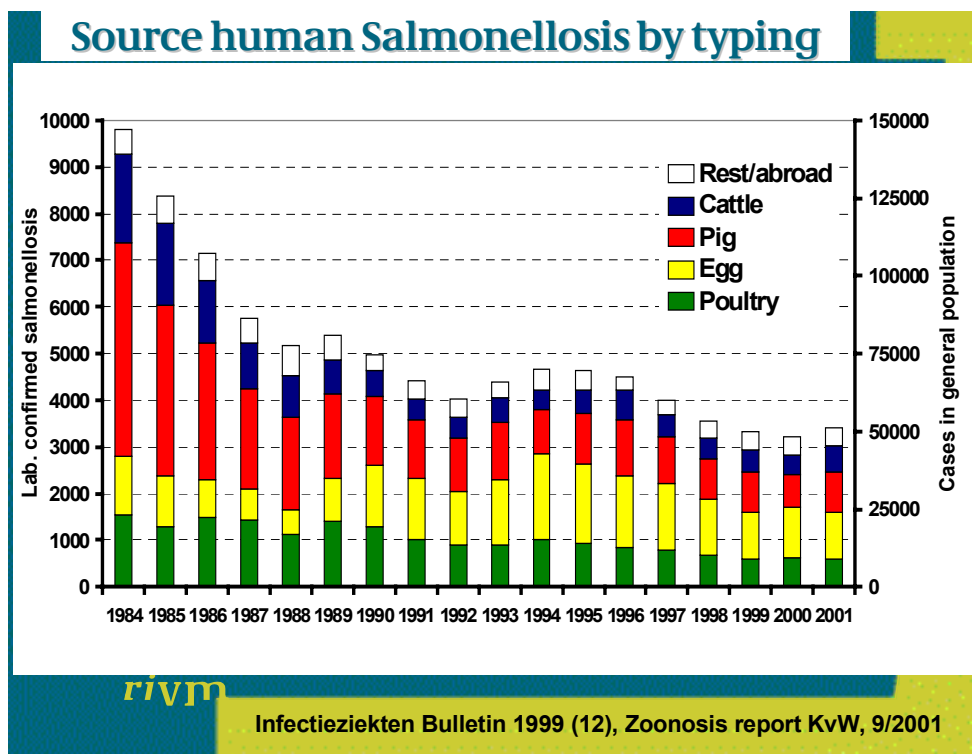
rivm
Rijksinstituut voor Volksgezondheid en Milieu

Onderzoek in dienst van mens en milieu

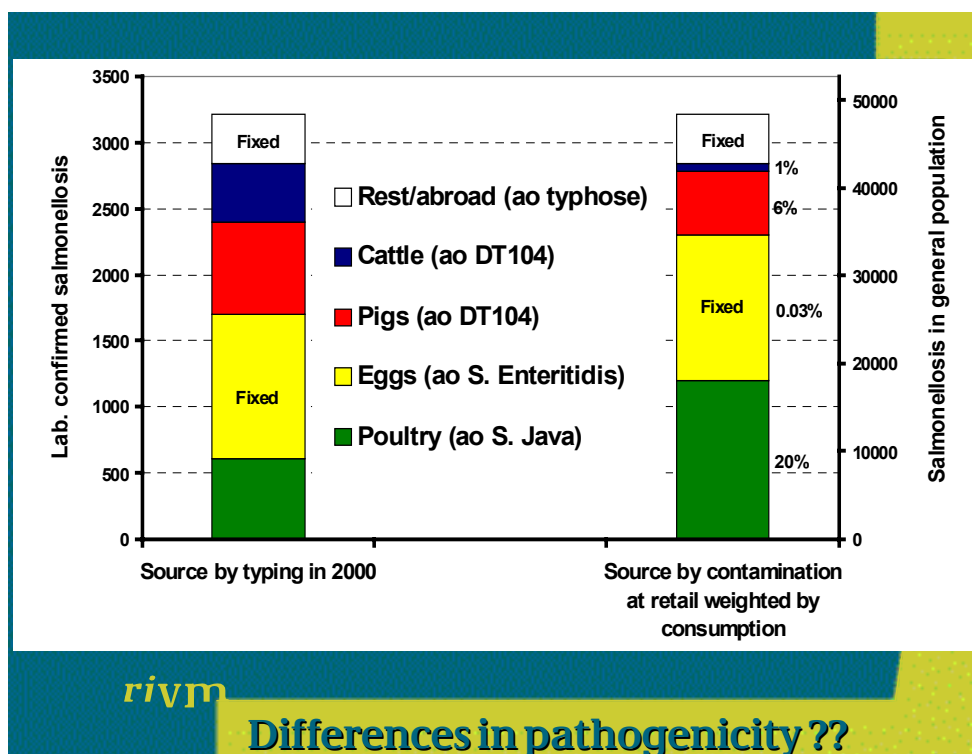
Slide 2



Slide 3



Slide 4



Slide 5

Selection to more pathogenic types/strains???

In **Pig / Cattle** multiresistent *S. Typhimurium* DT104 dominates. Appears to be more pathogenic in a Danish, and in a Dutch study spring 2001.

(Infectieziekten Bulletin 9 2001)

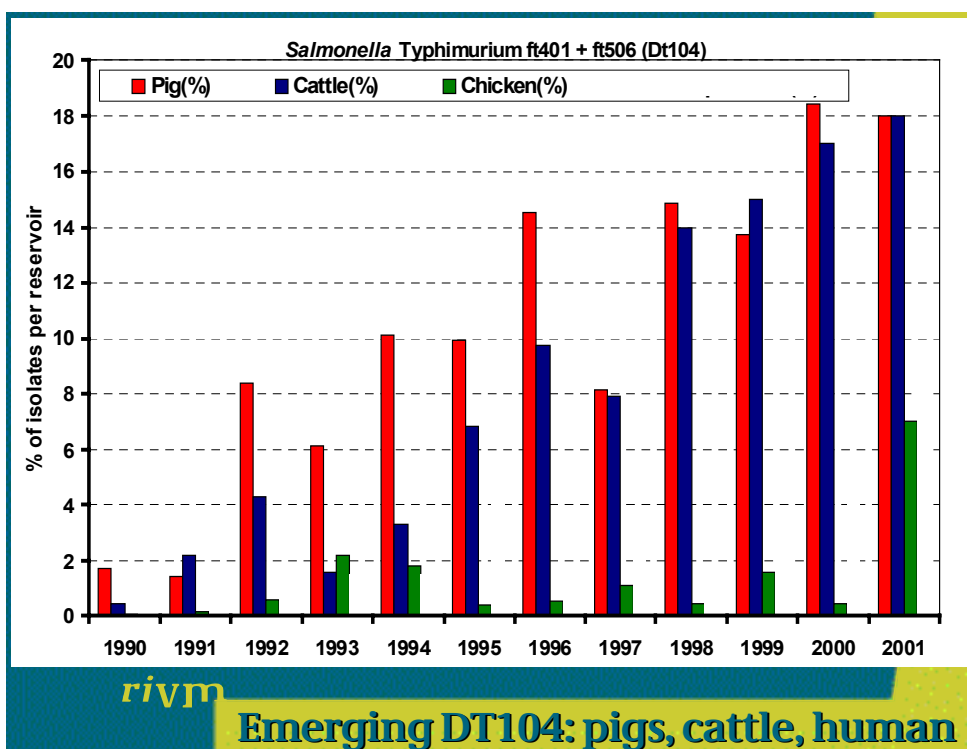
The same *S. type* from **eggs** is more acid-tolerant (easier passes the stomach) as from **poultrymeat**, especially if grown in environment with sugar (bavarois)

(Rob de Jonge, MGB/RIVM, Infectieziekten Bulletin 12 2001)

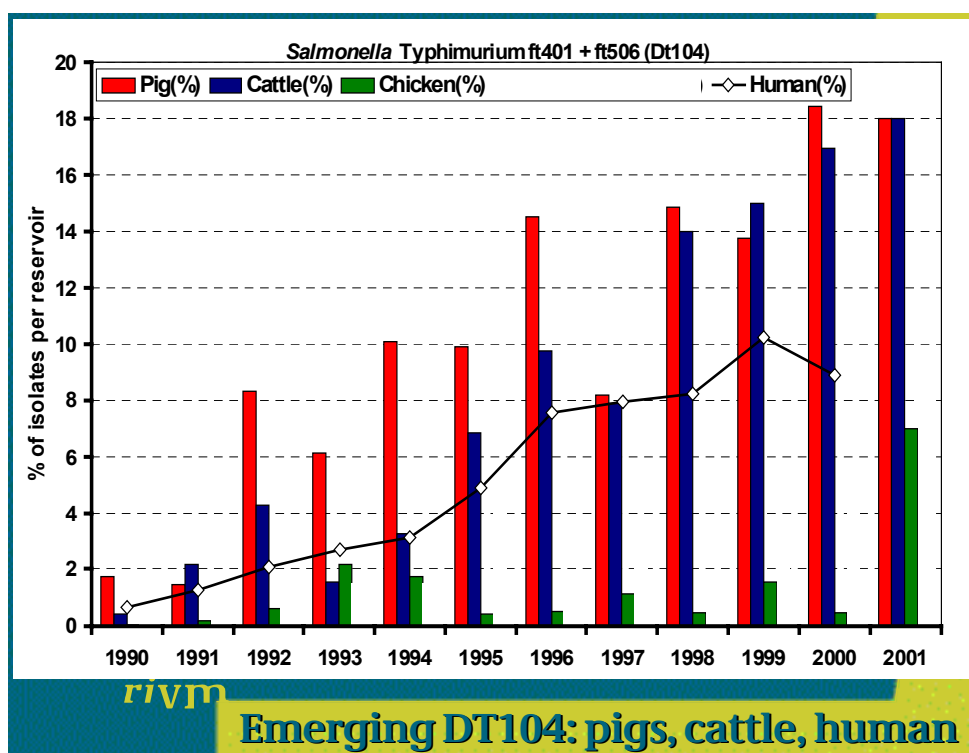
In **poultrymeat** *S. Paratyphi* B var Java dominates and seems hardly zoonotic (sofar?) (Infectieziekten Bulletin 6 2002)

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Slide 6



Slide 7



Slide 8

Case-control study diaryfarms
(J. Veling e.a., 2002)

Animal Healthcare Department:
increase "outbreaks" of *S. Typhimurium* (often DT104)
in diary farms 1998-1999

→ **case-control farms study 1999**
47 case farms (*S.T.* infections)
47 control farms (no infections)

extensive questionnaire

- opportunity for introduction of infection in the herd
- expression of the infection (clinical symptoms)

Slide 9

Case-control study diaryfarms

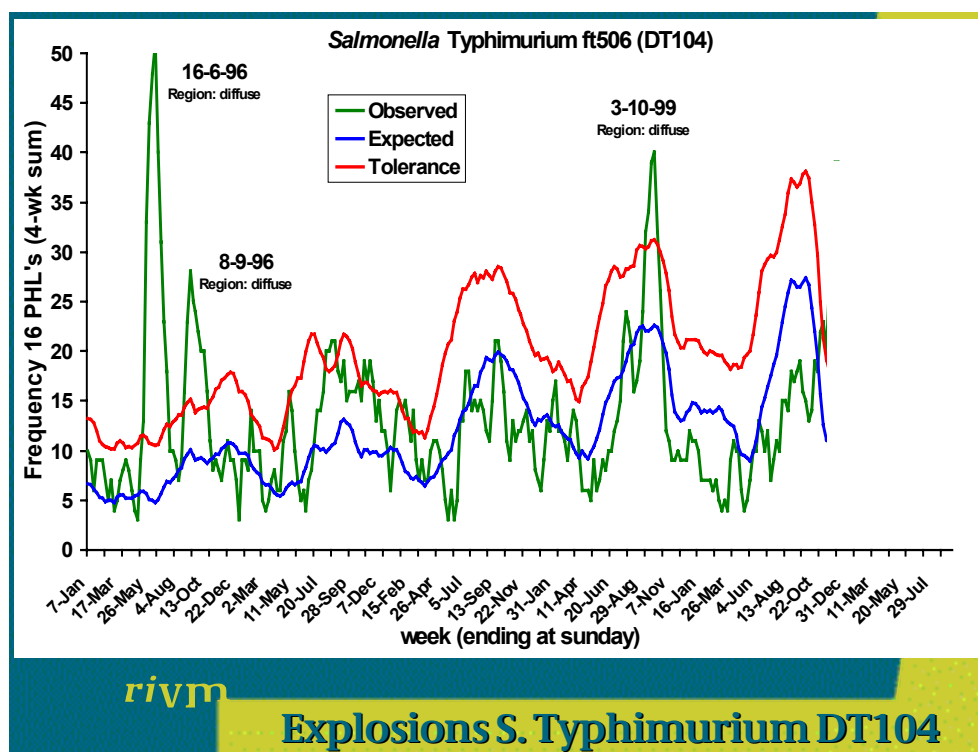
(J. Veling e.a., 2002)

Principal risk-factors:
 purchase of (pig-) manure (OR 21)
 factors for “crowding”; “modern dairy industry”

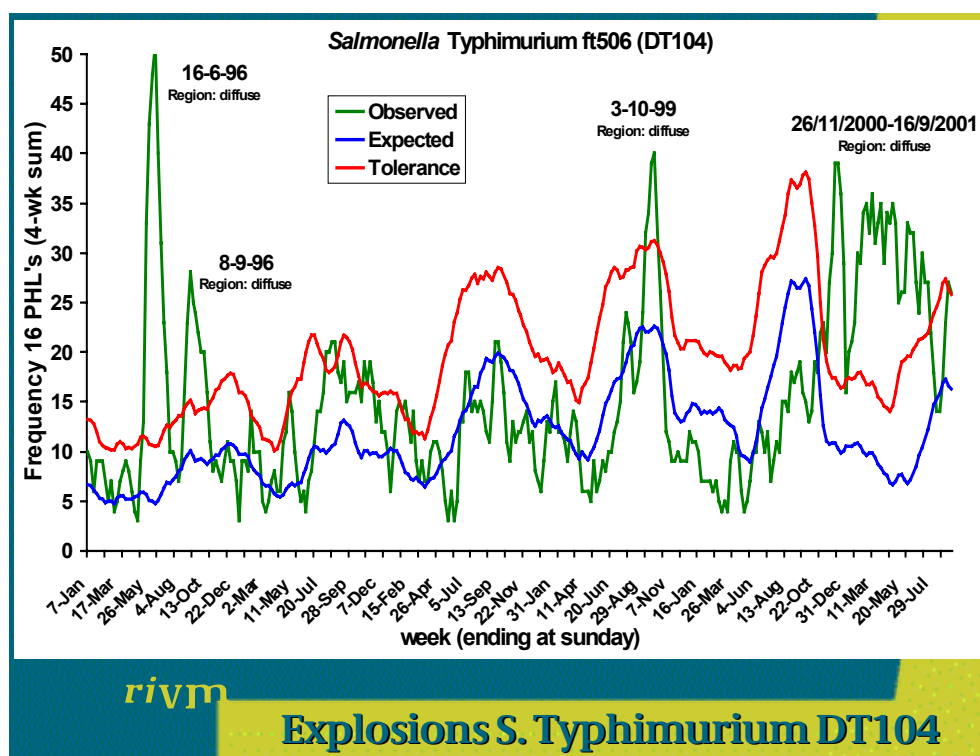
→ DT104 (13/47) especially related to abortions

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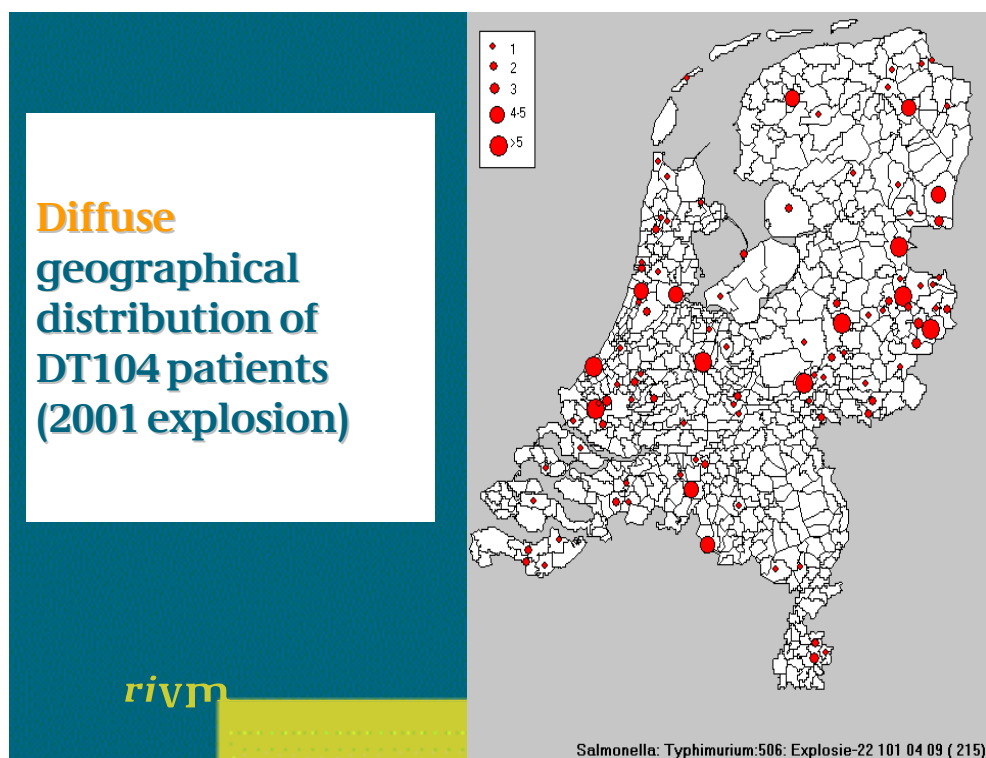
Slide 10



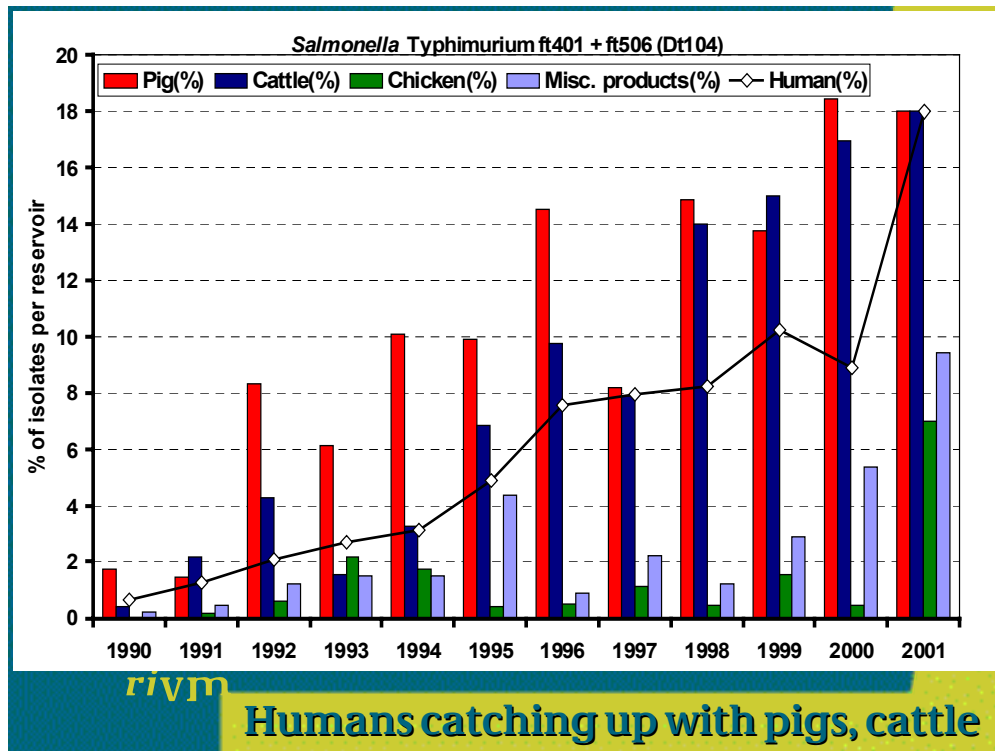
Slide 11



Slide 12



Slide 13



Slide 14

DT104 : other European countries, 1997-2000 (ENTER-net data)

DT104%Total

- Austria (1.5%); Denmark (4%)
- about 9%: **Holland**, E&W, Scotland
- Ireland (23%)

DT104%Typhimurium

- about 29%: **Holland**, Denmark, Germany, Austria
- about 65%: E&W, Scotland, Ireland

In 2001 levels Holland and Ireland highest in Europe!!

Slide 15

Clinical course DT104				
	Wall, '93	Mølbak, '98		Lab-surveillance spring-2001
	DT104 n=83	DT104 (i.q.r.) n=27		DT104 n=42
diarrhoea	100%			95%
abdominal pain	65%			80%
vomiting	42%			46%
fever	78%			84%
bloody stools	25%			56%
slimy stools				79%
hospital admissions	41%	44%		43%
hospital days (P50)	5			4
mortality	10/295(3.3%)	2/27(7.4%)		1/42(3.1%)
antibioticum use		48%		34%
feces from specialist				n=109 47%

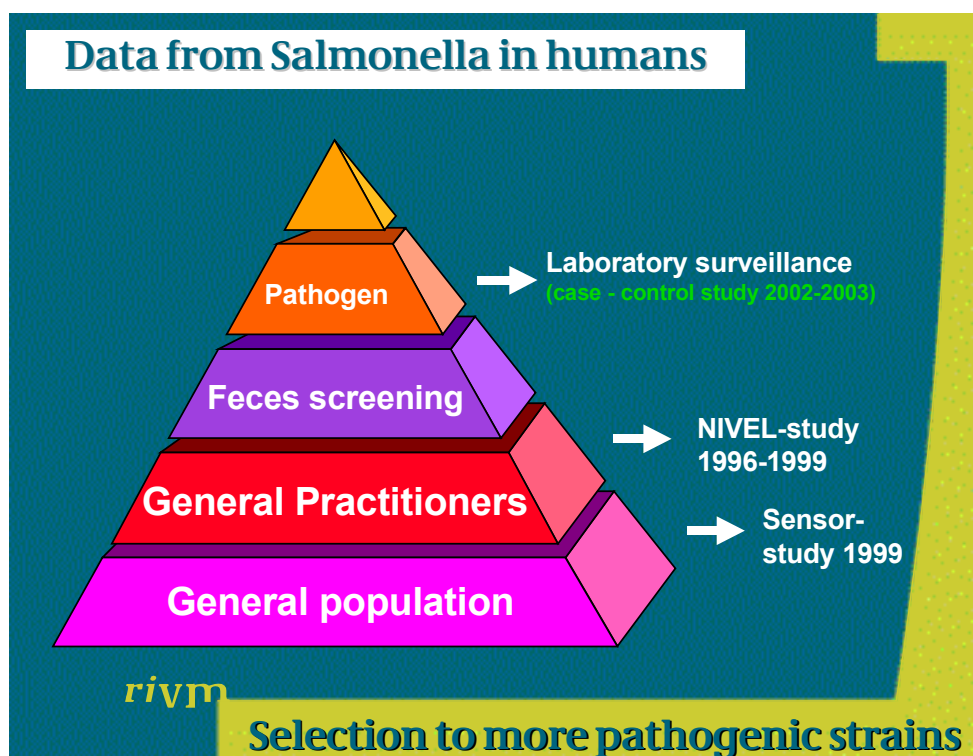
rivm Differences in pathogenicity??

Slide 16

Clinical course DT104						
			NIVEL-study (GP)	Lab-surveillance spring-2001		
			Salmonella n=33	DT104 n=42	Other T. Enteritidis n=28	Enteritidis n=27
diarrhoea			88%	95%	93%	100%
abdominal pain			91%	80%	79%	95%
vomiting			30%	46%	50%	38%
fever			67%	84%	71%	78%
bloody stools			21%	56%	52%	36%
slimy stools				79%	55%	57%
hospital admissions				43%	27%	35%
hospital days (P50)				4	5	5
mortality				1/42(3.1%)		
antibioticum use			12%	34%	39%	35%
feces from specialist				n=109 47%	n=68 34%	n=80 30%

rivm Differences in pathogenicity??

Slide 17



Slide 18

Excess mortality 2-years after Salmonella infection

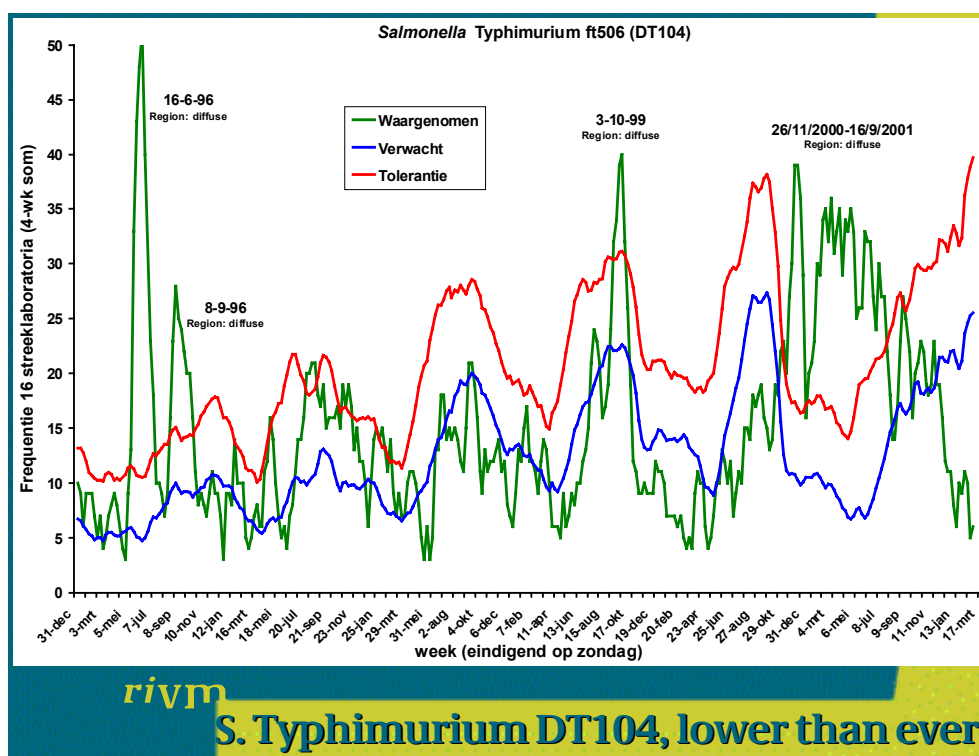
(Mortality register study Denmark: K. Mølbak, 2002)

Excess mortality:

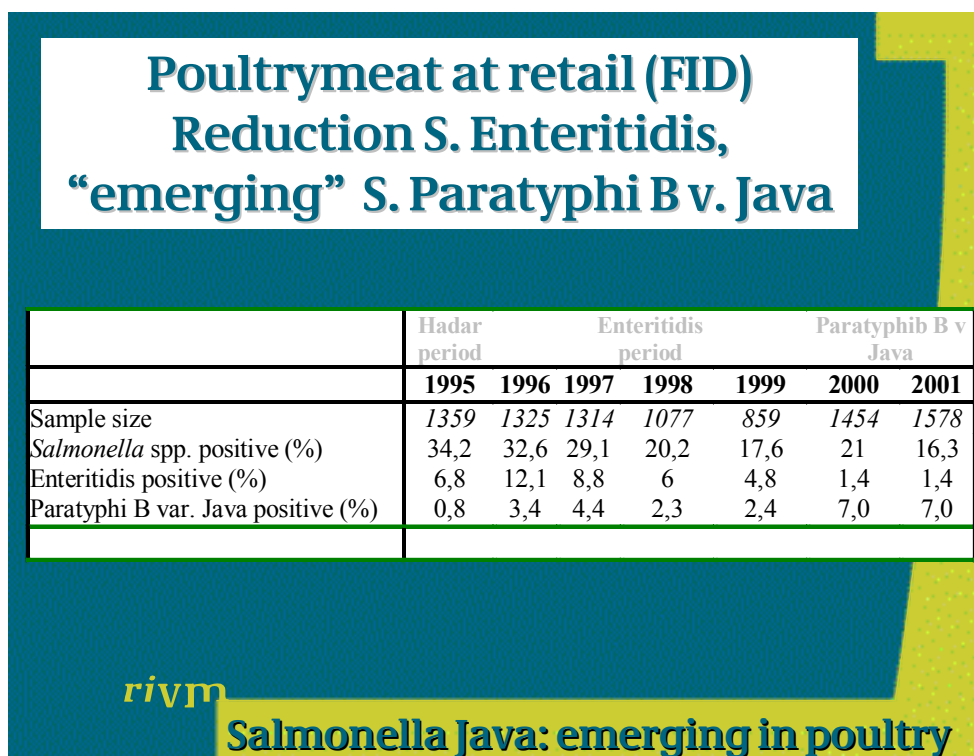
antibiotic sensitive Salmonellae:	2x
penta-resistant Salmonellae:	5x
penta-resistant + fluoroquinolone resistant:	10x

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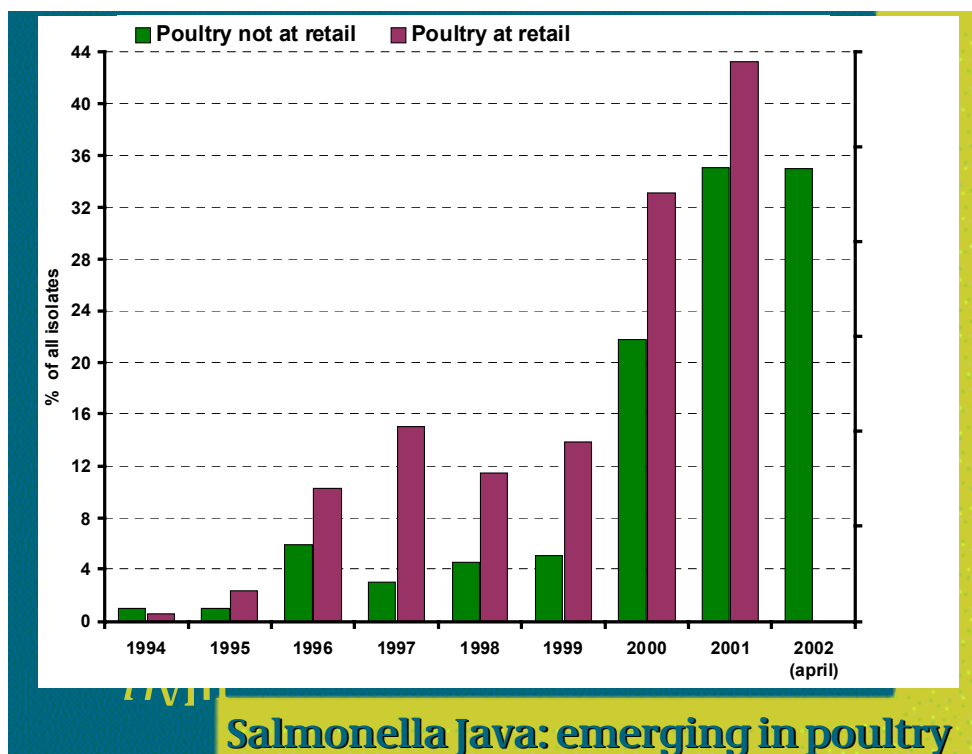
Slide 19



