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Editorial Note

Bilthoven, 6 July 2018

Dear colleague,

By the first of May we received the agreement from DG-SANTE on our work-programme for this year. Due to the late receipt of this agreement, we did not yet inform you about this years' work-programme in an earlier Newsletter. Therefore, the **work-programme of 2018** is included in this Newsletter. The lay-out of the work-programme has changed, following the task and duties described in Article 94(2) of Regulation (EU) 625/2017.

By mid-April we have sent the **annual technical report of the activities of EURL-*Salmonella* performed in 2017** to EC DG-SANTE. For your information, this report is also included in this Newsletter.

By the end of May we organised our annual **EURL-*Salmonella* workshop**. For the first time we organised the workshop in Uppsala, Sweden, with the kind help of the NRL-*Salmonella* in Sweden. A total of 45 participants were present at the workshop. The presentations given at the workshop are available at our website since 1 June 2018, see: https://www.eurlsalmonella.eu/Workshops/Workshop_2018

Talking about the **website**, we have to change to new software for management of the website (this is necessary for all 'RIVM websites'). This will result in a new lay-out of the website and in the fact that old documents (approx. > 5 years) will be approachable through a link to the archive of the website. Currently we are in an intermediate phase, meaning that the content of the old website has been migrated to the new version of the website, but the new website is not yet ready for publication. The old version of the website is still available, but will no longer be updated. Updates will be done to the new version of the website, but it may take a while until this is publicly available. Sorry for the inconvenience, but we hope to have the new version of the website life as soon as possible. The url will remain the same (www.eurlsalmonella.eu), so that the change should not affect your links.

After a transition year in 2017, we have now been able to change the order of the interlaboratory comparison studies for detection of *Salmonella* in 2018. Earlier this year the interlaboratory comparison study for detection of *Salmonella* in animal feed was organised and in October the **interlaboratory study for detection of *Salmonella* in samples from the primary production stage** is planned. The timetable of this study is included in this Newsletter. Also included is the timetable of the **23rd interlaboratory comparison study on typing of *Salmonella***, which will be organised in fall 2018 as usually. If we will receive a sufficient number of applications for it, this typing study will also include the first pilot study on MLVA typing.

In the past months, the EURL-*Salmonella* has been approached by EFSA and by an individual NRL-*Salmonella* to ask the help of the NRL network for **outbreak investigations**. You have received calls for molecular data of *S.* Bareilly, *S.* Typhimurium (MLVA 3-14-14-NA-311) and, more recently, *S.* Agona. Thank you all for your fast and helpful replies. Your information can be important in tracing the source(s).

In 2016, **EFSA** sent a **questionnaire** via the EURLs to the NRL networks, to make an inventory **on the use of Whole Genome Sequencing (WGS)** for food- and waterborne pathogens isolated from animals, food, feed and related environmental samples in EU/EFTA countries. Due to the large amount of data, it took a while to prepare the report. Recently the outcome of the questionnaire was reported and published at the EFSA website. Please find the report at the following link: https://www.efsa.europa.eu/en/supporting/pub/en-1432

Another questionnaire on the use of Whole Genome Sequencing (WGS)/Next Generation Sequencing (NGS) was prepared by the EURLs working group on NGS. Each of the 8 EURLs of this working group sent the survey to their NRLs by the end of March 2018. The outcome of all surveys was discussed at a meeting of the working group in June 2018 and it was agreed to perform some common analysis. A summary of the outcome of the survey held amongst the NRLs-*Salmonella* was presented at the EURL-*Salmonella* workshop in May 2018. The information can be found in the last presentation given at this workshop ('closure') and can be found at the EURL-*Salmonella* website: https://www.eurlsalmonella.eu/Workshops/Workshop_2018

From 18 to 22 June 2018, **the annual meeting of ISO/TC34/SC9 and CEN/TC275/WG6** was organized in Lausanne, Switzerland. A summary on relevant '*Salmonella*-related items' as discussed at these meetings will be published in the next Newsletter. For now, it is important to know that it has been agreed to draft an **amendment to EN ISO 6579-1:2017**, for the following items:

- To change the status of Annex D on detection of *Salmonella* Typhi and *Salmonella* Paratyphi from normative to informative. The normative status of the current Annex D causes confusion at several laboratories. Some laboratories have the impression that this Annex always has to be followed when analysing 'routine' samples, which is not the case. Annex D of EN ISO 6579-1 should only be followed if *S.* Typhi and/or *S.* Paratyphi is specifically sought (e.g. in case of outbreaks). To prevent from further confusion, it was decided to amend the status of this annex.
- To include a few corrections in Annex D, especially concerning the composition of Selenite cystine medium (broth) in Annex D.3. Currently it is indicated that 100 ml L-cystine solution (D.3.1.2) should be added to 1000 ml base medium, but this has to be only 10 ml.
- To indicate that for incubation of selective media also a temperature range of 34 to 38 °C can be used, like for incubation of non-selective culture media. This is the outcome of the comparison study performed by 9 laboratories in 2017. In this study a total of 855 (routine) samples were analysed for the detection of *Salmonella*, with incubation of the selective media at 35 °C and at 37 °C.

Reports published in May and June 2018:

Pol-Hofstad, I.E. and Mooijman, K.A. The 20th EU Interlaboratory comparison study in Primary Production (2017) - Detection of *Salmonella* in chicken faeces. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM Report no.: 2017-0083 (May 2018). The report was sent to the participants by the end of May 2018 and will soon also become available through the EURL-*Salmonella* website.

Mooijman, K.A. The 22nd EURL-*Salmonella* workshop – 29 and 30 May 2017, Zaandam, the Netherlands. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM Report no.: 2017-0080 (June 2018). https://www.rivm.nl/bibliotheek/rapporten/2017-0080.pdf

In the past year, two **publications** have been drafted related to the new EN ISO 6579-1:2017. The first publication gives information on the changes compared to EN ISO 6579:2002 and was published in May 2018:

Mooijman, K.A., 2018. The new ISO 6579-1: A real horizontal standard for detection of *Salmonella*, at last! Food Microbiology 71 (2018), 2-7. This publication was in press, available on line since 18-03-2017. http://dx.doi.org/10.1016/j.fm.2017.03.001 (special issue I3S 2016).

The second publication concerns the validation of EN ISO 6579-1 and has been accepted in May 2018. This article will be published, together with articles describing 14 other validation studies, in a special issue of the International Journal of Food Microbiology (validation studies performed under the CEN Mandate M/381):

Mooijman, K.A., Pielaat, A. and Kuijpers, F. A. Validation of EN ISO 6579-1 - Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 1 Detection of *Salmonella* spp. Revised paper submitted 17-11-2017 to International Journal of Food Microbiology (special issue CEN mandate). Paper accepted and on line since 12-05-2018: https://doi.org/10.1016/j.ijfoodmicro.2018.03.022

Best wishes,
Kirsten Mooijman
Coordinator EURL-*Salmonella*

Contribution of the EURL-*Salmonella*

Timetable EURL-*Salmonella* interlaboratory comparison study on the detection of *Salmonella* spp. in samples from the primary production stage (2018)

Week (2018)	Dates	Subject
37	In week of 10 September	Mailing of the protocol, lab code, and the questions of the web based test report to the NRLs by email.
38	In week of 17 September	Sending the link for the electronic results form to the participants by email.
39	24 September	Mailing of the parcels to the NRLs as Biological Substance Cat. B (UN3373) by DHL courier service Preparation of the media by the NRLs
40	1 October	Performance of the study
43	Before 26 October	Deadline for completing the electronic submission of results: **26 October** (23:59) After this deadline the electronic submission form will be closed.

Timetable of the 23rd interlaboratory comparison study (2018) on serotyping and optional PFGE typing and/or MLVA typing of *Salmonella* for NRLs-*Salmonella*

Week	Date	Topic
36	3-7 September	Request for participation PFGE typing *and/or Pilot MLVA typing* (serotyping is obligatory for EU NRLs).
43	22-26 October	Emailing of the protocol 2018.
45	5-9 November	Shipment of the parcels to the participants as Biological Substance Category B (UN 3373). If you did not receive the parcel by **9 November**, please contact the EURL-*Salmonella*.
45	5-9 November	Sending the link and the password for the web based test report on serotyping to the participants.
45	5-9 November	Sending the link for the dedicated web based test report on PFGE typing to the participants in a separate email. The pre-configured database and instructions for use in case of (optional) analysis of a provided gel in Bionumerics will be included in this email as well.
45	5-9 November	Sending the link for the dedicated web based test report on MLVA typing to the participants in a separate email.
45	5-9 November	*Upon receipt*: Starting the identification of the strains, according to the usual practice of the laboratory.
50	14 December 2018 at the latest	Deadline for completing the electronic submission of **serotyping** results: **14 December 2018** (23:59 h CET) After this deadline, the electronic submission form for serotyping results will be closed.
51	21 December 2018 at the latest	Deadline for completing the electronic submission of **PFGE typing** results: **21 December 2018** Deadline for completing the electronic submission of **MLVA typing** results: **21 December 2018**
	February 2019	Serotyping: Reporting of individual laboratory results and Interim Summary Report.
	April 2019	Pilot MLVA typing: Reporting of individual laboratory results and Summary Report.
	May 2019	PFGE typing: Reporting of individual laboratory results and Summary Report.
	Summer 2019	Final report.

EURL-*Salmonella* work-programme 2018

INTRODUCTION

In this document the activities of the EURL-*Salmonella* are described for the year 2018. These activities are based on the responsibilities and tasks described in Article 94 of Regulation (EU) 2017/625 for European Union reference laboratories.

Regulation (EU) 625/2017 Art 94(2):
European Union reference laboratories designated in accordance with Article 93(1) shall be responsible for the following tasks insofar as they are included in the reference laboratories' annual or multiannual work programmes that have been established in conformity with the objectives and priorities of the relevant work programmes adopted by the Commission in accordance with Article 36 of Regulation (EU) No 652/2014:
(taking into account Art 147 of (EU) 625/2017)

1 TO ENSURE AVAILABILITY AND USE OF HIGH QUALITY METHODS AND TO ENSURE HIGH QUALITY PERFORMANCE BY NRLs.

Please, provided activities related to Regulation (EU) 2017/625:
(Number of Sub-activity boxes can be adjusted by EURL)

- *Art. 94.2.a* ***Providing national reference laboratories with details and guidance on the methods of laboratory analysis, testing or diagnosis, including reference methods.***
- *Art. 94.2.b* ***Providing reference materials to national reference laboratories***
- *Art. 94.2.c* ***Coordinating the application by the national reference laboratories and, if necessary, by other official laboratories of the methods referred to in point (a), in particular, by organising regular inter-laboratory comparative testing or proficiency tests and by ensuring appropriate follow-up of such comparative testing or proficiency tests in accordance, where available, with internationally accepted protocols, and informing the Commission and the Member States of the results and follow-up to the inter-laboratory comparative testing or proficiency tests.***
- *Art. 94.2.l* ***Where relevant for their area of competence, cooperate among themselves and with the Commission, as appropriate, to develop methods of analysis, testing or diagnosis of high standards.***

Sub-activity 1.1 *Analytical methods*

Objectives:
- Standardisation of methods.
- Keep track of developments in (alternative) methods.
- Provide NRLs with information on developments of relevant (standardised/new) analytical methods.

Description:

Standardisation of methods

The EURL-*Salmonella* is involved (as project leader or as member of working groups or task advisory groups) in several activities of ISO and CEN. More specific in:

- ISO/TC34/SC9: International Standardisation Organisation, Technical Committee 34 on Food products, Subcommittee 9 – Microbiology of the food chain.
- CEN/TC275/WG6: European Committee for Standardisation, Technical Committee 275 for Food analysis – Horizontal methods, Working Group 6 for Microbiology of the food chain.

For the following groups of ISO/TC34/SC9 and CEN/TC275/WG6, staff members of EURL-*Salmonella* have the leadership. Activities for these groups will be continued in 2018:

ISO-WG3 'Method validation' –part 6 of EN ISO 16140 on 'validation of confirmation methods', project leader Wilma Jacobs and co-project leader Kirsten Mooijman.

The development of a procedure for validation of (proprietary) alternative confirmation and typing methods started in 2014. By the end of 2017/early 2018 it is expected that the voting for the Draft International Standard (DIS) of ISO 16140-6 will be launched. After this, the comments will need to be discussed with the members of WG3 and amendments will need to be introduced in a next draft version of ISO 16140-6, after which the voting for the Final Draft International Standard (FDIS) is likely to be launched in the second half of 2018.

ISO - Ad hoc group 'Checklist to avoid ambiguity in drafting standards in food microbiology', project leader Kirsten Mooijman and co-project leader Wilma Jacobs. At the annual meeting of 2013, the need of a checklist for writing standards for ISO/TC34/SC9 and CEN/TC275/WG6 was indicated, which would function as a checklist for convenors and project leaders to make sure that standards in food microbiology will become as uniform as possible. The document will become an internal guidance document for SC9 and WG6. As Kirsten Mooijman and Wilma Jacobs have much experience in writing standards, they were asked to become project leader and co-project leader respectively. Up to 2017, several draft versions have been prepared and discussed and it is expected that a more final version will become available by early 2018. However, this guidance document is likely to be a 'dynamic' document and may need regular updating to keep the information up to date.

