



**rivm**

National Institute  
for Public Health  
and the Environment

# **CRL-Salmonella**

23 April 2009

Kirsten Mooijman

# 'Old CRL'

- CRL-*Salmonella* was established in 1992 with as legal basis EU Directive 92/117/EC
- 92/117/EC: 'Measures for protection against specified zoonoses and specified zoonotic agents in animals and products of animal origin to prevent outbreaks of food-borne infections and intoxications'



# Legal basis CRL-*Salmonella* since 2003

- Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents;
- Regulation (EC) 2160/2003 on the control of *Salmonella* and other specified food-borne zoonotic agents;
- Regulation (EC) 882/2004 'Feed and food regulation'
  - Describes tasks and duties of CRLs and NRLs;
  - Annex VII: Official name of CRL-*Salmonella*: 'Community Reference Laboratories for the analysis and testing of **zoonoses** (salmonella)'

# Community Reference Laboratories

In 2007, 40 CRLs exists:

- 12 CRLs for biological risks
- 13 CRLs for chemical risks
- 13 CRLs for animal health
- 1 CRL GMO's
- 1 CRL for feed additives



# CRLs for biological risks (feed and food)

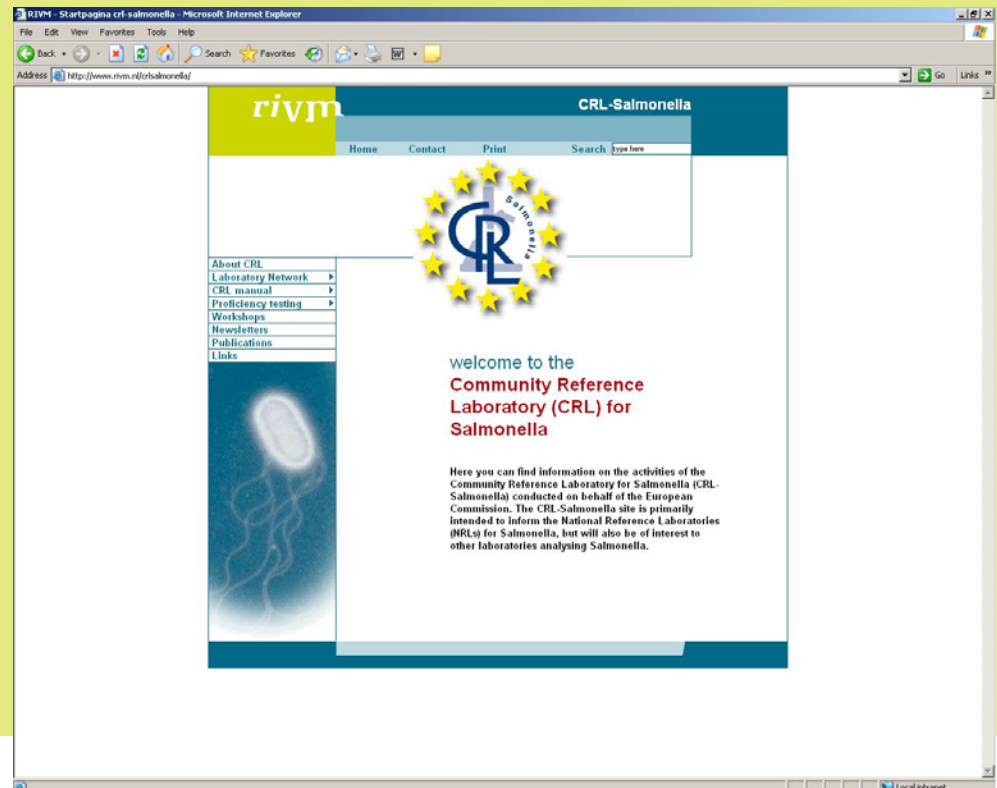
- Milk and milk products (raw) (France)
- Zoonoses (*Salmonella*) (The Netherlands)
- Marine Biotoxins (Spain)
- Bacteriological and viral contaminants of bivalve molluscs (UK)
- *Listeria monocytogenes* (France)
- Coagulase pos *Staphylococci*, incl. *S. aureus* (France)
- *Escherichia coli*, incl. VTEC (Italy)
- *Campylobacter* (Sweden)
- Parasites (Italy)
- Antimicrobial resistance (Denmark)
- Animal proteins in feeding stuffs (Belgium)
- Transmissible spongiform encephalopathies (TSE's) (UK)