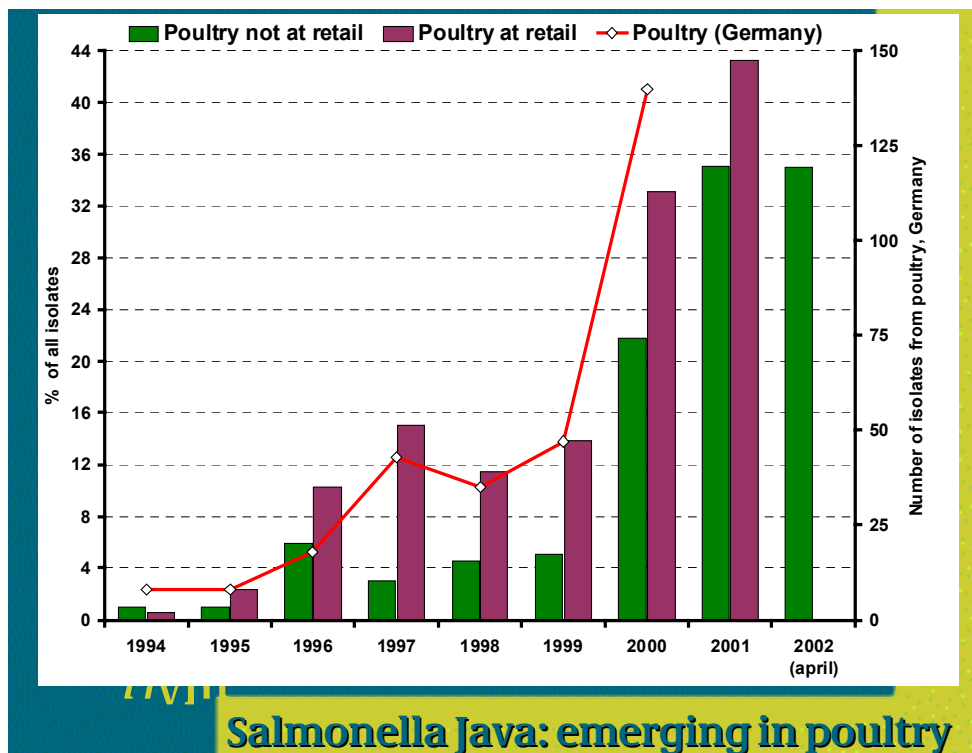
Slide 20



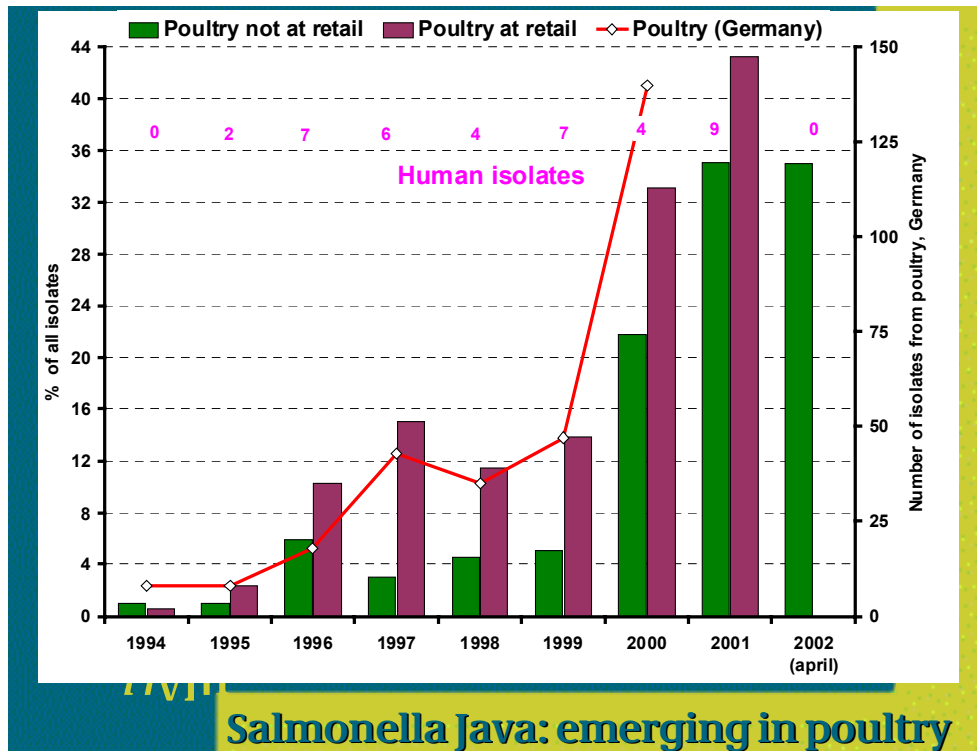
Slide 21



Slide 22



Slide 23



Slide 24

Literature

Salmonella Paratyphi B:

d-tartrate negative (dT-): typhose clinical symptoms, many outbreaks reported

d-tartrate positive (dT+) **var Java**: ordinary gastro-intestinal complaints, usually non-invasive

however:

several outbreaks of dT+ with typhose symptoms!!!

Germany, 1937; Kroatie, 1967; Mongolie 1999 (WHO-glob salm surv 2000)

Australië only in humans, not in poultry (other genotypes)

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Emerging since 1995/6 (?) in poultry

Slide 25

Genetic and phenotypic study of Java isolates 1960-2000 Miko, Dorn, Schroeter, Helmuth, 2002

	Source	Period	Genetic	Antibiotic
Group 1	no poultry	'60 beginning '90	divers	sensitive
Group 2	poultry	middle '90	a few clones	multiresistant (broad)
Group 3	poultry	end '90	one clone	multiresistant

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Development antibiotic resistance

Slide 26

Agar-diffusion (ROSCO-tablets)

	Poultry						Humans	
	All types, Java excluded			Only S. Paratyphi B var. Java			S. Paratyphi B var. Java	
	1984-1995	1996-1999	2000-2002	1984-1995	1996-1999	2000-2002	1984-1995	1996-2002
Isolates	N	N	N	N	N	N	N	N
Antibiotic tests	38506	3718	2222	52	255	1096	51	44
	30418	991	783	37	71	446	43	13
	%	%	%	%	%	%	%	%
Tetracycline	7	9	6	49	8	7	0	8
Chlooramfenicol	1	2	2	27	0	1	0	8
Neomycine	1	0	0	30	0	0	0	0
Ampicilline	6	9	9	8	23	47	0	44
Cotrimoxazol	2	2	3	49	48	66	0	30
Furazolidon	9	9	11	27	100	98	0	44
Flumequine	0	6	7	0	3	20	0	18

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Development antibiotic resistance

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MIC-values Java 1999/'00 - 2001/'02

MIC-value (µg/ml)	0,015	0,03	0,06	0,125	0,25	0,5	1	2	4	8	16	32	64	Res%
<i>Gentamycine</i> 1999-'00				14	44	2	1							0
2001-'02					96	14					4			4
<i>Ciprofloxacin</i> 1999-'00	15	33	11	1	1									0
2001-'02		67	13	5	11	14	4							0
<i>Flumequine</i> 1999-'00						36	11	13	1					0
2001-'02						59	14	11	1	15	8	5	1	25

MIC-value (µg /ml)	0,25/ 4,75	0,5/ 9,5	1/ 19	2/ 38	4/ 76	8/ 152	16/ 304	32/ 608	>32/ 608	Res%
<i>Cotrimoxazol</i> 1999-'00			1	1		5	4	13	37	89
(Trim./Sulf.) 2001-'02		3		1	1	3		2	104	93



Development antibiotic resistance

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Development antibiotic resistance Java

Flumequine and Cotrimoxazol widely used in poultry
current selection towards resistant strains

Gentamycin (gene pockets) hardly used, Furazolidon
(point mutation) forbidden
no recent selection result, byproduct of clonal
selection



Development antibiotic resistance

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Persistence S. Paratyphi B v. Java in poultry

Extremely **difficult to control** as compared to Enteritidis, Hadar, Virchow, Infantis, etc. [5 1996.....50-100 2000--]
Sofar, even with more draconic, expensive interventions, none of the contaminated farms has been Java-free for more than a few rounds!!!!

The RIVM logo, consisting of the letters 'rivm' in a stylized, lowercase, sans-serif font.

Persistence in poultryfarms

Slide 30

Findings Salmonella Java(1)

Java now **dominant in poultry** and poultrymeat (43%),
"replacing" Enteritidis (<7%).

Indications that it has been found in **parent flocks**

Same emerging as in **Germany**

NRL's and ENTER-net ---> unique Dutch-German problem

Fast becoming resistant to fluoroquinolones!!!

(treatment broiler flocks with f.q. 17% in 1999; 13% in 2000-2001)

The RIVM logo, consisting of the letters 'rivm' in a stylized, lowercase, sans-serif font.

Slide 31

Findings Salmonella Java(2)

What is the reason for the emerging, apparently since 1996

- **Imported hatching eggs** from Portugal en Spain?
 - types already seen before 1996
 - why not a problem there?
- Introduction through **poultryfeed**?
 - sporadic findings of g1/g2 types in feed of unclear origin (1996, 2000). Not in official monitoring statistics of animal feeds
 - why not a problem elsewhere??

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Reason emerging Java in poultry

Slide 32

Findings Salmonella Java(3)

The result of the intensive poultry industry itself?
(the German Hypothesis)

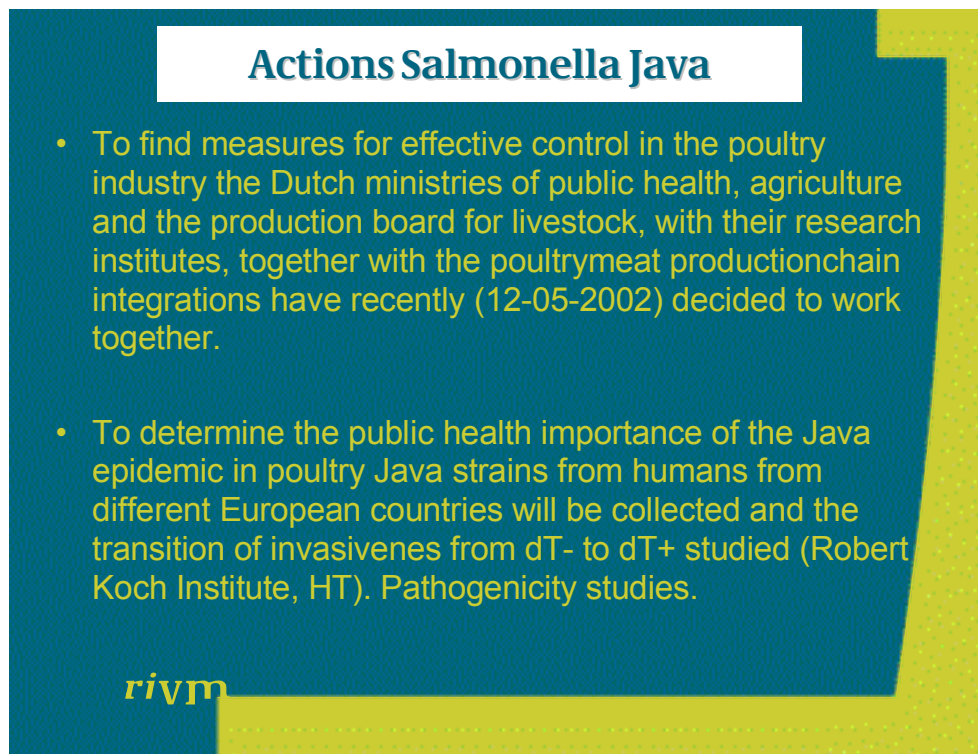
- Selection **multiresistant**, highly **poultry adapted** Java clones
 - antibiotic use fighting the Enteritidis epidemic '90
 - + reaching target contamination level at the end of 2000 in the Netherlands

Is it a zoonosis at all?
A blessing instead of a pest?
Difficult to treat if Cipro-resistant!!

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Reason emerging Java in poultry

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Actions Salmonella Java

- To find measures for effective control in the poultry industry the Dutch ministries of public health, agriculture and the production board for livestock, with their research institutes, together with the poultrymeat production chain integrations have recently (12-05-2002) decided to work together.
- To determine the public health importance of the Java epidemic in poultry Java strains from humans from different European countries will be collected and the transition of invasiveness from dT- to dT+ studied (Robert Koch Institute, HT). Pathogenicity studies.

rivm

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The End

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Appendix 7 Slides of presentation 3.1

Slide 1

Dutch National Reference Laboratory for Salmonella

Key investigators:

Arjen van de Giessen	projectleader / bacteriology
Edda van Raamsdonk	bacteriology
Wim Wannet	salmonella typing centre
Wilfrid van Pelt	epidemiology

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Slide 2

Main activities of the Dutch NRL Salmonella

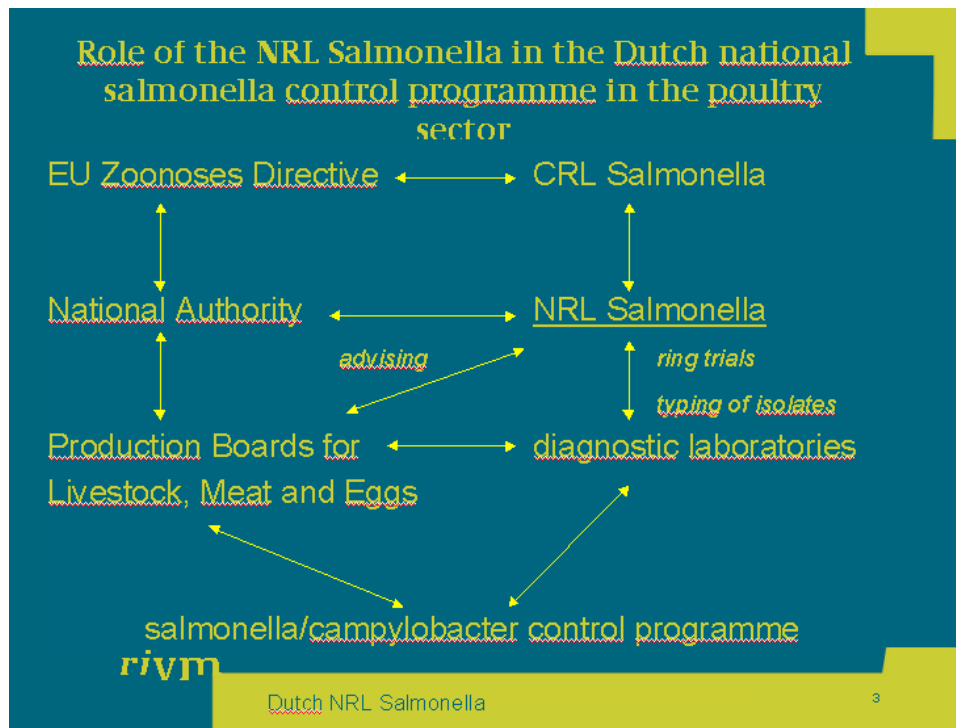
- Conducting research on new analytical methods
- Advising National Authorities and Production Boards on the application of analytical methods in national monitoring and control programmes
- Contributing to quality control of analytical methods applied by diagnostic laboratories in particular by organizing ring trials
- Typing and antibiotic resistance testing of salmonella isolates and analyses of results
- In the near future: supporting diagnostic laboratories to implement a limited typing scheme for identification of specific serotypes

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Dutch NRL Salmonella

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Slide 3



Slide 4

Dutch programme for control of salmonella (and campylobacter) in the poultry primary production chains

- **Objectives with respect to salmonella:**
 - poultry meat: < 5% pos. products / individual company / 1-1-2003
 - egg production: < 5% SE/ST pos. comm. layer flocks / 1-1-2003
- **Main components of the programme:**
 - Strict hygiene requirements for all links in the chain
 - Incoming and outgoing examinations at each production stage
 - Specific measures upon detection of infection
 - Open communication between the various production stages
 - Logistic slaughtering of broiler flocks
- **Objectives with respect to campylobacter**
 - Monitoring trends in the occurrence in broiler flocks
 - More research to provide a basis for intervention strategies

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Slide 5

Standardization of methods for detection of salmonella in poultry samples

- **National steering group advising on the application of analytical methods**
- **ISO 6579: not appropriate for faecal samples**
- **Criteria for a branch method:**
 - applicable to various poultry samples including fluff, inlay leaflets, faeces, caecal contents and neck skins
 - reliable (in terms of sensitivity, specificity, etc.)
 - easily applied by diagnostic laboratories
- **Comparative studies:**
 - selective enrichment: SC, RV, RVS, MSRV, DIASALM
 - selective agars: BGA, XLD
 - various types of samples
 - studies at the NRL and the Animal Health Service

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Slide 6

Comparison of selective enrichment media for detection of *Salmonella* spp. in poultry feces

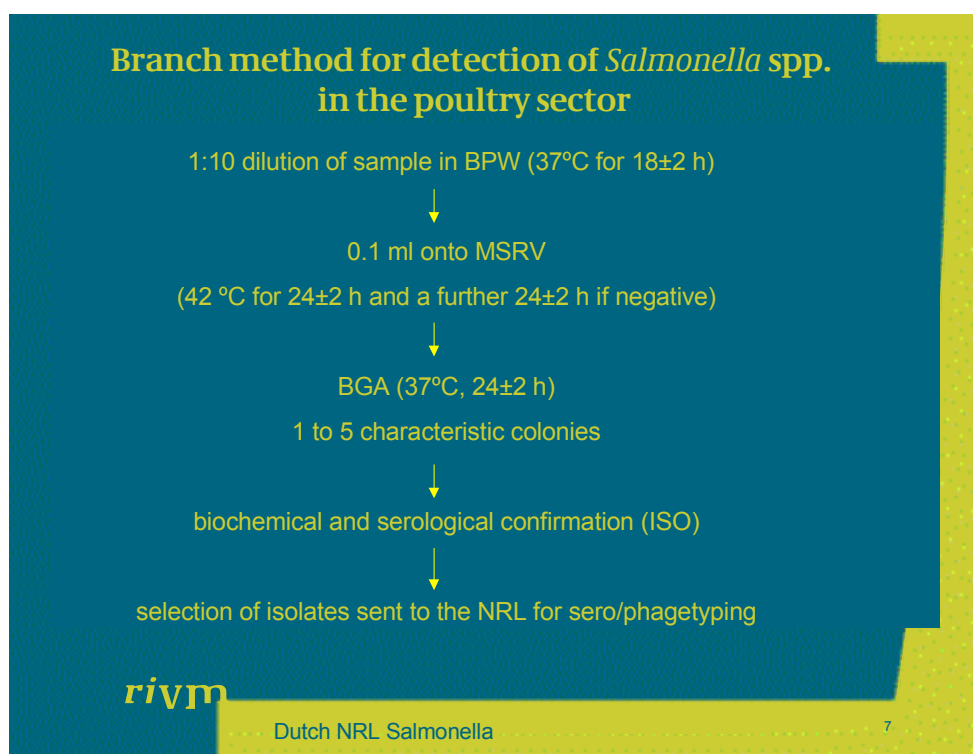
selective medium used	poultry layer flocks (n = 1022)		broiler flocks (n = 892)	
	no. of samples containing <i>Salm.</i> bacteria	no. of samples containing <i>Salm.</i> bacteria	no. of samples containing <i>Salm.</i> bacteria	no. of samples containing <i>Salm.</i> bacteria
	(% of total no. <i>Salm.</i> pos 48 h after 24 h)	(% of total no. <i>Salm.</i> pos 48 h after 48 h)	(% of total no. <i>Salm.</i> pos 48 h after 24 h)	(% of total no. <i>Salm.</i> pos 48 h after 48 h)
RV	46 (34.8)	54 (40.9)	61 (56.5)	65 (60.2)
DIASALM	115 (87.1)	122 (92.4)	88 (81.5)	95 (88.0)
MSRV	117 (88.6)	122 (92.4)	99 (91.7)	100 (92.6)
RV + DIASALM	119 (90.2)	126 (95.4)	96 (88.9)	102 (94.4)
RV + MSRV	120 (90.9)	126 (95.4)	104 (96.3)	106 (98.1)
DIASALM + MSRV	124 (93.9)	129 (97.7)	100 (92.6)	102 (94.4)
RV + DIASALM + MSRV	129 (97.7)	132 (100)	105 (97.2)	108 (100)

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Slide 7



Slide 8

Diagnostic laboratories

- **Criteria for participation in the Dutch salmonella monitoring and control programme in the poultry sector**
 - approval by the National Authority and the Production Boards
 - accreditation of the branch method
 - participation in ring trials organized by the NRL Salmonella
- **Participating laboratories in 2002:**
 - 17 from the Netherlands
 - 5 from Belgium
 - 1 from Germany

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Slide 9

Collaborative studies organized by the NRL Salmonella

- Studies on detection of *Salmonella* spp. in poultry faeces using 15 samples
 - meant for laboratories with satisfying results in previous trials
 - contribute to quality assurance of the branch method
- Studies on detection of *Salmonella* spp. in poultry faeces using 50 samples
 - meant for laboratories with unsatisfying results in previous trials and for laboratories participating for the first time
 - enable statistical evaluation of results
- Pilot study on detection of *Campylobacter* spp.
- Frequency: twice a year

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Dutch NRL Salmonella 9

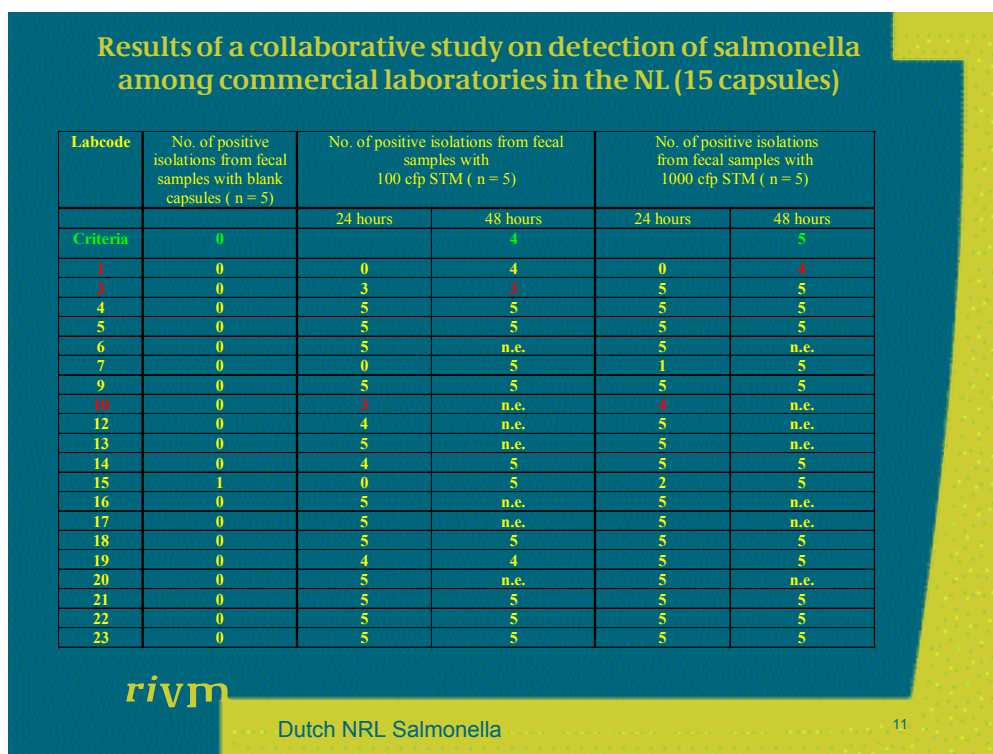
Slide 10

Collaborative studies: materials and methods

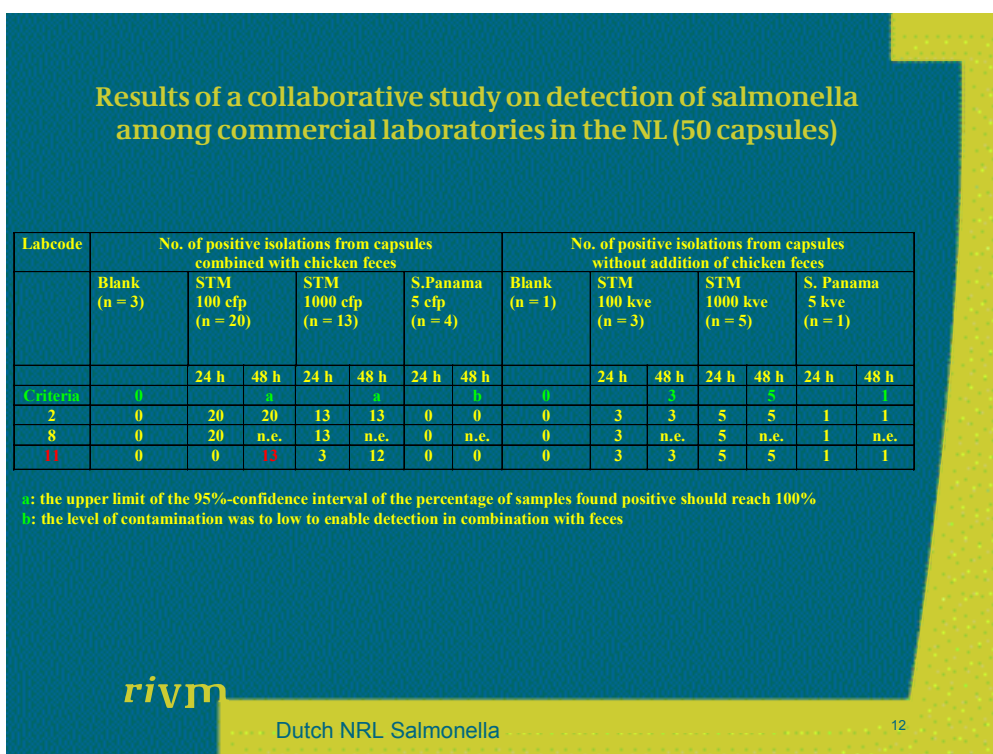
- Reference capsules containing:
 - S. Panama 5 cfp
 - S. Typhimurium 100 cfp
 - S. Typhimurium 1000 cfp
 - blanks
- Chicken faeces
 - derived from layer flocks tested negative for salmonella
 - examined for the absence of salmonella at the NRL
- Methods
 - standardized time schedule, transport conditions, protocol for performance of the study
 - criteria for the results set by the Production Boards

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Slide 11



Slide 12



Slide 13

Results of a pilot collaborative study on detection of campylobacter among commercial laboratories in the NL

Labcode	No. of positive isolations from swabs with <i>Campylobacter jejuni</i> (n = 7)	No. of positive isolations from swabs without <i>Campylobacter jejuni</i> (n = 3)
1	3	0
2	6	0
3	3	0
4	3	0
5	5	0
6	5	0
7	7	0
8	7	0
9	5	0
10	3	0
11	5	0
12	4	0
13	7	0
14	7	0
15	7	0
16	7	0
17	7	0
18	2	0
19	6	0
20	Not examined	Not examined
21	7	0
22	3	0
23	0	0

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Slide 14

Alternative methods for salmonella detection in poultry samples

- **Alternative methods can be allowed under the following conditions:**
 - Approval by the National Authority and Production Boards
 - Validation according to internationally recognized rules indicating that the alternative method offers equivalent results to those obtained by the branch method
 - Accreditation of the method
- **Approved alternative methods:**
 - PROBELIA™ PCR (BioRad) for salmonella detection in poultry faeces, fluff and neck skins
 - VIDAS-SLM (bioMérieux) for salmonella detection in fluff

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Slide 15

Serotyping of salmonella isolates from poultry

Present situation:

- Identification of SE and STM and of the main salmonella serogroups (B,C,D,E) is performed at a few diagnostic laboratories
- A selection of isolates is sent to the NRL for sero/phage typing and antibiotic resistance testing

Future situation:

- 90% of the isolates (top 10 ranking types) will be typed at a few diagnostic laboratories. The role of the NRL will be to:
 - provide a limited typing scheme for identification of the most prevalent serotypes
 - conduct training courses on serotyping of salmonella
 - organize collaborative studies on serotyping
- the remaining 'difficult' isolates will be sent to the NRL for typing

Appendix 8. Slides of presentation 3.2

Slide 1

Specificity using ISO 6579

- Growth levels and appearance of *Salmonella* strains
- growth levels and appearance of " enteric fever " *Salmonella*
- growing of non-*Salmonella* strains

Slide 2

Criteria of choice for *Salmonella* strains

- AOAC protocol = 100 to 200 strains
and groups IV, VI, V not to be tested
- Kauffmann - White scheme describe
46 somatic O groups and 2435 serovars
- ⇒ We selected strains to have a % of
subspecies and somatic groups
- ⇒ Strains were supplied by •AFSSA-Paris and
• Institut Pasteur, Salmonella reference lab.