ISO - Ad hoc group 'Harmonisation of incubation temperatures', project leader Kirsten Mooijman. During the drafting of the EN ISO documents for detection and serotyping of *Salmonella*, it was discussed whether the temperature range for incubation of media could be made broader (34-38 °C), to harmonise the different incubation temperatures used worldwide (e.g. 35 °C in USA and 37 °C in Europe). At the annual meeting in 2013, the broadening of the temperature ranges for incubation of non-selective media for culturing different bacteria (not only *Salmonella*) was agreed. For incubation of selective media at a broader temperature range, experiments were performed by members of ISO and CEN in 2016 and 2017. The first results of these experiments were presented by the project leader at the annual meeting of SC9 and WG6 in 2017. Additional analysis of confirmed data will be performed by the end of 2017/early 2018. The outcome of the final analysis will be reported and presented at the annual meeting of SC9 and WG6 in 2018.

CEN/ISO - TAG3/WG10 'ISO/TS 6579-4 PCR identification of monophasic *Salmonella* Typhimurium'. Project leaders for this activity are Burkhard Malorny (NRL-*Salmonella* Germany) and Kirsten Mooijman (EURL-*Salmonella*). A draft Technical Specification (TS) has been prepared by CEN-TAG3 in 2017. In this document 3 PCR procedures are described. For determination of the performance characteristics of the PCR procedures, a 'standard set of test strains' is needed. After a call for strains, the EURL-*Salmonella* received approximately 400 strains by spring 2017. In 2017 the identity of all strains was verified by the EURL and a further selection will be made before the end of 2017. This subset of strains will be used to verify the performance of the PCR procedures by the NRL-*Salmonella* in Germany and by the EURL-*Salmonella* in 2018. As a result of this verification study it may be necessary to update the draft document, after which the work is likely to be moved from CEN-TAG3 to ISO-WG10

(possibly in fall 2018). After agreement on the final draft document, an interlaboratory study will be organised with a selection of strains to determine the performance characteristics of the three PCR protocols. This interlaboratory study is not expected to be organised before 2019.

In the following groups in ISO and CEN, a staff member of EURL-*Salmonella* participates and contributes to the projects. Activities for these groups will be continued in the coming years:
CEN - TAG9 'Improvement of the pre-enrichment step to enhance the recovery of Gram negative bacteria'.
ISO - WG4 'Revision of ISO/TS 22117 on organisation of Proficiency Testing'.
ISO - WG25 'Whole-genome sequencing for typing and genomic characterization'.

The plenary meeting of both ISO/TC34/SC9 and CEN/TC275/WG6 will be organised in Switzerland in June 2018. One representative of the EURL-*Salmonella* will participate in these meetings.

Development of (alternative) methods
Several (proprietary) alternative (molecular) methods have been developed for the detection of *Salmonella*. The application of these methods depends on its validation. Certificates of validated methods (following ISO 16140-2) are published by the relevant validation organisations (Afnor validation, MicroVal). The EURL-*Salmonella* will keep track on developments in alternative methods by regularly checking the literature and the information from validation organisations.
For validation of alternative (proprietary) confirmation and typing methods no internationally accepted protocol existed up to 2017, meaning that no certificates of validated alternative confirmation or typing methods have been published so far. This may change after publication of ISO 16140-6, although it may last a few more years before the first certificates of validated alternative confirmation/typing methods will be published. For serotyping of *Salmonella* several alternative methods (already) exist (multiplex PCR, Whole Genome Sequencing) and some NRLs may have performed an internal validation to show the usefulness of these methods. When needed, the EURL-*Salmonella* can advise on the protocol for this internal validation until the full validation will be performed following ISO 16140-6.

Expected Output:
- DIS voting and FDIS voting of ISO 16140-6.
- Publication of first version of Guidance document for drafting standards in food microbiology.
- Report and presentation on the outcome of experiments in which incubation at 35 °C and at 37 °C is compared.
- Verification studies of the PCR protocols for identifying monophasic *Salmonella* Typhimurium with a selected set of test strains in two laboratories.
- Report annual meeting ISO/TC34/SC9 and CEN/TC275/WG6.
- Overview literature on new/alternative methods published in the EURL-*Salmonella* Newsletter, every 3 months.

Duration:
Whole 2018 and most ISO and CEN activities will continue after 2018.

Sub-activity 1.2 *EURLs working group on NGS*

Objectives:
- Promote the use of NGS across the EURLs' networks.
- Build capacity on producing and using NGS data within the EU.
- Ensure liaison with the work of the EURLs and the work of EFSA and ECDC on NGS.

Description:
In 2017 a working group of 8 EURLs was raised on Next Generation Sequencing (NGS). The working group will work on the following activities in relation to NGS:
1) Proficiency Testing
2) NGS laboratory procedures (SOPs)
3) Bioinformatics tools
4) NGS cluster analysis
5) Bench marking
6) Trainings on NGS
7) Reference and confirmatory testing using NGS
8) Follow-up of ISO activities on WGS
The lead for each activity is distributed over the 8 EURLs. EURL-*Salmonella* will have the lead of activity 7) and will participate in the other activities. For each activity, guidance documents will be drafted and discussed in the working group.
To have a better view on the needs of the NRLs concerning NGS, the working group will conduct a survey by the end of 2017/early 2018 among all NRLs in the 8 networks. For drafting the questions for this survey, the EFSA questionnaire on the availability of 'Whole Genome Sequencing (WGS) methods for food- and waterborne pathogens' conducted in 2016 will be used. The outcome of the survey will be discussed in the working group at the meeting in spring 2018.
Another meeting of the working group is foreseen in fall 2018.

Expected Output:
In relation to the whole working group:
- Conducting a survey on the needs concerning NGS among all NRLs of the 8 networks.
- Summary of the results of this survey.

In relation to activity 7):
- Draft guidance document for information needed for reference strains; for the use of and/or validation of the use of NGS for confirmatory testing and typing.

In relation to all activities:
- Summary on the progress and planning of the different activities.

Duration:
Whole 2018 and most activities will continue after 2018.

Sub-activity 1.3 *Interlaboratory comparison studies*

Objectives:
Organisation of interlaboratory comparison studies to gain information on the performance of the NRLs-*Salmonella* for detection and typing of *Salmonella.*

Description:
Organisation of 3 interlaboratory comparison studies:
1. One study on detection of *Salmonella* in samples from the primary production stage.
2. One study on detection of *Salmonella* in food or animal feed samples.
3. One study on typing of *Salmonella* (serotyping, PFGE and/or MLVA).

For the set-up of the detection studies, EN ISO/TS 22117 ('Microbiology of food and animal feeding stuffs – Specific requirements and guidance for proficiency testing by interlaboratory comparison') will be followed.
The choice of the *Salmonella* serovars, the contamination levels of the samples, the type of matrix, the number of samples, as well as the protocol for artificially contaminating the

samples will be established for each study. Whenever possible, the samples will be artificially contaminated individually at the laboratory of the EURL-*Salmonella.* Homogeneity and stability of the samples will be tested in advance of each interlaboratory study.
For the transport of the samples to the NRLs, the materials will be packed and shipped in accordance with the IATA rules for shipping UN 3373 materials (biological substance category B).
For the reporting of the results by the NRLs for *Salmonella* to the EURL, electronic (web-based) test reports will be used. These test reports are amended for each study.

The results of each NRL will be evaluated and compared with the pre-set definition of 'good performance' per study. In case of unexplainable 'poor performance', the follow-up will be discussed with the relevant NRL. A follow-up can exist of either one of the following activities, or of a combination of these activities:
- Sending additional samples, which need to be tested according to a prescribed protocol;
- Training at the EURL for *Salmonella*;
- Visiting the poor performing NRL by staff members of the EURL-*Salmonella*.

Additional to the judgement 'good performance', or 'poor performance', the results of an NRL can also be judged as 'moderate performance'. The criteria to define a performance as 'moderate' are described per study. The actions after moderate performance are less stringent than after poor performance. In case of moderate performance, the performance of the NRL over several consecutive studies is judged. If moderate performance is seen in three consecutive studies, the NRL will be contacted by the EURL to discuss a proper follow-up. The type of follow-up will be considered on a case by case basis depending on the nature of the moderate performance. A visit of a staff member(s) of the EURL-*Salmonella* to the NRL can be considered as a possible follow-up. In case of repeated moderate performance (like for poor performance), DG-Sante will be informed.

Additional to the NRLs of the 28 EU Member States, also NRLs-*Salmonella* of other countries regularly participate in one or more interlaboratory studies. It is proposed that the costs for shipment of samples to NRLs of the following countries will be part of the budget of EURL-*Salmonella* for 2018:
- Northern Ireland;
- EFTA countries Iceland, Norway and Switzerland;
- Candidate or potential candidate countries Bosnia and Herzegovina, Former Yugoslav Republic of Macedonia (FYROM) and Serbia

Expected Output:
Organisation of 3 interlaboratory comparison studies. Interim summaries (shortly after each study) and full reports (later) of the results of each interlaboratory comparison study.

Duration:
Preparation and testing of samples, organisation and reporting of the three studies will be divided over 2018. Some activities may continue in 2019 (like follow-up study, reporting).

2 TO PROVIDE SCIENTIFIC AND TECHNICAL ASSISTANCE TO NRLs

Please, provided activities related to Regulation (EU) 2017/625:
(Number of Sub-activity boxes can be adjusted by EURL)

- *Art. 94.2.d* ***Coordinating practical arrangements necessary to apply new methods of laboratory analysis, testing or diagnosis, and informing national reference laboratories of advances in this field.***
- *Art. 94.2.e* ***Conducting training courses for staff from national reference laboratories and, if needed, from other official laboratories, as well as of experts from third countries.***
- *Art. 94.2.g* ***Providing information on relevant national, Union and international research activities to national reference laboratories.***

Sub-activity 2.1 *Workshop*

Objectives:
Exchange of information on the activities of the NRLs-*Salmonella* and the EURL-*Salmonella*.
Exchange of information on (new) developments in the relevant work field.

Description:
Every year, the EURL-*Salmonella* organises a workshop for the NRLs for *Salmonella*. The workshops are generally organised in May and will last 1,5-2 days. For 2018 the workshop will be organised in Sweden, with the help of the NRL-*Salmonella* in Sweden.
The programme of the workshops may contain the following items:

- Introductory presentations (e.g., by EU representative and EURL-*Salmonella*);
- Zoonoses in Europe (EFSA, DG-Sante);
- Results of (research) activities of EURL-*Salmonella*;
- Results of interlaboratory comparison studies as organised by EURL-*Salmonella*;
- Experiences, problems, results in relation to monitoring surveys for *Salmonella*;
- Plans and results of (research) activities of the NRLs-*Salmonella*;
- Discussion on methods (e.g. typing, molecular, serological);
- Activities in ISO and CEN;
- Future working plan of EURL-*Salmonella*;
- Information on research in relation to *Salmonella* by one or more guest speakers.

Additional to the NRLs of the 28 EU Member States, also NRLs-*Salmonella* of other countries regularly participate in the workshops. These concern the NRLs of the same 'third' countries which participate in the interlaboratory studies (see 1.3). Therefore it is proposed that participation of one delegate per NRL of the following countries will be part of the budget of EURL-*Salmonella* for 2018:
- Northern Ireland;
- EFTA countries Iceland, Norway and Switzerland;
- Candidate or potential candidate countries Bosnia and Herzegovina, Former Yugoslav Republic of Macedonia (FYROM) and Serbia;
- Additional, 2-3 Guest speakers from different countries are foreseen;
- Additional, a delegate from the EURL for monitoring viral and bacteriological contamination of bivalve molluscs (EURL-BM) will be invited, as this EURL will discontinue on 31 December 2018 and its activities for *Salmonella* in bivalve molluscs shall be taken over by the EURL-*Salmonella*.

Expected Output:
- Publication of the presentations of the workshop at the EURL-*Salmonella* website (www.eurlsalmonella.eu) shortly after the workshop;
- Report of the workshop, including a summary of the discussion performed per item at the workshop and the outcome of the evaluation of the workshop.

Duration:
The workshop itself will last 1,5-2 days. Organisation and reporting will last several months (before and after the workshop). Finalisation of the report may continue in 2019.

Sub-activity 2.2 *Training courses*

Objectives:
To train NRLs-*Salmonella* in a specific work field.

Description:
The following training courses are foreseen:
1. Upon request of an NRL, the EURL can give a training course for a specific need of an NRL, which can be on detection and typing of *Salmonella* (including serotyping and molecular typing).
2. Upon advise of the EURL, an NRL will follow a training at the EURL or staff members of the EURL give a training at the laboratory of the NRL, especially in case of (repeated) poor performance of the NRL in interlaboratory comparison studies.
3. Training on the use of the software package BioNumerics for PFGE profile analysis. A 2-days' training session is foreseen on PFGE profile analysis, on the management of the metadata related with each isolate and on submission of the data to the EFSA molecular typing database. The training session will be organised, like in 2016 and 2017, in cooperation with the EURL-VTEC (ISS, Italy) and the EURL-*Listeria monocytogenes* (ANSES, France) at the premises of EURL-*Salmonella* (the Netherlands). For this a didactic room equipped with at least 12 computer workstations will be made available, and the course will be attended by representatives of 4 NRLs for VTEC, 4 NRLs for *Listeria monocytogenes* and 4 NRLs for *Salmonella*. The training will be given by staff members of the three EURLs. The aim is to train the participants in correctly performing band assignment and profile analysis and identification of relatedness between PFGE profiles.