# Tasks and duties CRLs (Reg 882/2004)

- Provide NRLs with details of analytical methods;
- Coordinate research on new methods (develop, validate and standardise methods);
- Coordinate application of methods by NRLs, by organising comparative testing;
- Conduct training for NRLs
- Scientific and technical assistance to European Commission
- Collaboration with laboratories in third countries

# Inform the NRLs

- Website: [www.rivm.nl/crlsalmonella](http://www.rivm.nl/crlsalmonella)
- Newsletter published every 3 months (through website)
- Annual workshop for all NRLs-*Salmonella*



# Provide NRLs with details of analytical methods, research on new methods

- Test 'new' methods (PCR, ELISA, 'new' media);
- Participation / being project leader in ISO/TC34/SC9 and CEN/TC275/WG6 (Feed and food microbiology):
  - Development of Annex D of ISO 6579 (2007): Detection of *Salmonella* in animal faeces and samples primary production;
  - Review of ISO 6579 (2002): Detection of *Salmonella* in food and animal feed;
  - Enumeration of *Salmonella* (mini-MPN);
  - Serotyping of *Salmonella*;
  - Validation of microbiological methods;
  - Proficiency testing in microbiology;
  - Project leader for validation Annex D of ISO 6579 (Detection of *Salmonella* in primary production('CEN-mandate')).





# Tasks and duties CRLs (Reg 882/2004)

- Provide NRLs with details of analytical methods;
- Coordinate research on new methods (develop, validate and standardise methods);
- Coordinate application of methods by NRLs, by organising comparative testing;
- Conduct training for NRLs
- Scientific and technical assistance to European Commission
- Collaboration with laboratories in third countries

# ‘Coordinate application of methods by NRLs, by organising comparative testing’

Organisation of 2-3 ring trials per year (approx. 30 NRLs):

- 2007:

- Typing of *Salmonella* (serotyping and phagetyping)
- Detection of *Salmonella* in minced beef;
- Detection of *Salmonella* in pig sera with serological methods.

- 2008:

- Detection of *Salmonella* in chicken faeces;
- Detection of *Salmonella* in chicken feed;
- Typing of *Salmonella*.



# Typing ring trials

- Organised in cooperation with Health Protection Agency, London for phagetyping;
- Pure cultures of different *Salmonella* serovars (blind samples);
- For serotyping: 20 different serovars of *Salmonella enterica* subsp. *enterica*. Serovars with public health significance and/or causing typing problems in earlier studies;
- For phage typing: 10 *Salmonella* Typhimurium strains and 10 *Salmonella* Enteritidis strains;
- Methods:
  - Kauffmann-White scheme (serotyping);
  - scheme HPA (phagetyping).



# Detection ring trials

- Food, animal feed or animal faeces negative for *Salmonella* (but containing background flora);
- 25 (blind) samples artificially contaminated with reference materials (RMs) containing *Salmonella* Typhimurium or *Salmonella* Enteritidis at low and high levels (e.g. SE10 & SE100; STM5 & STM50) and blanks & 10 controls (only RMs);
- RMs prepared by CRL: capsules with artificially contaminated milk powder at controlled contamination level;
- Methods: ISO 6579 and/or Annex D