Slide 3

species and subspecies	Number	Number tested
<i>S. enterica</i> subsp. <i>enterica</i> (I)	1425 (59%)	99 belonged to 32 O groups
	35 O groups	+ 10 atypical + 10 "enteric fever" (8 <i>S. Typhi</i> + 1 Paratyphi C + 1 Paratyphi B)
<i>S. enterica</i> subsp. <i>salamae</i> (II)	485 (20%)	14
<i>S. enterica</i> subsp. <i>arizonae</i> (IIIa)	94 (4%)	1
<i>S. enterica</i> subsp. <i>diarizonae</i> (IIIb)	321 (13%)	4
<i>S. enterica</i> subsp. <i>houtenae</i> (IV)	69 (3%)	5
<i>S. enterica</i> subsp. <i>indica</i> (VI)	11 (0.5%)	0
<i>Salmonella bongori</i> (V)	20 (0.8%)	1
all Salmonella	2435 serovars 46 O groups	total = 144 tested

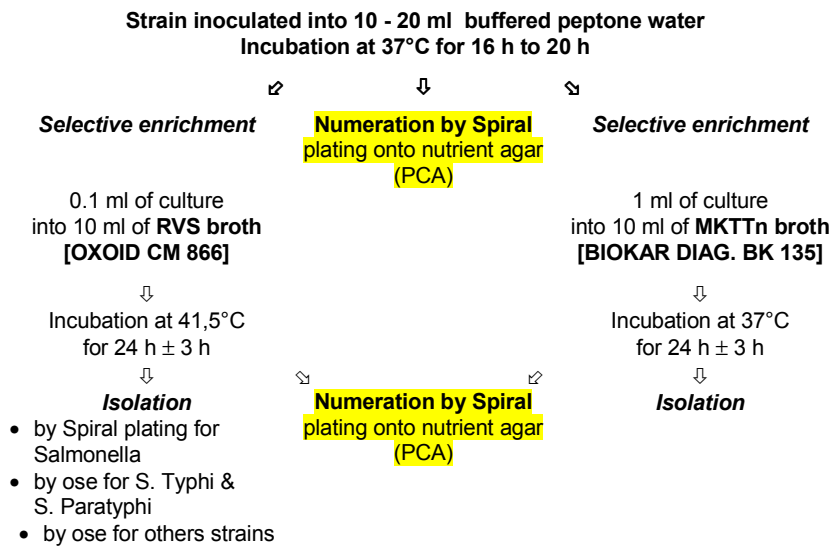
Slide 4

List of atypical strains

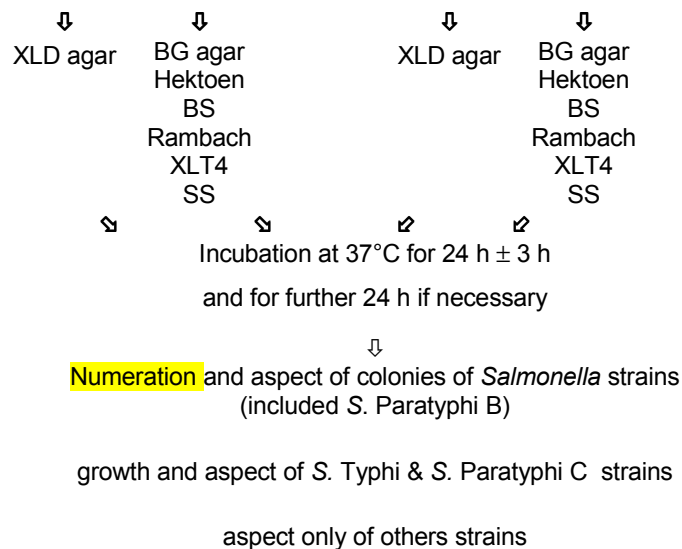
- malonate + : *S. Sofoa*
- H₂S- : *S. Dublin*; *S. Montevideo*;
S. Gallinarum biovar Pullorum
- saccharose + (= sucrose+) : *S. Panama* (x2)
- LDC- : *S. Panama*
- lactose + & LDC- : *S. Senftenberg*
- saccharose + & LDC- : *S. Senftenberg*
- lactose + & H₂S- : *S. Senftenberg*

Slide 5

**Diagram procedure for strains
(excluded S. Typhi and S. Paratyphi A)**



Slide 6



Slide 7

Salmonella: “growth” levels after incubation

	BPW	RVS (41.5°C)	MKTTn (37°C)
group I <i>S. Virchow</i> <i>S. Dublin</i>	3 to 9 $\times 10^8/\text{mL}$	$\approx 1 \times 10^8/\text{mL}$ \uparrow from 10^6 to 10^8 $4-9 \times 10^7/\text{mL}$ 6×10^6 to 6×10^7	$\approx 2 \times 10^8/\text{mL}$ \uparrow from 10^7 to 10^8 2×10^6 to 3×10^7
<i>S. Typhi</i> (x 8 strains)	2 – 5 $\times 10^8/\text{mL}$	1×10^3 to 4×10^6 \downarrow to 3 log or \uparrow 0 to 0.1 log	1×10^6 to 2×10^7 \downarrow 0.1 to 1 log
<i>S. Paratyphi C</i> (x 1 strain)	$4 \times 10^8/\text{mL}$	$2 \times 10^4/\text{mL}$ \downarrow 2 log	$< 10^4/\text{mL}$
<i>S. Paratyphi B</i> (x 1 strain)	like others <i>Salmonella enterica</i> subsp. <i>enterica</i> (group I)		

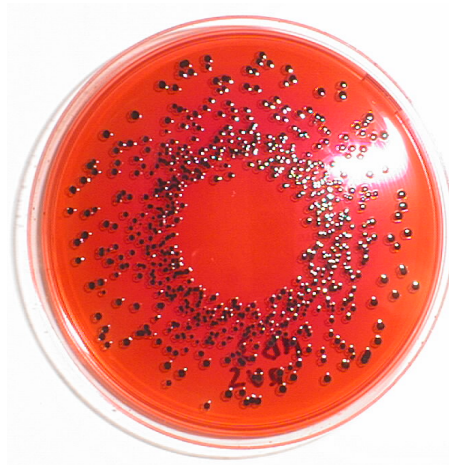
Slide 8

XLD : appearance of Salmonella colonies

- **Typical** = pink colonies with large glossy black centers, or almost completely black colonies
- **A2** = yellow/white colonies with small black centers
- **A3** = pink colonies without black centers
- **A4** = yellow/white colonies without black centers

S. Paratyphi C : no growth

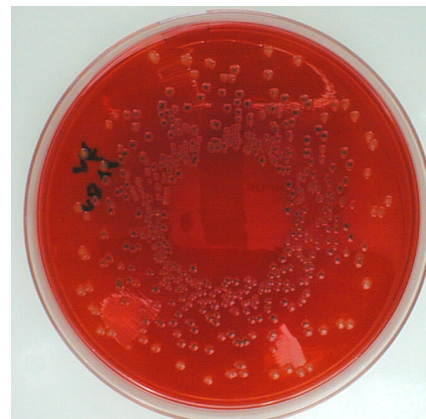
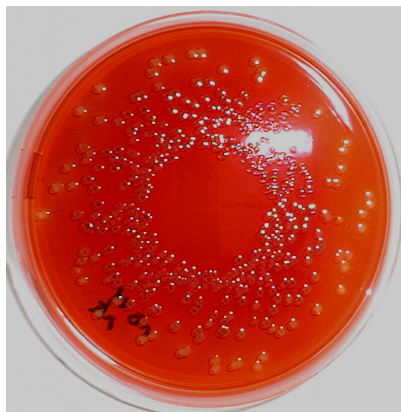
Slide 9



According to me : the edge of colonies appear pink but it is the color of the agar. The colonies are transparent/translucent

XLD / T : pink colonies with large, glossy black centers

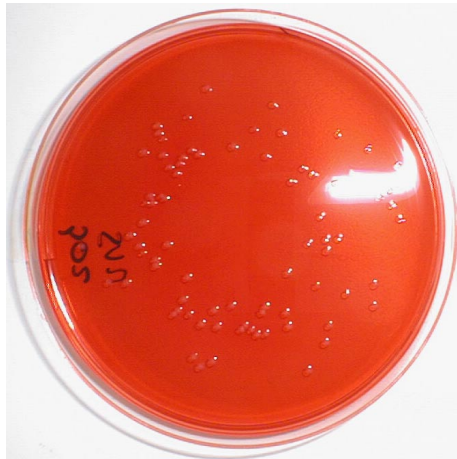
Slide 10



XLD / A2 :
Yellow/white with
small black
centers

ωS. Heidelberg ; ωS. Quentin ; ωS. Saphra ;
ωS. Carmel; ωS. Chicago ; ωS. Aschersleben ;
ω S. Perth ;ωgroupII O:4 ; ω O:50 ; ω O:53 ;
ωS. group IV O:51 ; ω S. groupIV O:44 ;
ωS. Sofoa malonate+ ;
ωS. Montevideo H₂S-

Slide 11



XLD type A3 :

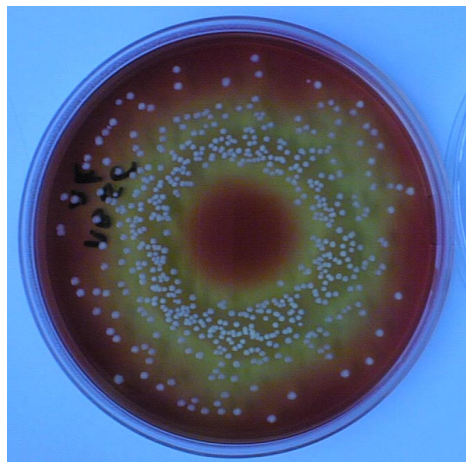
pink colonies
without black centers

(according to me, transparent)

S. Choleraesuis ; *S. Antonio* ; *S. Crossness* ; *S. Dublin* H₂S-
group IV serovar 40:

S. Typhi (3 strains)

Slide 12



- *S. Regent*
- *S. Liverpool*
- 1 strain, group II O:6,14
- *S. Panama*
saccharose+ (the 2 strains)
- *S. Senftenberg* lact+ & H₂S-
- *S. Typhi* (5 strains)

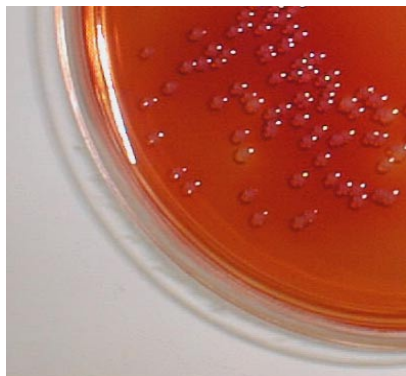
XLD / A4 : yellow/white colonies without black centers

Slide 13

Phenol red brilliant green agar (BGA) : aspect of *Salmonella* colonies

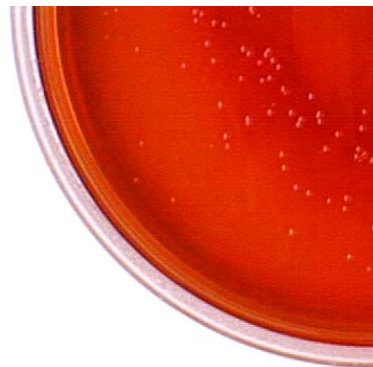
- Typical = mostly smooth, very low convex, moist pink/red, surrounded by bright red medium
- A2 = lightly transparent, yellow color due to the indicator change
- A3 = like T but smaller

Slide 14



BGA / T : according to me = lightly translucent, reddish color due to the agar

S. Typhi colonies are typical
S. Paratyphi C : on a plate there are 2 sizes typical colonies (T & A3)



BGA / A3 = typical but small

- *S. enterica* subsp. *salamae* (goup II) serovar 50: z:e,n,x
- *S. Sofoa* malonate +
- *S. Gallinarum* biovar Pullorum H2S-

Slide 15



BGA / A2 = lightly transparent,
yellow color due to the indicator
change

S. Regent

S. Liverpool

S. Aschersleben

S. Panama saccharose + (= sucrose+)

S. Senftenberg lactose + & H₂S -

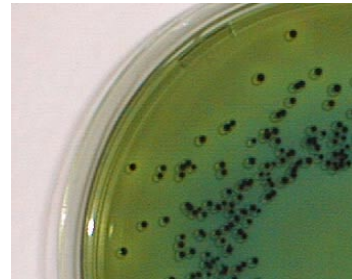
Slide 16

Hektoen : appearance of salmonella colonies

- Typical = blue-green to blue colonies with large glossy black centers or almost completely black colonies
- A2 = yellow-green colonies without black centers
- A3 = orange colonies with small black centers , sometime surrounding orange
- A4 = yellow small colonies with dark centers
- A5 = translucent colorless colonies
(only *S. Gallinarum* biovar *Pullorum* H₂S-)
- no growth of *S. Paratyphi* C

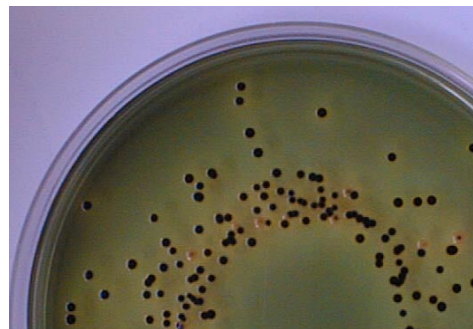
Slide 17

HE / Typical



HE / T & A3

(IIIb) 38:r,z:-
 (IIIb) 61:i,z53
 (IV) 17:z29:-
 (IV) 1,42:g,z51:-
 S. Panama sucrose+
 S. Panama LDC-



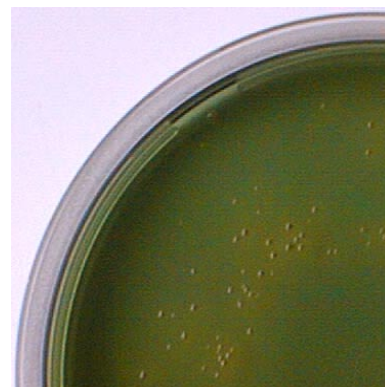
Slide 18



A2 : yellow-green, without
 black center, with or without
 dark center

S. Heidelberg ; S. Quentin ; S. Saphra ;
 S. Carmel ; S. Aschersleben ; S. Perth ;
 (II) 4,12:g,z62:- ; (II) 53:z4,z24:- ;
 (IV) 51:a:- ; (IV) 44:a:- ; S. Sofoa
 malonate+ ; S. Montevideo H₂S-

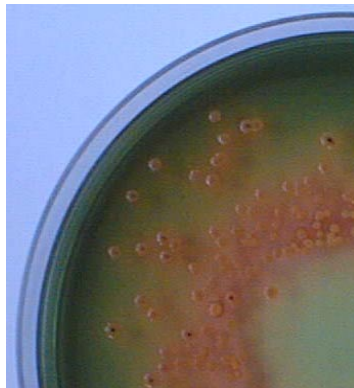
HE



HE / A4 : like A2 but small

S. Antonio
 S. (II) 50:z:e,n,x
 S. Dublin H₂S-

Slide 19



HE

A3: orange with small black center,
sometime surrounding orange

S. Regent
S. Liverpool
S. Senftenberg lact+ & H₂S-
S. Panama sucrose+

A5 : [no photo] : transparent
colorless colonies

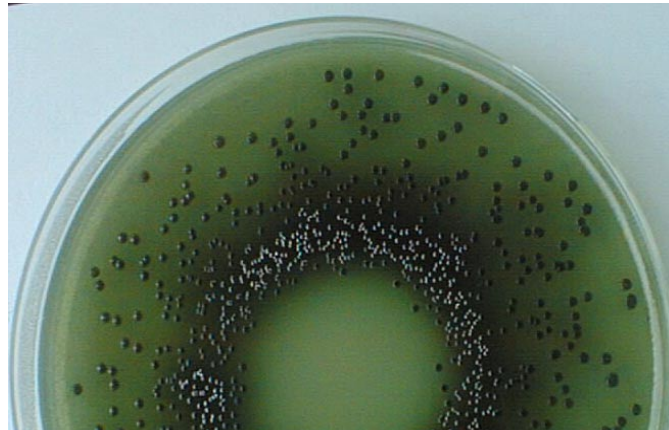
S. Gallinarum biovar Pullorum H₂S-

Slide 20

Bismuth sulfate agar (BS a): appearance of
Salmonella colonies

- Typical = brown, gray or black, with or without metallic sheen, surrounding brown at first then turning black
- A2 = green with darkening edge
- A3 = small colorless
- A4 = light green small
- no growth of *S. Paratyphi* C

Slide 21

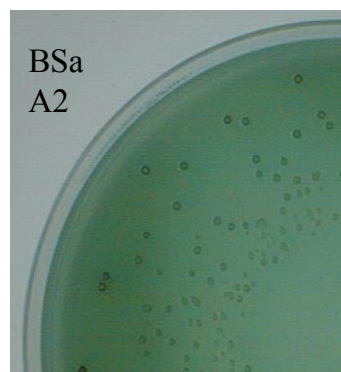


BSA

Typical = brown, gray or black, sometimes with metallic sheen.
Surrounding medium is usually brown at first, turning black with increasing incubation time

S. Typhi

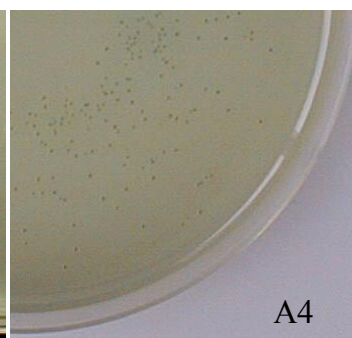
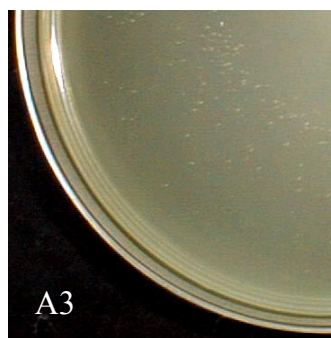
Slide 22



A2 : *S. Heidelberg* ;
S. Choleraesuis

A3 : *S. Djakarta*

A4 : 2 strains group II ;
1 group IV ; 1 group V ;
S. Sofoa malonate+



Slide 23

Non-Salmonella growth levels and colony description (possible confusion with Salmonella colonies)

after ISO 6579 enrichment and isolation onto various agar plates :

results of 35 strains

Slide 24

	RVS	MKTT+n	plates
<i>E. coli</i> (x3)	2×10^7 to 2×10^8 / mL	N D	no growth or atypical
<i>Yersinia enterocolitica</i>	1×10^5 / mL	N D	XLD: A BGa: ng , A3 BSa: ng
<i>Pseudomonas stutzeri</i>	<20 / mL	1×10^7 / mL	XLD: ng, A3 BGa: ng , A3 BSa: ng
<i>Enterococcus faecalis</i>	4×10^5 / mL	200 /mL	XLD: A, BGa: ng BSa: ng
<i>Staphylo. Kocuria</i> (x5)	<20 / mL	<20 / mL	XLD: ng, BGa: ng BSa: ng

Slide 25

	RVS	MKTT+n	plates
<i>Proteus</i> , <i>Providencia</i> , <i>Morganella</i>	<20 / mL to 3×10^5 / mL	Morganella 8×10^7 / mL	XLD: ng, A, A2, A3, A4 BGa: ng, A3 BSa: ng, A
<i>Citrobacter</i> (x 9 strains)	4×10^3 to 3×10^8 / mL	8×10^7 to 3×10^8 / mL	XLD: ng, A BGa: I, A, A3 BSa: ng, A
<i>Klebsiella</i> , <i>Enterobacter</i> <i>Serratia</i> (X5)	200 / mL to 3×10^7 / mL	(K & E.) 2 to 6 $\times 10^7$ / mL	XLD: ng, A BGa: ng, A, A3 BSa: ng, A, I
<i>Hafnia alvei</i> (x 5)	3×10^4 to 3×10^7 / mL	4×10^7 to 2×10^8 / mL	XLD: A, A3 BGa: ng, A, A3 BSa: A

Slide 26

Salmonella

- RVS :
 - 1 strain do not growth in RVS (to be confirm)
 - level of 9 strains < 6×10^7 / mL
- MKTT+n :
 - level of 6 strains < 6×10^7 / mL
- choice of second agar
 - all appearances of *Salmonella* must be well-known

Slide 27

This study does not describe the growth in mix cultures, just the best level that can be obtained

Performance testing of RVS, MKTTn various agar should be done individually

Appendix 9. Slides of presentation 3.3

1. ISO/DIS 6579 (1999/2000)

Microbiology of food and animal feeding stuffs –
Horizontal method for the detection of *Salmonella* spp.

- Pre-discussion on 5 February 2001 with Dk, B, D, NL, USA, Fr
- Discussion at ISO/TC34/SC9 meeting in Bern (Switzerland) on 19-21 June 2001

2. Points of discussion (from 5/2 meeting):

⇒ **Selective enrichment:**

- **RVS:** Incubation time now 24 h; some prefer 2 x 24 h.
Extra info will be sent to 'cie and discussed in Bern
- **MKTTn:**
 - Selenite Cystine broth was deleted because of toxicity;
 - MKTTn is found difficult to prepare;
 - A small trial will be organised to compare MKKTn with TT (AOAC medium) at 37 °C, results will be discussed in Bern

⇒ **Isolation agar (plating-out):**

- **XLD:**
 - Medium was originally prepared for detection of *Shigella* (not for *Salmonella*);
 - Not easy to prepare;
 - Some prefer BGA instead of XLD;
 - Additional data on isolation media will be sent to 'cie and discussed in Bern.

⇒ **Biochemical confirmation:**

- Prefer to keep TSI-agar
- Add interpretation table to the ISO of biochemical and serological tests

⇒ **Serological confirmation:**

- Maintain only polyvalent sera in the ISO

Advise from EU-validation study:

‘To aim at defining a common AOAC and ISO method for the detection of *Salmonella* in foods.’

3. ISO/FDIS 6579: 2002

Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.

4. ‘Introduction

Because of the large variety of food and feed products, this horizontal method may not be appropriate in every detail for certain products. In this case, different methods, which are specific to these products, may be used if absolutely necessary for justified technical reasons. Nevertheless, every attempt should be made to apply this horizontal method as far as possible.

When this International Standard is next reviewed, account will be taken of all information then available regarding the extent to which this horizontal method has been followed and the reasons for deviations from this method in the case of particular products.’

Scope

..... Subject to the limitations discussed in the Introduction, this International Standard is applicable to products intended for human consumption and the feeding of animals.’

Biochemical confirmation

‘If shown to be reliable, commercially available identification kits for the biochemical examination of *Salmonella* may be used.’

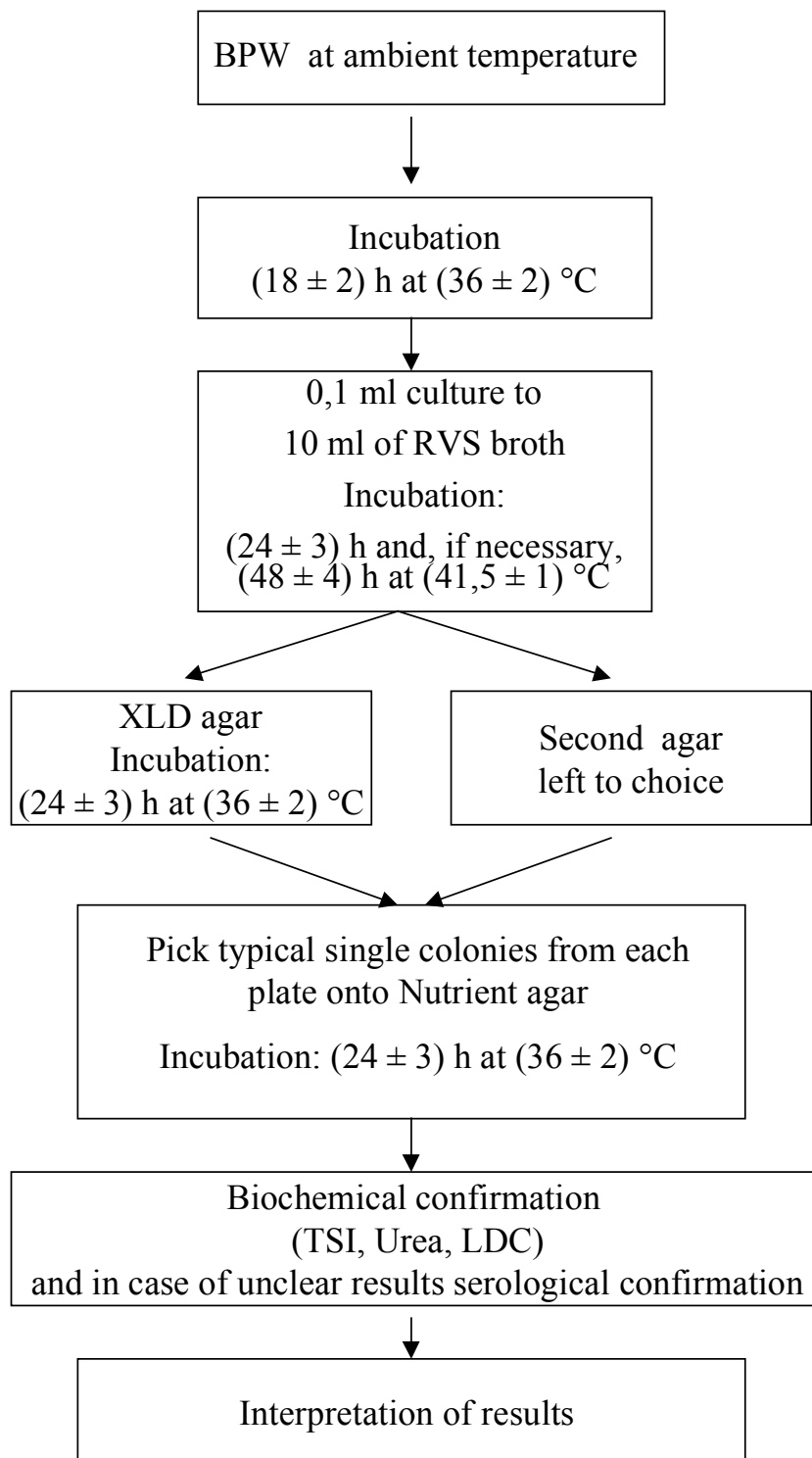
The pure culture from nutrient agar is inoculated on (and incubated at $(37 \pm 1) ^\circ\text{C}$ for (24 ± 3) h):

- Triple sugar/iron agar (TSI);
- Urea agar;
- L-Lysine decarboxylation medium;
- Medium for detection of β -galactosidase;
- Medium for Voges-Proskauer (VP) reaction;
- Medium for indole reaction.

Serological confirmation

‘The detection of the presence of *salmonella* O-, Vi and H-antigens is tested by slide agglutination with the appropriate sera, from pure colonies (from nutrient agar).’

ISO/DIS 6340 (2002) Water quality- Detection and enumeration of Salmonella



5. Working Group

In 2001 a European (ad hoc) Working group was set up named 'Salmonella-criteria for evaluation'. This Working group was requested to advise the European Commission on criteria to be applied in the evaluation of new methods for Salmonella detection, with the aim to determine whether they are equivalent to the authorised standard methods.¹ The Working group has met three times in 2001 and evaluated several international procedures on validation and comparison of methods. Results of the discussions were summarised in a draft report and will be finalised in the 'Scientific Committee on Veterinary Measures relating to Public Health'. After agreement of the Scientific Committee the opinion will be sent to the European Commission.

¹: According to Council Decisions 97/278/EC and 98/227/EC (testing of meat samples, swab samples and/or samples from faeces, meconium or chick organs for presence of *Salmonella*), other methods which are considered to be equivalent to ISO 6579:1993 and NMKL No. 71 or their latest versions may be authorised using the comitology procedure.

The following **recommendation** is made in the report:

‘It is desirable that the validation of the alternative method follows an official procedure. The Committee would favour the procedure currently presented collectively by the CEN and the ISO (prEN ISO/FDIS 16140²) once adopted’.

According to the scope of prEN ISO/FDIS 16140, ‘the procedure can be applied for validation of alternative methods in the field of microbiological analysis of food, animal feeding stuffs and environmental and veterinary samples.’

²:prEN ISO/FDIS 16140 (2000): Microbiology of food and animal feeding stuffs. Protocol for the validation of alternative method.

Appendix 10. Slides of presentation 3.4

Slide 1



Quantification of *Salmonella* spp. using a miniaturisation of MSRV enrichment medium : mini-MSRV

P. Fravallo, M. Leroux, Y. Hascouët, S. Queguiner, M. Le Fellic, J. Petton and G. Salvat

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Slide 2



Quantification of *Salmonella* spp.

Food safety :

- **retrospective studies of outbreaks** : could allow to rapidly identify the highly contaminated part of a control meal

Epidemiology

- identify the **at risk steps** in a primary production chain
- **reveal the efficiency** of partial decontamination procedure in highly contaminated environments

Slide 3



What exists?

- **Direct count :** Immunofluorescent or DNA probes
 - sensibility specificity antibodies (all serovars?)
 - technical steps (filtration...) micro-colonies
 - official method in AOAC and BAM (FDA)
- **Culture techniques :**
 - Direct plating on selective solid medium :
 - selectivity against competitive gut flora
 - need selective concentration of inhibitors : selective against *Salmonella* ?
 - selective media dedicated (Dulcitol-Bile-Novobiocine)
 - Culturability ? (stressed *Salmonella* cells) overlay procedure,
 - Most probable number methods...

Slide 4



Most probable number methods...

- Theory assumes two conditions :
 - organisms are randomly distributed throughout the solution
 - each sample from the solution which contains at least one organism is able to exhibit growth in the culture medium
- Both hypothesis are false (overall for *Salmonella*)
 - underline the need to well homogenised the solutions
 - select the most sensitive detection technique.

Slide 5



Most probable number methods...

- Principe : Based on repetition of serial dilutions of a sample - Generate a characteristic number
- Calculation of the MPN :
 - using statistical assumption each characteristic number is related to a MPN with confidence limits
- Increase confidence of the result :
 - increase the number of repetition on the right dilution only if always almost the same level of contamination... so necessity to quantify?

Slide 6



Most probable number methods...