Additional to the NRLs of the 28 EU Member States it is desirable to offer training courses to NRLs-*Salmonella* of one or more of the following (third) countries:
- EFTA countries Iceland, Norway and Switzerland;
- Candidate or potential candidate countries Bosnia and Herzegovina, Former Yugoslav Republic of Macedonia (FYROM) and Serbia.

Expected Output:
The training courses intend to lead to improved performance of the NRLs in the relevant work field. Details on each training course as well as the results of the evaluation will be summarised in the annual technical report of the EURL-*Salmonella*.
Duration:
The duration of training courses 1 and 2 will depend on the set up of the course and the needs of the NRLs, but in general will vary between 2 and 5 days.
Training course 3 will last 1.5-2 days, but the organisation and reporting will last several weeks (before and after the training course).

Sub-activity 2.3 *Scientific advice and support of NRLs*

Objectives:
Provide scientific and technical assistance to the NRLs-*Salmonella* for the relevant work field. Perform confirmatory testing for NRLs when needed. Maintenance of the EURL-*Salmonella* website and keeping the information on the website up to date. Inform the NRLs of the activities of the EURL and other parties in the relevant work field, as well as of developments in this field.

Description:

The EURL-*Salmonella* is regularly contacted by various parties, i.e. NRLs-*Salmonella*, other institutes in Member States, (potential) Candidate Member States or (other) third countries, with requests for information or for participation in activities being organised. Whenever possible, the EURL-*Salmonella* will provide assistance to the parties concerned.

Information relevant for the NRLs for *Salmonella* as well as for other parties is published on the website of the EURL-*Salmonella*, www.eurlsalmonella.eu. Every three months the EURL-*Salmonella* publishes a newsletter with information from the EURL-*Salmonella,* from the NRLs-*Salmonella* and/or other information related to the work field. Also, a literature search of developments in the work field is included in each newsletter covering the previous 3-months period. The NRLs will be notified by email when new information is published.

In 2017 the EURL *Listeria monocytogenes* proposed to develop, together with other biological EURLs, a harmonised guidance document on outsourcing part of proficiency tests organised by NRLs for national networks. NRLs in the different EURL/NRL networks expressed their need for such a guidance document. In 2018 further development of this joint document will be performed.

The EURL-*Salmonella* will perform confirmation and/or typing of samples/isolates from NRLs-*Salmonella* for e.g. second opinion and information on subtype, whenever needed.

Expected Output:
- Scientific and technical support of NRLs and other parties in the relevant work field.
- An up to date website.
- Publication of 4 newsletters (per year) through the website.
- Final (draft) harmonised guidance document on outsourcing part of proficiency tests organised by NRLs for national networks.
- Confirmation of samples/isolates when applicable.

Duration:
Continuous activities divided over 2018. Some activities may continue in 2019.

3 TO PROVIDE SCIENTIFIC AND TECHNICAL ASSISTANCE TO THE EUROPEAN COMMISSION AND OTHER ORGANISATIONS

Please, provided activities related to Regulation (EU) 2017/625:
(Number of Sub-activity boxes can be adjusted by EURL)

- *Art. 94.2.f* ***Providing scientific and technical assistance to the Commission within the scope of their mission.***
- *Art. 94.2.h* ***Collaborating within the scope of their mission with laboratories in third countries and with the European Food Safety Authority (EFSA), the European Medicines Agency (EMA) and the European Centre for Disease Prevention and Control (ECDC).***
- *Art. 94.2.i* ***Assisting actively in the diagnosis of outbreaks in Member States of foodborne, zoonotic or animal diseases, or of pests of plants, by carrying out confirmatory diagnosis, characterisation and taxonomic or epizootic studies on pathogen isolates or pest specimens.***

Sub-activity 3.1 *Scientific advice and support of European Commission and other organisations*

Objectives:
Provide scientific and technical assistance to EC DG SANTE for the relevant work field. Provide assistance to DG SANTE, EFSA and (NRLs of) Member States in case of (international) *Salmonella* outbreaks. Collaborate with EFSA and ECDC for the relevant work field. Cooperation with other biological EURLs.

Description:
The EURL-*Salmonella* will provide ad-hoc scientific and technical assistance to DG SANTE on different subjects in relation to *Salmonella* (e.g. amendment of legislation, methods for detection and typing of *Salmonella*, validation of (alternative) methods).

The EURL-*Salmonella* will participate in relevant (expert) working group and scientific committees of DG SANTE and EFSA.

The EURL-*Salmonella* will assist DG SANTE, EFSA, NRLs, and (if relevant) ECDC in case of (international) *Salmonella* outbreaks. Amongst others this may include: keeping close contact with the NRL network (e.g. asking NRLs for information, (sub)typing data, isolates for further (sub)typing, sharing information); (sub)typing suspect isolates, using for example Multiple Locus Variable number of tandem repeat Analysis (MLVA) and Whole Genome Sequencing (WGS)/Next Generation Sequencing (NGS) technologies and by helping with analysis and interpretation of the data.

The EURL-*Salmonella* will perform curation of PFGE data which will be uploaded in the EFSA database intended for the collection of molecular typing data from pathogens isolated from food, animal feed and animals and its environment. The criteria for judging the quality of PFGE data are summarised in an EFSA-SOP, which was drafted in a joint cooperation with EURL-*Salmonella*, EURL-VTEC, EURL-*Listeria monocytogenes*, EFSA (for non-human strain profiles), ECDC and the curator of the ECDC molecular database (for human strain profiles). The three EURLs and the ECDC curator will meet, at least, once a year to exchange information in relation to the curation of molecular data and to keep harmonised curation procedures. The curation meeting of 2018 will be organised in conjunction with the BioNumerics training course (see 2.2) in Bilthoven, the Netherlands. The management of the joint EFSA-ECDC molecular typing database is performed by the joint EFSA-ECDC Steering Committee, in which members of the organisations mentioned above participate. This Steering Committee will meet twice a year, generally in the country of the chair of that year (the chairmanship alternates between EFSA and ECDC). In 2018, the chairmanship is in hands of ECDC so that it is expected that the meetings will be organised in Stockholm, Sweden.

Due to the planned departure of the United Kingdom from the European Union, the EURL-bivalve molluscs will cease to exist by 01-01-2019 and its activities will be distributed over other EURLs. For that reason, *Salmonella* in bivalve molluscs will become part of the activities of EURL-*Salmonella* from 01-01-2019. In 2018, it will be investigated in more detail what activities this will concern and what activities may possibly be combined with other EURL(s). For example, currently the EURL-bivalve molluscs organises comparative tests for which some samples contain both *E. coli* and *Salmonella*. It will be investigated if such combined studies can be organised after 2018 as well, by a cooperation of EURL-*Salmonella* and EURL-*E. coli.* In preparation to the new activities, it is foreseen to meet twice with the relevant EURLs and the EURL-bivalve molluscs in 2018. Additional it is foreseen that the EURLs which will take over the activities, will participate in the workshop of the EURL-bivalve molluscs as well, to get acquainted with the NRL network and vice versa.

Expected Output:
- Scientific and technical advices when needed.
- Summary of (substantial) advices in the annual technical report.
- Assistance in case of outbreaks, including (sub)typing of isolates when needed.
- Curation of PFGE data of the EFSA molecular database.
- Minutes of curators meeting.
- Minutes of meetings EFSA-ECDC steering committee.
- Plan for transfer of activities from EURL-bivalve molluscs to EURL-*Salmonella* (and other EURLs concerned).

Duration:
Continuous activities and meetings divided over 2018.

4 REAGENTS AND REFERENCE COLLECTIONS

Please, provided activities related to Regulation (EU) 2017/625:
(Number of Sub-activity boxes can be adjusted by EURL)

- *Art. 94.2.*j ***Coordinating or performing tests for the verification of the quality of reagents and lots of reagents used for the diagnosis of foodborne, zoonotic or animal diseases and pests of plants.***
- *Art. 94.2.k* ***Where relevant for their area of competence, establishing and maintaining:***
  - ***i. reference collections of pests of plants and/or reference strains of pathogenic agents;***
  - ***ii. reference collections of materials intended to come into contact with food used to calibrate analytical equipment and provide samples thereof to national reference laboratories;***
  - ***iii. up-to-date lists of available reference substances and reagents and of manufacturers and suppliers of such substances and reagents.***

Sub-activity 4.1 *Reference strains and reference materials*

Objectives:
Supply information on available culture collections and suppliers of microbiological reference materials.

Description:
Information on the *Salmonella* serovar names and formulas is available in the so-called White-Kauffmann-Le Minor scheme, which has been published by the WHO collaborating Centre for Reference and Research on *Salmonella*, situated at Institute Pasteur, Paris in 2007 ('Antigenic formulae of the *Salmonella* serovars'). A link to this scheme is available at the website of the EURL-*Salmonella*. Supplements to the White-Kauffmann-Le Minor (WKLM) scheme (new serovars) are published in a journal of Institute Pasteur. It is necessary to regularly check the accessibility of the WKLM scheme and to update the information on published supplements.

Culture collections of reference strains are available from different organisations, like the National Collection of Type Cultures (NCTC, UK), the American Type Culture Collection

(ATCC, USA), the Collection de l'Institut Pasteur (CIP, France). These organisations maintain the strains in a controlled way, making sure that properly defined strains are available for a user. The EURL-*Salmonella* website will be updated with information on culture collections.
The EURL-*Salmonella* also stores an 'in-house' collection of *Salmonella* strains which were collected from different projects performed at the National Institute for Public Health and the Environment (RIVM). New/interesting strains will regularly be added to this collection. This collection is mainly intended for 'in-house' use, e.g. for use in interlaboratory comparison studies and testing/verification of methods. Occasionally, strains of this 'in-house' collection will be provided to NRLs when needed for specific tests.

Microbiological reference materials for use in, for example, first line quality control are produced by different organisations. Examples of reference material producers are given at the EURL-*Salmonella* website. Additional producers (when available) will be added to this information on the website.

Expected Output:
Up to date information on reference strains and reference materials at the EURL-*Salmonella* website. This sub-activity is considered to be part of the sub-activity for keeping all information at the EURL-*Salmonella* website up to date. Therefore the planning and output of this sub-activity 4.1 is merged with sub-activity 2.3.

Duration:
Continuous activities divided over 2018.

Technical report on activities of the EURL-*Salmonella* 2017

K.A. Mooijman
5 April 2018

National Institute for Public Health and the Environment (RIVM)
Centre for Zoonoses and Environmental microbiology (Z&O)

Letter-report 038/2018 Z&O Mo/km
RIVM project-number: E/114506/17

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Introduction

The work plan of the EURL-*Salmonella* for the year under review, 2017, was submitted to the European Commission in September 2015 as part of a two-year work programme (2016 and 2017). This report details the activities of the EURL-*Salmonella* according to the agreed work plan for 2017.

General

In 2017, the following activities were carried out:

1. Organisation of three interlaboratory comparison studies
2. Organisation of a workshop with the NRLs-*Salmonella*
3. Performance of supporting activities
4. Giving assistance to the Commission and ad hoc activities
5. Communication
6. Training
7. Molecular typing of *Salmonella* spp.
8. Missions

Deliverables

Reports

In 2017, the following reports were published:
Pol-Hofstad, I.E. and Mooijman, K.A. The 19th EU Interlaboratory comparison study in Primary Production (2016) - Detection of *Salmonella* in chicken faeces adhering to boot socks. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM Report no.: 2016-0044. The interim summary of this interlaboratory comparison study was published in April 2016 and is available through the EURL-*Salmonella* website:
http://www.eurlsalmonella.eu/dsresource?type=pdf&disposition=inline&objectid=rivmp:315274&versionid=&subobjectname
The final report was published in February 2017 (and some errata in March 2017) and is available through the EURL-*Salmonella* website:
https://www.rivm.nl/dsresource?objectid=9dae5131-3a49-41f7-9d67-e3617bf4f295&type=pdf&disposition=inline

Kuijpers A.F.A. and Mooijman, K.A. EU Interlaboratory comparison study food VII (2015) - Detection of *Salmonella* in whole liquid chicken egg. National Institute for Public Health and the Environment, Bilthoven, The Netherlands. RIVM Report no.: 2016-0042. The interim summary of this interlaboratory comparison study

was published in November 2015 and is available through the EURL-*Salmonella* website:
http://www.eurlsalmonella.eu/dsresource?type=pdf&disposition=inline&objectid=rivmp:293851&versionid=&subobjectname
The final report was published in May 2017 and is available through the EURL-*Salmonella* website: https://www.rivm.nl/bibliotheek/rapporten/2016-0042.pdf

The following report was published early 2018:
Kuijpers A.F.A. and Mooijman, K.A. EURL-*Salmonella* 8th interlaboratory comparison study Food 2016 - Detection of *Salmonella* in minced chicken meat. The interim summary of this interlaboratory comparison study was published in November 2016 and is available through the EURL-*Salmonella* website:
http://www.eurlsalmonella.eu/dsresource?type=pdf&disposition=inline&objectid=rivmp:325328&versionid=&subobjectname
The final report was published in February 2018 and is available through the RIVM website: https://www.rivm.nl/bibliotheek/rapporten/2017-0081.pdf

The following reports are in the pipeline for publication in spring 2018:
Pol-Hofstad, I.E. and Mooijman, K.A. The 20th EU Interlaboratory comparison study in Primary Production (2017) - Detection of *Salmonella* in chicken faeces. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM Report no.: 2017-0083. The interim summary of this interlaboratory comparison study was published in June 2017 and is available through the EURL-*Salmonella* website:
https://www.eurlsalmonella.eu/dsresource?type=pdf&disposition=inline&objectid=rivmp:331946&versionid=&subobjectname
The final report is likely to be published in spring 2018.

