National Institute
for Public Health
and the Environment

Letter report 330604016/2009
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Quality assurance of serotyping of Salmonella isolates

obtained from the baseline study on breeding pigs-2008

CRL-*Salmonella* report

Quality Assurance by CRL-*Salmonella* of the serotyping of isolates obtained from the 'Survey on the prevalence of *Salmonella* spp. in herds of breeding pigs in the Member States' (2008)

RIVM letter report 330604016/2009
Annex to letter 088/2009 LZO Mo/km

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12 June 2009

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This investigation has been performed by order and for the account of the European Commission, Directorate-General for Health and Consumer Protection and the Laboratory for Zoonoses and Environmental Microbiology (LZO) of the National Institute for Public Health and the Environment (RIVM) within the framework of RIVM project V/330604/08/CS and V/330604/09/CS by the Community Reference Laboratory for *Salmonella*.

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Introduction

In September 2007 the Commission Decision 'concerning a financial contribution from the Community towards a survey on the prevalence of *Salmonella* spp. in herds of breeding pigs to be carried out in the Member States' (2007/636/EC) was published. In the technical specification (Annex 1) of this Decision, the more practical aspects of this study are worked out. One of these aspects concerns serotyping of *Salmonella* isolates. In the technical specifications it is indicated that all strains isolated and confirmed as *Salmonella* spp. shall be serotyped according to the Kaufmann-White scheme (Popoff, 2001). For quality assurance of the serotyping, a maximum of 16 typable strains and 16 non-typable isolates of the one year study had to be sent to the CRL-*Salmonella*. The results of the quality assurance are reported here.

Materials and Methods

Each National Reference Laboratory (NRL) could send a maximum of 16 typable strains and 16 non-typable isolates to the CRL-*Salmonella* during the one year baseline study (January 2008 – January 2009).
For this purpose each NRL-*Salmonella* received 16 submission forms for typable strains and 16 forms for non-typable strains. Each form contained a unique code indicating the strain and the country as follows:

- For typable strains: country abbreviation/ bp (of breeding pig) T-1 until bpT-16;
- For non-typable strains: country abbreviation/ bpNT-1 until bpNT-16.

For example, the form to be used for submission of the third typable strain of NRL-Latvia should be coded LV/bpNT-3. Examples of the forms are given in Annex 1.

All strains isolated by the NRLs-*Salmonella* had to be stored using the normal method for NRL culture collection.
Every 3 months a selection of the isolates (typable and non-typable) were sent to the CRL-*Salmonella*. The selection of typable isolates had to be representative of the (variety of) serotypes found during the baseline study.
For the mailing of the isolates, strains were cultured in tubes using the NRL's regular method for mailing of strains.
The serotyping by the CRL-*Salmonella* was performed in accordance with the Kauffman-White scheme (Popoff, 2001). As soon as the serotyping was performed at the CRL-*Salmonella*, the relevant NRL was informed on the results. In case of (large) differences between the serotyping results of an NRL and of the CRL, the results were further discussed.

Results

Up to April 2009, 378 *Salmonella* isolates (typable and non-typable) were received at the CRL-*Salmonella* and further serotyped.
In Tables 1 and 2 a summary is given of the number of typable strains (Table 1) and the number of non-typable strains (Table 2) as sent in for quality assurance by the NRLs. In

these tables it is also indicated how many of the isolates were typed differently by the CRL-*Salmonella* and also the serovar names (when possible) as found by the CRL are given. In both tables the NRLs are indicated by labcodes. For this report the same labcodes were used as for the interlaboratory comparison study on typing of *Salmonella* 2008.

From six NRLs (labcodes 2, 10, 11, 15, 17 and 23) no isolates were received by the CRL. Laboratories 2 and 11 indicated that they had not found *Salmonella* isolates during the baseline study. Laboratory 23 indicated that the baseline study was not performed in their country. Laboratories 10, 15 and 17 gave no further clarification why they did not sent isolates.

Discussion and conclusions

Of the 294 typable strains 17 strains (5,8%) were serotyped differently by the CRL-*Salmonella*. This is a slight improvement in comparison to the baseline study on slaughter pigs (2006-2007) in which 9,4% of the typable isolates were serotyped differently by the CRL-*Salmonella* (Mooijman et al., 2008).
Of the 84 non-typable strains, the CRL-*Salmonella* was able to further identify 18 strains to serovar names.