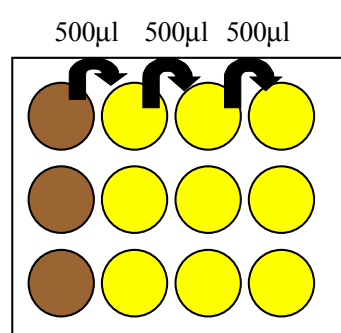
- Limits :
 - time and labor consuming
 - uncertain results
 - In case of low numbers, , is quantification needed ?
- Assuming this, we propose a MPN technique :
 - rapid and able to work with high numbers of samples (at least 75 samples per week and person)
 - based on miniaturised MSRV enrichment
 - Able to discriminated highly from poorly contaminated samples



Slide 7



Mini-MSRV

- Sample diluted 1/10 in PBW : stomaching at least one minute



 2 mL EPT
 2,5 ml initial suspension

Serial dilution 1/5 with multi-channel pipette

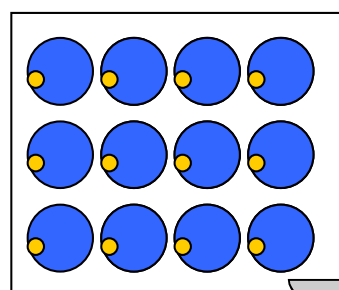
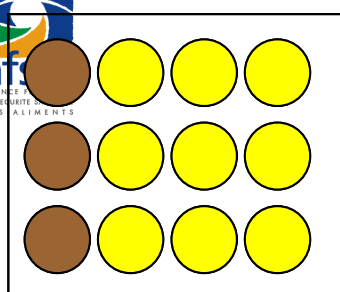
Incubation 16 h 37°C

Slide 8



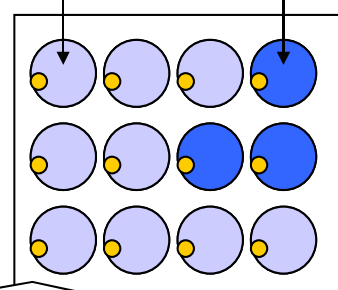
Mini-MSRV

Replication of the pre-enriched plate 20µl/well with multichannel microtitration pipette



Incubation
24 - 36h
41,5°C

Migration No migration



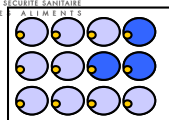
Slide 9



Mini-MSRV

- Streak each positive well on Rambach agar
- Biochemical characterisation of typical colonies
- Serogroup determination using polyspecific serums
- Determination of the characteristic number of the sample

Slide 10



Mini-MSRV

Characteristic number : 3321

MPN deducted : $1,33 \cdot 10^2$ (CI : $4,9 \cdot 10^1$ - $4,3 \cdot 10^2$)

Salmonella per g of sample

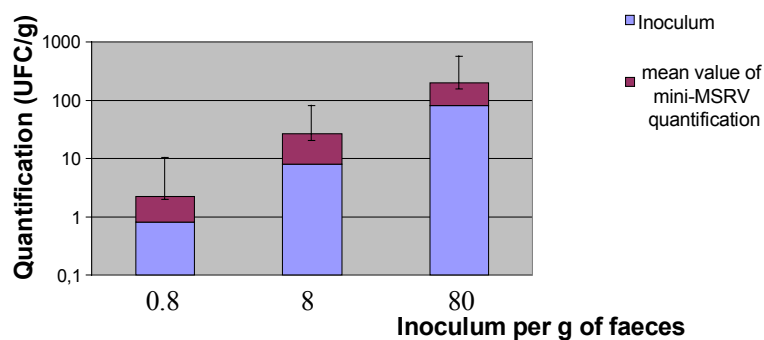
	mini	Maxi
Characteristic nb	1000	3332
MPN(<i>Salmonella</i>/g)	1,2	$5,7 \cdot 10^2$
CI	0,17-8,65	$1,5 \cdot 10^2$ - $2,1 \cdot 10^3$

Slide 11



Mini-MSRV

**Mean values obtained after mini-MSRV
quantification of artificially contaminated
pig faeces**



N = 7 samples independantly inoculated for each inoculum

Slide 12



Mini-MSRV Applications

- Quantification in pig fecal samples
- Quantification in surface samples in lairage
- Quantification in turkey neck skin samples

Slide 13



Mini-MSRV Applications

- Quantification in pig fecal samples

	Distribution of samples	<i>Salmonella</i> MPN per g	
	181	0,0	← 2 pigs populations
	25	1,0	
	1	3,5	
	4	6,4	
	4	$3,3 \cdot 10^1$	
	3	$1,8 \cdot 10^2$	←
	6	$8,9 \cdot 10^3$	
TOTAL	224		

2 different risk levels for the production chain

Slide 14



Mini-MSRV Applications

- Quantification in surface samples in lairage
 - 300cm² per swab
 - 192 samples, 27 detected by mini-MSRV method
 - 24 positive samples present less than 1 *Salmonella* per cm² (MPN=0.82 CI : 0.11 - 5.8)
- Cleaning procedures are efficient, disinfection must be improved

Slide 15



Mini-MSRV Applications

- Quantification in turkey neck skin samples
 - samples from a positive herd
 - 10 g of neck skin
 - 122 samples, 14 positives, 9 detected by mini-MSRV method

Slide 16



Mini-MSRV Applications

Positive sample	MPN Salmonella /g of skin	IC	
1	0.1	0.5	4.2
2	0.1	0.6	4.2
3	0.1	0.6	4.2
4	0.1	0.6	4.2
5	0.5	1.4	5.4
6	0.7	2.1	6.9
7	1.3	3.5	9.8
8	1.4	3.9	10.7
9	3.1	8.3	21.9

Few numbers of bacteria on the skin of this positive flock

Slide 17



Conclusion

- Interesting when :
 - *First investigations of a risk assessment
 - *Monitoring a quantitative criteria
- Number of dilutions and replicates must be fitted to the supposed contamination level
- Convenient quantification method for high numbers of samples
- Improvement: more convenient if we need just to confirm the more diluted positive well

Appendix 11. Slides of presentation

Slide 1



Slide 2



Slide 3

Discussion on Bacteriological Collaborative Study VI (2002)

	Set-up of study V	ISO 6579
Pre-enrichment	BPW (pre-heated at 37°C)	BPW (pre-heated at 37°C)
Selective enrichment	RV and MSRV	RVS and MKTT
Plating-out	XLD and BGA	XLD and BGA
Biochemical confirmation	Urea, TSI and LDC	Urea, TSI and LDC

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Appendix 12. Slides of presentation 4.1

Slide 1

Test results of Salmonella typing by NRLs and ENLs

Collaborative study VI (2001)

CRL - Salmonella Hans Korver

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the Environment

Research for man and environment

Slide 2

Questionnaire

Results serotyping

Results phage typing

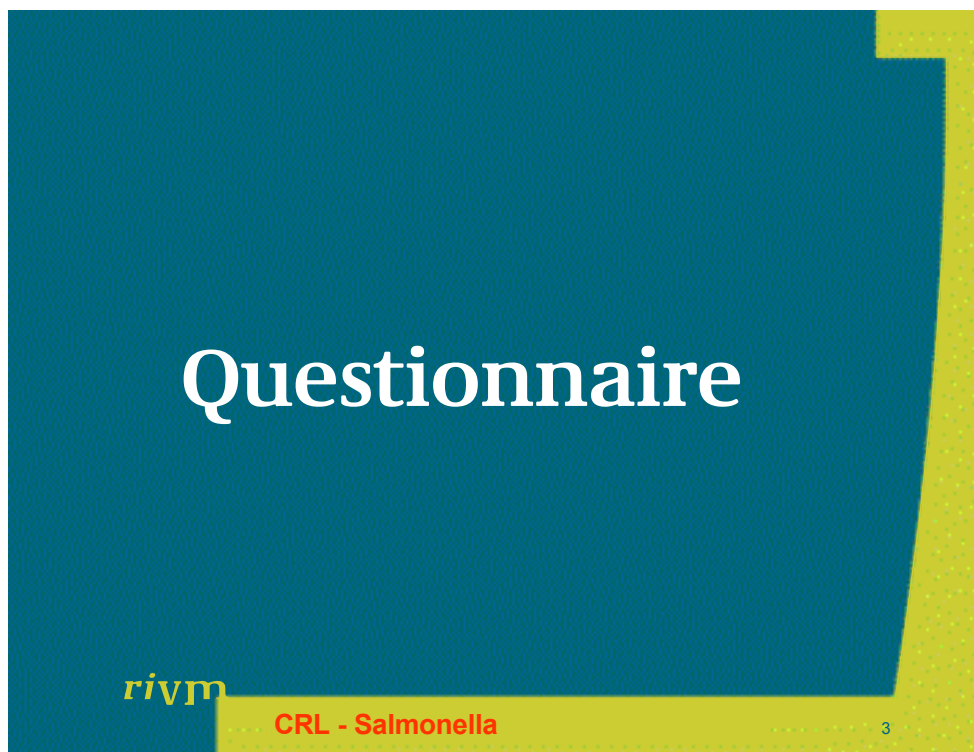
Antimicrobial susceptibility testing

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Slide 3

A presentation slide with a dark teal background and a yellow border on the right and bottom. The word "Questionnaire" is centered in a large, white, serif font. In the bottom left corner, the "rivm" logo is displayed in yellow. In the bottom center, the text "CRL - Salmonella" is written in red. A small white number "3" is in the bottom right corner.

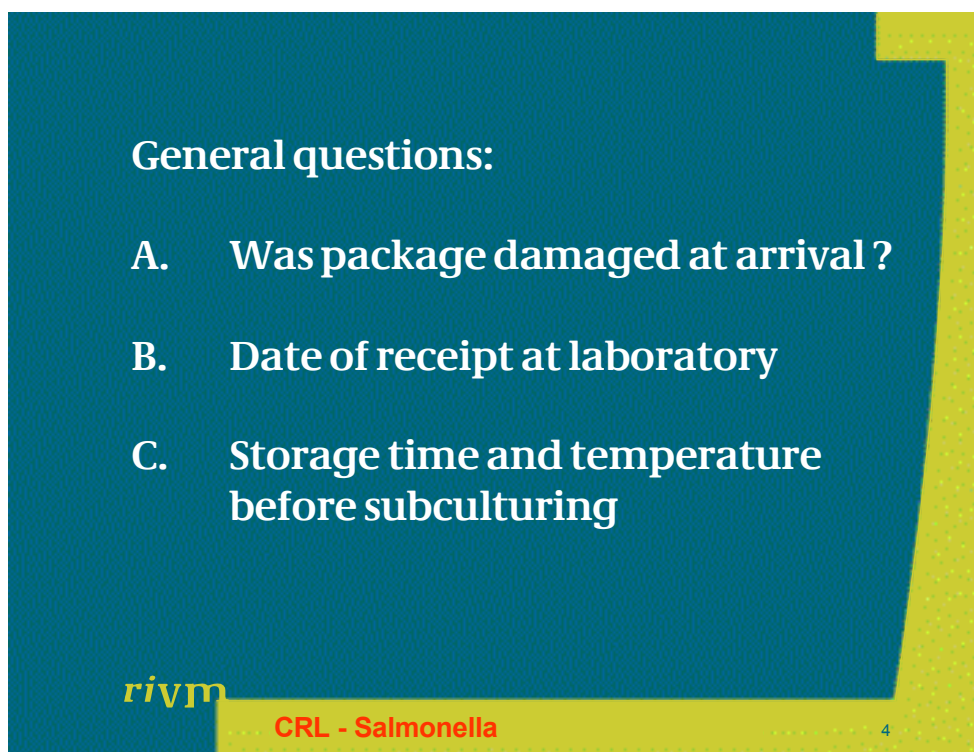
Questionnaire

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Slide 4

A presentation slide with a dark teal background and a yellow border on the right and bottom. The text "General questions:" is centered at the top in a white, serif font. Below it, three questions are listed, each preceded by a letter (A, B, C) and a period, in a white, serif font. In the bottom left corner, the "rivm" logo is displayed in yellow. In the bottom center, the text "CRL - Salmonella" is written in red. A small white number "4" is in the bottom right corner.

General questions:

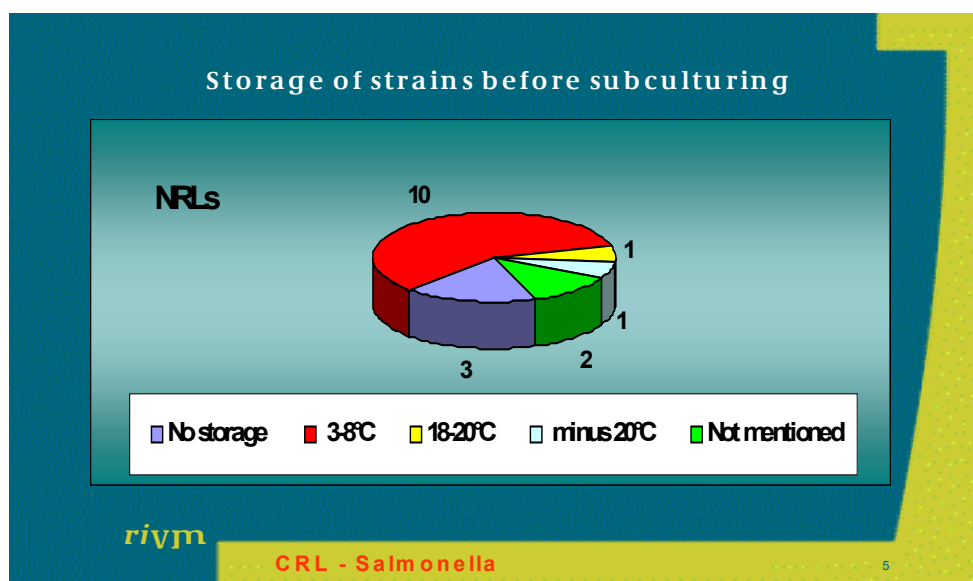
- A. Was package damaged at arrival ?
- B. Date of receipt at laboratory
- C. Storage time and temperature before subculturing

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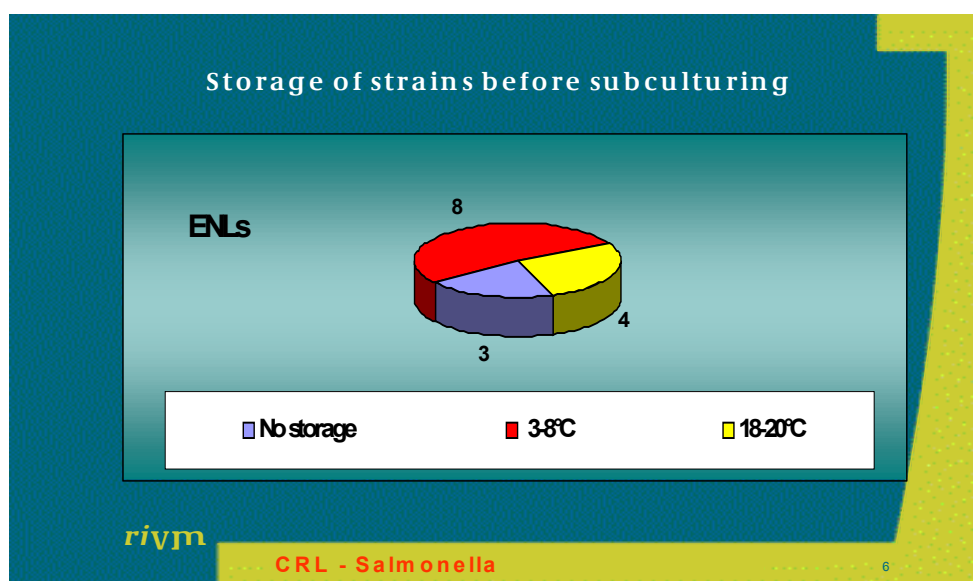
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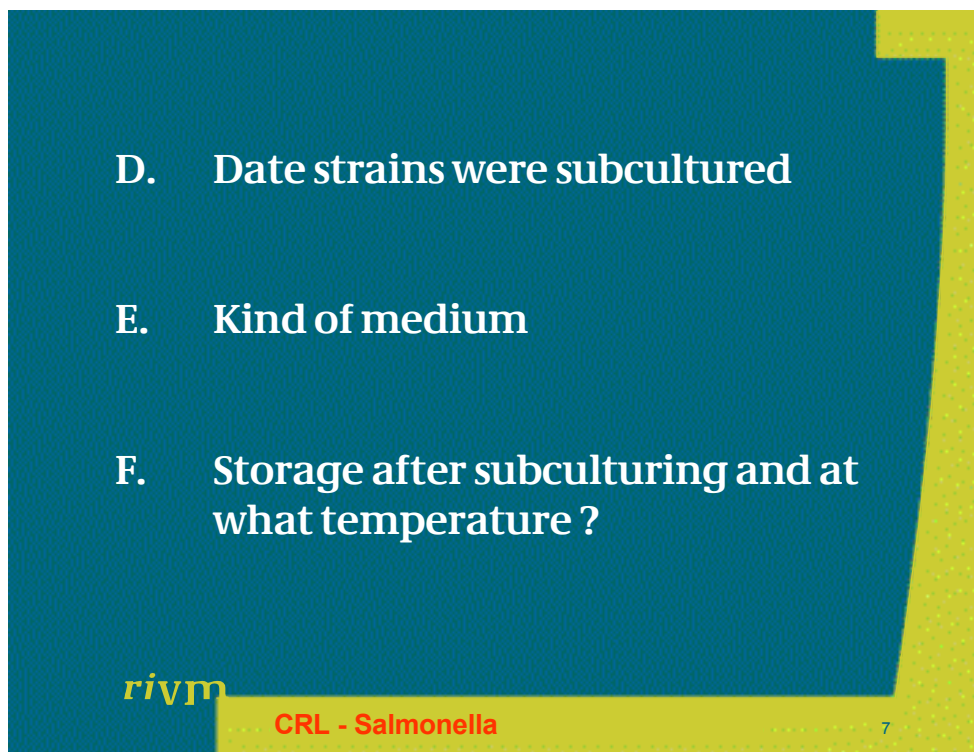
Slide 5



Slide 6



Slide 7



D. Date strains were subcultured

E. Kind of medium

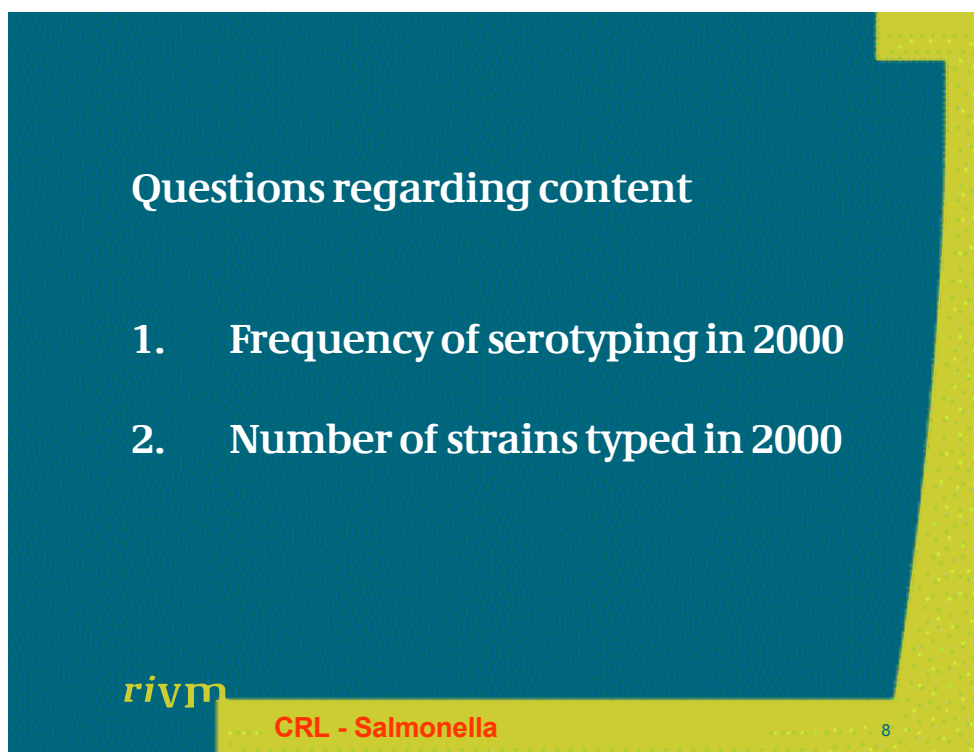
F. Storage after subculturing and at what temperature ?

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Slide 8



Questions regarding content

- 1. Frequency of serotyping in 2000**
- 2. Number of strains typed in 2000**

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Slide 9

Frequency and number of strains serotyped in 2000

Labcode NRLs	Typing frequency	Number of strains typed in 2000	Labcode ENLs	Typing frequency	Number of strains typed in 2000
1	Daily	11,191	A	Daily	14,000
2	Weekly	1,750	B	Daily	2,300
3	Daily	15,070	C	Daily	9,123
4	80 per month	800	D	Twice a week	980
5	Weekly	1,000	E	Daily	850
6	Daily	5,000	F	Monthly	70
7	Twice a week	289	H	Daily	3,580
8	Daily	1,036	J	Daily	9,015
9	Daily	1,337	K	Thrice a week	5,526
10	Twice a month	??	L	Daily	2,767
11	Weekly	6,000	P	Daily	8,000
12	??	??	R	Daily	479
13	At arrival	437	T	Daily	1,214
14	Daily	900	V	Daily	200
15	Twice a week	950	W	Daily	2,600
16	Daily	9,000			
17	Daily	2,050			

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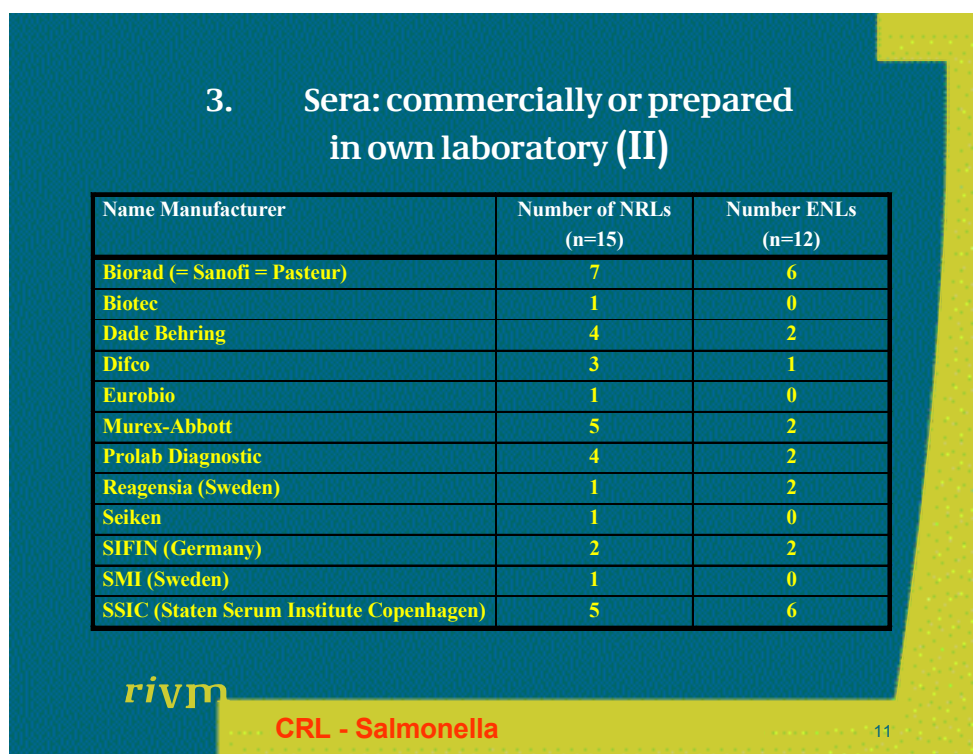
Slide 10

3. Sera: commercially or prepared in own laboratory (I)

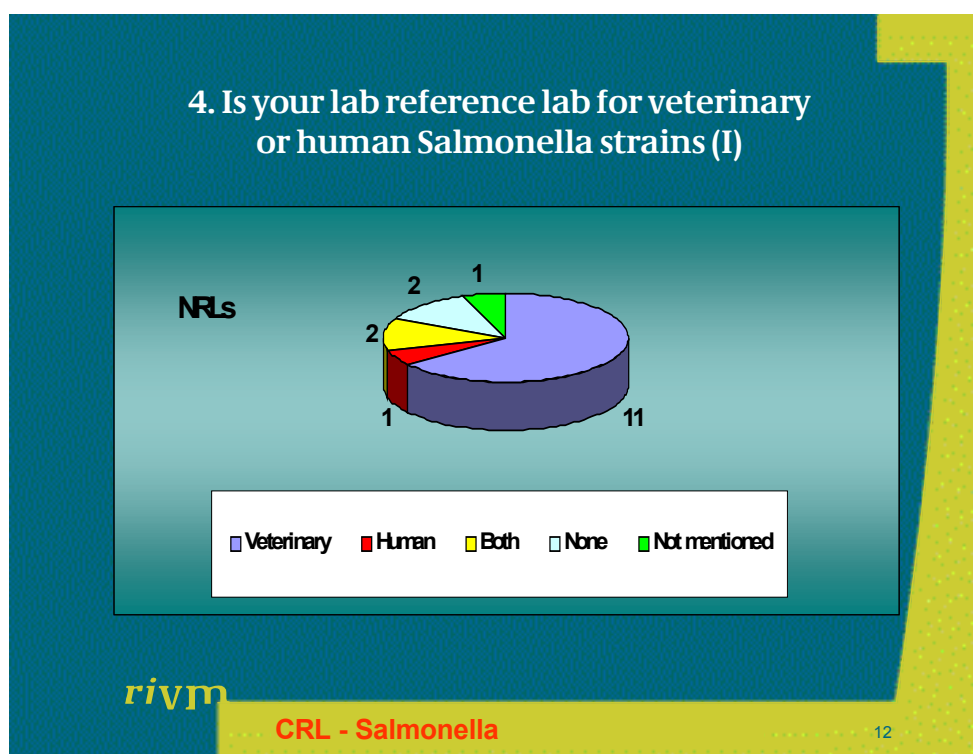
Number of manufacturers	Number of NRLs	Number of ENLs
From 1 manufacturer	2	6
From 2 manufacturers	8	1
From 3 manufacturers	2	4
From 4 manufacturers	3	1
Not mentioned	2	3
Preparation own laboratory	4	4

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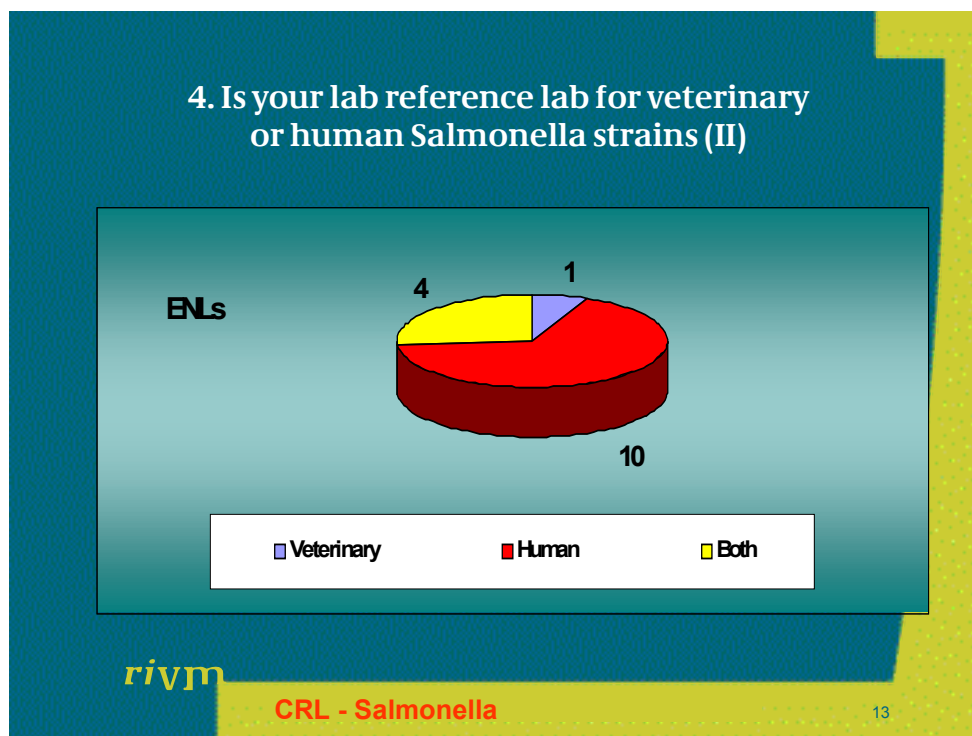
Slide 11



Slide 12



Slide 13



Slide 14



Slide 15

No	Serovar	O antigens	H antigens	Origin of strains
1	<i>S. Blockley</i>	6, 8	k : 1, 5	Human faeces
2	<i>S. Agona</i>	<u>1</u> , 4, [5], 12	f, g, s : [1, 2]	Human faeces
3	<i>S. Rissen</i>	6, 7, <u>14</u>	f, g : -	Human faeces
4	<i>S. Brazzaville</i>	6, 7	b : 1, 2	Human faeces
5	<i>S. Kiambu</i>	<u>1</u> , 4, 12	z : 1, 5	Chicken
6	<i>S. Typhimurium</i>	<u>1</u> , 4, [5], 12	i : 1, 2	Human faeces
7	<i>S. Goldcoast</i>	6, 8	r : l, w	Human faeces
8	<i>S. Kottbus</i>	6, 8	e, h : 1, 5	Human faeces
9	<i>S. Blockley</i>	6, 8	k : 1, 5	Human faeces
10	<i>S. Yoruba</i>	16	c : l, w	Animal feed

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No.	Serovar	O antigens	H antigens	Origin of strains
11	<i>S. Grumpensis</i>	<u>1</u> , 13, 23	d : 1, 7	Human faeces
12	<i>S. Heidelberg</i>	<u>1</u> , 4, [5], 12	r : 1, 2	Human faeces
13	<i>S. spp.arizonae</i>	41	z4, z23 : -	Human faeces
		41 : z4, z23 : -		
14	<i>S. Enteritidis</i>	<u>1</u> , 9, 12	g, m : -	Human faeces
15	<i>S. Newport</i>	6, 8, <u>20</u>	e, h : 1, 2 : [z67]	Human faeces
16	<i>S. Dublin</i>	<u>1</u> , 9, 12	g, p : -	Human faeces
17	<i>S. Muenchen</i>	6, 8	d : 1, 2 : [z67]	Human faeces
18	<i>S. Lexington</i>	3, 10 [15][15, 34]	z10 : 1, 5	Environmental sample
19	<i>S. Waycross</i>	41	z4, z23 : [e, n, z15]	Human faeces
20	<i>S. Llandoff</i>	1, 3, 19	z29 : [z6]	Animal feed

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Guidelines for evaluation of serotyping results