Mooijman, K.A. The 22nd EURL-*Salmonella* workshop – 28 and 30 May 2017, Zaandam, the Netherlands. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM Report no.: 2017-0080. The presentations were published on the EURL-*Salmonella* website on 2 June 2017: https://www.eurlsalmonella.eu/Workshops/Workshop_2017. The draft report was sent to DG-Sante in February 2018. The final report is likely to be published in spring 2018.

The following reports are under preparation:
Jacobs-Reitsma, W.F., Maas, H.M.E., Bouw, E. and Mooijman, K.A. 20th EURL-*Salmonella* interlaboratory comparison study (2015) on typing of *Salmonella* spp. RIVM report no.: 2016-0043. The interim summaries (one for serotyping and one for PFGE typing) of this interlaboratory comparison study were published in February 2016 and are available through the EURL-*Salmonella* website:
Serotyping:http://www.eurlsalmonella.eu/dsresource?type=pdf&disposition=inline&objectid=rivmp:304857&versionid=&subobjectname
PFGE:https://www.eurlsalmonella.eu/dsresource?type=pdf&disposition=inline&objectid=rivmp:315273&versionid=&subobjectname
The draft report was finalised in October 2017. The final report is under preparation.

Jacobs-Reitsma, Verbruggen, A., Bouw, E. and Mooijman, K.A. 21st EURL-*Salmonella* interlaboratory comparison study (2016) on typing of *Salmonella* spp. RIVM report no.: 2017-0082. The interim summaries (one for serotyping and one for PFGE typing) of this interlaboratory comparison study were published in February 2016 and are available through the EURL-*Salmonella* website:
Serotyping:https://www.eurlsalmonella.eu/dsresource?type=pdf&disposition=inline&objectid=rivmp:327826&versionid=&subobjectname
PFGE:https://www.eurlsalmonella.eu/dsresource?type=pdf&disposition=inline&objectid=rivmp:334494&versionid=&subobjectname

The draft report was finalised in January 2018. The final report is under preparation.

Pol-Hofstad, I.E. and Mooijman, K.A. Combined Interlaboratory comparison study for Food and Primary Production stage (2017) - Detection of *Salmonella* in contaminated hygiene swabs. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM Report no.: 2018-0021 The interim summary of this interlaboratory comparison study was published in December 2017 and is available through the EURL-*Salmonella* website:
https://www.eurlsalmonella.eu/dsresource?type=pdf&disposition=inline&objectid=rivmp:337240&versionid=&subobjectname
The report is under preparation.

Publications
In March 2017 a paper of EFSA, ECDC and three EURLs on the ECDC-EFSA molecular typing database was published:
Rizzi, V., Da Silva Felicio, T., Felix, B., Gossner, C.M., Jacobs, W., Johansson, K., Kotila, S., Michelon, D., Monguidi M., Mooijman, K., Morabito, S., Pasinato, L., Torgny Björkman, J., Torpdahl, M., Tozzoli, R and Van Walle, I. The ECDC-EFSA molecular typing database for European Union public health protection. Euroreference 2, March 2017.
http://euroreference.mag.anses.fr/sites/default/files/17%2003%20ED%20ER%2002%201_RIZZI.pdf

In 2016 a presentation was given at the I3S symposium on the new ISO 6579-1. A manuscript on this presentation was submitted to Food Microbiology in December 2016. After minor revision the manuscript was accepted for publication (likely to be published in May 2018):
Mooijman, K.A. The new ISO 6579-1: A real horizontal standard for detection of *Salmonella*, at last! Food Microbiology (2017), in press, available on line since 18-03-2017. http://dx.doi.org/10.1016/j.fm.2017.03.001 (special issue I3S 2016).

As a result of the validation study of EN ISO 6579-1 performed for the CEN mandate M/381, a manuscript was submitted to International Journal of Food Microbiolgy in July 2017 (special issue CEN mandate). After major revisions, the revised version of the manuscript was submitted in November 2017:
Mooijman, K.A., Pielaat, A. and Kuijpers, A.F.A. Validation of EN ISO 6579-1 - Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 1 Detection of *Salmonella* spp.

ISO and CEN
A consolidated report of 7 EURLs (coordinated by the EURL-*Salmonella*) of the meetings of ISO/TC34/SC9 and CEN/TC275/WG6 held in Tokyo, Japan on 19-23 June 2017, was sent to DG-Sante on 18 July 2018.

ISO 6579-1: Detection of *Salmonella*
A second FDIS voting of ISO 6579-1 was launched on 31 October 2016 and finished on 26 December 2016. The final version of EN ISO 6579-1 was published in February 2017.

ISO 16140-6: Validation of confirmation and typing methods
The voting on DIS 16140-6 was launched on 15 December 2017 and finished on 9 March 2018.

In 2016 and 2017 several updates of the draft document 'Template and guidance for drafting ISO/CEN Standard methods for microbiology of the food chain' were prepared.

A fifth Working Draft (WD5) was prepared in August 2016 and further updated in January-March 2017. After comments from the ISO editor, WD6 was prepared in fall 2017.

1. Interlaboratory comparison studies

General

Since 2013, the matrices under analyses for the different interlaboratory comparison studies are artificially contaminated with a diluted culture of a *Salmonella* serovar at the laboratory of the EURL-*Salmonella*. These types of samples mimic well 'real-life' samples and are easy in use for the NRLs for *Salmonella*.
For the set-up of the studies the directions of CEN ISO/TS 22117:2010 are followed, which indicate that for comparative tests of qualitative methods each participant should test at least 18 samples in total. These 18 samples consist of six replicates of three different levels of the target strain: blank (matrix) samples; low level (matrix) samples (close to the detection limit of the method); high level (matrix) samples (5-10 times higher than the low level samples). It is expected that when samples with a contamination level close to the detection limit are tested, approximately 50% of the samples will be tested negative.

For the reporting of the results of the interlaboratory comparison studies by the NRLs-*Salmonella,* web based test reports are used. The test reports have been improved over the years and the number of questions on general laboratory information has been reduced as this information can be requested at the NRL in case of deviating results.

Up to and including 2016, the order of the interlaboratory studies for detection of *Salmonella* was: February/March a study for samples from the primary production stage (PPS) and September/October a study for food or feed samples. However, repeatedly we have had problems with the choice of the matrix for the PPS study due to Avian Influenza outbreaks in poultry flocks. Avian Influenza problems are generally related to migration of wild birds in fall and winter and may affect in this way the interlaboratory study in February/March. For that reason it was decided to change the order of the PPS and food/feed studies. To avoid problems with changing the order of the studies (like two studies with similar matrix would be organised closely after each other; in one year, a study for either PPS or food/feed would not be organised) a combined Food-PPS study was organised in October 2017. Next, the order of the studies can be changed in 2018.

Details on the performance of third countries in the EURL-*Salmonella* interlaboratory comparison studies are reported annually to DG-Sante. In 2017 this report was prepared and sent to DG-Sante in October.

Follow-up study of interlaboratory comparison study on typing of *Salmonella* organised in 2016

In November 2016 the 21st interlaboratory comparison study on typing of *Salmonella* spp. was organised. This study contained a compulsory part on serotyping and an optional part on PFGE (Pulsed Field Gel Electrophoresis) typing. In total 34 NRLs for *Salmonella* participated: 29 NRLs from the (28) EU Member States and 5 NRLs from third countries (EU candidate MS or potential EU candidate MS and member countries of the European Free Trade Association (EFTA)).
All (34) NRLs participated in the serotyping part of the study. For this, 20 different serovars of *Salmonella enterica* subsp. *enterica* had to be analysed. Additionally a 21st strain of an uncommon *Salmonella* serovar was added for serotyping. The serotyping of this latter serovar was optional, and the results were not taken into account for the evaluation of the performance of the NRLs.

Fifteen NRLs participated in the PFGE part of the study. For this, 10 different *Salmonella* strains had to be analysed. Like for the typing study organised in 2015, the *Salmonella* strains for this part of the study were also selected in close cooperation with the Statens Serum Institute (SSI; Copenhagen, Denmark), who organises EQA schemes for PFGE typing for the laboratories analysing human samples of the ECDC-FWD network.
The NRLs reported the results of the serotyping before mid-December 2016. The PFGE results were reported separately by December 2016/January 2017.
The analyses of the serotyping results were performed in January 2017 after which the NRLs received their own results and an interim summary report, including the results of all laboratories in February 2017. Two laboratories (non-EU MS) did not fulfil the criteria of good performance for the serotyping. One NRL performed an internal study to find possible explanations for the deviating results and concluded this to be likely caused by high work pressure of laboratory staff at the time of the interlaboratory study. This NRL decided not to participate in the follow-up study. The second NRL with poor performance participated in the follow-up study organised in May 2017, but unfortunately did again not fulfil the criteria of good performance for serotyping. EC DG-Sante was informed accordingly.
The results of the study on PFGE typing were analysed in spring 2017 and reported to the participants in September 2017. The results of all laboratories for both typing methods (serotyping and PFGE) were presented at the EURL-*Salmonella* workshop in May 2017 and the full report of this study is likely to be published in spring 2018.

Interlaboratory comparison studies on detection of *Salmonella* organised in 2017

In March 2017, the 20th interlaboratory comparison study on the detection of *Salmonella* in samples from the primary production stage was organised.
In this study, 36 NRLs for *Salmonella* participated: 29 NRLs from the (28) EU Member States and 7 NRLs from third countries (member countries of the EFTA, (potential) EU candidate Member States and one NRL from a non-European country).
Each NRL had to analyse a total of 20 samples: 18 samples of 25 g chicken faeces artificially contaminated with three different levels (blank, low and high level) of *Salmonella* Infantis and 2 control samples. The inoculation levels were: 17 cfu/sample and 55 cfu/sample.
The prescribed method for analyses was Annex D of ISO 6579 (2007) or ISO 6579-1:2017, with selective enrichment on Modified Semi-solid Rappaport Vassiliadis (MSRV) agar. All NRLs reported the results before mid-April 2017, after which the analysis of the results was performed. In June 2017, the participants received information on their performance as well as an interim summary report including the results of all participants. One laboratory scored a 'moderate performance' for making an error in labelling the control samples. One laboratory (non-EU MS) scored a 'poor performance' for detecting *Salmonella* in three of the six blank samples. This laboratory did not provide any possible explanation for their problems, and was not able to participate in the follow-up study for unknown reasons. The results of the study were presented at the EURL-*Salmonella* workshop in May 2017. The final report is likely to be published in spring 2018 (see 'Introduction').

In October 2017, a combined interlaboratory comparison study for Food and Primary Production stage was organised. The matrix under analysis concerned artificially contaminated hygiene swabs. In this study both NRLs-*Salmonella* for analysis of food samples as for analysis of samples from the primary production stage were invited to participate. In total 56 NRLs participated in the study, of which 33 NRLs-*Salmonella* for food and 23 NRLs-*Salmonella* for PPS. The participants originated from the 28 EU Member States (MS), 4 NRLs from third

countries within Europe (EU (potential) candidate countries and EFTA countries) and one NRL from a non-European country.
Each NRL analysed in total 20 samples: 18 hygiene swabs artificially contaminated with three different levels (blank, low and high level) of *Salmonella* Typhimurium and 2 control samples. The inoculation levels were: 5 cfu/sample and 107 cfu/sample.
The prescribed method for analyses was the EN ISO method for detection of *Salmonella* and preferably the latest version, being ISO 6579-1:2017, following the relevant procedure for the relevant NRL. Thus if the swabs were analysed by an NRL for PPS, MSRV agar should be used as the only selective enrichment medium. If the swabs were analysed by an NRL for food, two selective enrichment media had to be used: Muller Kauffmann Tetrathionate broth with novobiocin (MKTTn) and MSRV agar or Rappaport Vassiliadis with Soya (RVS) broth.
All NRLs reported the results by November 2017. In December 2017, the participants received information on their performance as well as an interim summary report including the results of all participants. One laboratory scored a 'moderate performance' for making an error in reporting the positive control sample being negative for *Salmonella*. One laboratory scored a 'poor performance' for falsely detecting *Salmonella* in two blank hygiene swab samples. This laboratory was asked for possible clarifications for the deviating results. A follow-up study will be organised in spring 2018.