For many differences some general explanations could be given:

- The use of different culture media, which may cause differences in some antigen reactions;
- The use of a microtiter method by the CRL-*Salmonella*, which may be more sensitive than an agglutination reaction on slide;
- Because of storage and transport a typable strain may become non-typable;
- The availability of specific antisera. For some NRLs it is difficult to obtain a complete set of specific antisera in their country;
- A few isolates concerned mixed cultures and were thus difficult to identify further;
- During the baseline study on slaughter pigs (2006-2007) 'monophasic *Salmonella* Typhimurium' (4, [5], 12 : i : -) was often found by the NRLs (Mooijman et al., 2008). During the present study this isolate was also found and by some NRLs indicated as *Salmonella* Typhimurium. However, as the second phase of the H-antigen is not expressed, the isolate can serologically not be called *Salmonella* Typhimurium (antigenic formula: 4, [5], 12 : i : 1, 2). During the present study the strain was either less often sent to the CRL for quality assurance or it was found less often when compared to the baseline study on slaughter pigs.

As a follow-up of this quality assurance study, a selection of the strains causing problems in the baseline study will be used in interlaboratory comparison studies on typing. In case of repeated problems extra follow-up studies will be organised and/or NRLs will be invited for a training at the CRL-*Salmonella*.

*Table 1 Number of **typable** strains as sent by the NRLs-Salmonella for quality assurance of the serotyping by the CRL-Salmonella*

Labcode	Number of isolates sent by NRL	Different from CRL			
		Number	Serovar found by NRL	Serovar found by CRL	Matrix
1	16	0	-	-	-
2	0	-	-	-	-
3	16	0	-	-	-
4	10	3	*S.* Typhimurium (2x)	*S.* subsp. *enterica*	Faeces
			S. Kaapstad	Mixed culture of *S.* Bredeney and *S.* Kottbus	Faeces
5	16	0	-	-	-
6	16	0	-	-	-
7	15	0	-	-	-
8	16	0	-	-	-
9	16	0	-	-	-
10	0	-	-	-	-
11	0	0	-	-	-
12	16	0	-	-	-
13	2	0	-	-	-
14	16	0	-	-	-
15	0	-	-	-	-
16	13	3	*S.* Virginia	*S.* Muenchen	Faeces
			S. Hindmarsh	*Salmonella* autoagglutinating	Faeces
			S. Ohio	*S.* Infantis	Faeces
17	0	-	-	-	-
18	6	0	-	-	-

Table 1 *Number of **typable** strains as sent by the NRLs-Salmonella for quality assurance of the serotyping by the CRL-Salmonella (continued)*

Country	Number of isolates sent by NRL	Different from CRL			
		Number	Serovar found by NRL	Serovar found by CRL	Matrix
19	1	1	*S.* Typhimurium	*S.* subsp. *enterica* rough	swab
20	16	0	-	-	-
21	16	1	*S.* Typhimurium	Mixed culture of *S.*London and *S.* subsp. *enterica*	Faeces
22	16	3	*S.* Llandoff	*S.* Broughton	Faeces
			S. Panama	*S.* Typhimurium	Faeces
			S. Eko	*S.* Anatum	Faeces
23	0	0	-	-	-
24	4	0	-	-	-
25	4	0	-	-	-
27	16	3	*S.* Agona (3x)	*S.* Derby (3x)	Swab
28	16	2	*S.* Typhimurium (2x)	*S.* subsp. *enterica* (2x)	Faeces
29	15	1	*S.* Gloucester	*S.* Wien	Feaces
30	16	0	-	-	-
Total	294	17			

*Table 2 Number of **non-typable** strains as sent by the NRLs-Salmonella for quality assurance of the serotyping by the CRL-Salmonella*