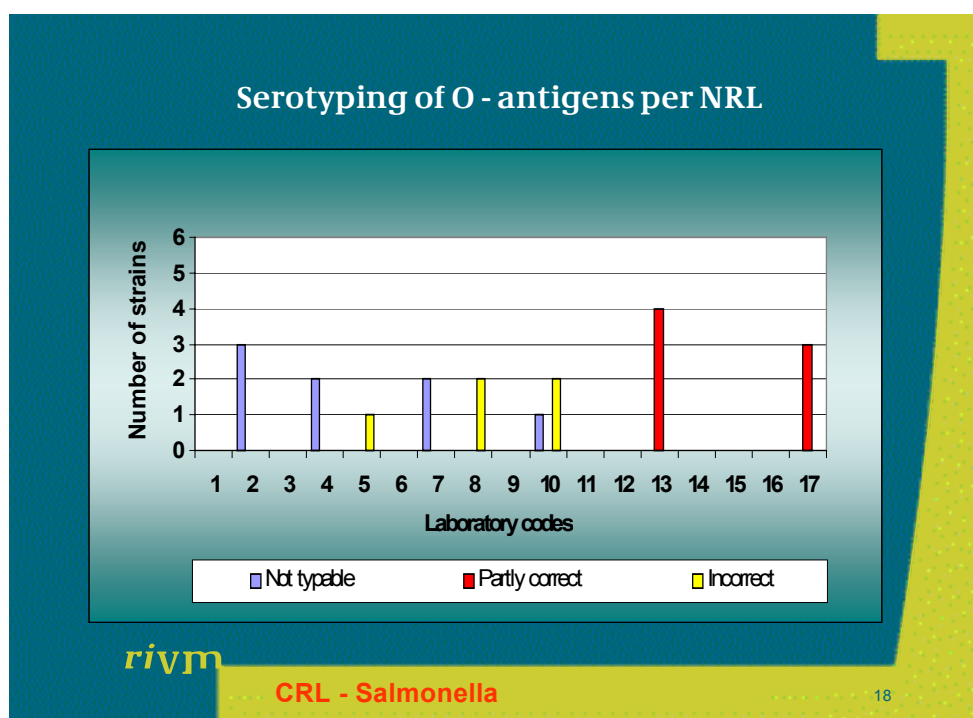
Results of serotyping	Evaluation
<ul style="list-style-type: none"> • Auto agglutination • Incomplete set of antisera (outside the range of antisera) 	nt = not typable
<ul style="list-style-type: none"> • Partly typable due to incomplete set of antisera • Part of the formula (for the name of the serovar) 	+/- = partly correct
<ul style="list-style-type: none"> • Wrong serovar or mixed sera formula 	- = incorrect

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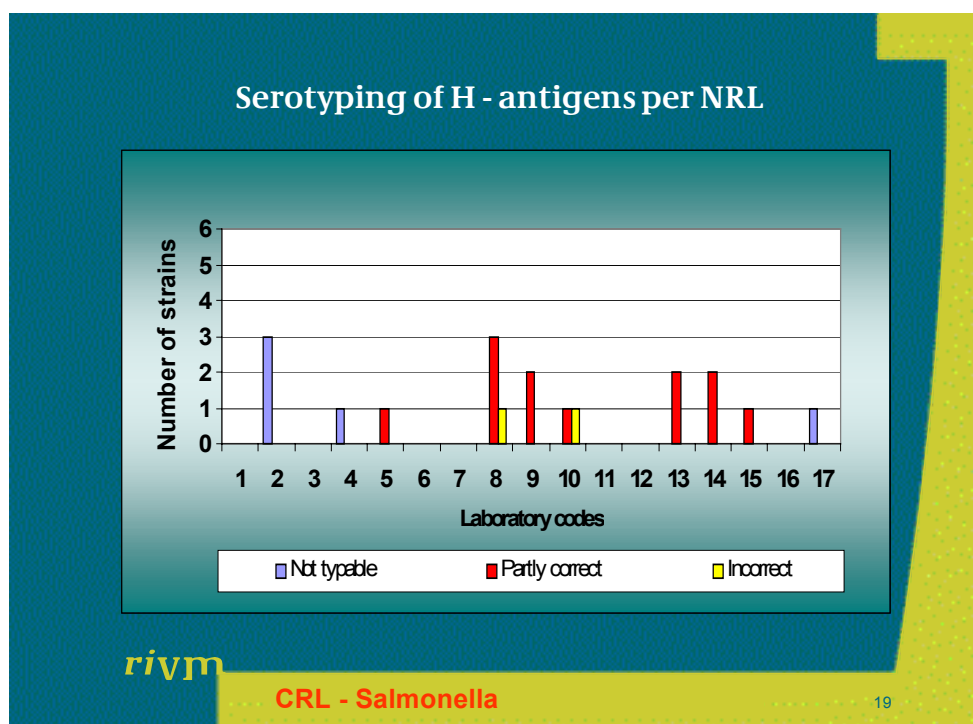
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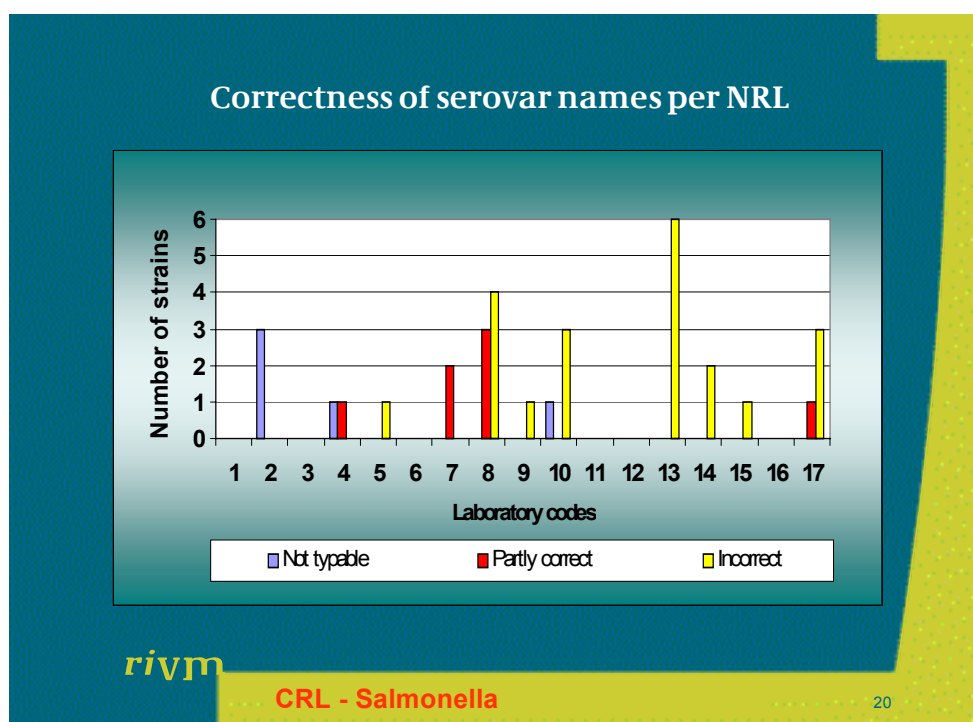


Slide 19



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Slide 20



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Slide 21

Serotyping per strain for NRLs

Strain No.	Serotype	O antigen detected				H antigen detected				Name serovar			
		+	nt	+/-	-	+	nt	+/-	-	+	Nt	+/-	-
3	<i>S. Rissen</i>	17				16		1		16			1
4	<i>S. Brazzaville</i>	17				16		1		16			1
5	<i>S. Kiambu</i>	17				17				16			1
7	<i>S. Goldcoast</i>	16		1		17				16			1
8	<i>S. Kottbus</i>	16		1		14		3		12			5
9	<i>S. Blockley</i>	15		2		17				15			2
10	<i>S. Yoruba</i>	15	1		1	14	1	2		14	1		2
11	<i>S. Grumpensis</i>	15			2	17				17			
12	<i>S. Heidelberg</i>	17				16		1		16			1
13	<i>S. spp. arizonae</i> 41:z4,z23:-	13	4			13	2	2		12	3	2	
15	<i>S. Newport</i>	15		2		17				15			2
16	<i>S. Dublin</i>	17				16		1		16			1
17	<i>S. Muenchen</i>	16		1		16		1		15		1	1
19	<i>S. Waycross</i>	13	3		1	16	1			12	1	3	1
20	<i>S. Llandoff</i>	16			1	14	1		2	14		1	2

+ = correctly; nt = not typable; +/- = partly correct; - = incorrect

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Identifications causing problems

	Strain 8	Strain 10	Strain 13	Strain 19	Strain 20
Correct typing	<i>S. Kottbus</i> 6,8: e,h: 1,5	<i>S. Yoruba</i> 16: e: 1,w	<i>S. spp. arizonae</i> 41: z4, z23:-	<i>S. Waycross</i> 41: z4,z23: [e,n,z15]	<i>S. Llandoff</i> 1,3,19: z29: [z6]
Labcode 2		??	??	??	
Labcode 4			<i>spp. arizonae</i> Polyvalent 11+: -----	?? Polyvalent 11+: z4,z23	
Labcode 7			<i>spp. 111b:</i> z4,z23:-	<i>spp. enterica:</i> z4,z23:-	
Labcode 8	<i>S. Cremieu</i> 6,8: e,h: 6	11 3,9: e	?? 41: z4,z23:-	?? 41: z4,z23:-	<i>S. Cannstatt</i> 3,10,19: m, t
Labcode 9		<i>S. Vancouver</i> 16: e: 1,5			
Labcode 10	<i>S. Manhattan</i> 6,8: e,h: 1,5		?? ??: z4	<i>S. Parera</i> 11: z4,z23:-	<i>S. Simsbury</i> 1,3,19: z27
Labcode 13	<i>S. Tshingwe</i> 6,8: e,h:e,n,z15				
Labcode 15	<i>S. Tshingwe</i> 6,8: e,h:e,n,z15				
Labcode 17	<i>S. Lomita</i> 6,7: e,h: 1,5				<i>spp. enterica</i> 3,19: Poly H ph 1+2

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Results phage typing

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SE phage typing by NRLs

Phage type of each laboratory

Strain	PT	1	3	6	9	11	13	16
E1	6	6	6	6	6	6	6	6
E2	1	1	1	1	1b	1	1b	1
E3	21	21	21	21	21	21	21b	21
E4	4b	4b	4b	4b	4b	4b	4b	4b
E5	14b	14b	14b	14b	14b	14b	14b	24
E6	4	4	4	4	4	4	4	4
E7	25	25	25	25	25	25	25	11
E8	8	8	8	8	8	28	29a	8
E9	6a	6a	6a	6a	6a	6a	6a	6a
E10	11	9a	9a	11	11	11	9a	9a

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STM phage typing by NRLs

Phage type of each laboratory

Strain	PT	1	3	6	9	11	13	16
M11	36	36	36	36	36	NT	36	36
M12	8	8	8	8	9	NT	115	8
M13	18	18	18	18	18	NT	18	18
M14	41	41	41	41	41	NT	41A	41
M15	U302	U302	U302	U302	U302	NT	U302	U302
M16	193	193	193	193	193	NT	193	193
M17	12	12	12	12	12	NT	104A	12
M18	104(L)	104L	104	104L	104	NT	104L	104L
M19	208	208	208	208	208	NT	208	208
M20	170	170	170	170	170	NT	104A	170

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Antimicrobial susceptibility testing

1. Minimal Inhibitory Concentration (MIC)
2. Disc diffusion

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Strains/ Antibiotics	MIC testing in µg/ml					
	Laboratory codes					
	3	6	15	C	H	T
6/AMP	AMP>32	AMP>32	AMP>32	AMP>16	AMP>50	AMP>32
6/CHL	CHL>64	CHL>64	CHL>16	CHL>32	CHL>20	CHL>32
6/FEN	FEN>64	FEN64	FEN16	nt.	nt.	nt.
6/SIR	SIR>64	SIR>64	SIR128	SIR>64	SIR>20	SIR>64
6/TET	TET>32	TET>32	TET64	nt.	TET>10	TET>32
8/CIP			nt.			CIP>4
8/NAL	NAL>128	NAL>128	NAL>128	NAL>32	NAL>40	NAL>64

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Strains/ Antibiotics	Disc diffusion testing by NRLs					
	Laboratory codes					
	Lab 10	Lab 11	Lab 12	Lab 14	Lab 16	Lab 17
2/AMP				AMP10		
2/CEN		nt.	nt.	CEN10		
2/SIR	SIR100*	nt.		SIR10*		SIR10
2/SXT	SXT2452	SXT2452	SXT25	SXT25	SXT25	
2/TET	TET80	TET80	TET30	TET30	TET10	TET10
2/TMP	nt.	nt.	nt.	nt.	nt.	TMP5
3/TET	TET80	TET80	TET30	TET30	TET10	

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Achievements by NRLs in %					
Labcodes	O-antigens	H-antigens	Serovar names	SE Phage	STM Phage
1	100	100	100	90	100
2	85	85	85		
3	100	100	100	90	100
4	90	95	90		
5	95	95	95		
6	100	100	100	100	100
7	90	100	90		
8	90	80	65		
9	100	90	95	90	90
10	85	90	80		
11	100	100	100	90	N.T.
12	100	100	100		
13	80	90	70	60	60
14	100	90	90		
15	100	95	95		
16	100	100	100	70	100
17	85	95	80		

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Achievements in % by ENLs					
	O-antigens	H-antigens	Serovar names	SE Phage	STM Phage
A	100	95	95	90	90
B	100	100	100	90	100
C	100	100	100	100	100
D	100	90	90		
E	100	100	95	100	90
F	95	80	70	80	90
H	100	100	100	100	100
J	100	100	100	80	100
K	100	100	100	90	100
L	100	95	95		
P	100	100	100	90	40
R	100	100	100		
S				70	80
T	100	95	95		
V				100	90
W	95	100	95	70	60

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Conclusions:**Serotyping**

O-antigens: minor problems
H-antigens: more problems

Phage typing

Overall results were good
Two labs: problems with STM

**Antimicrobial
Susceptibility**

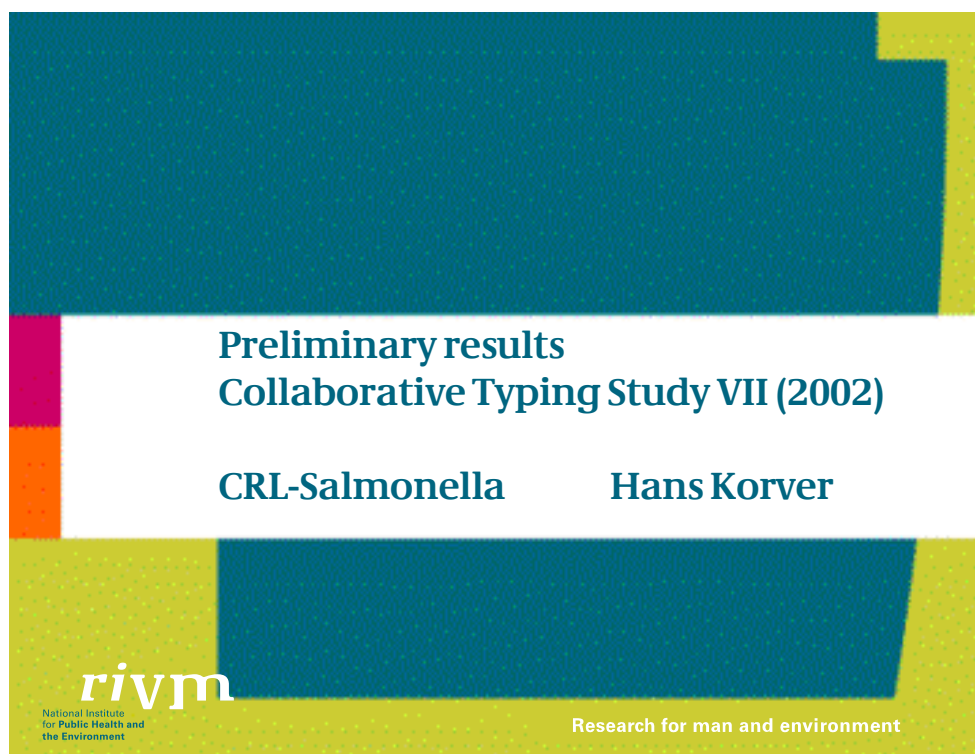
Two methods (MIC and disc)
Too many antibiotics were used
Standardisation

The RIVM logo, consisting of the letters 'rivm' in a stylized, lowercase, yellow font.**CRL - Salmonella**

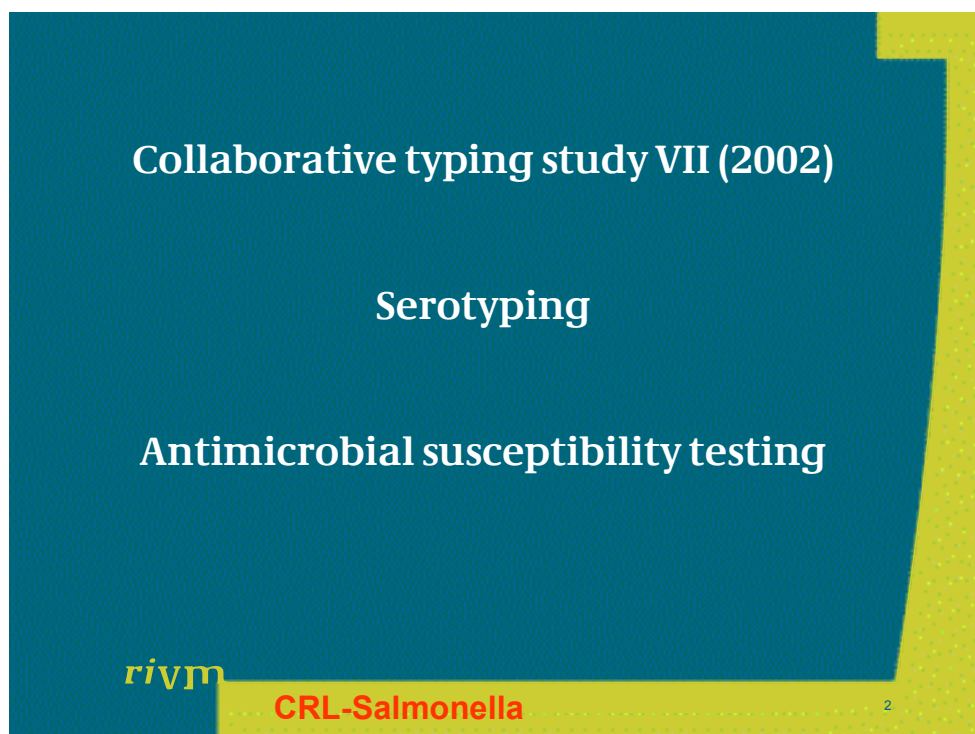
31

Appendix 13. Slides of presentation 4.2

Slide 1



Slide 2



Slide 3

No.	Serovar	O-antigens	H-antigens
1	<i>S. Typhimurium</i>	<u>1</u> , 4, [5], 12	i : 1, 2
2	<i>S. Jangwani</i>	17	a : 1, 5
3	<i>S. Kapemba</i>	9, 12	l, v : 1, 7
4	<i>S. Brandenburg</i>	4, [5], 12	l, v : e, n, z15
5	<i>S. Enteritidis</i>	<u>1</u> , 9, 12	g, m : -
6	<i>S. Paratyphi B/Java</i>	<u>1</u> , 4, [5], 12	b : 1, 2
7	<i>S. Bareilly</i>	6, 7, <u>14</u>	y : 1, 5
8	<i>S. Manhattan</i>	6, 8	d : 1, 5
9	<i>S. Give</i>	3, 10 [<u>15</u>] [<u>15</u> , <u>34</u>]	[d], l, v : 1, 7
10	<i>S. Paratyphi B/Java</i>	<u>1</u> , 4, [5], 12	b : 1, 2

rivm CRL-Salmonella 3

Slide 4

No.	Serovar	O-antigens	H-antigens
11	<i>S. Vinohrady</i>	28	m, t : [e, n, z15]
12	<i>S. Derby</i>	<u>1</u> , 4, [5], 12	f, g : [1, 2]
13	<i>S. London</i>	3, 10, [<u>15</u>]	l, v : 1, 6
14	<i>S. Adelaide</i>	35	f, g : -
15	<i>S. Bovismorbificans</i>	6, 8, <u>20</u>	r, [i] : 1, 5
16	<i>S. Oranienburg</i>	6, 7, <u>14</u>	m, t : [z57]
17	<i>S. Llandoff</i>	1, 3, 19	z29 : [z6]
18	<i>S. Stanley</i>	<u>1</u> , 4, [5], 12, <u>27</u>	d : 1, 2
19	<i>S. Agona</i>	<u>1</u> , 4, [5], 12	f, g, s : [1, 2]
20	<i>S. Kedougou</i>	<u>1</u> , 13, 23	i : l, w

rivm CRL-Salmonella 4

Slide 5

Evaluation of serotyping per NRL (I)

Correctness of O-antigens	Number of laboratories
20	12
19	3
18	0
17	0
16	0
15	1

rivm

CRL-Salmonella

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Slide 6

Evaluation of serotyping per NRL (II)

Correctness of H-antigens	Number of laboratories
20	6
19	5
18	3
17	0
16	2

rivm

CRL-Salmonella

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Slide 7

Evaluation of serotyping per NRL (III)

Correctness of serovar names	Number of laboratories
20	6
19	5
18	3
17	0
16	0
15	1
14	1

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CRL-Salmonella

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Slide 8

Evaluation of serotyping per strain (I)

Strains	Serovar names	O-antigens	H-antigens
1	16	16	16
2	15	15	15
3	16	16	15
4	15	16	15
5	15	15	16
6	13	15	13
7	16	16	16
8	16	16	16
9	16	16	16
10	12	15	12

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CRL-Salmonella

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Slide 9

Evaluation of serotyping per strain (II)

Strains	Serovar names	O-antigens	H-antigens
11	12	14	13
12	16	16	16
13	16	16	16
14	15	15	15
15	16	16	16
16	11	16	13
17	16	16	16
18	15	16	15
19	16	16	16
20	15	15	15

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CRL-Salmonella

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Slide 10

Antimicrobial susceptibility testing for the
Collaborative Study VIII (2003)Newsletter September 2002
(Questionnaire)

Variables

Standardisation

rivm

CRL-Salmonella

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Slide 11

Antibiotics	NRLs (n=17)	ENLs (n=10)	Antibiotics	NRLs (n=17)	ENLs (n=10)
Amikacin	2	1	GENTAMYCIN (GEN)	14	10
Amoxicillin	1	0	Imipenem	0	1
Amox+Clavulanate	11	1	KANAMYCIN (KAN)	7	9
AMPICILLIN (AMP)	16	10	Marbofloxacin	1	0
Ampicillin+Sulbactam	0	1	Mecillinam	0	2
Apramycin	5	1	Mezlocillin	0	1
Cefalotin	4	2	Mezloc.+Sulfalactam	0	1
Cefoperazone	2	0	Minocyclin	0	1
Cefotaxim	4	9	NALADIXIC ACID (NAL)	14	10
Cefotiam	0	1	NEOMYCIN (NEO)	11	0
Cefoxitine	1	1	Netilmicin	0	1
Ceftazidime	2	3	Nitrofurantoin	1	2
Ceftiofur	4	1	Nourseothricin	0	1

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Slide 12

Antibiotics	NRLs (n=17)	ENLs (n=10)	Antibiotics	NRLs (n=17)	ENLs (n=10)
Cefuroxim	2	0	Oxolinic Acid	1	0
Cephazolin	1	0	Oxytetracyclin	0	1
CHLORAMPHENICOL (CHL)	17	9	Polymyxin	0	1
CIPROFLOXACIN (CIP)	10	10	Spectinomycin	3	5
Colistin	6	0	STREPTOMYCIN (STR)	16	8
Co-sulphonamides	3	2	Sulfamerazin	0	1
Co-trimoxazole	0	1	Sulfamethoxazole	3	2
Doxycyclin	2	0	SULPHAM.+TMP (SXT)	14	4
Enrofloxacin	8	0	Sulfisoxazole	1	1
FLORFENICOL (FFN)	4	0	Sulfonamides	4	4
Flumequin	3	0	TETRACYCLIN (TET)	17	9
Framycetin	1	0	TRIMETHOPRIM (TMP)	7	7
Furazolidone	4	1	Triple sulphonamides	1	0

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CRL-Salmonella

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Slide 13

List of antibiotics for study VIII (2003)

- Ampicillin
- Chloramphenicol
- Ciprofloxacin
- Florfenicol
- Gentamycin
- Kanamycin
- Naladixic Acid
- Neomycin
- Streptomycin
- Sulphonamide/Trimethoprim
- Tetracyclin
- Trimethoprim

rivm **CRL-Salmonella** 13

Slide 14

Questionnaire / Variables

- Discs or MIC
- Load ($\mu\text{g/ml}$)
- Free areas in mm (diameter)
- Maximum number of antibiotics per plate
- Thickness medium
- Method inoculating plates
- Drying time
- Incubation time
- Incubation temperature
- Size of inoculum
- Measurement of inoculum size

rivm **CRL-Salmonella** 14

Slide 15

Questionnaire / Variables

Breakpoint per antibiotic and per country

Breakpoints in relation to
resistant/intermediate/sensitive

Diameter bacterial free zones in relation to
resistant/intermediate/sensitive

rivm

CRL-Salmonella

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Slide 16

Proposal

Questionnaire in Newsletter

Presentation new plan for 2003

Strains to be tested : 10
(different from serotyping)

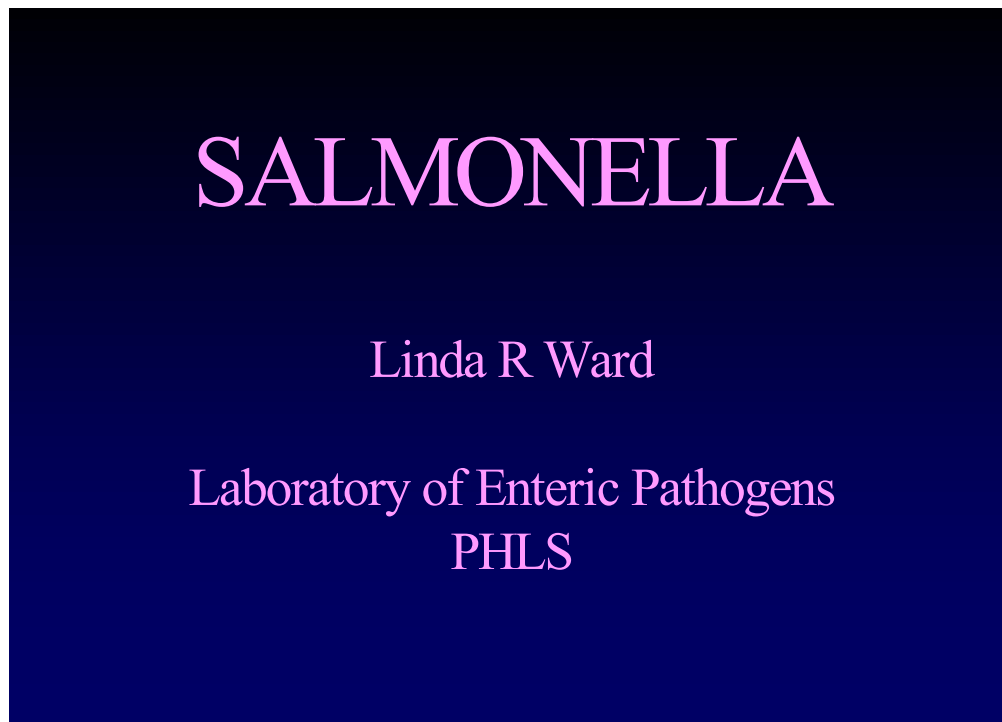
rivm

CRL-Salmonella

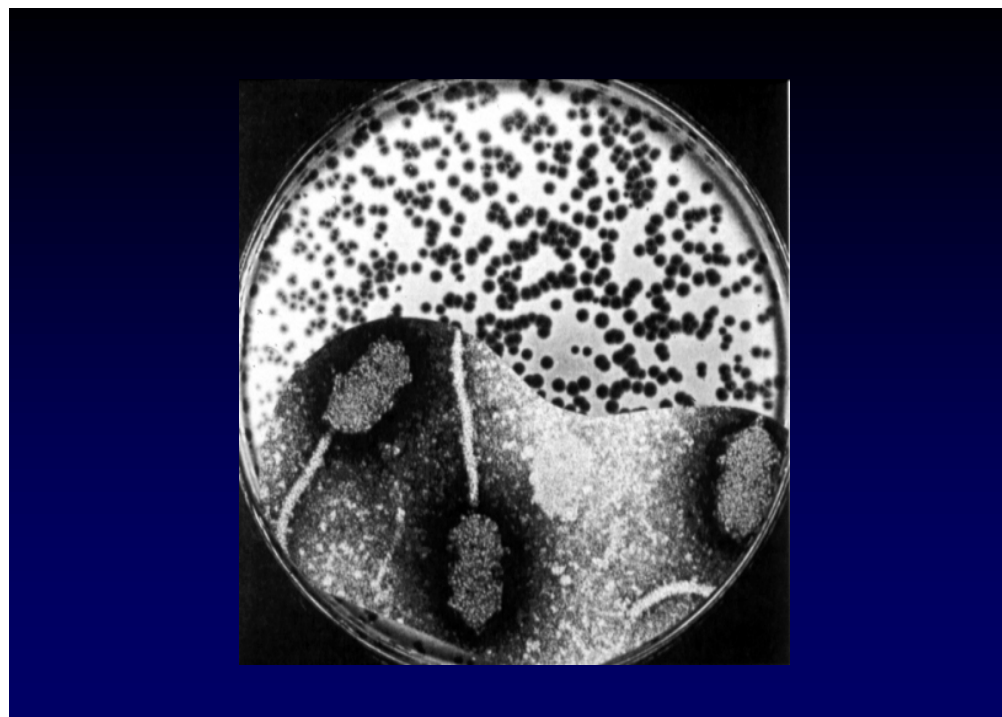
16

Appendix 14 Slides of presentation 4.3

Slide 1



Slide 2



Slide 3



Slide 4

SALMONELLA PHAGE-TYPING SCHEMES

SEROTYPE	NO. OF TYPING PHAGES	NO. OF PHAGE TYPES
<i>S. typhi</i>	110	110
<i>S. paratyphi A</i>	9	15
<i>S. paratyphi B</i>	12	53
<i>S. java</i>		
<i>S. enteritidis</i>	16	80
<i>S. typhimurium</i>	38	280
<i>S. hadar</i>	9	62
<i>S. virchow</i>	15	68
<i>S. thompson</i>	10	38
<i>S. pullorum</i>	5	13
<i>S. agona</i>	9	38