Interlaboratory comparison study on typing of *Salmonella* organised in 2017

In November 2017 the 22nd interlaboratory comparison study on typing of *Salmonella* spp. was organised. This study contained a compulsory part on serotyping and an optional part on PFGE (Pulsed Field Gel Electrophoresis) typing.
In total 35 NRLs for *Salmonella* participated: 29 NRLs from the (28) EU Member States and 6 NRLs from third countries (EU (potential) candidate MS, EFTA countries and one non-EU country).
All (35) NRLs participated in the serotyping part of the study. For this, 20 different serovars of *Salmonella enterica* subsp. *enterica* had to be analysed. Additionally a 21st strain of an uncommon *Salmonella* serovar was added for serotyping. The serotyping of this latter serovar was optional, and the results were not taken into account for the evaluation of the performance of the NRLs.
Fifteen NRLs participated in the PFGE part of the study. For this, 10 different *Salmonella* strains had to be analysed. Like for the typing study organised in 2016, the *Salmonella* strains for this part of the study were also selected in close cooperation with SSI.
The NRLs reported the results of the serotyping before mid-December 2017. The PFGE results were reported separately by December 2017/January 2018.
The analyses of the serotyping results were performed in January 2018 after which the NRLs received their own results and an interim summary report, including the results of all laboratories in February 2018. All NRLs fulfilled the criteria of good performance for the serotyping. The results of the study on PFGE typing are under analysis.

2. Workshop

On 29 and 30 May 2017, the annual EURL-*Salmonella* workshop was organised in Zaandam, the Netherlands.
A total of 46 participants were present at the workshop:
35 participants from the 28 EU-MS
3 participants from the EFTA countries
2 participants from EU candidate MSs or potential EU candidate MSs
3 participants from EURL-*Salmonella*
1 guest speaker
1 participant from EFSA
1 participant from DG-Sante

Three participants of NRLs from one EU Member State, and two (potential) candidate countries, were unable to come to the workshop due to lack of staff.

Presentations were given on the following subjects:

- Results of the interlaboratory comparison studies as organised by the EURL-*Salmonella* since the previous workshop (June 2016);
- Proposals for new interlaboratory comparison studies;
- *Salmonella* monitoring data and food-borne outbreaks for 2015 in the EU;
- Update on the joint EFSA/ECDC molecular typing database;
- Preliminary results of EFSA survey on the use of WGS for typing *Salmonella;*
- Multi country outbreak of *Salmonella* Enteritidis related to Polish eggs;
- Outbreak of a new serotype *S. enterica enterica* 11:z_{41}:e,n,z_{15} in Greece;
- *S. enterica diarizonae* 61:k:1,5,(7) in Swiss sheep herds;
- Update on activities in ISO and CEN;
- Validation of alternative microbiological methods – ISO 16140 series
- The new Official Control Regulation (revision of Regulation 882/2004);
- 5 NRLs presented their activities for being NRL-*Salmonella;*
    - Work-programme of the EURL-*Salmonella* for the coming year.

During the workshop an evaluation form about the workshop was distributed and the participants were requested to complete it (anonymously). The evaluation form was handed over to 44 participants of the workshop (excluding the organisers) and 41 completed forms were returned, which is a response rate of 93%. From the answers of the respondents, it could be concluded that the participants were satisfied with the workshop and considered the scientific programme as interesting.

More details on the presentations, discussion and evaluation of the workshop is summarised in the report of the workshop. The draft version of this report was sent to EC DG-Sante in February 2018 and the final report is likely to be published in spring 2018 (see 'Introduction'). All presentations were placed on the EURL-*Salmonella* website (https://www.eurlsalmonella.eu/Workshops/Workshop_2017) on 2 June 2017.

3. Supporting activities

Activities in ISO and CEN

EURL-*Salmonella* is involved (as project leader/convenor or as member of (working) groups) in several activities of ISO and CEN. More specific in:

- ISO/TC34/SC9: International Standardisation Organisation, Technical Committee 34 on Food products, Subcommittee 9 – Microbiology.
- CEN/TC275/WG6: European Committee for Standardisation, Technical Committee 275 for Food analysis – Horizontal methods, Working Group 6 – Microbiology of the food chain.

Both groups organised their annual meeting in Tokyo, Japan from 19 to 23 June 2017.
Kirsten Mooijman and Wilma Jacobs of the EURL-*Salmonella* are (co-)project leaders of groups in CEN and ISO dealing with methods for *Salmonella*, validation of typing methods and drafting of a guidance document for drafting ISO/CEN standard methods. Kirsten presented the progress of these groups at the plenary meeting of SC9 and of WG6. A consolidated report of 7 EURLs (coordinated by the EURL-*Salmonella*) on the meetings was sent to DG-Sante on 18 July 2017 (see 'Introduction').

EN ISO 6579-1: Detection of* Salmonella *(activity in CEN/TC275/WG6)
From November 2015 to January 2016, the first FDIS voting on EN ISO 6579-1 took place, and included additional to editorial comments some technical

comments which had to be taken into account. For that reason a second FDIS voting was needed. Before doing so, the members of CEN-WG6 and ISO-SC9 were consulted for their approval to launch the amended draft document for second FDIS voting (13 July – 11 August 2016). The outcome of this consultation was positive with a few comments. After introducing the last remarks into the document, the second FDIS voting (ISO FDIS 6579-1.2) was launched on 31 October 2016 and finished at 26 December 2016. The outcome was positive (26 approvals (96%) and 1 disapproval (Canada)) and again a few editorial comments were added. In January and February 2017 the last amendments were introduced into the document and in February 2017 the final version of ISO 6579-1 was published. In March 2017 the document was published by CEN as well, becoming EN ISO 6579-1.

Standardisation of PCR method(s) for identification of monophasic* Salmonella *Typhimurium (activity in CEN/TC275/WG6 TAG3 in cooperation with ISO/TC34/SC9 WG10)

In 2016, several draft versions of the standard have been prepared by Burkhard Malorny (NRL-*Salmonella* Germany) and discussed with the EURL-*Salmonella* and the experts of CEN-TAG3. Earlier it has been agreed that the performance characteristics of the standard will be determined in an interlaboratory study with a 'standard set of strains', to be organised by the EURL-*Salmonella*. In November 2016, the EURL-*Salmonella* has made a call for test strains to create this 'standard set of strains'. By March 2017, the EURL received approximately 400 strains. The identity of all strains has been verified by the EURL. In fall 2017, 172 strains out of the 400 have been selected (target strains as well as non-target strains) which will be used to verify the 3 PCR procedures described in draft ISO/TS 6579-4 by the NRL-*Salmonella* in Germany and by the EURL-*Salmonella* (to be started in early 2018). These experiments are performed to not only test the PCR protocols, but also to decide on a further selection of strains for use in the validation study.

Draft EN ISO 16140-6 Validation of confirmation and typing methods (activity in ISO/TC34/SC9 WG3)

The project group leader, Wilma Jacobs, incorporated the comments of the Committee Draft (CD) voting into the amended document and the first draft DIS (Draft International Standard) version of EN ISO 16140-6 was sent to the members of ISO-WG3 in October 2016 for further comments and discussion at the meeting of ISO-WG3 in December 2016. After this meeting a second draft DIS version has been prepared and sent to the members of ISO-WG3 for further comments (25 February 2017 until 31 March 2017). Next a third draft DIS version was prepared which was discussed at a meeting with the members of WG3 in May 2017. Finally in December 2017 the official ISO/DIS voting and CEN enquiry was launched for a period of 3 months. The results of voting came available in March 2018 and showed 100% positive votes in ISO, with comments. In 2018, the document will be updated incorporating the comments from the DIS voting after which the last voting step before final publication (Final Draft International Standard - FDIS) will be launched.

ISO/TC34/SC9 Ad hoc group on template and guidance for drafting microbiological ISO/CEN standard methods

In 2014, it was decided to raise an ad hoc group for drafting a guidance document for the drafting of microbiological ISO/CEN standards. This document is intended to (further) harmonise the content and layout of standards for microbiology of the food chain. Kirsten Mooijman and Wilma Jacobs became respectively project leader and co-project leader of this group because of their extensive experiences with drafting ISO/CEN documents. The document will become an internal guidance document for convenors and project leaders of SC9 and WG6. Since 2014, several draft versions of the guidance document have been prepared.

In fall 2016 a fifth draft document was prepared and was sent to ISO/SC9 secretariat and the ISO editor for comments in April 2017. Useful comments were received and a sixth draft document was prepared in January 2018. Some comments of the ISO editor would affect many existing EN ISO documents for microbiology of the food chain (concerning the way definitions are formulated) and this needs to be discussed at the next annual meeting of ISO/SC9 and CEN/WG6 in June 2018. Before this meeting, the latest version of the guidance document will be distributed to the members of SC9 and WG6.

ISO/TC34/SC9 ad hoc group on harmonization of incubation temperatures

In 2014, at an annual meeting of ISO/TC34/SC9 and CEN/TC275/WG6, it was agreed to use a broader temperature range for incubation of non-selective media (34-38 °C instead of 37 °C ± 1 °C). For accepting a broader temperature range for the incubation of selective media, data were needed showing no effects on the results when incubating at this broader temperature range. In 2014-2015, a laboratory in France performed some experiments to test the influence of incubation temperature (35 °C or 37 °C) on the growth of *Salmonella* and on several *Enterobacteriaceae* species. These experiments showed no difference in growth of *Salmonella* spp. at both temperatures, but some impact on the growth of some (other) *Enterobacteriaceae* species. Therefore, it was decided to draft a protocol to test the influence of the incubation temperature with a larger group of laboratories (members of ISO and CEN), especially to test the influence on the growth of *Enterobacteriaceae*. In 2016 a protocol was prepared for comparing the growth of *Salmonella* and of background flora in MKTTn broth and on subsequent plating out media (XLD and second plate) after incubation at 37 °C and at 35 °C. The members of ISO and CEN were invited to perform experiments, following the protocol. By mid-2017, study results were received from 9 laboratories from 6 different countries, representing 850 test results of which 236 were positive for *Salmonella*. Results confirmed for the presence or absence of *Salmonella* were analysed and interpreted following the information for the sensitivity study according to ISO 16140-2:2016. The outcome of the first analysis was presented at the meeting of ISO/SC9 and CEN/WG6 (June 2017). At this meeting it was agreed that the EURL-*Salmonella* would perform some additional analysis and will report the final outcome at the meeting of ISO/SC9 and CEN/WG6 in June 2018.

ISO/TC34/SC9 WG4 Revision of ISO/TS 22117 Proficiency testing (PT)

ISO/TS 22117 was published in 2010 and it was decided in 2014 to revise the document for several reasons (e.g. to make it a full standard, to include PT schemes for viruses, parasites, primary production, yeasts and moulds, and molecular methods). The involvement of the EURLs in this working group is considered important as they have much experience with the organisation of PT schemes. In November 2017 a meeting was organised to discuss the amendment of the different parts of the document. The EURL-*Salmonella* is member of this working group but was not able to attend this meeting.

ISO/TC34/WG25 WG 25 Whole-genome sequencing for typing and genomic characterization

In 2016 and 2017 this working group has been busy with the preparation of a first draft document containing information on:

- Wet laboratory sequencing and analysis of sequence data;
- Validation of data and methods;
- Metadata and sequence repository (not to develop databases, but to give guidance on how to control the quality of databases and pipelines).

The original plan of WG25 was to draft a standard in three parts, but while drafting the document it was noticed that there was overlap between the three parts and that it would be better to merge the three parts into one document.

Early 2018, the draft document was launched as new work item proposal and members of ISO/SC9 can comment on it until May 2018.
The EURL-*Salmonella* is member of this ISO working group and comments to the draft documents and participates in the teleconferences and meetings when possible.

CEN/TC275/WG6 TAG 9 on pre-enrichment

The CEN Task group, TAG9, was set up in 2012 to try to come to an optimal pre-enrichment medium for detection of several (mainly Gram negative) pathogenic bacteria, in order to resuscitate stressed or damaged cells.
The group is working on a protocol to evaluate pre-enrichment media performance characteristics. The objective of this protocol is to evaluate the performance characteristics of pre-enrichment media (mainly raw ingredients, composition, etc.) during the development stage and not as routine control. In this protocol, information will be given on stressing strains and the minimum concentration (cfu/ml) to be obtained after pre-enrichment. The target organisms are *Salmonella*, *Enterobacteriaceae*, STEC, *Cronobacter*, and *Listeria*. For that reason, convenors or project leaders of ISO/CEN working groups dealing with the relevant pathogens became member of this TAG9.
The rough ideas of the protocol have been discussed at a meeting in March 2017 (to which the EURL-*Salmonella* could not attend) and further discussed in a teleconference in February 2018.
TAG9 is also working on a second protocol to evaluate neutralizing procedures/ingredients (given for example in EN ISO 6887-4:2017) to be used when inhibitory substances are present in the sample during pre-enrichment.