labcode	Number of isolates sent by NRL	Different from CRL			
		Number	Serovar found by NRL	Serovar found by CRL	Matrix
1	15	1	*S.* subsp. *enterica* (4,12 : d:-)	*S.* Stanley (1,4,[5],12,27 : d:1,2)	Faeces
2	0	-	-	-	-
3	6	0	-	-	-
4	0	-	-	-	-
5	2	0	-	-	-
6	1	0	-	-	-
7	0	-	-	-	-
8	9	0	-	-	-
9	3	3	No info	*S.* Infantis	Faeces
			No info	*S.* Derby	Faeces
			No info	*S.* Derby	Faeces
10	0	-	-	-	-
11	0	-	-	-	-
12	7	7	2x Unknown (4,12 : -:-)	2x *S.* Brandenburg (4,[5],12 : l,v:e,n,z_{15})	Faeces
			1x Unknown (4,12 : l,v:-)	1x *S.* Brandenburg (4,[5],12 : l,v:e,n,z_{15})	Faeces
			1x Unknown (4 : f,g:-)	*S.* Derby (1,4,[5],12 : f,g:[1,2])	Faeces
			2x Unknown (8,20: r:-)	*S.* Altona (8,20 : r,[i]i:z_6)	Faeces
			1x Unknown (6,8,20: r:-)	*S.* Altona (8,20 : r,[i]i:z_6)	Faeces
13	0	-	-	-	-
14	2	0	-	-	-
15	0	-	-	-	-
16	2	0	-	-	-

*Table 2 Number of **non-typable** strains as sent by the NRLs-Salmonella for quality assurance of the serotyping by the CRL-Salmonella (continued)*

labcode	Number of isolates sent by NRL	Different from CRL			
		Number	Serovar found by NRL	Serovar found by CRL	Matrix
17	0	-	-	-	-
18	6	0	-	-	-
19	2	0	-	-	-
20	0	0	-	-	-
21	0	-	-	-	-
22	5	4	Unknown (3,10: h:-)	*S.* Blockley, but mixed culture	Faeces
			Unknown (3,10: l,v:-)	*S.* London	Faeces
			Unknown (6,8: -:-)	*S.* Bovismorbificans	Faeces
			Unknown (4: -:-)	*S.* Typhimurium	faeces
23	0	0	-	-	-
24	0	-	-	-	-
25	0	-	-	-	-
27	2	0	-	-	-
28	2	1	Unknown (4 : b:-)	*S.* Schleissheim (4,12,27 : b:-)	Faeces
29	15	0	-	-	-
30	5	2	*S.* subsp. *enterica* (rough : f,g:-)	*S.* Derby (1,4,[5],12 : f,g:[1,2]	Faeces
			S. subsp. *enterica* (rough : b:e,n,x)	*S.* Ohio (6,7,14 : b:l,w)	Faeces
Total	84	18			

References

Commission Decision Decision 2007/636/EC of 3 October 2007, concerning a financial contribution from the Community towards a survey on the prevalence of *Salmonella* spp. in herds of breeding pigs to be carried out in the Member States (notified under document number C(2007) 4434).

Mooijman K.A., Veenman C. and Maas, H.M.E. Quality assurance of serotyping of Salmonella isolates obtained from the baseline study on slaughter pigs (2006-2007). National Institute for Public Health and the Environment, Bilthoven, The Netherlands. RIVM letter report, 330604008/2008, 31 March 2008

Popoff, M.Y, 2001. Antigenic formulas of the *Salmonella* serovars. WHO Collaborating Centre for Reference and Research on *Salmonella*. Institute Pasteur, Paris, France.

Annex 1 Submission forms (examples)

Shipping date:	Date of arrival at CRL:

Quality assurance serotyping of typable strains isolated from breeding pigs in the context of the baseline study on the prevalence of *Salmonella* in breeding pigs in the EU

Project number CRL-*Salmonella*	V/330604/08/CS
Study number strain Note your country abbreviation before the slash	LV /bpT-3

Country	
Name contact person	
E-mail address	

Salmonella strain was isolated from:

- o Swab with faecal material
- o Faeces
- o Other, namely……..

Formula according to the Kaufmann-White scheme:

O - antigens:

H - antigens:

Remarks:

Shipping date:	Date of arrival at CRL:

Quality assurance serotyping of non-typable strains isolated from breeding pigs in the context of the baseline study on the prevalence of *Salmonella* in breeding pigs in the EU

Project number CRL-*Salmonella*	V/330604/08/CS
Study number strain Note your country abbreviation before the slash	LV /bpNT-3

Country	
Name contact person	
E-mail address	

Salmonella strain was isolated from:

- Swab with faecal material
- Faeces
- Other, namely……..

Formula according to the Kaufmann-White scheme:

O - antigens:

H - antigens:

Remarks:

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