



National Institute
for Public Health
and the Environment

Letter report 330604008/2008

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Quality assurance of serotyping of Salmonella isolates

obtained from the baseline study on
slaughter pigs (2006-2007)

CRL-*Salmonella* report

Quality Assurance by CRL-*Salmonella* of the serotyping of isolates obtained from the 'Baseline study on the prevalence of *Salmonella* in slaughter pigs in the EU Member States' (2006 – 2007)

RIVM letter report 330604008/2008
Annex to letter 049/2008 LZO Mo/km

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31 March 2008

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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/330604/07/CS by the Community Reference Laboratory for *Salmonella*.

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Introduction

In September 2006, the Decision ‘concerning a financial contribution from the Community towards a baseline survey on the prevalence of *Salmonella* in slaughter pigs to be carried out in the Member States’ (2006/668/EC), was formally adopted by the Commission. In the technical specifications (Annex I) of this Decision, the more practical aspects of this study were worked out. One of these aspects concerns serotyping of the isolates. In the technical specifications it was indicated that all strains isolated and confirmed as *Salmonella* spp. had to be serotyped, following 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 (October 2006 – October 2007).

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/p (of pigs) T-1 until pT-16;
- For non-typable strains: country abbreviation/pNT-1 until pNT-16.

For example, the form to be used for submission of the third typable strain of NRL-France has been coded FR/pT-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 January 2008, *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 the detection of *Salmonella* in Food-II (2007).

From four NRLs (labcodes 13, 16, 18 and 27) no isolates were received by the CRL. Laboratory 13 gave no further clarification why they did not send isolates. Laboratory 16 was not able to send strains due to lack of resources. The NRL with labcode 18 indicated that they had not found *Salmonella* isolates during the baseline study. Laboratory 27 became EU Member State after the baseline study had started (1 January 2007) and was probably not aware of the need to send isolates to the CRL-*Salmonella*.

Discussion and conclusions

Of the 340 typable strains 32 strains were serotyped differently by the CRL-*Salmonella*. Of the 84 non-typable strains, the CRL-*Salmonella* was able to further identify 17 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;
- The carcass swab isolates were relatively often found to be contaminated or being mixed cultures and thus difficult to identify further;
- An isolate which was found by many NRLs was: 4, [5], 12 : i : -. The antigenic formula of this isolate is close to the antigenic formula of *Salmonella* Typhimurium (4, [5], 12 : i : 1, 2). Consequently, the naming of this isolate varied, although the NRLs refer to the same strain: *Salmonella* Typhimurium (DT120), monophasic *Salmonella* Typhimurium, *S. subsp. enterica*.

Specific problems:

The NRL with labcode 8 sent the strains in two sets. The first set included the strains pT1 up to and including pT6 and pNT1 up to and including pNT5. From the results it became clear that the NRL had a problem with typing *S. Brandenburg*. This was communicated to the NRL. Obviously the NRL had taken some actions as in the next set of strains (pT7-16 and pNT6-7) *S. Brandenburg* was typed correctly by the NRL. Other typing differences between the CRL and this NRL indicated a lack of (specific) antisera by the NRL.

Possible explanations of 2 differently typed typable strains and of 1 non-typable strain of the NRL with labcode 9 might be the fact that the NRL had some problems with finding the second phase of these 3 strains.

The NRL with labcode 19 was able to correctly type the O-antigens but showed to have some problems with the typing of the H-antigens. Either the NRL did not possess a complete set of antisera or the antisera were not sufficiently specific.

The NRL with labcode 22 found for 10 of the 16 typable strains different results and was not able to completely type 5 strains ('non-typable isolates'). Through e-mail contact it was tried to give the NRL some advises to improve the serotyping, like the need of the NRL to expand the panel of more specific antisera and/or to order the antisera at another supplier.

As a follow-up of this study, a selection of the strains causing problems in the baseline study will be used in a next interlaboratory comparison study on typing (to be organised in fall 2008).

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	3	<i>S. Typhimurium</i> (2x)	<i>S. subsp. enterica</i> (2x)	lymph nodes
			<i>S. Choleraesuis</i> var. Kunzendorf	<i>S. subsp. enterica</i>	lymph nodes
2	15	1	<i>S. Agona</i>	<i>S. Bredeney</i>	Carcass swab
3	16	1	<i>S. Goldcoast</i>	<i>S. Infantis</i>	Carcass swab
4	16	0	-	-	-
5	10	0	-	-	-
6	16	0	-	-	-
7	12	0	-	-	-
8	16	4	<i>S. Bredeney</i> (3x)	<i>S. Brandenburg</i>	Lymph nodes
			<i>S. Typhimurium</i>	<i>S. subsp. enterica</i>	Lymph nodes
9	16	2	<i>S. Blockley</i>	<i>S. Kottbus</i>	Lymph nodes
			<i>S. Menden</i>	<i>S. Mbandaka</i>	Lymph nodes
11	16	0	-	-	-
12	16	4	<i>S. Typhimurium</i>	<i>S. subsp. enterica</i>	Lymph nodes
13	0	-	-	-	-
15	16	1	<i>S. Choleraesuis</i>	<i>S. Infantis</i>	Lymph nodes
16	0	-	-	-	-
17	16	0	-	-	-
18	0	0	-	-	-
19	16	4	<i>S. Heidelberg</i>	<i>S. Agona</i>	Lymph nodes
			<i>S. Fyris</i>	<i>S. Coeln</i>	Lymph nodes
			<i>S. Typhimurium</i>	<i>S. subsp. enterica</i>	Lymph nodes
			<i>S. Virchow</i>	<i>S. Infantis</i>	Lymph nodes