Samples for interlaboratory comparison studies

Samples for interlaboratory comparison study primary production, March 2017

For the study on detection of *Salmonella* in samples from the primary production stage (PPS) of March 2017, it was decided to use chicken faeces. For the artificial contamination of 25 g chicken faeces samples, two different strains of *Salmonella* Infantis were tested for their stability in the samples when stored at 5 °C and at 10 °C. The pre-studies performed in fall 2016 showed better stability for one strain of *S.* Infantis and this strain was used in further studies. The high contaminated samples (approx. 50 cfu/25 g) were all positive (5/5) after 3 weeks of storage at +5 °C, and when stored at +10 °C 5/5 samples were still positive after 2 weeks of storage. At 5 °C still 3/5 low contaminated samples (approx. 8 cfu/25 g) were positive after storage for 2 weeks. However, at 10 °C these low contaminated samples were less stable: after 1 week of storage only 1/5 samples were tested positive for *Salmonella*. Still it was decided to use the *Salmonella* Infantis strain to inoculate the chicken faeces samples for the interlaboratory study, but it was aimed to inoculate the low contaminated samples with a slightly higher level than the one of the pre-test.
Information on the contamination levels of *Salmonella* Infantis in the chicken faeces samples used for the interlaboratory study is given in Table 1. In this Table information is given on the inoculation levels of *Salmonella* Infantis at the day of preparing the samples as well as on the number of *Salmonella* in the samples at the day of the study. For this latter an MPN format (Most Probable Number) of Annex D of ISO 6579 (2007) was used.
The amount of background flora in the chicken faeces was determined shortly after arrival at the laboratory of the EURL and in the week of the interlaboratory study after storage at 5 °C. For this the total aerobic count (following ISO 4833-1:2013) as well as the number of *Enterobacteriaceae* (following ISO 21528-2:2004) were tested. The results are summarised in Table 2.

Table 1 Salmonella *Infantis concentrations in inoculum and in chicken faeces samples after storage at 5 °C, used in the EURL-*Salmonella *PPS interlaboratory study, organised in March 2017*

Date of testing	Low level *S.* Infantis (cfu/25 g)	High level *S.* Infantis (cfu/25 g)
7 March 2017 Inoculation level diluted culture	17	55
20 March 2017 (date of the study; after storage at 5 °C). MPN of artificially contaminated chicken faeces (95 % confidence limit)	22 (8.5 -56)	35 (11 – 110)

Table 2 Amount of background flora in the chicken faeces, tested immediately after receipt and after storage at 5 °C

Date of testing	Number of aerobic bacteria (cfu/g)	Number of *Enterobacteriaceae* (cfu/g)
7 March 2017	4.2×10^8	8.7×10^4
20 March 2017(date of the study; after storage at 5 °C).	1.0×10^8	5.5×10^4

Samples for combined interlaboratory comparison study Food-PPS, October 2017

For the combined interlaboratory study for Food and primary production, hygiene swabs were the samples of choice. Several experiments were performed to test the optimal inoculum strains and levels for the artificial background flora as well as for the *Salmonella* serovar. Individually packed hygiene swabs (in a plastic bag) were moistened with 10 ml peptone saline solution and left to saturate at room temperature. These hygiene swabs were originally sterile; therefore the swabs were artificially contaminated with both background flora and *Salmonella*. After moistening the swabs, 1 ml of a mixture of *Escherichia coli* and *Citrobacter freundii* (approx. 10^6 cfu/ml) was added to each swab. Next, *Salmonella* Typhimurium was added to the swabs with background flora to obtain low and high contaminated samples. Blank samples were not inoculated with *Salmonella* but did contain background flora. In pre-tests the low contaminated samples (at a level of approx. 11 cfu/sample) were tested for their stability when stored at 5 °C. After 3 months of storage at 5 °C, still 6/7 samples were tested positive for *Salmonella*. Some additional stability tests were performed with even lower contaminated hygiene swabs (approx. 6 cfu/sample) stored at 5 °C and at 10 °C. After 2 weeks of storage at 10 °C, still 5/5 samples were positive for *Salmonella* and after 3 weeks of storage at 5 °C, still 4/5 samples were positive. These stability tests showed that the artificially contaminated hygiene swabs were sufficiently stable for use in the interlaboratory comparison studies.

Information on the contamination levels of *Salmonella* Typhimurium (STM) in the hygiene swabs used for the interlaboratory study is given in Table 3. In this Table information is given on the inoculum levels of *Salmonella* Typhimurium at the day of preparation of the samples as well as on the number of *Salmonella* in the samples at the day of the study, after storage at 5 °C. For this latter an MPN format (Most Probable Number) of ISO 6579-1:2017 was used (using only MSRV agar as selective enrichment medium).

The amount of background flora in the hygiene swabs was also determined at the day of preparation of the samples as well as at the day of the study, after storage at 5 °C. This was tested for the number of *Enterobacteriaceae* (following ISO 21528-2:2004) for the three types of samples (blank, low and high contaminated). The results are summarised in Table 4.

Table 3 Salmonella *Typhimurium concentrations in inoculum and in hygiene swabs used in the combined EURL-*Salmonella *Food-PPS interlaboratory comparison study, organised in October 2017.*

Date of testing	Low level *S.* Typhimurium (cfu/sample)	High level *S.* Typhimurium (cfu/sample)
28 September 2017 Inoculation level diluted culture	5	107
9 October 2017 (date of the study; after storage at 5 °C). MPN of artificially contaminated hygiene swabs (95 % confidence limit)	7 (2.3-22)	92 (28-300)

Table 4 Number of Enterobacteriaceae *in the high, low and blank* Salmonella *hygiene swab samples, tested at the day of preparation and at the start of the the interlaboratory comparison study in October 2017 (after storage at +5 °C)*

Date of testing	Blank samples (cfu/sample)	Low level STM samples (cfu/sample)	High level STM samples (cfu/sample)
28 September 2017	7.7×10^5	1.4×10^6	7.3×10^7
9 October 2017 (date of the study; after storage at 5 °C).	7.1×10^6	1.4×10^4	4.7×10^6

Samples for interlaboratory comparison study animal feed, March 2018

For the study on detection of *Salmonella* in animal feed to be organised in spring 2018, it was decided to use chicken feed. For the artificial contamination of the chicken feed two different *Salmonella* serovars (*S.* Typhimurium and *S.* Mbandaka) were tested for their stability in the samples when stored at 5 °C and at 10 °C. The pre-studies performed in fall 2017 showed comparable stability for both serovars and it was decided to choose the *Salmonella* serovar originally isolated from animal feed: *Salmonella* Mbandaka. Different types of chicken feed were tested and the one with the highest amount of background flora (approx. 10^3 cfu/g *Enterobacteriaceae*) was chosen to perform further experiments in fall 2017. Samples of 25 g chicken feed were artificially contaminated with a low level culture of *Salmonella* Mbandaka (approx. 12 cfu/25 g) and stored at 5 °C and at 10 °C. After 3 weeks of storage at 5 °C still 5/6 samples were tested positive for *Salmonella*. After 2 weeks of storage at 10 °C also still 5/6 samples were tested positive. It was concluded that the samples were fit for use in the interlaboratory comparison study.

4. Giving assistance to the Commission and ad hoc activities

Several questions were received from several parties (European Commission DG-Sante, NRLs-*Salmonella*, European Food Safety Authority - EFSA, European Centre for Disease Prevention and Control – ECDC, and other institutes inside and outside the EU) on the following subjects (list not exhaustive):

- Content and interpretation of the new ISO 6579-1 for detection of *Salmonella*;
- Validation of (alternative) methods;
- Advise EFSA for the reporting of analytical methods in the EFSA catalogues;
- Share information on PCR protocols for identification of monophasic *Salmonella* Typhimurium;
- Reporting of *Salmonella* serovars;

- Analysis of egg shells;
- Preparation of cocoa containing samples (interpretation of ISO 6887-4);
- Information on (availability of) reference materials;
- Organisation of interlaboratory comparison studies;
- Sampling of different matrices (dust, animal feed);
- Pooling of samples;
- Disposal of presumptive *S.* Typhi or *S.* Paratyphi A;
- Confirmation of strains (serotyping/genotyping) of NRLs-*Salmonella*, e.g. serotyping of two *Salmonella* isolates for the NRL in Croatia; sequencing and cluster analysis of 14 strains of *Salmonella* Give for NRL-*Salmonella* in Malta;
- Information on different *Salmonella* serovars;
- Advise DG-Sante with the amendment of Regulation 2073/2005;

The EURL-*Salmonella* received several questions every week, varying from simple to complex. All questions were answered as quickly as possible. Depending on the complexity of the questions, answers could be given immediately by the experts of the EURL-*Salmonella*, or further information was gained from other experts (inside or outside the RIVM) or from literature.

Regularly the EURL receives requests from laboratories for participation in the comparative tests and/or in the EURL workshops or trainings. If these questions come from non-NRL laboratories, most of the time the EURL rejects these requests because of lack of capacity.

Early 2017, the NRL-*Salmonella* from Lithuania asked the EURL-*Salmonella* to help with MLVA and WGS typing of 5 *Salmonella* Enteritidis strains isolated from mice (as feed for reptiles) and poultry to investigate possible correlations. Earlier there have been some cases in Lithuania connected with EU outbreaks of reptile salmonellosis (2015-2016). The primary hypothesis was that infection was associated with exposure to pet snakes or their feed (specifically frozen mice) from Lithuania. In April 2017 the molecular typing results were reported to the NRL in Lithuania showing a relation between the three mice isolates, but no correlation between the mice isolates and the two poultry isolates.

In April 2017, a staff member of the EURL-*Salmonella* participated in a task force meeting in Poland. The purpose was to analyse the *Salmonella* control programmes implemented in Poland and to try to find issues and propose solutions to improve the efficacy of the programmes.

Staff members of the EURL-*Salmonella* were asked to give advice for the organisation of an interlaboratory study for the use of WGS for serotyping of *Salmonella*. This study was organised as part of the EFSA project ENGAGE. Further details are given in clause 7.

In July 2017, delegates from the Food and Drug Administration (FDA), USA visited the EURL-*Salmonella* as part of the investigations of EU food safety systems, to determine if these systems can be recognised as comparable to the US systems. The FDA had expressed an interest in the role, functioning and responsibilities of the EU Reference labs and for that reason visited, amongst other EURLs, the EURL-*Salmonella*.

In June and September 2017, a staff member of EURL-*Salmonella* participated in meetings (teleconference and face-to-face) with DG-Sante and the EURLs for bivalve molluscs, foodborne viruses, *E. coli* and biotoxines to discuss the distribution of activities of EURL-bivalve molluscs after the Brexit.

In October 2017, a staff member of EURL-*Salmonella* explained draft ISO (DIS) 16140-6 on validation of alternative confirmation and typing methods at a meeting of the working group of DG-Sante on Microbiological criteria.

In October 2017, a staff member of EURL-*Salmonella* participated in a joint network meeting of EFSA and ECDC in Parma, Italy. Important subjects discussed at this meeting were multi-country outbreaks.

5. Communication

Every three months a newsletter is published through the EURL-*Salmonella* website. In each newsletter, a selection of the most recent publications in relation to *Salmonella* is published. Additionally the following information was included:

- In March 2017, volume 23 no 1 of the newsletter was published, which included the summary results of the questionnaire amongst NRLs-*Salmonella* on the use of MLVA and the technical report on the activities of the EURL performed in 2016.
- In June 2017, volume 23 no 2 of the newsletter was published including the timetables of the combined interlaboratory study for Food and Primary Production stage on detection of *Salmonella* spp. in hygiene swabs (October 2017) and of the interlaboratory comparison study on typing of *Salmonella* (November 2017). Additionally, a summary was given of the '*Salmonella*-items' as discussed at the annual meetings of ISO/TC34/SC9 and CEN/TC275/WG6 in Tokyo, Japan in June 2017.
- In September 2017, volume 23 no 3 of the newsletter was published, which included again both time tables of the interlaboratory comparison studies of October and November 2017. Additionally, the updated suggested (guidance) 'Protocol for management of underperformance in comparative testing or lack of collaboration of NRLs' was included for information.
- In December 2017, volume 23 no 4 of the newsletter was published, which included the time table of the 4th interlaboratory comparison studies on the detection of *Salmonella* spp. in chicken feed (February/March 2018).

Other relevant information is also published through the website: www.eurlsalmonella.eu. One staff member of the EURL regularly keeps the information on the website up to date.

6. Training activities

On 3 and 4 July 2017, a joint training course of three EURLs (*Listeria monocytogenes*, STEC and *Salmonella*) was organised on the use of BioNumerics software to analyse PFGE data. The training was organised at the premises of the EURL-*STEC*, Rome, Italy. Of each EURL network, 4 NRLs participated, resulting in a total of 12 participants. From EURL-*Salmonella*, two staff members (Wilma Jacobs and El Bouw) were part of the group of trainers at this course. The evaluation of this training course is summarised in Annex 1.