LEP data

Slide 5

Salmonella enteritidis Development of phage-typing scheme - 1960-1998			
Year	Human isolates	Typing phages	Phage types
1960	236	6	9
1977	442	8	15
1987	6858	10	29
1993	20,254	15	53
1996	18,296	16	65
1998	16,196	16	71

Slide 6

Phage type	Year defined	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	1960	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
1b	1993	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	1960	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
4b	1995	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
6	1960	-	+	-	+	-	+	-	+	+	+	-	-	-	-	-	-
6a	1987	-	+	-	+	-	+	-	-	+	-	-	-	-	-	-	-
8	1960	-	-	+	+	+	+	+	+	+	+	+	+	+	-	-	-
13	1960	-	-	-	+	-	+	-	-	+	-	-	-	-	-	-	-
13a	1981	-	-	-	+	-	+	-	-	+	+	+	-	-	-	-	-
21	1985	+	+	-	+	-	+	(+)	+	+	+	-	-	-	+	-	-
21b	1995	(+)	(+)	-	+	-	+	(+)	+	+	+	-	-	-	(+)	-	-
26	1987	-	-	-	-	-	-	-	-	(+)	-	+	-	-	-	-	-
29	1988	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
30	1988	-	-	-	-	-	-	-	-	-	-	(+)	-	+	-	-	-
33	1988	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
34	1988	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-
34a	1996	-	-	-	-	-	-	-	(+)	-	(+)	-	-	-	-	-	-

Salmonella enteritidis
typing scheme -
Phage type examples

+ Full lysis
(+) Reduced lysis
- No lysis

Slide 7

Salmonella Enteritidis New Phage Types

Phage Type	Country	England & Wales Outbreaks
44 (71)	Canary Islands	–
5c (40)	Teneriffe	Fish cakes Egg mayonnaise sandwich Egg mayonnaise bagels*
()% foreign travel * 23 year old female fatal following appendectomy		

Slide 8

SALMONELLA TYPHIMURIUM**SCHEME 1
Felix and Callow 1943**

Typing phages	Types
11	12

Slide 9

SALMONELLA TYPHIMURIUM	
SCHEME 2 Callow 1959	
Typing phages	Types
29	34

G593

Slide 10

SALMONELLA TYPHIMURIUM TOP TEN PHAGE TYPES						
Rank	1968	1971	1976	1978	1985	1993
1	32	49	104	104	49	104
2	29	104	49	193	10	193
3	1	56	32	49	12	208
4	95	32	44	204	204	170
5	104	1	12a	12a	141	12
6	12a	12a	204	44	104	10
7	8	160	193	10	193	108
8	132	44	1	20	110	49
9	40	74	56	56	204c	110
10	14	6	95	104b	170	204

SOURCE: ERL, DEP & LEP DATA

G593

Slide 11

Salmonella Typhimurium
Human England and Wales 2001

Rank	Phage Type	Number	%
1	104	832	38
2	193	194	9
3	104b	118	5
4	U310	80	4
5	U302	77	4
6-7	49	53	2
	193a	53	2
8-9	141	51	2
	U311	51	2
10	208	46	2
Total 10 phage types		1,555	72
Total remaining phage		610	28
TOTAL		2,165	100

Slide 12

S. typhimurium DT104 from humans



Slide 13

SALMONELLA TYPHIMURIUM

PHAGE TYPE 104 AND RELATED TYPES

TYPE	8	12	13	18	20	27	28	32	35	E10*
104 (High)	+	CL	CL	CL	+	+	+	+	+++	OL
104 (Low)	+	+++	+++	+++	-	+	-	-	+	OL
104a	-	CL	CL	-	+	+	+	-	+	OL
104b (High)	-	-	-	CL	+	+	-	-	+	OL
104b (Low)	-	-	-	+++	-	+	-	-	+	OL
104c	CL	CL	CL	CL	+	+	CL	+	+	OL
U302	-	-	-	-	-	-	-	-	-	OL

E = experimental phage

Slide 14

SALMONELLA TYPHIMURIUM

Provisional Phage Type	Additional Phages			
	10	10v1	10v2	10v3
U302	OL	OL	OL	OL
U309	+	OL	-	-
U310	+	+	OL	-
U311	+	+	+	OL

Slide 15

Salmonella Paratyphi B var Java
Emergence of Phage type Dundee var 1 variant,
Resistant Strains**

	Human England & Wales	Chicken* Live / Food
1997	1	2
1998	—	2
1999	4	1
2000	7	6
2001	8	41
*UK and Ireland		
**Dominant Resistance ASSuTmFu		

Slide 16

ACKNOWLEDGEMENTS

Food Safety Microbiology Laboratory

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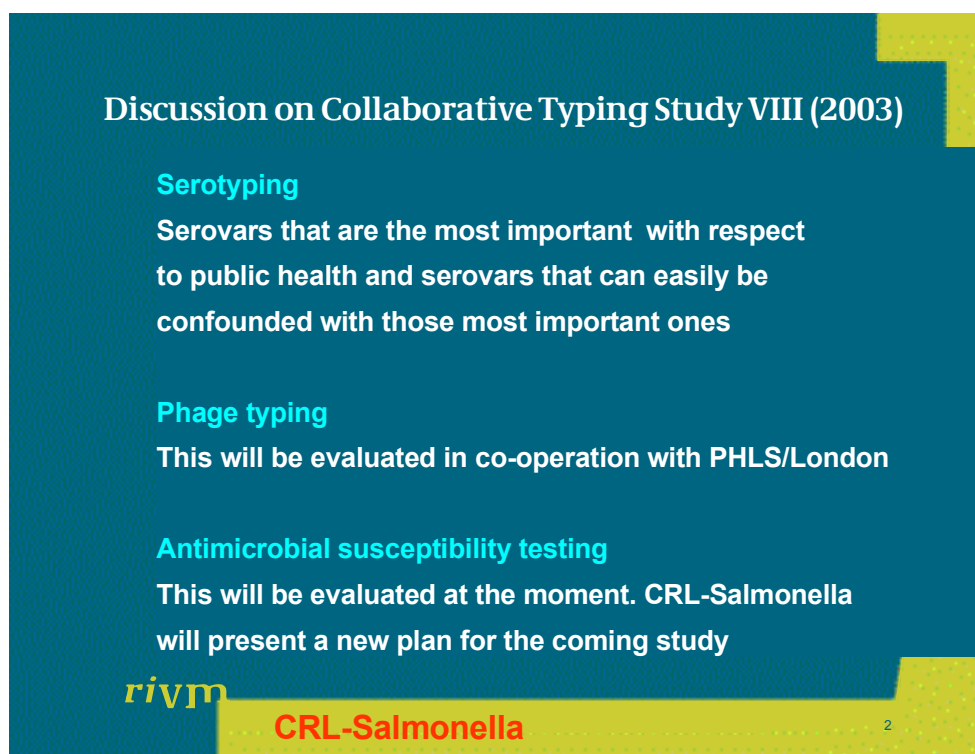
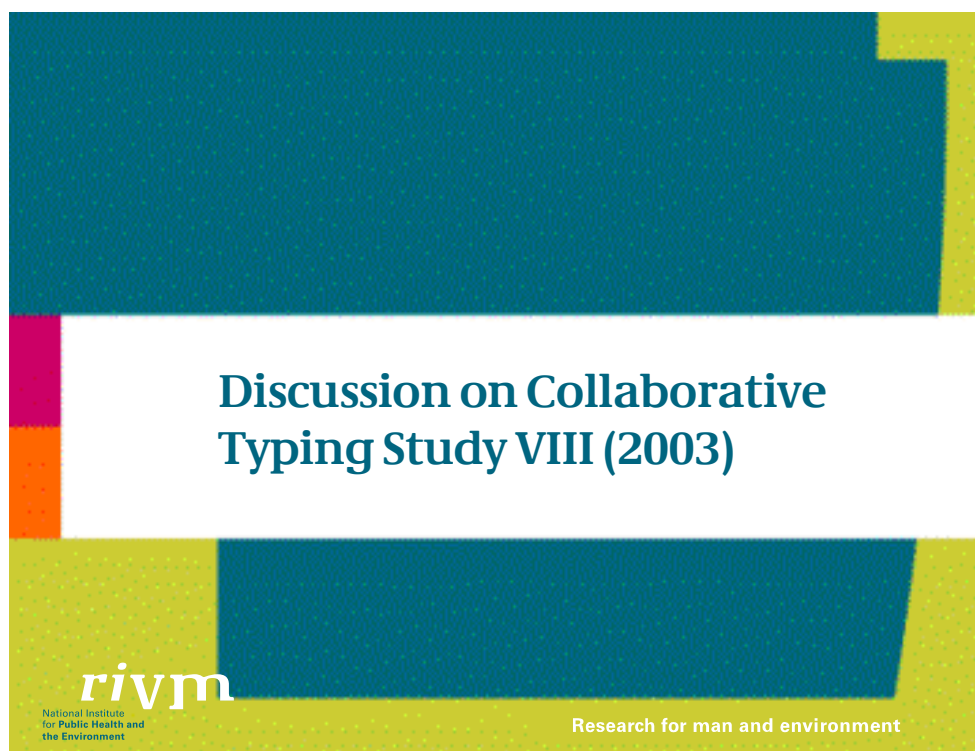
Laboratory of Enteric Pathogens

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All members of the *Salmonella* Reference Unit,
past and present

Appendix 15. Slides of presentation 4.4


Slide 1



6. Slide 2

Appendix 16. Slides of presentation 5.1

Slide 1




Epidemiological Studies of Multiresistant *Salmonella* Typhimurium in Pigs (OZ0134)

Contractor: VLA
Project Leader: Ian McLaren (initially Rob Davies)
Start Date: 01/04/97 End Date: 31/03/00
Project Team: Ian McLaren: Control measures, molecular analysis
Rob Davies: Longitudinal studies/feedmills/abattoirs/communications
Sue Bedford, Steve Shankster, Harvey Russell: General field and laboratory work
Carol Clouting: *Salmonella* ELISA
Nigel Woods (Private veterinarian): Integrated company study

H.Davies-Pig OZ0134 FBZ Review 2002.ppt Jan 2002

Slide 2




Background/Policy Relevance

- Danish *Salmonella* Control Initiative (*S. Infantis* outbreak)
- High level *S. Typhimurium*/multiple resistance (esp. DT104) in pig clinical isolates
- No information on subclinical carriage of *Salmonella* in pigs in UK
- No information on sources, transmission routes, persistence
- Need to assess potential for monitoring and control options in UK pig industry

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Slide 3




Main Objectives / Approaches

- **Longitudinal studies of farms with multiresistant STM**
 - 23 farms: 3-5 year follow up (+ extras)
 - intensive sampling - pigs/environment (inc. post C&D)
 - enhanced culture methods
 - effect of management changes/control measures
- **Cross-sectional survey of nursery and finishing farms in large integrated pig company**
 - 161 herds
 - pooled pen faeces/analytical questionnaire
- **Detailed Abattoir studies (2 abattoirs)**
 - carcase/tissue prevalence
 - effect of process/cross-contamination
- ***Salmonella* ELISA**
 - development & evaluation - individual/herd
 - extended antigenic range/various tissue fluids
- **Survival and disinfection studies**
 - various substrates/conditions
- **Application of *Salmonella* differentiation techniques**
 - serotyping; phage typing; antibiogram; plasmid profile; PFGE

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Slide 4




Incidence of *Salmonella* in Adult Pigs (% positive bulked pen faeces)

Gilt Pens	Boar Pens	Service Pens	Farrowing Pens	Dry Sow Pens
29	27	27	19	15

Infections persistent throughout study (unlike cattle)
Usually several serotypes/STM phage types on each farm

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Slide 5




Incidence of *Salmonella* in Rearing and Finishing Pigs (% positive bulked pen faeces)

	Weaner Pens	Nurse Pens	Grower Pens	Finishing Pens	Sick Pens	Disinfected Pens
Breeder/ Finishers	47	59	48	51	63	27
Specialist Finishers	-	-	53	37	30	27

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Slide 6




Incidence of *Salmonella* in Wildlife Vectors on Pig Breeder/Finisher Farms

Rats	Mice	Birds	Foxes	Badgers	Flies
30	52	34	33	8	48

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Slide 7



Periodic Intensive Sampling on Farm A


Salmonella isolation from bulked faeces from various pig categories and environment: Growers/Finishers
No. of STM isolates [No. of other serovars]/No. samples taken (% STM) [% total *Salmonellas*]

Year	Weaners	Nurse Pens	Growers	Finishers	Sick Pens
1	21[0]/29 (72.4) 104(B)	3[0]/3 (100.0) 104B	31[1]/42 (73.8) [76.2] 104(B), Derby	20[0]/25 (80.0) 104(B)	2[0]/2 (100.0) 104
2	5[5]/47 (10.6) [21.2] 104B, Livingstone, Derby	5[0]/5 (100.0) 104B	8[0]/55 (14.5) 104B, Untyp, 30	4[3]/45 (8.8) [15.5] 104B, Derby, Agona, Alachua	4[1]/5 (80.0) [100.0] 104B, Untyp, Livingstone
3	1[1]/15 (6.7) [13.4] U302, Livingstone	6[0]/6 (100.0) U302, 104B	23[0]/49 (46.9) U302, 104B, 193	3[0]/17 (17.6) 104B	0/2
4(a)*	4[0]/59 (6.8) 104B	0/1	6[2]/46 (13.0) [17.3] 104B, Derby	1[4]/36 (2.8) [13.9] 104B, Derby	0/2
4(b)**	2[0]/44 (4.5) (disinfected pens) 104B, U302	NS	0/33 (disinfected pens)	1[0]/22 (4.5) (104B: disinfected pens)	NS
5***	0/8	NS	0/48	N/A	0/10
6	17[0]/29 (58.6) STM DT104	NS	23[0]/45 (51.1) STM DT104	N/A	11[0]/14 (78.6) STM DT104

NS - not sampled
 * mainly depopulated/washed 3 months
 ** depopulated 3 months post cleaning and disinfection
 *** partially repopulated

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Slide 8




Farm A - *Salmonella* Isolation from Bulked Faeces Samples from Replacement Breeding Stock after Depopulation

Sampling date	Pig Category	No. samples positive for <i>Salmonella</i> / No. samples taken (%)	<i>Salmonella</i> serovars and definitive types
12/03/99	gilts	1/4 (25.0)	S.Derby
19/03/99	gilts	1/4 (25.0)	S.Typhimurium DT104
31/03/99	boars	0/4	
01/04/99	gilts	0/4	
09/04/99	gilts	0/4	
16/04/99	boars	0/4	
16/04/99	gilts	1/4 (25.0)	S.Typhimurium DT104
26/04/99	gilts	0/4	
04/05/00	gilts	0/4	
10/05/99	gilts	1/4 (25.0)	S.Typhimurium DT104
17/05/99	gilts	0/4	
24/05/99	gilts	2/4 (50.0)	S.Typhimurium DT104
01/06/99	gilts	0/4	
07/06/99	gilts	1/4 (25.0)	S.Typhimurium DT104
14/06/99	gilts	1/4 (25.0)	S.Derby
21/06/99	gilts	0/4	
28/06/99	gilts	0/4	
02/07/99	gilts	0/4	
30/03/00	gilts	0/9	
30/05/00	gilts	1/6 (16.6)	S.Derby
22/06/00	gilts	0/6	
31/07/00	gilts	0/6	
19/08/00	gilts	0/5	
10/11/00	gilts	3/5 (60.0)	S.Derby

H/Davies: Pig 020134 FBZ Review 2002.ppt Jan 2002

Slide 9




Disinfection of Pig Units

	Continuous Occupation	All-in/All-out
Pens before	52/118 (44.1)	177/509 (34.8)
Pens after	29/91 (31.9)	7/339 (2.1)
Pigs before	19/79 (24.1)	8/96 (8.3)
Pigs after	7/135 (5.2)	33/529 (6.2)

H.Davies: Pig OZ01134 FBZ/ Review 2002.ppt Jan 2002

Slide 10




Fermented Liquid Feed (pH4.0) Trial

No. pigs positive for *Salmonella*/No. pigs sampled (%)

	Treated Group	Controls
Round 1	5/56(8.9)	0/56
Round 2	8/61(13.1)	4/61(6.5)
Round 3	2/74(2.7)	5/74(6.7)

H.Davies: Pig OZ01134 FBZ/ Review 2002.ppt Jan 2002

Slide 11




Acidified Feed Trial

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

	Bulked Faeces		Rectal Swabs	
	Weaners	Finishers	Weaners	Finishers
Before Treatment	12/30(40.0)	6/28(21.4)	6/75(8.0)	0/70
After Treatment	11/22(50.0)	13/28(46.4)	6/75(8.0)	2/70(2.8)

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Slide 12




Other 'Interventions'

<p><u>Not Successful</u></p> <p>Depopulation (2 farms)</p> <p>Vaccination (1 farm)</p> <p>Naturally fermented liquid feed (1 farm)</p> <p>3 site production (1 farm)</p> <p>Improved rodent control alone (2 farms)</p> <p>Change to organic production (1 farm)</p>
<p><u>Partially Successful</u></p> <p>Withdrawal of tetracycline medication of breeding stock</p> <p>Change of outdoor production site</p> <p>Improved disinfection of finishing pens</p>
<p><u>Largely Successful</u></p> <p>Closed herd (3 smaller farms)</p>

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Slide 13




Survey for *Salmonella* in an Integrated Pig Company

- 161 farms - 20 x 15-20g pooled pen faeces/farm
 - analytical questionnaire
- 69% farms positive (73% nurseries, 67% finishing farms)
- DT104 found in wheat stores in feed mills - not finished products
- Mostly *S. Typhimurium* DT104 complex (+ 14 other serotypes)
- Statistically significant risk factors - length of time used as pig farm
 - nursery > finishing farms
 - herd size (for STM)

H.Davies: Pig Q20134 FBZ Review 2002 ppt Jan 2002

Slide 14




Comparative *Salmonella* Isolation from Various Tissues

	Large Intestinal Contents	Carcase Swabs	Mesenteric Lymph Nodes	Spleen	Tonsil
No samples positive for <i>Salmonella</i> /No samples taken (%)	36/394 (9.1)	29/400(7.2)	23/398(5.8)	0/344	5/391(3.8)
No of batches of pigs positive (%)	12/29 (41.4)	16/29(55.1)	8/29(27.6)	0/29	8/29(27.6)
No of batches where specified tissue was only positive sample	1 <5>	8	1[3]	0	0

< > = no of batches positive in large intestinal contents but not in mesenteric lymph nodes.
 [] = no of batches positive in mesenteric lymph nodes but not in large intestinal contents.

H.Davies: Pig Q20134 FBZ Review 2002 ppt Jan 2002

Slide 15




Comparison of *Salmonella* Isolation from Pigs Laired Overnight or not Laired Overnight (No samples positive for *Salmonella* /No samples taken (%))

Visit No	Large Intestinal Contents		Carcase Swabs	
	Laired Pigs	Non-laired Pigs	Laired Pigs	Non Laired Pigs
1	NS	31/160(19.4)	NS	25/160(15.6)
2	3/60(5.0)	14/219(6.4)	0/60	9/219(4.1)
3	3/68(4.4)	25/209(12.0)	1/68(1.5)	19/209(9.1)
4	13/61(21.3)	16/166(9.6)	5/61(8.2)	7/166(4.2)
5	10/62(16.1)	25/227(11.0)	1/62(1.6)	35/227(15.4)
6	12/79(15.2)	46/191(24.1)	2/79(2.5)	19/191(9.9)
7	3/87(3.4)	19/222(8.5)	1/87(1.1)	2/222(0.9)
8	0/28	36/366(9.8)	1/28(3.6)	28/372(7.5)
TOTALS	44/445(9.9)	212/1760(12.0)	11/445(2.5)	144/1766(8.1)

NS = not sampled
*p = <0.001

H.Davies Pig Q20134 FBZ Review 2002 ppt Jan 2002

Slide 16




Abattoir B: Distribution of *Salmonella* in Batches of Pigs from Different Farms

Batch No	Large intestinal contents	<i>Salmonella</i> serotypes	Phage types	Carcase swabs	<i>Salmonella</i> serotypes	Phage types
1	2/13 (15.4)	<i>S. derby</i>		0/13		
2	10/17 (58.8)	<i>S. derby</i> (9), <i>S. typhimurium</i> (1)	104L	4/17 (23.5)	<i>S. derby</i>	
3	11/17 (64.7)	<i>S. typhimurium</i> (8), <i>S. derby</i> (2) <i>S. manhattan</i> (1)	UNTYP	4/17 (23.5)	<i>S. typhimurium</i> (3), <i>S. derby</i> (1)	UNTYP
4	0/8			6/8 (75.0)	<i>S. typhimurium</i>	UNTYP
5	0/8			4/8 (50.0)	<i>S. typhimurium</i>	UNTYP
6	1/18 (5.5)	<i>S. infantis</i>		2/18 (11.1)	<i>S. typhimurium</i> (1), <i>S. manhattan</i> (1)	UNTYP
7	0/10			1/10 (10.0)	<i>S. typhimurium</i>	UNTYP
8	0/4			0/4		
9	1/7 (14.3)	<i>S. derby</i>		0/7		
10	1/20 (5.0)	<i>S. typhimurium</i>	104L	4/20 (20.0)	<i>S. typhimurium</i>	104L, UNTYP
11	1/8 (12.5)	<i>S. typhimurium</i>	104L	0/8		
12	1/10 (10.0)	<i>S. typhimurium</i>	104L	0/10		
13	3/9 (33.3)	<i>S. typhimurium</i>	104L, 120 104B	0/9		
14	0/3			0/3		
15	0/3			0/3		
16	0/5			0/5		
Totals	31/160 (19.4)			25/160 (15.6)		

H.Davies Pig Q20134 FBZ Review 2002 ppt Jan 2002

Slide 17




Salmonella Meat Juice Elisa - Neck Muscle. Sensitivity and Specificity, Assuming Caeca is the Gold Standard

	Caecum			ii) Meat Juice Elisa > 40% OD	
MJE	Neg	Pos	Total	% pos MJE & caeca	
Negative	1642	396	2038	sensitivity	28.9
Positive	204	161	365	specificity	88.9
Total	1846	557	2403	predictive value	44.1

H.Davies Pg Q20134 FBZ Review 2002 ppt Jan 2002

Slide 18



Survival of DT104 on Building and Bedding Material


STM 104 survival: > 31m on brick/concrete/straw/
plastic/wood

survival levels ↑ by faeces, esp. sterile faeces
 ↑ by dessication
 ↓ by moisture

Poorer survival in: feed (<3m)
 rusty metal (<3m)
 6m pure faeces (9m if pasteurised)
 1 year in pure tap water (↓ by faeces)

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Slide 19



Effect of Agricultural Disinfectants


20 disinfectants Wooden dowel + 1g dried
pig faeces + 10⁶g DT104

1% formaldehyde successful

Others not successful - even at 2 x G.O. rate

H.Davies: Pig Q20134 FBZ Review 2002 ppt Jan 2002

Slide 20




Molecular Genetic Strain Differentiation Studies

- Great diversity of plasmid profile types in DT104
- PFGE (X ba1) Ribotyping not very discriminatory. PFGE with Bln1 better
- Distinct PPs trackable through abattoir
- Persistence and strain turnover/evolution both occurring on most farms

H.Davies: Pig Q20134 FBZ Review 2002 ppt Jan 2002

Slide 21



Overall Conclusions


- *Salmonella* appears to be widespread in UK pig production
- Slaughter process markedly reduces carcase contamination
- Control on farm is difficult and costly in UK pig industry - ? Incentives
- More research required:
 - contribution to human disease
 - structured intervention studies
 - cost/benefit analysis
 - 'vertical' transmission
 - effective vaccination policies
 - abattoir best practice/carcase decontamination

} OZ0316

11 Davies Pig Q20134 FBZ Review 2002 ppt Jan 2002

Appendix 17. Slides of presentation 5.2

Slide 1




Veterinary Surveillance for Antimicrobial Resistance in *Salmonella* and *E.Coli*

Rob Davies
Department of Bacterial Diseases
Veterinary Laboratories Agency Weybridge
New Haw
Addlestone
Surrey KT15 3NB
UK

[Data and observations derived from DEFRA/MLC funded surveillance]

Veterinary Laboratories Agency

Slide 2




Agenda

- Significance of *Salmonella* and *E.Coli*
- *Salmonella* and *E.Coli* - antimicrobial resistance surveillance trends
- Antimicrobial resistance in *Salmonella* and *E.Coli* from abattoir surveys
- Conclusions

Veterinary Laboratories Agency

Slide 3



Significance of *Salmonella* and *E.Coli* from Food Animals

Salmonella: True zoonotic pathogen

- gastroenteritis
- blood poisoning
- mortality

} medical/social costs

- surveillance
- investigations
- product recalls


} economic loss

E.Coli: Some strains toxigenic pathogens
 Opportunist wound, blood & urinary infections
 Transient intestinal coloniser
 Potential source of antimicrobial resistance genes

} Magnitude unknown

Antimicrob_Panels2.ppt

Slide 4



Antimicrobial Susceptibility Test Panel: *Salmonella*/Survey *E.Coli*

	Antimicrobial	Concentration	Code
1	Nalidixic acid	30 µg	NA
2	Tetracycline	10 µg	T
3	Neomycin	10 µg	N
4	Ampicillin	10 µg	AM
5	Furazolidone	15 µg	FR
6	Cefuroxime	30 µg	CX
7	Sulphamethoxazole/trimethoprim	25 µg	TM
8	Chloramphenicol	10 µg	C
9	Amikacin	30 µg	AK
10	Amoxycillin/clavulanic acid	30 µg	AMC
11	Gentamicin	10 µg	CN
12	Streptomycin	25 µg	S
13	Sulphonamide compounds	300 µg	SU
14	Cefoperazone	30 µg	CF
15	Apramycin	15 µg	APR
16	Colistin	25 µg	CT

Antimicrob_Panels2.ppt

Slide 5


All *Salmonellas*: Antimicrobial Sensitivity 2000

Origin	No of cultures	Percentage of isolates resistant to at least one of the 16 antimicrobial panel	Percentage of cultures resistant to:											
			S	SU	T	N	AM	FR	TM	C	APR	NA	GEN*	
Cattle	1623	20.9	16.7	18.2	18.4	0.3	16.2	0.1	4.8	15.9	-	1.9	-	
Sheep	166	19.3	14.5	15.7	18.7	0.6	15.1	-	3.0	14.5	-	-	-	
Pigs	426	88.0	42.0	62.7	82.6	4.2	43.0	1.2	35.0	31.0	6.1	4.9	5.9	
Poultry	1439	42.8	10.5	24.9	13.0	1.5	9.9	0.5	16.2	9.1	0.3	10.7	0.4	
Horses	51	39.2	35.3	37.3	37.3	-	27.5	-	11.8	25.5	-	-	-	
Other species	114	27.2	16.7	19.3	17.5	1.8	12.3	-	10.5	9.6	0.9	4.4	-	
Feed	607	10.0	2.0	9.2	5.3	0.2	1.8	0.2	6.6	1.3	-	0.3	-	
Environment	178	61.8	12.4	51.7	19.1	0.6	21.3	-	42.1	9.6	-	6.7	-	
Total	4604	34.3	15.1	24.7	21.1	1.1	15.0	0.3	13.0	12.9	0.7	4.9	0.7	

*Provisional data

Antimicrob 14/02/02

Slide 6




Antimicrobial Resistance in *E.Coli*/Coliforms in Diagnostic Cases (All ages) (% Resistant strains 1999* (1998))

	<u>Cattle</u>	<u>Sheep</u>	<u>Pigs</u>	<u>Poultry</u>
Multiple resistance	24 (35)	13 (9)	23 (30)	11 (7)
Ampicillin 10 µg	51 (66)	33 (32)	45 (41)	37 (31)
Amoxycillin/Clavulanic 20/10 µg	14 (21)	6 (9)	NT (NT)	NT (NT)
Tetracyclines 10 µg	57 (71)	51 (39)	83 (74)	56 (39)
Neomycin 10 µg	29 (40)	19 (20)	18 (20)	14 (8)
Apramycin 15 µg	5 (8)	3 (2)	15 (7)	3 (6)
Trimethoprim/Sulphonamide 25 µg	29 (39)	19 (14)	49 (44)	26 (14)
Enrofloxacin 5 µg	1 (1)	0.2 (0)	3 (3)	1 (6)

* Provisional Data
Antimicrob 14/02/02

Slide 7




Abattoir Survey Design

Pig	Cattle	Sheep
March 99 - Feb 2000	Jan 99 - Feb 2000	Jan 99 - Feb 2000
32 abattoirs (>80% GB)	118 abattoirs (c.50% GB)	118 abattoirs (c.50% GB)
Structured sampling - max 5/day	Structured sampling - max 5/day	Structured sampling - max 5/day
Ligated caecum	Ligated rectum	Ligated rectum
Carcase swab		
2509 pigs sampled	891 samples	973 samples