# General scheme for ring trials

Week	Date	Topic
7	9 - 13 February	Mailing of the protocol, standard operating procedure and test report to the NRLs- <i>Salmonella</i>
10	2 - 6 March	Mailing of parcels to NRLs as biological substance cat. B (UN3373) by door-to-door courier service Storage capsules at -20 °C, faeces at 5 °C
11	9-13 March	Preparation of media by NRLs (own ingredients)
12	16-20 March	Performance of the study
14	Max. 3 April	Completion of the test report and returning it to the CRL <i>Salmonella</i>
15	6 -10 April	Data input at CRL- <i>Salmonella</i> and sending data for check to NRLs
17-	20-30 April	Preparation of interim summary with results of all NRLs, send to NRLs
	May - July	Follow-up, in case of poor performance Full report of study

# Mailing of samples

- Samples mailed (and packed) as 'Biological substances, category B (UN3373)', by door-to-door courier service (TNT):
  - For typing studies one package, non-cooled;
  - For detection studies: several packages in one box, cooled with cooling elements; temperature check with electronic temperature recorder.



# How to define 'good performance'?



## For serotyping

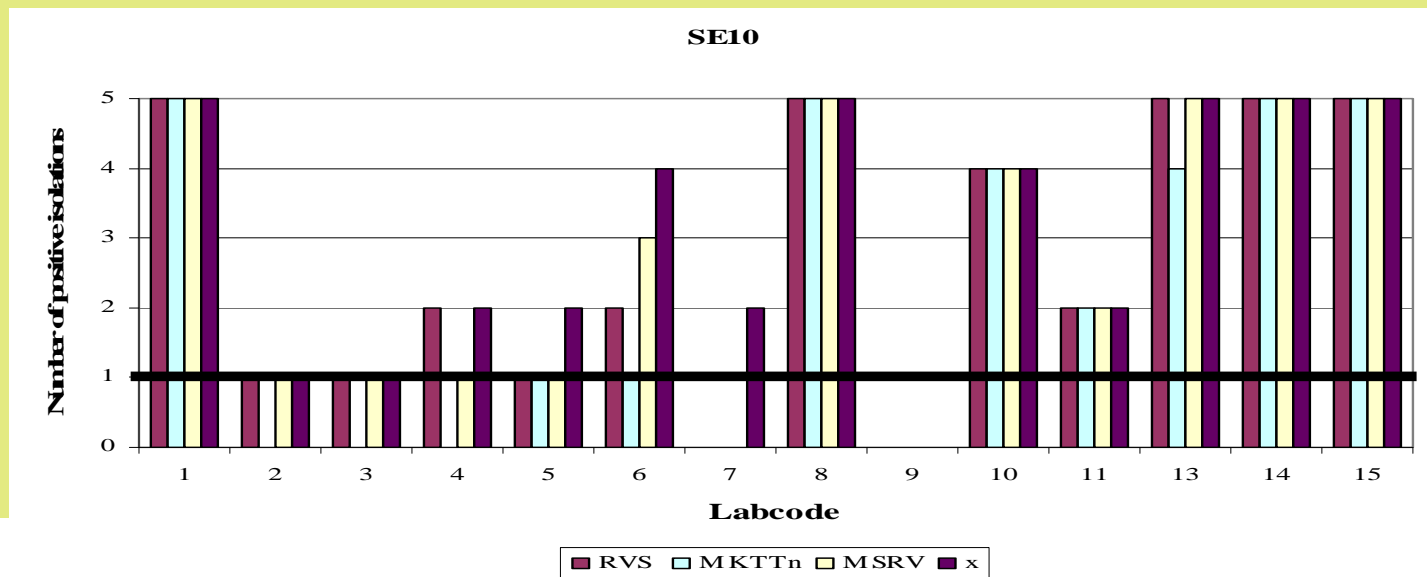
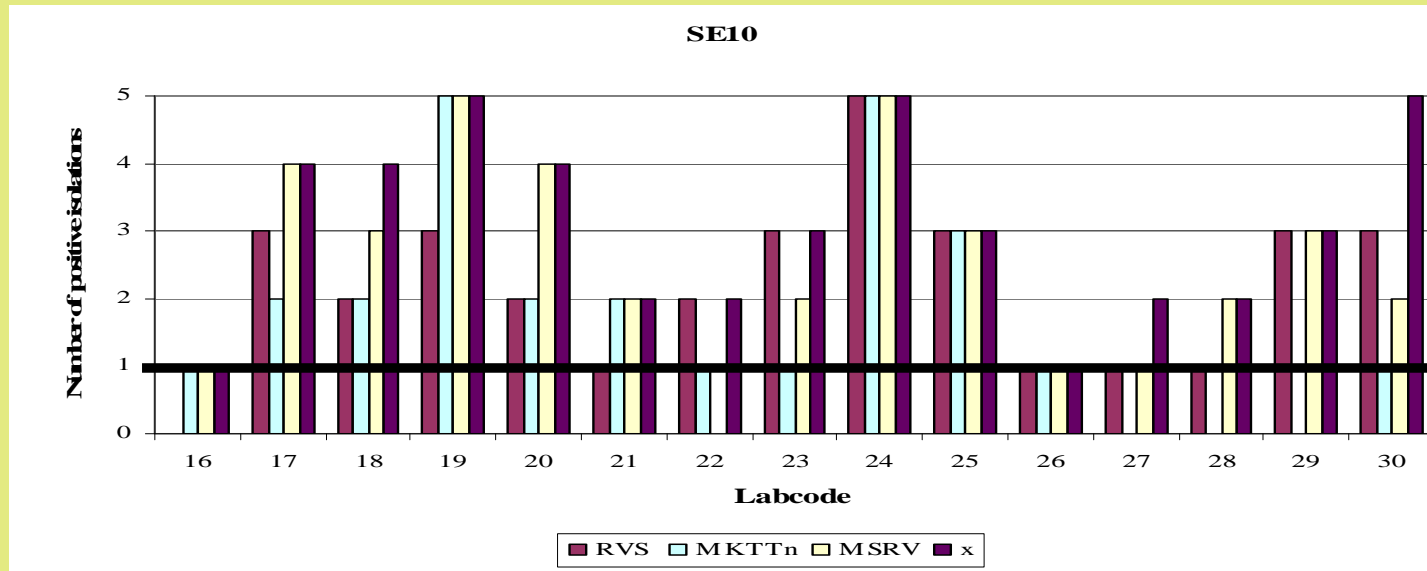
- 4 penalty points:
  - Incorrect typing of *S. Enteritidis*, *S. Typhimurium*, *S. Hadar*, *S. Infantis* or *S. Virchow*
  - Assigning the serovar names of *S. Enteritidis*, *S. Typhimurium*, *S. Hadar*, *S. Infantis* or *S. Virchow* to another strain
- 1 penalty point:
  - Incorrect typing of other strains
- Determining total amount of penalty points per NRL
- Good performance: <4 penalty points