7. Molecular typing of *Salmonella*

In relation to molecular typing of *Salmonella*, the following activities were performed in 2017:

- Throughout 2017, monthly meetings were organised with members of two centres of the RIVM: the Centre for Zoonoses and Environmental Microbiology (Z&O; the centre where the coordination of the EURL-*Salmonella* is hosted) and the Centre for Infectious diseases, Diagnostics and Screening (IDS; the centre performing the typing of strains). The group was raised in 2013 to regularly exchange information in relation to (molecular) typing of *Salmonella*.
- One member of the EURL-*Salmonella* participated in meetings of the joint EFSA-ECDC steering committee on 'the collection and management of molecular typing data from animal, food, feed and the related

environment, and human isolates.' In 2017, the steering committee organised two physical meetings (in March and December 2017) in Parma, Italy , in which Kirsten Mooijman participated.

- The EFSA pilot database for the collection of molecular data was activated in December 2014, but for *Salmonella* little activities were employed so far. To a large extent this was caused by the fact that agreement on and signature of the collaboration agreement by all parties lasted until April 2016. Additionally, each Member State needs to agree for its own country which molecular typing data are suitable for uploading in the database and who in the MS is allowed and able to do so. To help the NRLs-*Salmonella* with the discussions in their MS, they have regularly been updated on the EFSA-ECDC database, e.g. at the EURL-*Salmonella* workshops.
- In July 2017, Wilma Jacobs and El Bouw of the EURL-*Salmonella* were part of the group of trainers of the joint training course for NRLs on the use of BioNumerics software (see 6. 'Training activities'). Next to this training, a meeting was organised for the curators of the three EURLs (*Listeria monocytogenes*, STEC and *Salmonella*), as well as for the ECDC database (SSI) to discuss and harmonise curation of molecular (PFGE) data.
- In November 2016, PFGE was included for the fourth time as optional typing method in the EURL-*Salmonella* interlaboratory comparison study on typing of *Salmonella.* Fifteen NRLs for *Salmonella* participated in the PFGE part of the study. The *Salmonella* strains for this part of the study were selected in close cooperation with Statens Serum Institute (SSI; Copenhagen, Denmark), who organises EQA schemes for PFGE typing for the laboratories analysing human samples of the ECDC-FWD network (FWD: Food- and Waterborne Diseases). The NRLs reported their results by December 2016/January 2017. The NRLs were asked to send their PFGE images, and additionally it was possible to send results after analysis of the gel in BioNumerics. The evaluation of the PFGE results was done by 3 staff members of the EURL to make sure that the evaluation was performed in a harmonised way. The quality grading of the PFGE images was done according to the PulseNet International/ECDC guidelines. The results of the individual laboratories were sent to the participants in September 2017. The overall results were presented at the EURL-*Salmonella* workshop in May 2017 and will be summarised in the report of this study (see 'Introduction'). All PFGE images were scored for 7 parameters. Each parameter was given a score from 1 (poor) to 4 (excellent) and also for each participant the total score for the 7 parameters was calculated. Like in earlier studies, there was some variation in results between the participants. Over time some improvements in the quality of the PFGE images were seen: the number of poor scores decreased and the number of good to excellent scores increased.
- In November 2017, PFGE was again included as optional typing method in the EURL-*Salmonella* interlaboratory comparison study on typing of *Salmonella.* Fourteen NRLs for *Salmonella* participated in the PFGE part of the study. The *Salmonella* strains for this part of the study were also selected in close cooperation with SSI. The NRLs reported their results by December 2017/January 2018 and the results are currently being evaluated.
- The activities for standardisation of PCR methods for identification of monophasic *Salmonella* Typhimurium is described in 3. 'Supporting activities'.
- In January 2017 a questionnaire was sent to the NRLs-*Salmonella* to investigate the use of MLVA by the NRLs. The outcome of this questionnaire was summarised and published in the EURL-*Salmonella* Newsletter of March 2017: https://www.eurlsalmonella.eu/dsresource?type=pdf&disposition=inline&objectid=rivmp:329474&versionid=&subobjectname and also given in this

report in Annex 2. In short: 10 NRLs-*Salmonella* indicated to perform MLVA for *Salmonella* Typhimurium and 7 NRLs for *Salmonella* Enteritidis.

- At a meeting of the biological EURLs and DG-Sante in May 2017, it was decided that a working group would be established to promote the use of Next Generation Sequencing (NGS) across the EURLs' networks, to build capacity towards the use of this analytical technology within the EU and ensure liaison with the work of EFSA and ECDC on the Whole Genome Sequencing (WGS) mandate sent by the Commission. The WG includes all the EURLs for microbiological hazards and also involves the Commission and the agencies EFSA and ECDC as observers. The first meeting of the working group was organised in Brussels in November 2017. Two staff members of EURL-*Salmonella* participated in this meeting. At this meeting it was agreed to draft a questionnaire in order to get an updated view of the NRL capacity of NGS to define the activities of the WG and to target the actions on the actual needs of the NRLs.
- In February 2017, the EURL-*Salmonella* was asked to help in setting up an interlaboratory study for the use of WGS for serotyping of *Salmonella* organised by an EFSA project called ENGAGE. The EURL advised on the number and type of strains (target and non-target), following the set-up of ISO/DIS 16140-6:2017, and provided the organisers in ENGAGE with sequence data of some strains. The interlaboratory study was organised in April 2017 and (10) participants were members of the ENGAGE network, and from RIVM (including EURL-*Salmonella*). Sequence data were sent to the participants concerning 18 isolates of 6 target *Salmonella* serovars (top 5 serovars, and monophasic *S.* Typhimurium), 5 non-target *Salmonella* serovars and 4 isolates of non-target genus strains. Each participant used its own tools to predict the serovar names and the analysis was performed on species level (yes/no *Salmonella*) and at serovar level. When analysing the data in accordance with ISO/DIS16140-6:2017, the evaluation of results at species level showed to be within the acceptability limits, but at serovar level they exceeded these limits. This latter was mainly caused by the fact that in 9 incidences the *Salmonella* serovar of the target strains could not be identified. A detailed report was drafted by the project leader of ENGAGE and reported to EFSA.

8. Missions

*The following missions in relation with the EURL-*Salmonella *activities and budget were performed in 2017.*

Related to activity 3. 'Supporting activities'

- Meetings ISO/TC34/SC9 – WG3 on validation of microbiological methods (including draft ISO 16140-6 on validation of confirmation/typing methods)
  17-19 May 2017: Berlin, Germany
  Participant: Wilma Jacobs (project leader drafting ISO 16140-6 and member WG3)
  21-22 September 2017: Utrecht, the Netherlands
  Participant: Wilma Jacobs
- Annual meetings of CEN/TC275/WG6 and ISO/TC34/SC9 (Microbiology of the food chain)
  19-23 June 2017: Tokyo, Japan
  Participant: Kirsten Mooijman

Related to activity 4. 'Giving assistance to the commission and ad hoc activities

- Meetings EURLs for distribution of activities EURL-bivalve molluscs after Brexit
  12 June 2017: Teleconference
  Participant: Kirsten Mooijman
  21 September 2017: Brussels, Belgium

Participant: Kirsten Mooijman
- Meeting working group microbiological criteria EC DG-Sante
  2 October 2017: Brussels, Belgium
  Participants (trainers): Wilma Jacobs
- Joint network meeting EFSA-ECDC (funded by EFSA)
  16-17 October 2017: Parma, Italy
  Participant: Kirsten Mooijman
- Directors meeting EURLs (funded by EC DG-Sante)
  1 December 2017: Brussels, Belgium
  Participant: Kirsten Mooijman

Related to activity 6. 'Training activities' and 7. 'Molecular typing of Salmonella'
- Joint training course on the use of BioNumerics, followed by curators meeting
  3-5 July 2017: Rome, Italy
  Participants (trainers): Wilma Jacobs, El Bouw

Related to activity 7. 'Molecular typing of Salmonella'
- Meetings of the EFSA-ECDC steering committee on the collection and management of molecular typing data
  27-28 March 2017: Parma, Italy
  13-14 December 2017: Parma, Italy
  Participant: Kirsten Mooijman (all meetings)
- Meeting biological EURLs (including discussion on activities for WGS)
  11 May 2017: Brussels, Belgium
  Participants: Kirsten Mooijman (funded by EC DG-Sante), Angela van Hoek (funded by EURL-*Salmonella*)
- First meeting working group NGS
  14 November 2017: Brussels, Belgium
  Participants: Kirsten Mooijman, Indra Bergval

Mrs. Drs. K.A. Mooijman,
Head EURL-*Salmonella*
Bilthoven, 5 April 2018

Abbreviations

BPW	Buffered Peptone Water
CEN	European Committee for Standardization
CI	Confidence Interval
cfu	colony forming units
DG-Sante	Directorate-General for Health and Food Safety
DIS	Draft International Standard
EC	European Commission
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
EFTA	European Free Trade Association
EURL	European Union Reference Laboratory
FDIS	Final Draft International Standard
FWD	Food- and Waterborne Diseases
ISO	International Standardization Organization
MKTTn	Mueller Kauffmann Tetrathionate novobiocin broth
MLVA	Multi-Locus Variable number of tandem repeats Analysis
monoSTM	monophasic *Salmonella* Typhimurium
MPN	Most Probable Number
MS	Member State
MSRV	Modified Semi-solid Rappaport Vassiliadis
NRL	National Reference Laboratory
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
PPS	Primary Production Stage
RIVM	National Institute for Public Health and the Environment
RVS	Rappaport Vassiliadis broth with Soya
SC	Sub Committee
SSI	Statens Serum Institute
STEC	Shiga toxin-producing *Escherichia coli*
TAG	Technical Advisory Group
TC	Technical Committee
WG	Working Group
WGS	Whole Genome Sequencing

References

EN ISO 4833-1:2013, Microbiology of the food chain – Horizontal method for the enumeration of microorganisms – Part 1: Colony-count technique at 30 °C by the pour plate technique.

EN ISO 6579:2002. Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.

EN ISO 6579:2002/Amd1:2007. Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp. - Annex D: Detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage.

EN ISO 6579-1: 2017. Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* – Part 1: Detection of *Salmonella* spp.

ISO 6887-4:2017. Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 4: Specific rules for the preparation of miscellaneous products

EN-ISO 21528-2:2004. Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of *Enterobacteriaceae* - Part 2: Colony-count

CEN ISO/TS 22117:2010, Microbiology of food and animal feeding stuffs – Specific requirements and guidance for proficiency testing by interlaboratory comparison.

EN ISO 16140-2:2016. Microbiology of the food chain - Method validation - Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method.

ISO/DIS 16140-6:2017 Microbiology of the food chain – Method validation - Part 6: Protocol for the validation of alternative (proprietary) methods for microbiological confirmation and typing procedures.

EC, 2004. European Regulation EC No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. Official Journal of the European Union L 165: 30 April 2004. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32004R0882&qid=1458664362396&from=EN (access date 05/04/2018)

Annex 1 Evaluation joint training course organized by 3 EURLs on the use of BioNumerics (3-4 July 2017)

Evaluation Joint Training Course of 3 EURLs on the use of BioNumerics Software to analyse PFGE data of STEC, *Salmonella* and *Listeria monocytogenes*
3-4 July 2017. Location: EURL-STEC, Rome, Italy
Only NRLs-*Salmonella* participating in the training completed the evaluation form for EURL-*Salmonella*

Number of participants training course	12
Number of participants completing evaluation	6 NRLs-*Salmonella*
Participating countries (NRLs-*Salmonella*)	Slovak Republic, Latvia, Belgium, France, Cyprus, Romania
What did you expect to learn from this training (on forehand)?	- Correct picture normalisation, cluster analysis; - To be able to work with PFGE data in BioNumerics without constantly looking at a reference manual; - A standardised method for analysing and comparing profiles; - Cluster analysis, how to manage the software; - How to use the BioNumerics software to analyse PFGE data of *Salmonella*; - Differences between old version of BioNumerics and new version of this software.
Were the trainees able to fulfil your expectations	
Yes	6
No	-
Comments	- It would have been better to use smaller data sets as it was difficult also for the trainer to find the correct entries/files; - Great instructions; - Very competent.
Was the time sufficient for your training?	
Yes	5
No too short	1
Can you please describe (in short) what you have learned during the training?	- Quality assessment of TIFF files, analyse TIFF files, cluster analysis; - Learned to process PFGE images for use in BioNumerics and to make a simple comparison and dendrogram from it; - A standardised method for analysing and comparing profiles and new tricks in comparing gels/profiles; - The data base curation, image analysis and the importance of this step; - Image acquisition, analysis and evaluation, profiles analysis and interpretation; - Normalisation, cluster analysis, the main points for visual evaluation of PFGE gel images.
Is what you have learned during the training applicable in your laboratory?	
Yes	6
No	-
Overall, did the training fulfil your expectations?	

Yes	6
No	-
Any other comments?	- It would have been useful to have some time to study the relationship between database entries, references, standards, experiment types, files, etc. Now I can do the analysis of PFGE data but the overall structure of BioNumerics data and workflow is still confusing; - Great initiative with different trainers with attention to personal needs/interests of trainees; - Thank you very much for this training course.