Antoonie Huisman

Slide 8

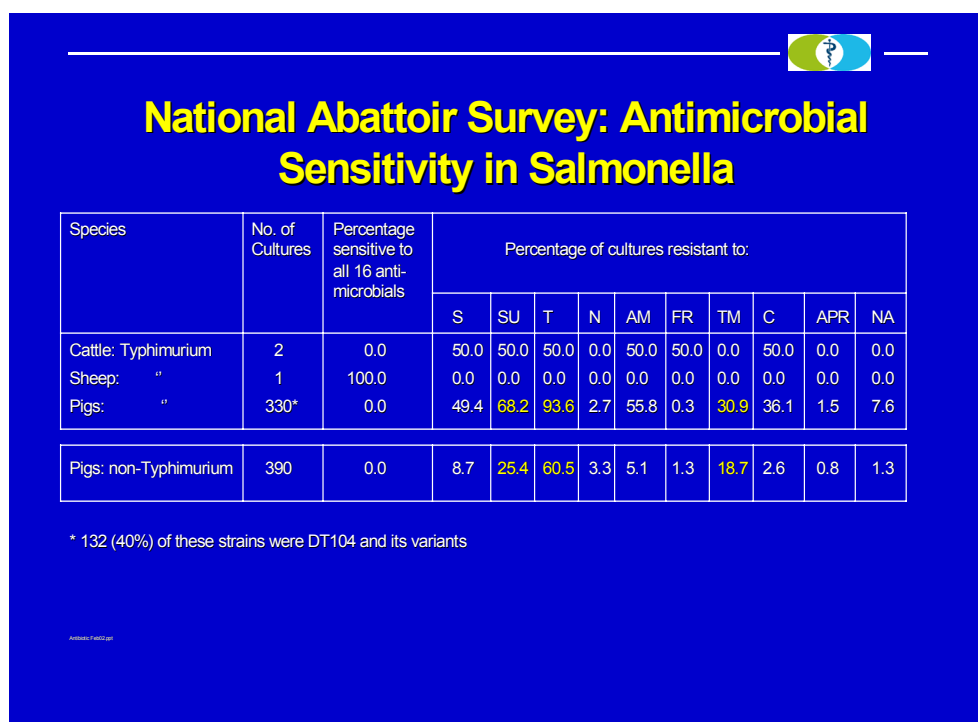


Prevalence of *Salmonella* in Cattle, Sheep and Pigs at Slaughter in GB

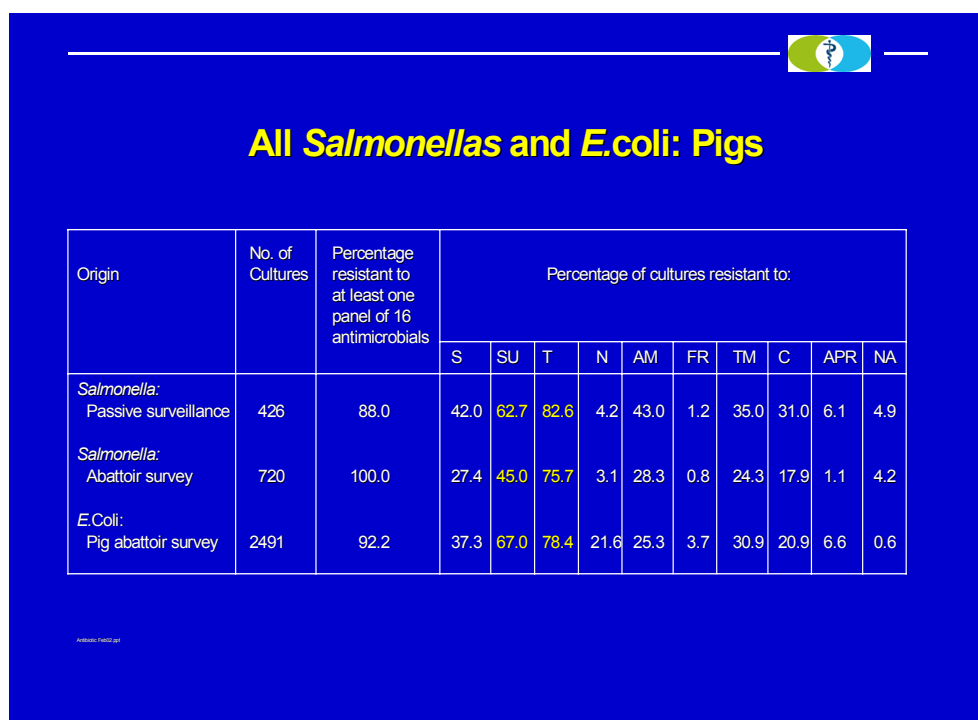
	Cattle		Sheep		Pigs			
					Caecum		Carcase swab	
	No. tested	% positive	No. tested	% positive	No. tested	% positive	No. tested	% positive
<i>Salmonella</i> spp.	891	0.2	973	0.1	2509	23.0	2509	5.3
<i>S. Enteritidis</i>	-	0.0	-	0.0	-	0.1	-	0.0
<i>S. Typhimurium</i>	-	0.2	-	0.1	-	11.1	-	2.1
<i>S. Derby</i>	-	0.0	-	0.0	-	6.3	-	1.6
<i>S. Kedougou</i>	-	0.0	-	0.0	-	1.0	-	0.04
Other serotypes	-	0.0	-	0.0	-	4.8	-	1.7

Antoonie Huisman

Slide 9



Slide 10



Slide 11

		Percentage of Resistant Isolates by Antimicrobial Tested															
Organism	Number of Isolates	NA	T	N	AM	FR	CX	TM	C	AK	AMC	CN	S	SU	CF	APR	CT
Cattle																	
VTEC O157	186	0.5	7.0	0	0	0	0	0	0	0	0	0	0	7.0	0	0	0
Commensal <i>E. coli</i>	855	0.1	4.0	0.6	1.2	0	0	0.6	0.1	0	0	0.1	0.7	2.6	0.1	0	0.2
Sheep																	
VTEC O157	70	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Commensal <i>E. coli</i>	895	0	2.1	0.6	0.9	0	0	0.3	0.1	0	0.2	0	0.3	1.3	0	0	0
Pigs																	
VTEC O157	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Commensal <i>E. coli</i>	2491	0.6	78.4	21.6	25.3	3.7	0.1	30.9	20.9	0	0.2	1.0	37.3	67.0	1.6	6.6	0*

*1998 isolates tested
H Davies/Comparison of Antimicrobial Resistance in Salmonella.pdf/Jun 2002


Slide 12

		1993	1994	1995	1996	1997	1998	1999	2000
Therapeutic antimicrobials - food animals		392	445	486	533	495	433	383	437
Antimicrobial growth promoters		83	88	122	96	69	89	28	24
Total antimicrobials - food animals		475	533	608	629	564	522	411	461
Therapeutic antimicrobials - non-food animals *		20	24	32	30	32	32	37	29
Total antimicrobials - food animals and non-food animals		495	557	640	659	596	554	448	490

* Horses, dogs, cats, etc

(VMD DATA)

Slide 13




Sales of therapeutic antimicrobials (tonnes active ingredient) by species, 1993 to 2000

Species	1993	1994	1995	1996	1997	1998	1999	2000
Cattle	10	12	14	11	9	11	11	10
Sheep	<1	<1	<1	<1	<1	<1	<1	0.3
Pigs	89	91	109	117	121	90	89	96
Poultry	10	16	17	20	17	14	11	24
Fish (salmon and trout)	10	7	7	7	9	5	4	2
Multispecies *	273	319	339	378	339	313	267	304
Totals †	392	445	486	533	495	433	383	436

* A combination of two or more of the following species: cattle, pigs, sheep and poultry
 † Does not include growth promoters, all of which are multispecies

(VMD DATA)

Slide 14




Trends in individual Antimicrobial use in GB in 2000

- Tetracyclines - increased by 36 tonnes
- Trimethoprim/sulphonamides - increased by 12 tonnes
- Macrolides - increased by 12 tonnes
- Pig and poultry production fell

56% antibiotics in medicated feed; 36% water/oral; 6% injectables

Slide 15



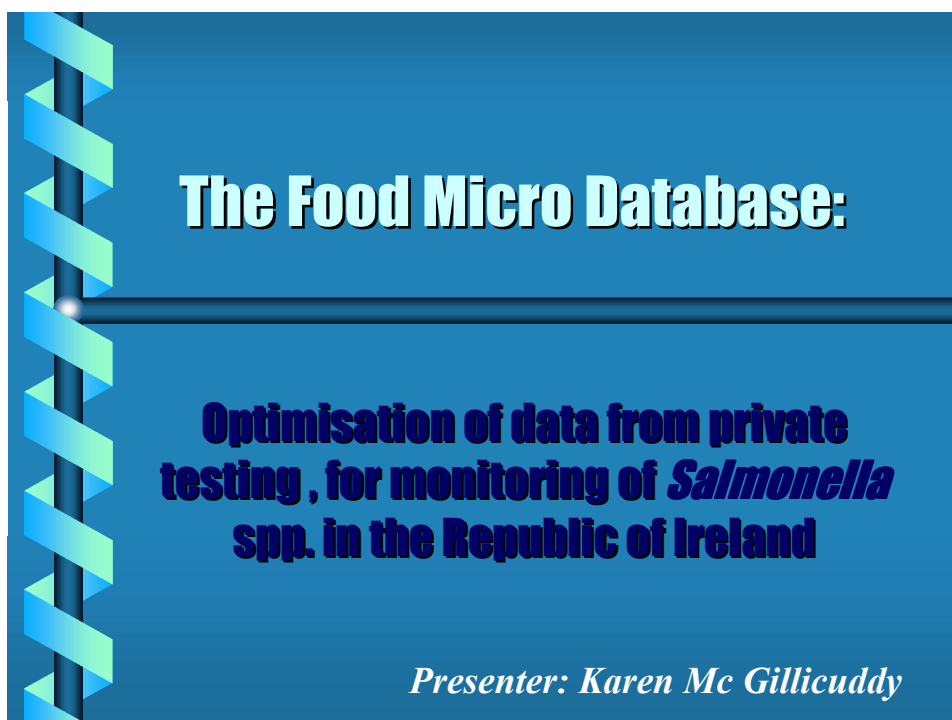
Conclusions

- Overall, antimicrobial resistance in *Salmonella* and *E.Coli* is improving
- Pigmeat production has highest prevalence of resistant organisms
- Industry and veterinary prudent use initiatives will lead to further improvements
- Not all resistance due to drug use - spread of particular resistant strains (e.g. *S.Typhimurium* DT104) is important
- International harmonised surveillance and test methods required
- Antimicrobial resistance is shared veterinary and medical responsibility

Antimicrob. Resist. 2010

Appendix 18. Slides of presentation 5.3

Slide 1

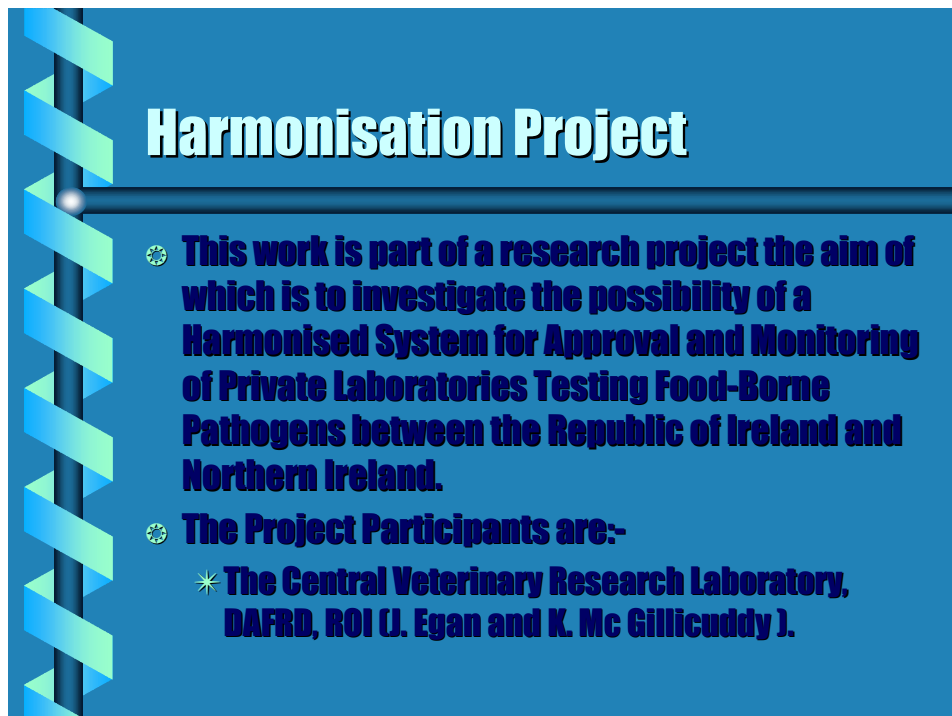


The Food Micro Database:

Optimisation of data from private testing, for monitoring of *Salmonella* spp. in the Republic of Ireland

Presenter: Karen Mc Gillicuddy

Slide 2



Harmonisation Project

- **This work is part of a research project the aim of which is to investigate the possibility of a Harmonised System for Approval and Monitoring of Private Laboratories Testing Food-Borne Pathogens between the Republic of Ireland and Northern Ireland.**
- **The Project Participants are:-**
 - * **The Central Veterinary Research Laboratory, DAFRD, ROI (J. Egan and K. Mc Gillicuddy).**


Slide 3



Harmonisation Project contd...

- * **Central Meat Control Laboratory, DAFRD, ROI (P. Rafter).**
- * **Veterinary Science Division, DARD, NI. (S. Mc Dowell).**
- * **Faculty of Veterinary Medicine, UCD, ROI. (N. Leonard).**
- **The Project is co-funded by the Food Safety Promotion Board (FSPB)**

Slide 4



Background

- **Salmonella is a the 2nd most common cause of bacterial food poisoning in ROI**
- **A control program for salmonellae in poultry was adopted in Ireland in 1988**
- **Subsequently the control programme was applied as outlined in Directive 92/117/EEC and accepted by commission decision 96/389/EEC**

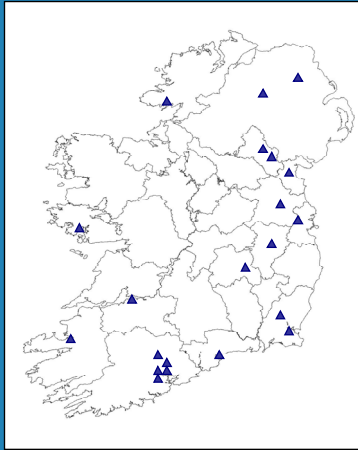
Slide 5

National Plan for Salmonella Monitoring (1996)

- **The control program involves a two-tier sampling programme:**
 - * **Official samples are taken by DAFRD officials are tested in the CVRL**
 - * **Producer/Processor sampling carried out by industry is tested in DAFRD approved private laboratories.**
- **Private/ commercial laboratories play an important role in *Salmonella* monitoring.**

Slide 6

DAFRD Approved Private Labs



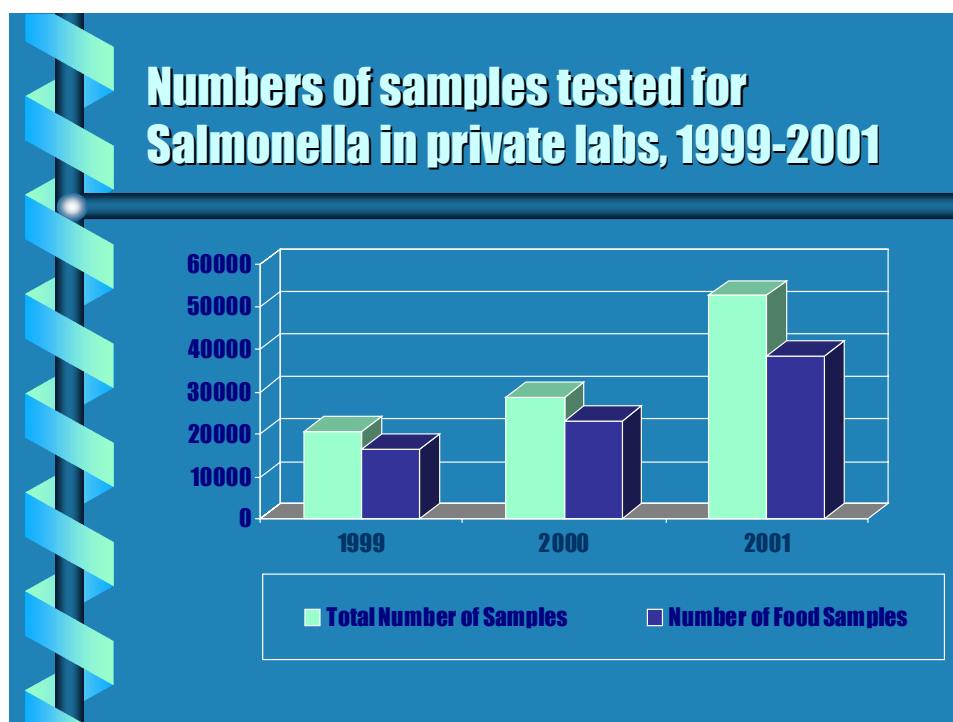
- **Currently there are 21 private laboratories approved by DAFRD.**
 - * **19 in ROI**
 - * **2 in NI, (both labs test samples from ROI)**

Slide 7

Private Laboratories

- One of the conditions of DAFRD approval is the submission of summary data for *Salmonella* testing on a monthly basis.
- The laboratory reports varied in the type of information they provided, this made storage and analysis of the data difficult.
- During 2001 each of the laboratories was visited and asked to help improve the reporting system.

Slide 8



Slide 9

New Data Management System

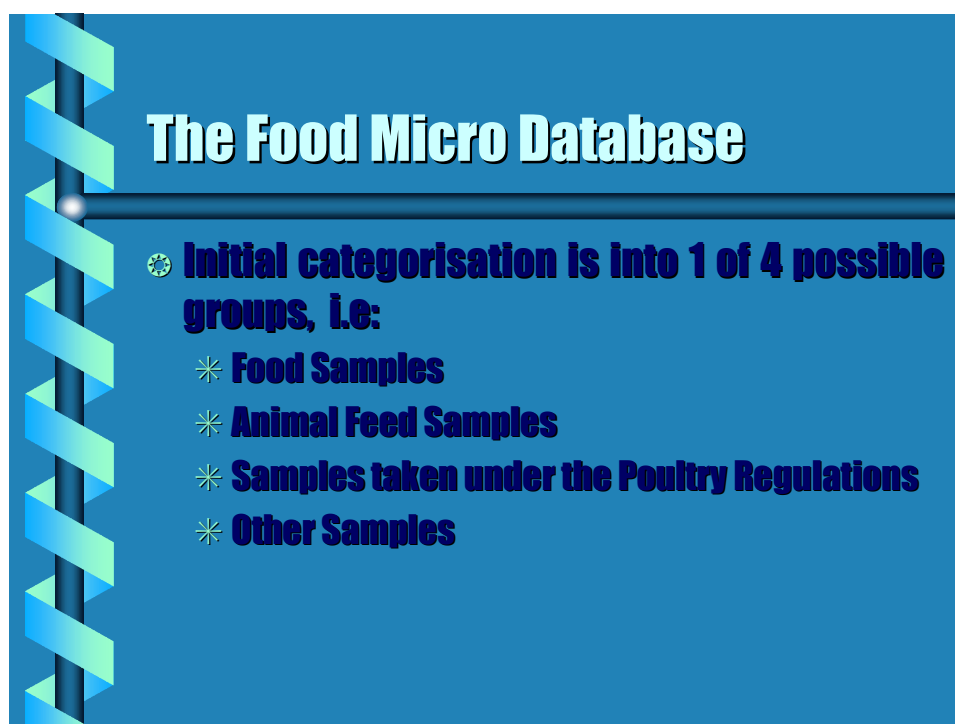
- Later in 2001 a database was developed to store the information from the monthly reports from private laboratories.
- In December 2001 a template for reporting was developed to compliment the database.
- This form was circulated to the approved laboratories In January 2002.

Slide 10

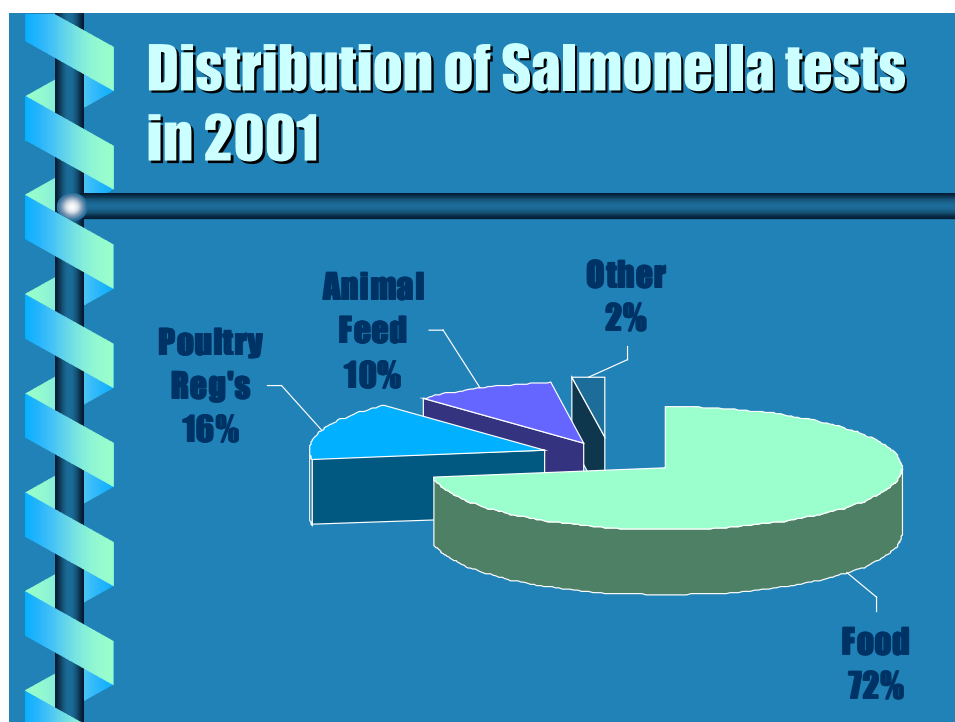
Template for Monthly Reports

I FOOD SAMPLES			
<i>1.1 Raw Meat</i>			
		<i>Salmonella spp.</i>	
SAMPLES	TOTAL NO. TESTED	NO. OF POSITIVES	REF. NO.'s FOR POSITIVES
Bovine (Beef, Veal etc)			
Porcine (Pork, Ham, Bacon etc)			
Chicken			
Turkey			
Ovine (Lamb, Mutton etc)			
Raw Meat-not specified			
Edible fat/Dripping			

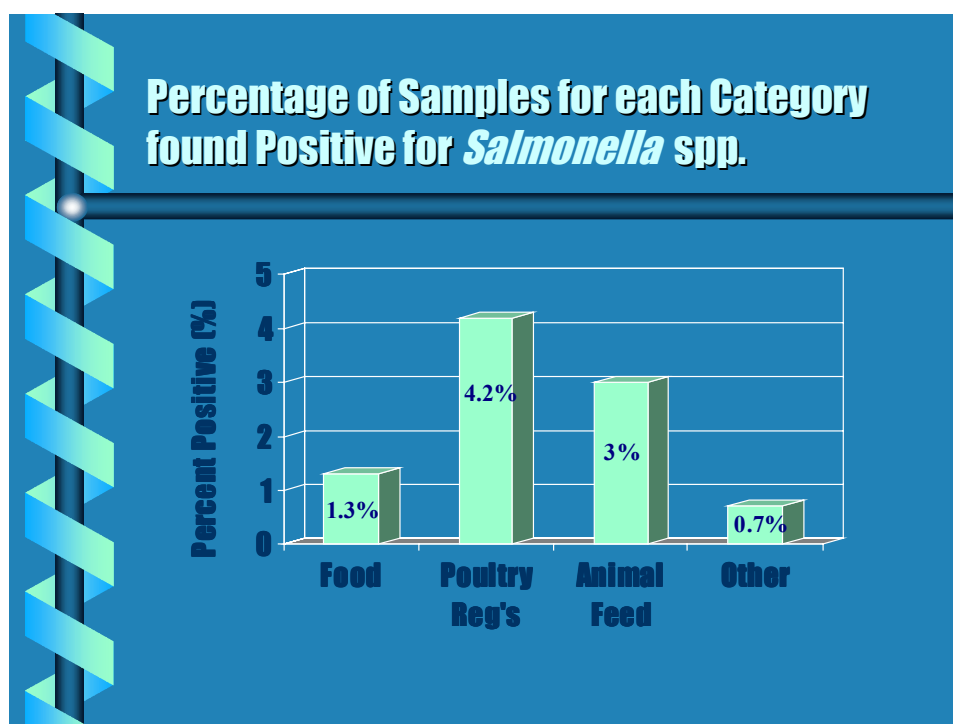
Slide 11



Slide 12



Slide 13



Slide 14

Food Sample Categories

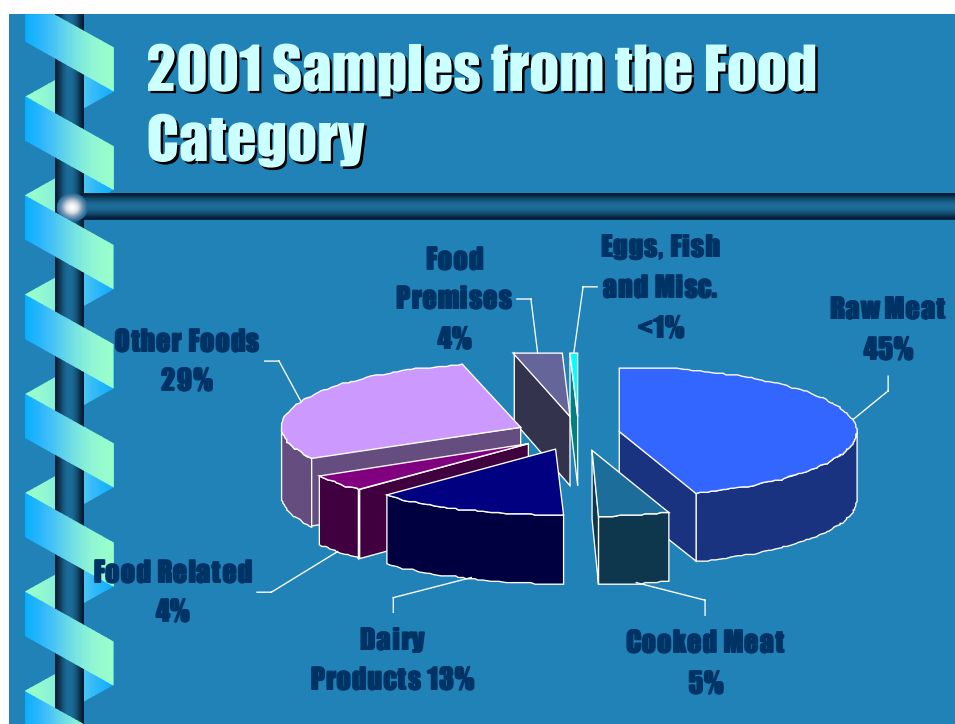
Each of these categories are further broken down, e.g. Food Samples are sub-divided into:

- * Raw Meat
- * Raw Meat Products
- * Cooked Meat and Cooked Meat Products
- * Fish and Fish Products
- * Eggs and Egg Products

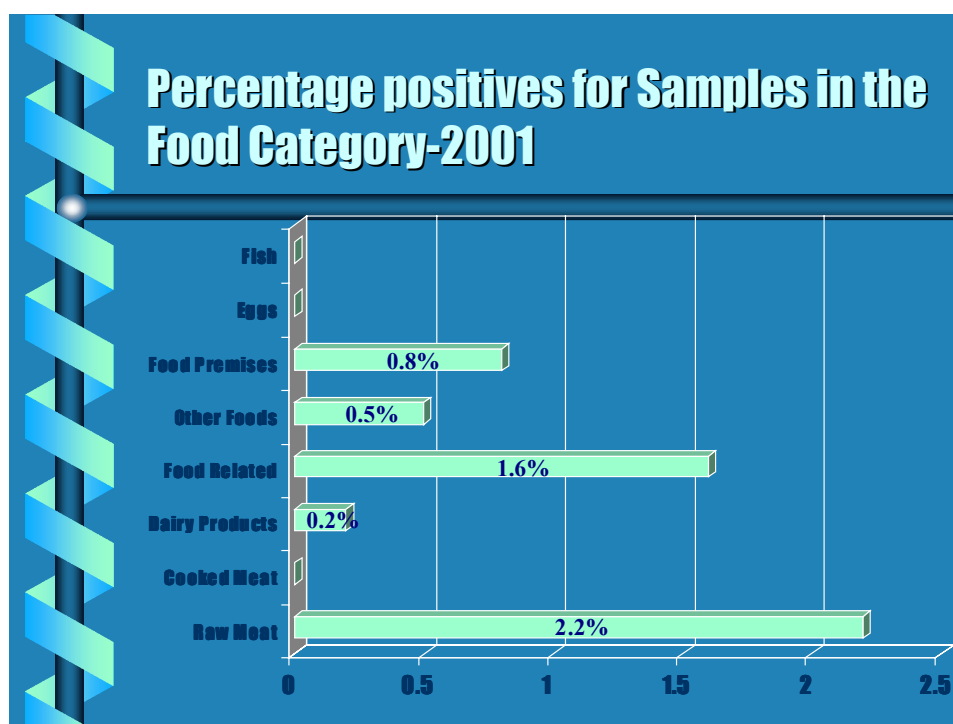
Slide 15



Slide 16



Slide 17

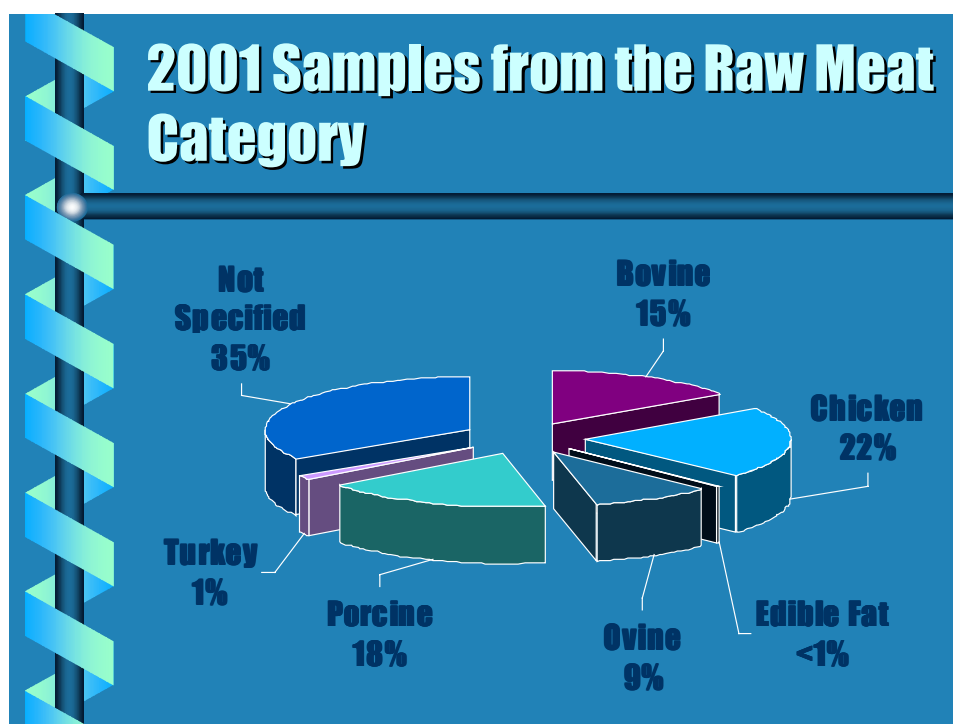


Slide 18

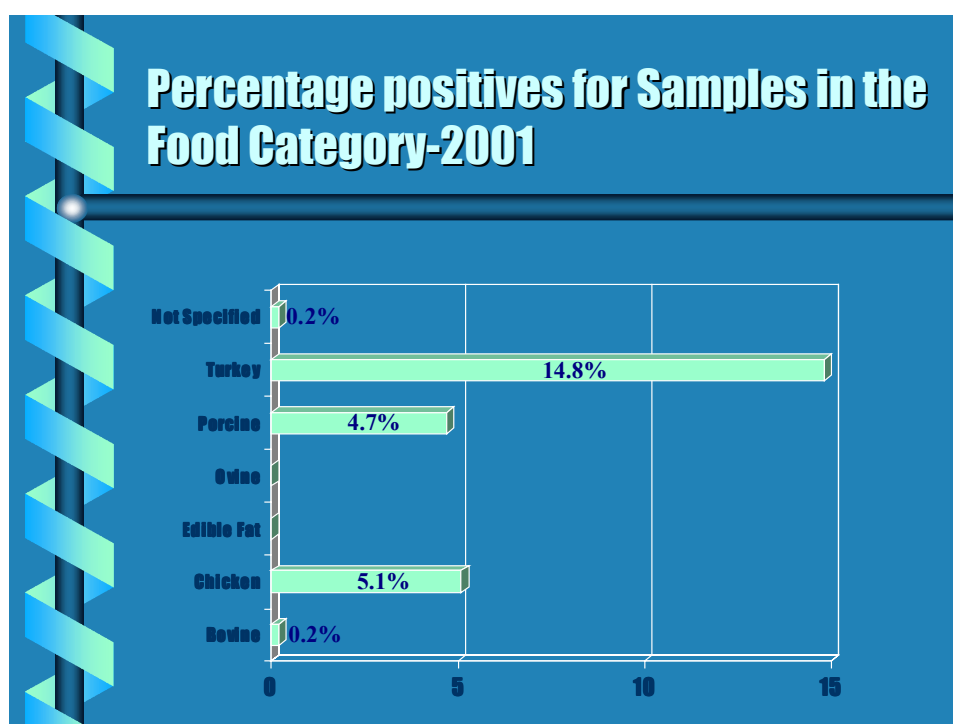
Raw Meat Samples

- Raw Meat samples, for example, are even further categorised into:-
 - * Bovine (Beef, Veal etc)
 - * Porcine (Pork, Ham, Bacon etc)
 - * Chicken
 - * Turkey
 - * Ovine (Lamb, Mutton etc)
 - * Raw Meat - Not Specified
 - * Edible Fat/Dripping