# How to define 'good performance' of detection studies?

	Minimum result	
	% pos	No pos / total
<b>Control (capsules, no matrix)</b>		
Blank	Max 0 %	0 / 2
Span5, SE10, SE20	50 %	1 / 2
STM5	60 %	2 / 3
SE100	100 %	1 / 1
<b>Samples (capsules + matrix)</b>		
Blank	Max 20 %	1 / 5
SE10	20 %	1 / 5
STM5, SE20	50 %	2-3 / 5
STM50, SE100	80 %	4 / 5



# Examples presentation results Food study (2007)



# Examples presentation results Food study (2007)



# What to do in case of no good performance?

- Contact the NRL and ask for possible technical problems;
- Organise a follow-up study with number and type of samples dependent on the problems of the NRL;
- In case of no good performance in follow-up study:
  - Contact NRL for possible technical problems;
  - Offer training;
  - Inform DG-Sanco.



# Tasks and duties CRLs (Reg 882/2004)

- Provide NRLs with details of analytical methods;
- Coordinate research on new methods (develop, validate and standardise methods);
- Coordinate application of methods by NRLs, by organising comparative testing;
- Conduct training for NRLs
- Scientific and technical assistance to European Commission
- Collaboration with laboratories in third countries

# ‘Conduct training’

Trainings are given on request and focus on the needs of the specific NRL:

- In 2007: 3 trainings of 1-2 weeks of 3 NRLs (2 persons per NRL) on detection and/or serotyping and/or molecular typing of *Salmonella*;
- In 2008: 4 trainings of 3 NRLs (4x2 persons per NRL).



# Tasks and duties CRLs (Reg 882/2004)

- Provide NRLs with details of analytical methods;
- Coordinate research on new methods (develop, validate and standardise methods);
- Coordinate application of methods by NRLs, by organising comparative testing;
- Conduct training for NRLs
- Scientific and technical assistance to European Commission
- Collaboration with laboratories in third countries

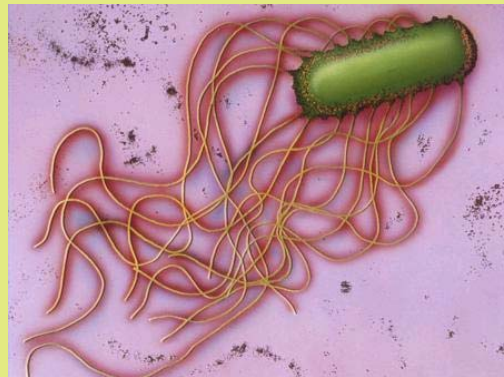
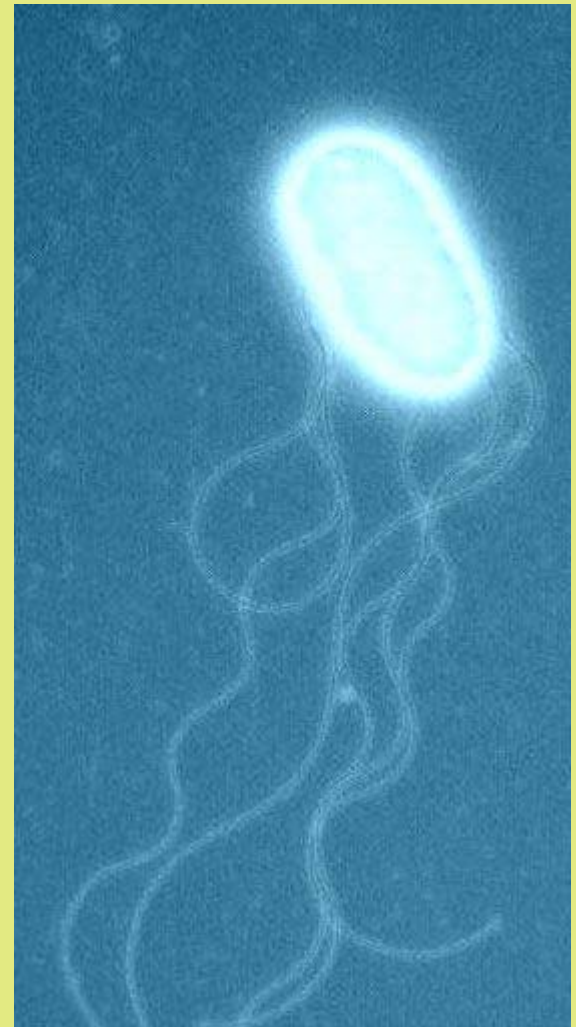
# **‘Scientific and technical assistance to EC’;** **‘Collaboration with third countries’**

- Participation in Working groups of DG-Sanco and EFSA, e.g. for setting-up regulations for baseline studies/ monitoring programmes;
- Help DG-Sanco with ‘ad-hoc’ questions;
- Help NRLs with ‘ad-hoc’ questions;
- NRLs of non-EU members participate in ring trials and some in workshops (for own costs). Inside Europe: Iceland, Norway, Switzerland, Croatia, Republic of Macedonia, Turkey. Outside Europe: Tunisia, Israel.





**Thank you for your attention!**



**rivm**

National Institute  
for Public Health  
and the Environment