Table 1 Number of **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
20	16	1	<i>S. Lomita</i>	<i>S. subsp. enterica</i>	Lymph nodes
22	16	10	<i>S. Essen</i>	no growth	lymph nodes
			<i>S. Essen</i>	<i>S. Typhimurium</i>	lymph nodes
			<i>S. Choleraesuis</i> var. <i>Kunzendorf</i>	<i>S. subsp. enterica</i>	lymph nodes
			<i>S. Typhimurium</i>	<i>S. subsp. enterica</i>	lymph nodes
			<i>S. Bonariensis</i> (2x)	<i>S. Kottbus</i> (2x)	lymph nodes
			<i>S. Agona</i>	<i>S. Derby</i>	lymph nodes
			<i>S. Colindale</i> (2x)	<i>S. Infantis</i> (2x)	lymph nodes
			<i>S. Give</i>	<i>S. Senftenberg</i>	lymph nodes
23	16	0	-	-	-
24	16	1	<i>S. Bradford</i>	<i>S. Infantis</i>	Carcass swab
25	16	0	-	-	-
26	9	0	-	-	-
27	0	-			
28	16	0	-	-	-
29	6	0	-	-	-
30	16	0	-	-	-
Total	340	32			

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	1	0	-	-	-
2	0	-	-	-	-
3	11	1	Unknown (6,7 : -:-)	<i>S. Oranienburg</i> (6,7,14 : m,t:-)	Lymph nodes
4	3	0	-	-	-
5	3	1	Auto agglutinating*	<i>S. Typhimurium</i>	Carcass swab
6	16	1	<i>Salmonella</i> rough strain	<i>S. subsp. enterica</i> (4,[5],12 : i:-)	Lymph nodes
7	0	-	-	-	-
8	7	7	5x Unknown (4 : -:-)	5x <i>S. Brandenburg</i> (4,[5],12 : l,v:e,n,z ₁₅)	Carcass swab
			1x Unknown (4 : -:-)	1x <i>S. Kimuenza</i> (1,4,12,27 : l,v:e,n,x)	Lymph nodes
			1x Unknown (8 : -:-)	<i>S. Bovismorbificans</i> (6,8,20 : r:1,5)	Lymph nodes
9	12	2	<i>S. subsp. enterica</i> (- : b:l,w)	<i>S. Ohio</i> (6,7,14 : b:l,w)	Lymph nodes
			<i>S. subsp. enterica</i> (6,7 : -:1,5)	<i>S. Thompson</i> (6,7,14 : k:1,5)	Lymph nodes
11	0	-	-	-	-
12	0	-	-	-	-
13	0	-	-	-	-
15	0	-	-	-	-
16	0	-	-	-	-
17	0	-	-	-	-
18	0	-	-	-	-
19	4	0	-	-	-
20	3	1	Unknown (18 : -:-)	<i>S. Cerro</i> (6,14,18 : z ₄ ,z ₂₃ : [1,5])	Lymph nodes
22	5	5	<i>S. group C1</i> (6,7: r)	<i>S. Infantis</i> (6,7,14: r:1,5)	Lymph nodes

*: By molecular typing (PCR and PFGE) the isolate was typed as *S. Typhimurium* by the NRL

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
23	3	0	-	-	-
24	0	-	-	-	-
25	0	-	-	-	-
26	0	-	-	-	-
27	0	-	-	-	-
28	1	0	-	-	-
29	0	-	-	-	-
30	15	0	-	-	-
Total	84	18			

References

Commission Decision Decision 2006/668/EC of 29 September 2006, concerning a financial contribution from the Community towards a baseline survey on the prevalence of *Salmonella* in slaughter pigs to be carried out in the Member States (notified under document number C(2006) 4306).

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

Project number CRL- <i>Salmonella</i>	V/330604/07/CS
Study number strain	FR/pT-3

NRL	France
Name contact person	
E-mail address	

Salmonella strain was isolated from:

- Lymph nodes
- Carcass swabs
- Other, namely.....

Serotyping result of NRL (name serovar):

O – antigens:

H – antigens:

Remarks:

Shipping date:

Date of arrival at CRL:

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

Project number CRL- <i>Salmonella</i>	V/330604/07/CS
Study number strain	FR/pNT-3

NRL	France
Name contact person	
E-mail address	

Salmonella strain was isolated from:

- Lymph nodes
- Carcass swabs
- Other, namely.....

Serotyping result of NRL (name serovar):

O – antigens:

H – antigens:

Remarks:



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