Slide 19



Slide 20

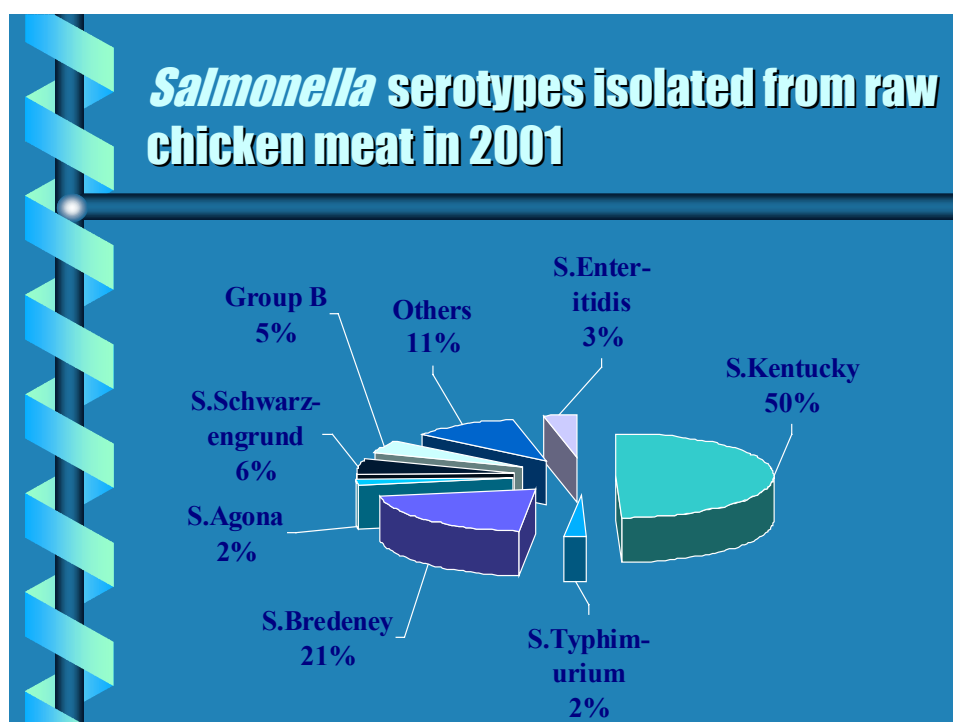


Slide 21

Serotype Information

- All *Salmonella* isolates recovered by private labs are submitted to the CVRL.
- These isolates are serotyped by the CVRL and if necessary sent for phage typing.
- The results from typing are also stored in the Food Micro database.
- The cultures are maintained in the CVRL as a culture collection for *Salmonella* spp.

Slide 22



Slide 23

The approved labs are initially assessed and monitored using Ring Trials

2001 Ring Trial

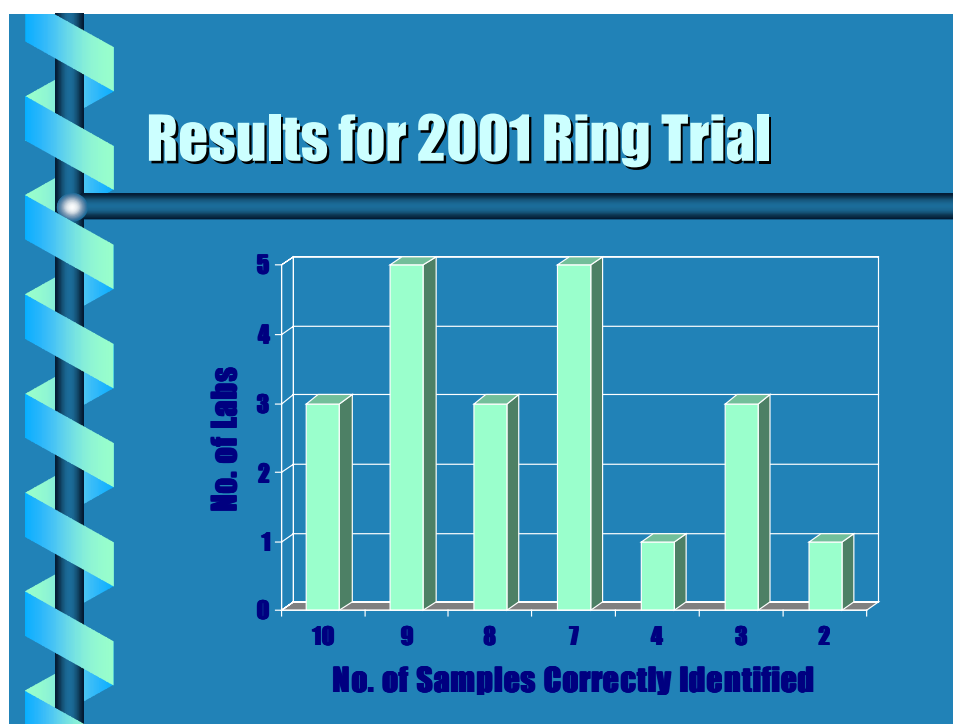
- * **10 Swabs**
- * ***Salmonella* Goldcoast or *S.Typhimurium***
- * **Varying levels of *S.Goldcoast***
- * **Some in the presence of a competitive microflora**

Slide 24

Sample details for the 2001 Salmonella Ring Trial

<u>Sample No.</u>	<u>Salmonella Serotype</u>	<u>Cfu/ml</u>	<u>Competitive Flora</u>
1	S.Typhimurium	>1000	Natural
2	None	0	Yes
3	S.Goldcoast	10	Yes
4	S.Goldcoast	10	Yes
5	S.Goldcoast	750	Yes
6	S.Goldcoast	750	Yes
7	S.Goldcoast	10	No
8	S.Goldcoast	10	No
9	S.Goldcoast	1000	Yes
10	S.Goldcoast	1000	Yes

Slide 25



Slide 26

Laboratory Evaluation

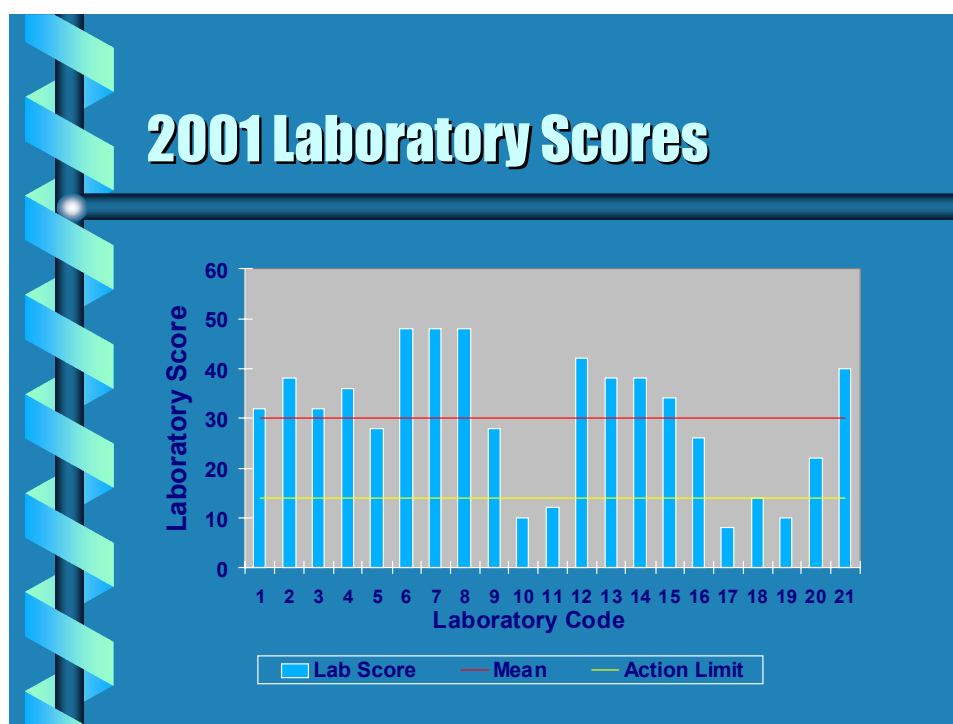
- **Currently developing a scoring system for the ring trial results.**
- **Samples are graded on the basis of the difficulty of isolating Salmonella.**
- **Sum of all samples scores gives each laboratory a score.**
- **Lab scores can be compared and monitored over time.**

Slide 27

Proposed Scoring System

Sample No.	Expected Difficulty	Correct ID Score	Incorrect ID Score
1	Easy	2	0
2	Easy	2	-8
3	Hard	8	0
4	Hard	8	0
5	Moderate	4	0
6	Moderate	4	0
7	Moderately hard	6	0
8	Moderately hard	6	0
9	Moderate	4	0
10	Moderate	4	0

Slide 28



Slide 29



Future Plans

- **Since March 2002 this 2002reporting system has been expanded for some laboratories to also supply information on:**
 - * *Listeria* spp.
 - * *Campylobacter* spp.
 - * Verotoxigenic *Escherichia coli*
- **In July propose to organise a similar Ring Trial to 2001, to allow for comparison of the laboratories.**

Slide 30



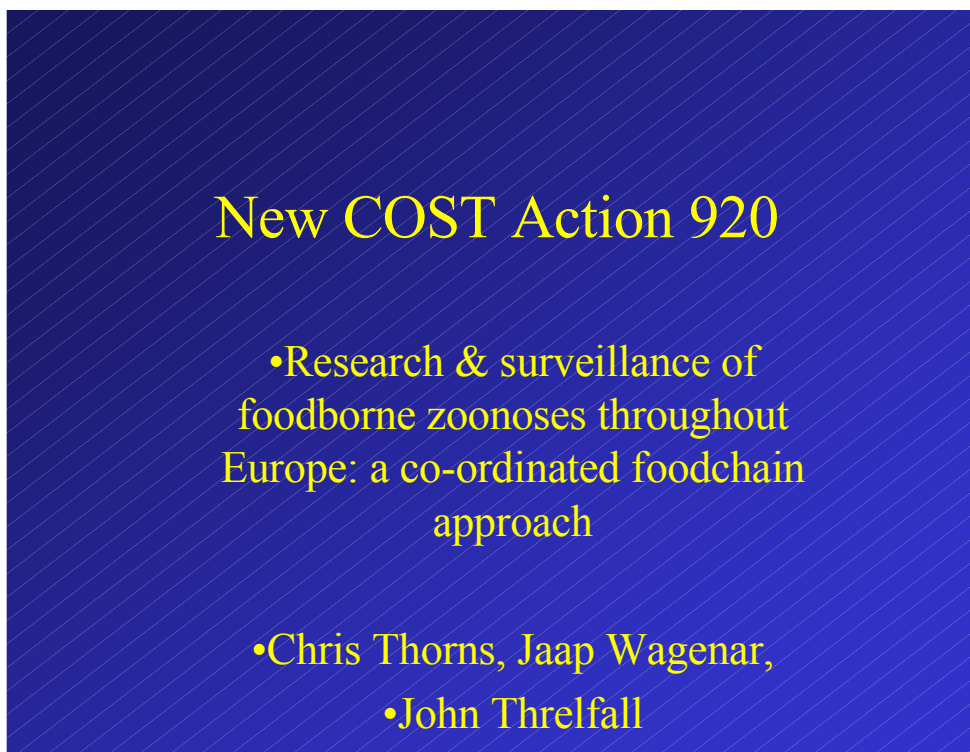
Acknowledgements

- **Thanks to John Ward, Bernard Bradshaw, Ann Murphy, Ger Murray, Aine O'Doherty and all in the Central Veterinary Research Laboratory.**
- **Thanks also to the 21 DAFRD approved laboratories.**

Appendix 19

Slides of presentation 5.4

Slide 1

A blue slide with a diagonal line pattern. The title 'New COST Action 920' is in yellow. Below it, two bullet points in yellow describe the research focus and the lead researchers.

New COST Action 920

- Research & surveillance of foodborne zoonoses throughout Europe: a co-ordinated foodchain approach
- Chris Thorns, Jaap Wagenaar,
• John Threlfall

Slide 2

A blue slide with a diagonal line pattern. The title 'Objectives & benefits' is in yellow. Below it, two main bullet points in yellow describe the goals, with a sub-bullet list for the second point.

Objectives & benefits

- Increase the coordinated, transfer of knowledge & expertise to influence & contribute to future pan-european policy initiatives to control foodborne zoonotic infections.
- By improving:
 - Comparability of surveillance data
 - Proactive approach to new & emerging pathogens
 - Integration of risk assessment into foodchain activities
 - In-depth understanding of the hazards

Slide 3

Scientific Programme

- Harmonisation of diagnostic typing methods
- New & emerging foodborne pathogens
- Quantitative foodchain risk assessment
- Survival of zoonotic pathogens through the foodchain


Slide 4

Working group 1

Diagnostic & typing

- Review detection & typing methods in EU
- Prioritise methods for harmonisation
- Inter-comparability ring trials
- Training & exchange of methods
- **Benefits:**
 - Comparability of surveillance data

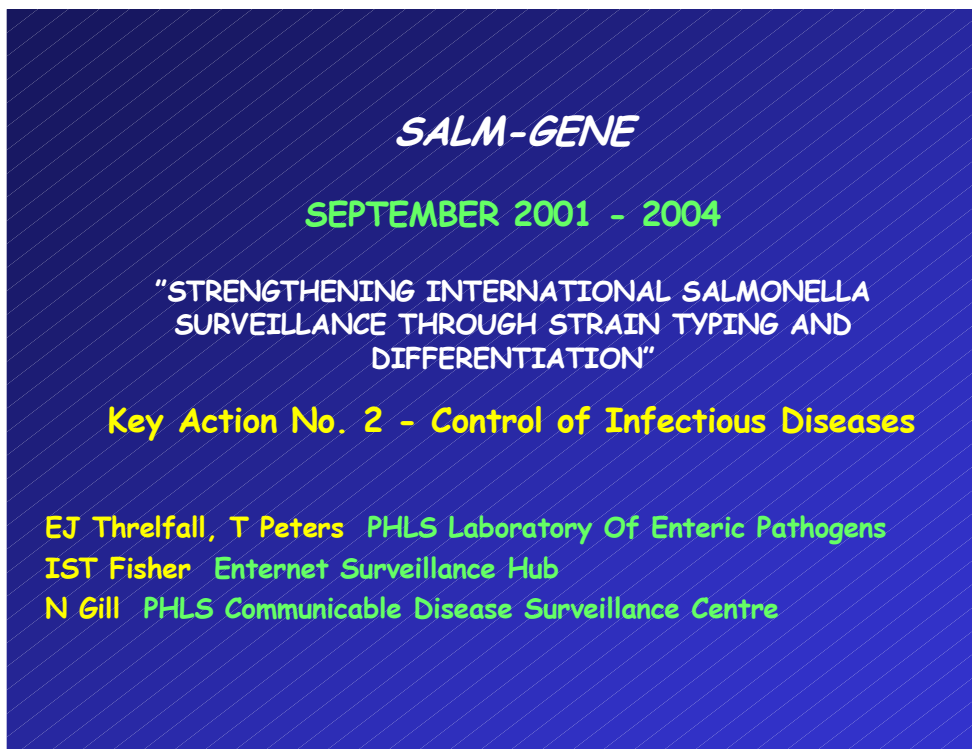
Slide 5

A blue rectangular slide with a diagonal line pattern. The title 'SALM-GENE' is centered at the top in white italicized font. Below it, the names and affiliations of the project members are listed in yellow and green text.

SALM-GENE

EJ Threlfall, T Peters PHLS Laboratory Of Enteric Pathogens
IST Fisher Enternet Surveillance Hub
N Gill PHLS Communicable Disease Surveillance Centre

Slide 6

A blue rectangular slide with a diagonal line pattern. The title 'SALM-GENE' is centered at the top in white italicized font. Below it, the project period 'SEPTEMBER 2001 - 2004' is shown in green. The project's goal is stated in white, followed by 'Key Action No. 2 - Control of Infectious Diseases' in yellow. At the bottom, the names and affiliations of the project members are listed in yellow and green text.

SALM-GENE

SEPTEMBER 2001 - 2004

"STRENGTHENING INTERNATIONAL SALMONELLA
SURVEILLANCE THROUGH STRAIN TYPING AND
DIFFERENTIATION"

Key Action No. 2 - Control of Infectious Diseases

EJ Threlfall, T Peters PHLS Laboratory Of Enteric Pathogens
IST Fisher Enternet Surveillance Hub
N Gill PHLS Communicable Disease Surveillance Centre

Slide 7

PROJECT DETAILS

DG RESEARCH-FUNDED: PROJECT No. QLRT-2000-01940

EC CONTRIBUTION: 1,194,035 Euros
DURATION: 39 MONTHS
STARTING DATE: SEPTEMBER 1st 2001
CONTRACT TYPE: SHARED-COST

Slide 8

PROJECT DETAILS
OBJECTIVES

- **Develop standard protocols for genotyping of salmonella subtypes**
- **Create on-line searchable database of genotypic information (24,000 strains)**
- **Establish external quality assurance scheme for laboratory methods used**
- **Real-time genotyping of large sample of salmonella strains from several countries using selection criteria that maximise outbreak recognition power**
- **Develop recommendations for incorporating genotyping into national and international surveillance of salmonella strains**

Slide 9

- Salm-gene partners include:
 - The Laboratory of Enteric Pathogens, Central Public Health Laboratory, UK (acting as the Project Co-ordinator together with the Enter-net Surveillance Hub, Central Disease Surveillance Centre, UK)
- The other national reference laboratories participating in this project are those from:



*Software compatability advisor

Slide10

METHODS

- Pulsed-field gel electrophoresis (PFGE)
- Amplified fragment length polymorphism
 - Fluorescent - fAFLP
 - Single enzyme - sAFLP
- Others (DNA sequence-based)
- Database - Bionumerics

Slide 11

SALM-GENE MEETING BILTHOVEN, NOVEMBER 2001

- Standard protocol for PFGE (rapid, real-time)
- Enzyme(s)
- Nomenclature
- Quality assurance
- Plasmid profile
- Strains to be included (retrospective, prospective)
- Database specifications and dataflow
- Future developments

Slide 12

Nomenclature - pattern designation

- PFGE patterns submitted to the Salm-gene project will be given a code:
 - SENTXB.0001 or STYMXB.0003
- a one-letter genus designation
- a three-letter serotype designation
- a two-letter restriction enzyme designation
- Finally, each new pattern is given a four-digit numerical identifier starting with .0001 and finishing at .9999

Slide 13

STANDARD PROTOCOL FOR PFGE (RAPID, REAL-TIME)

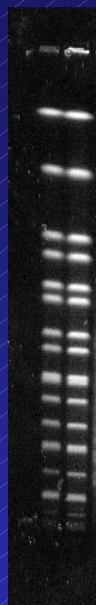
- Short lysis time
- Hot washes
- Ramp - initial 2s; final 64s; 6V/cm; 22h (CHEF DRII), 20h (CHEF DRIII)
- Reference strain: *S. Braenderup*
 - CDC Atlanta / Pulse-Net

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QUALITY ASSURANCE

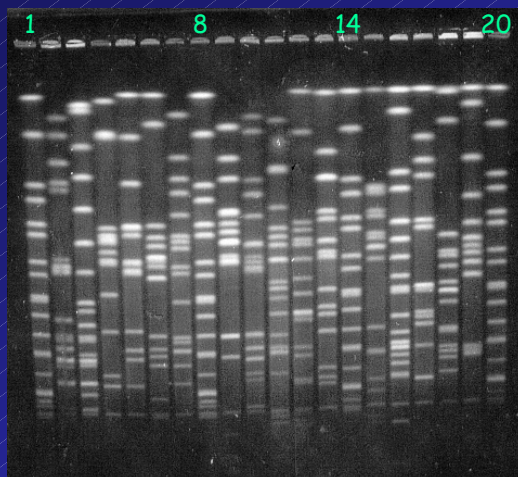
- PANEL - 16 strains + reference strain
- Panel to be held in a culture collection
- REFERENCE STRAIN: *S. BRAENDERUP*
 - CDC Atlanta / Pulse-Net
- EQA - every six months

Slide 15

S. enterica Braenderup (PulseNet, H9812)

- PFGE plugs prepared at CDC, Atlanta compared to plugs prepared at LEP, London
- Run at 6 V/cm, 22 hours, ramp 2 - 64 s

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Salm-gene EQA panel for *S. enterica*

Lane 1, 8, 14, 20:
S. enterica Braenderup (H9812, PulseNet)

- 2: Typhimurium, DT 104
- 3: Typhimurium, DT 208
- 4: Enteritidis, PT 4
- 5: Enteritidis, PT 6a
- 6: Hadar, PT 11
- 7: Virchow, PT 47
- 9: Agona, PT 15
- 10: Heidelberg
- 11: Indiana
- 12: Montevideo
- 13: Mbandaka
- 15: Livingstone
- 16: Anatum
- 17: London
- 18: Senftenberg
- 19: Poona

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PROGRAMME

- **PHASE 1**
RETROSPECTIVE: January - June 2002
- **PHASE 2**
PROSPECTIVE: To commence in second half of 2002
- **QA:** Every six months
- **INVENTORY OF LABORATORY CAPACITY**
- **PHASE 3:** New methods
Years 2 / 3

Slide 18

STRAINS

PHASE 1

JANUARY - JUNE 2002

RETROSPECTIVE:

500 strains per country, as follows:

50	<i>S. Enteritidis</i> PT 4
50	<i>S. Enteritidis</i> - other phage types (1, 5c)
50	<i>S. Typhimurium</i> DT 104
50	<i>S. Typhimurium</i> - other phage types
50	<i>S. Virchow</i>
50	<i>S. Hadar</i>
200	Other serotypes ("Top ten")

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STRAINS

PHASE 1 (continued)

JANUARY - JUNE 2002

RETROSPECTIVE:

500 strains per country, as follows:

100	<i>S. Enteritidis</i> - any phage type
100	<i>S. Typhimurium</i> - any phage type
50	<i>S. Virchow</i>
50	<i>S. Hadar</i>
200	Other serotypes ("Top ten")

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STRAINS

PHASE 2

JULY 2002

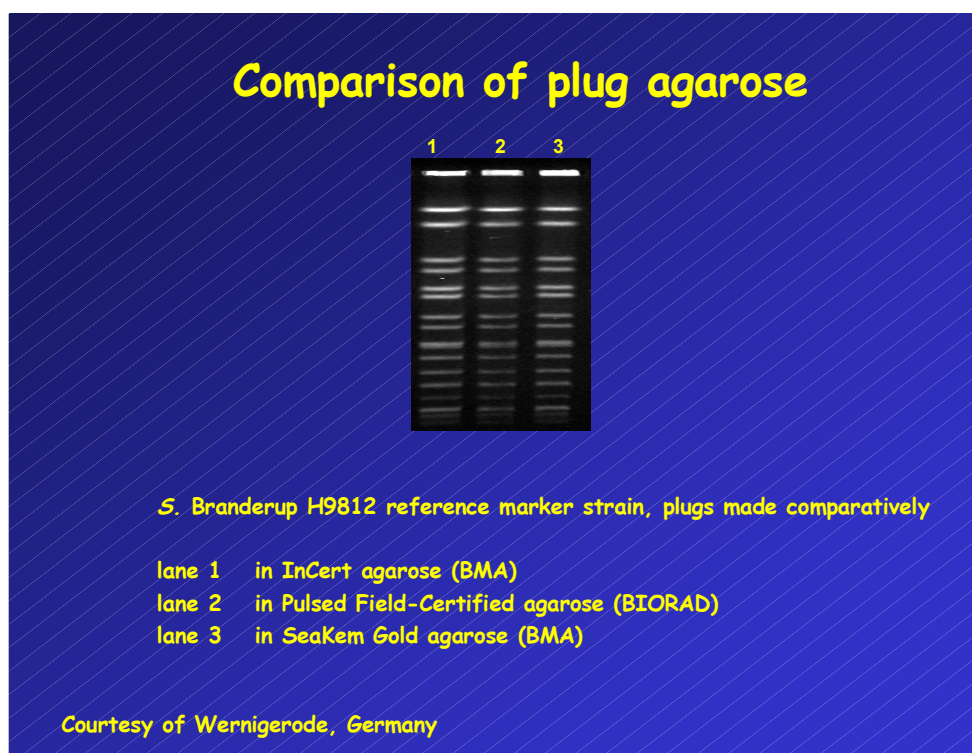
PROSPECTIVE:

THE ANALYSIS OF PROSPECTIVE STRAINS WILL COMMENCE IN JULY 2002

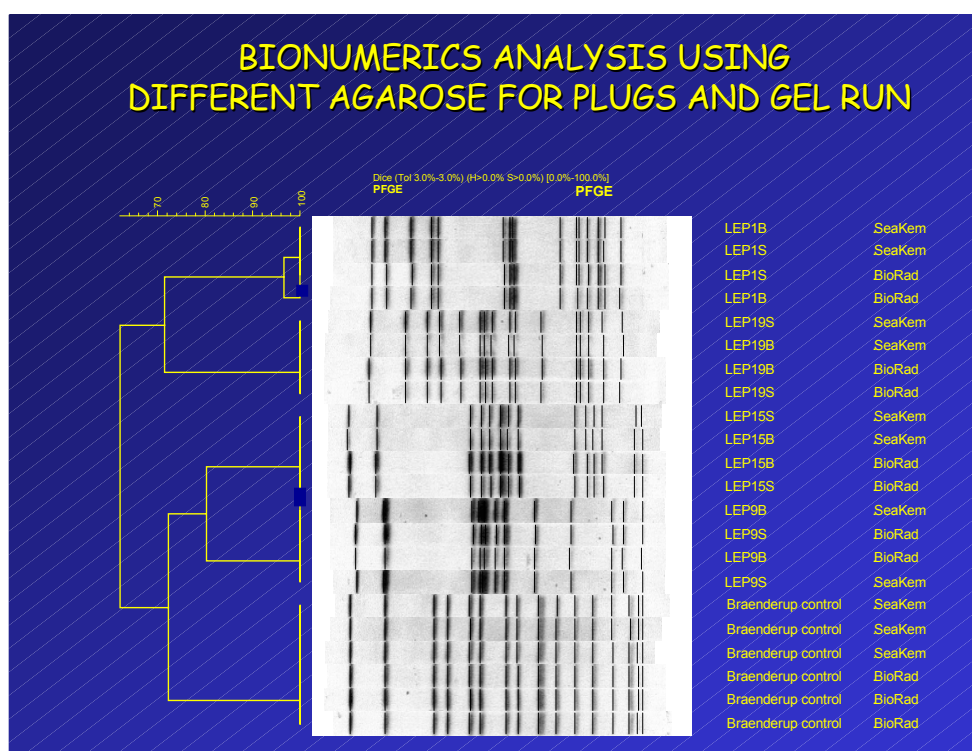
To include:

- Serotypes and phage types as above
- Current outbreak strains

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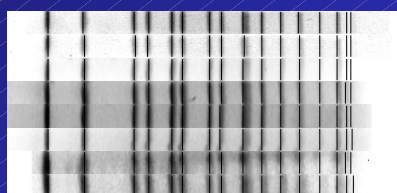
Slide 22



Slide 23

BIONUMERICS ANALYSIS FOR *S. BRAENDERUP* -comparison between countries

Dice (Tot 3.0%-3.0%) (H=0.0% S=0.0%) (0.0%-100.0%)
PFGE PFGE



Braenderup control	England	11.01.02
Braenderup control	England	11.01.02
Braenderup control	Scotland	22.02.02
Braenderup control	Scotland	22.02.02
Braenderup control	Scotland	22.02.02
Braenderup control	Scotland	22.02.02
Braenderup control	Denmark	11.03.02
Braenderup control	Denmark	11.03.02

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FUTURE DEVELOPMENTS

RESEARCH-BASED PROJECT

- Other methods of typing
 - AFLP
 - DNA sequence-based
- Interaction with all Enternet laboratories
- Interaction with Pulse-Net and Pulse-Net North
- Veterinary isolates
- Food chain