Annex 2 Summary results questionnaire amongst NRLs-*Salmonella* on the use of MLVA

The questionnaire was open from 26 January until 24 February 2017.
The questionnaire was sent by EURL-*Salmonella* to NRLs-*Salmonella* in 28 EU Member States (+ Northern Ireland), 3 EFTA countries and 4 (potential) EU candidate countries: in total 36 countries.
Number of replies (countries): 25 (69%)

Question	Number of replies (countries)
For which work field are you NRL-*Salmonella*?	
Animal feed	20
Food	17
Primary production – cattle	20
Primary production – pigs	21
Primary production – poultry	21
Typing	23
Does your laboratory (either internally or by outsourcing) perform MLVA typing of *Salmonella* isolates from food, feed, and/or animals?	
No	15 (CY, CZ, EE, FI, GR, LV, LT, NO, PL, PT, RO, SK, SI, ES, CH)
Yes	10 (AT, BE, BG, DK, FR, DE, IT, NL, SE. UK)
For which *Salmonella* serovar(s) do you perform MLVA?	
Salmonella Typhimurium	10
Salmonella Enteritidis	7
Other	2 (monophasic *S.* Typhimurium, *S.* Dublin, *S.* Derby)
Do you perform MLVA typing on a routine basis or occasionally?	
On a routine basis	6
Occasionally	4
Where do the isolates come from?	
Samples related to official controls, national control programmes or surveys carried out by competent authorities	8
Samples related to outbreak investigations	8
Samples related to research	8
Which MLVA protocol do you use for *Salmonella* Typhimurium?	
EFSA SOP (2014), incl. set of standardisation strains http://dx.doi.org/10.2903/sp.efsa.2014.EN-703	4
EFSA SOP (2014), **not** incl. set of standardisation strains	-
ECDC SOP (2011), incl. set of standardisation strains http://dx.doi.org/10.29000/56328	6
ECDC SOP (2011), **not** incl. set of standardisation strains	-
Which MLVA protocol do you use for *Salmonella* Enteritidis?	
ECDC SOP (2016), incl. set of standardisation strains http://dx.doi.org/10.29000/973540	7
ECDC SOP (2016), **not** incl. set of standardisation strains	-

Question	Number of replies (countries)
Which MLVA protocol do you use for other *Salmonella* serovars?	
Not applicable	9
Other	Internal method for *S.* Dublin and *S.* Derby
How many *Salmonella* isolates did you MLVA type in 2016?	
0	1
10-100	3
100-500	2
500-1000	3
>1000	1 (including human isolates)

Remarks

- Estonia (EE): In case of outbreaks the public health laboratory will decide which kind of molecular typing is used. Usually they use subcontracting from Finnish Public Health Laboratory.
- Finland (FI): Certain *Salmonella* Typhimurium and *Salmonella* Enteritidis strains (beforehand defined in method instructions) have been sent to the National Institute for Health and Welfare (THL) for phage typing. During the years THL has typed by MLVA part of the strains.
- Norway (NO): When we need a MLVA-typing on our non-human isolates, we send them to Norwegian Institute of Public Health.
- Romania (RO): We intend to implement the MLVA technique in our laboratory in this year.
- Switzerland (CH): We planned the implementation of MLVA for *S*. Typhimurium and *S*. Enteritidis about two years ago. Unfortunately we do not get resources for that task from our government until now, but we are still engaged in that field.
- Belgium (BE): CODA-CERVA is no longer NRL for *Salmonella* from January 2017. This competency has been transferred to WIV-ISP.
- Bulgaria (BG): We do not carry out research on MLVA for technical reasons related to the sequencer machine. I rule MLVA profiles of our strains in Denmark. I was supported by a grant of the Bulgarian Ministry of Education, Youth and Science, 2012.
- Denmark (DK): From January 1, 2017 DTU Food, Denmark has stopped using MLVA for molecular typing of *Salmonella* from food, feed and animal samples. Instead isolates are analysed by WGS.
- France (FR): we submitted in journal of Frontiers in Microbiology a MLVA subtyping method for *Salmonella* Dublin suitable for inter-laboratory surveillance. *Salmonella* Dublin is one of the most frequently encountered *Salmonella* in cattle in the EU. We defined a MLVA method with 6 VNTRs and a list of reference strains. To normalize the MLVA results we published in 2016 a pipeline, MLVA_normalizer (Bachelerie, et al., 2016. MLVA_Normalizer: Workflow for Normalization of MLVA Profiles and Data Exchange between Laboratories. Journal of Proteomics & Bioinformatics 09(02), 25-27. doi: 10.4172/jpb.1000385).
- Germany (DE): We are going to shut down MLVA within the next two years with establishing of WGS for outbreak and other epidemiological studies.
- United Kingdom (UK) – APHA: We would only do MLVA if specifically asked by government or a customer. WGS is now the routine method for outbreak investigation and it is likely that the use of MLVA will be completely discontinued soon as it is not economic to maintain the capability.
- United Kingdom (UK) – PHE: The information in this survey is based on the activity of the Scottish *Salmonella* Reference Laboratory (SSRL) (Scottish Microbiology Reference Laboratories, Glasgow, Scotland), who also receive mostly clinical strains. *Salmonella* isolates from England, Wales, and Northern Ireland are served by the Gastrointestinal Bacteria Reference Laboratory in

Public Health England, where whole genome sequencing is now performed, and MLVA has been discontinued. The SSRL also intend to implement WGS for *Salmonella* in the current year.

From the Literature

Salmonella-related Literature from Scopus: April – June 2018

Savran, D., Pérez-Rodríguez, F., Kadir Halkman, A.
Modelling survival of Salmonella Enteritidis during storage of yoghurt at different temperatures
(2018) International Journal of Food Microbiology, 271, pp. 67-76.
ABSTRACT: The aim of this study was to evaluate the behaviour of Salmonella Enteritidis during the storage of yoghurt at different temperatures (4, 12, 20, and 25 °C), and to develop mathematical models to predict the behaviour of this bacterium as a function of storage temperature. Results indicated that Salmonella was able to survive longer during storage when temperature was low (e.g. 304 h at 4 °C, 60 h at 25 °C). The Geeraerd model with log-decrease and tailing was selected as the most suitable model to describe survival. To evaluate the effect of storage temperature on kinetic parameters such as death rate (kmax) secondary models were developed. The kmax was maximum at 25 °C and minimum at 4 °C with kmax = 0.28 and 0.039 h−1, respectively. The residual population (Nres) ranged 0.5 and 1.8 log CFU/g but there was no temperature dependency of this parameter. A probabilistic example was conduced based on the developed model to assess the exposure to Salmonella by consumption of traditional Turkish yoghurt.
ISSN: 01681605

Marshall, K.E.H., Tewell, M., Tecle, S., Leeper, M., Sinatra, J., Kissler, B., Fung, A., Brown, K., Wagner, D., Trees, E., Hise, K.B., Chaturvedi, V., Schlater, L.K., Morningstar-Shaw, B.R., Whitlock, L., Holt, K., Becker, K., Nichols, M., Williams, I.T., Jhung, M., Wise, M.E., Gieraltowski, L.
Protracted outbreak of salmonella Newport infections linked to ground beef: Possible role of dairy cows — 21 states, 2016–2017
(2018) Morbidity and Mortality Weekly Report, 67 (15), pp. 443-446.
ABSTRACT: What is already known about this topic? Previous outbreaks of salmonellosis were linked to contaminated ground beef produced from slaughtered dairy cows. What is added by this report? Contaminated ground beef was the likely source of a protracted outbreak of 106 Salmonella Newport infections, 42 hospitalizations, and one death in 21 states during October 2016–July 2017. While no direct link was found, whole genome sequencing suggests dairy cows were the ultimate outbreak source. What are the implications for public health practice? Foodborne outbreak investigations could be enhanced by improvements in the traceability of cows from their originating farms or sale barns, through slaughter and processing establishments, to ground beef sold to consumers. ISSN: 01492195

Han, J., Pendleton, S.J., Deck, J., Singh, R., Gilbert, J., Johnson, T.J., Sanad, Y.M., Nayak, R., Foley, S.L.
Impact of co-carriage of IncA/C plasmids with additional plasmids on the transfer of antimicrobial resistance in Salmonella enterica isolates
(2018) International Journal of Food Microbiology, 271, pp. 77-84.
ABSTRACT: Background: Antimicrobial resistance in Salmonella enterica is often plasmid encoded. A key resistance plasmid group is the incompatibility group (Inc) A/C plasmids that often carry multiple resistance determinants. Previous studies showed that IncA/C plasmids were often co-located with other plasmids. The current study was undertaken to evaluate the impact of plasmid co-carriage on antimicrobial resistance and plasmid transfer. Methods: A total of 1267 Salmonella isolates, representing multiple serotypes and sources were previously subjected to susceptibility testing and 251 isolates with resistance to at least 5 antimicrobial agents were identified for further study. Each isolate was subjected to PCR-based replicon typing, and those with IncA/C plasmids were selected for plasmid isolation, PCR-based mapping of IncA/C plasmid backbone genes, and conjugation assays to evaluate resistance plasmid transferability. Results: Of the 87 identified IncA/C positive isolates, approximately 75% carried a plasmid with another identified replicon type, with the most common being I1 (39%), FIA, FIIA, FIB and HI2 (each 15%). PCR-based mapping indicated significant diversity in IncA/C backbone content, especially in regions encoding transfer-associated and hypothetical proteins. Conjugation experiments showed that nearly 68% of the isolates transferred resistance plasmids, with 90% containing additional identified plasmids or larger (>50 kb) non-typeable plasmids. Conclusions: The majority of IncA/C-positive strains were able to conjugally transfer

antimicrobial resistance to the recipient, encoded by IncA/C and/or co-carried plasmids. These findings highlight the importance of co-located plasmids for resistance dissemination either by directly transferring resistance genes or by potentially providing the needed conjugation machinery for IncA/C plasmid transfer. ISSN: 01681605

Yan, T., O'Brien, P., Shelton, J.M., Whelen, A.C., Pagaling, E.
Municipal Wastewater as a Microbial Surveillance Platform for Enteric Diseases: A Case Study for Salmonella and Salmonellosis
(2018) Environmental Science and Technology, 52 (8), pp. 4869-4877.
ABSTRACT: Municipal wastewater (MW) contains a conglomeration of human enteric microbiota from a community and, hence, represents a potential surveillance tool for gastrointestinal infectious disease burden at the community level. To evaluate this, the concentration of Salmonella in MW samples from Honolulu, Hawaii, was monitored over a 54-week period, which showed positive and significant linear and rank correlation with clinical salmonellosis case numbers over the same period. Salmonella isolates were obtained from the MW samples and then compared with clinical isolates obtained by the Hawaii Department of Health State Laboratories over the same period. The MW isolate collection contained 34 serotypes, and the clinical isolate collection contained 47 serotypes, 21 of which were shared between the two isolate collections, including nine of the 12 most commonly detected clinical serotypes. Most notably, nine Salmonella strains, including one outbreak-associated Paratyphi B strain and eight other clinically rare strains, were shared and concurrently detected between the MW and the clinical isolate collections, indicating the feasibility of using enteric pathogens in the MW as a timely indication of community enteric disease activity. ISSN: 0013936X

Mastrorilli, E., Pietrucci, D., Barco, L., Ammendola, S., Petrin, S., Longo, A., Mantovani, C., Battistoni, A., Ricci, A., Desideri, A., Losasso, C.
A comparative genomic analysis provides novel insights into the ecological success of the monophasic Salmonella serovar 4,[5],12:i:-
(2018) Frontiers in Microbiology, 9 (APR), art. no. 715, .
ABSTRACT: Over the past decades, Salmonella 4,[5],12:i:- has rapidly emerged and it is isolated with high frequency in the swine food chain. Although many studies have documented the epidemiological success of this serovar, few investigations have tried to explain this phenomenon from a genetic perspective. Here a comparative whole-genome analysis of 50 epidemiologically unrelated S. 4,[5],12:i:-, isolated in Italy from 2010 to 2016 was performed, characterizing them in terms of genetic elements potentially conferring resistance, tolerance and persistence characteristics. Phylogenetic analyses indicated interesting distinctions among the investigated isolates. The most striking genetic trait characterizing the analyzed isolates is the widespread presence of heavy metals tolerance gene cassettes: most of the strains possess genes expected to confer resistance to copper and silver, whereas about half of the isolates also contain the mercury tolerance gene merA. A functional assay showed that these genes might be useful for preventing the toxic effects of metals, thus supporting the hypothesis that they can contribute to the success of S. 4,[5],12:i:- in farming environments. In addition, the analysis of the distribution of type II toxin-antitoxin families indicated that these elements are abundant in this serovar, suggesting that this is another factor that might favor its successful spread. ISSN: 1664302X

Dayuti, S.
Antibacterial activity of red algae (Gracilaria verrucosa) extract against Escherichia coli and Salmonella typhimurium
(2018) IOP Conference Series: Earth and Environmental Science, 137 (1), art. no. 012074
ABSTRACT: Red alga was widely used in several fields, including food, feed, phamacy and industrial point of view. The chemical analysis showed that red alga contained terpenoid, acetogenic, and aromatic compounds, which have a wide range of biological activities, such as anti-micobial, anti-inflammatory and anti-viral. The objectives of this research was to evaluate the effect of extraction solvent and time on antibacterial activity of red alga (Gracilaria verrucosa), and to explore the bioactive compound contained within Gracilaria verrucosa. The method in this study used descriptive reseach. These findings revealed that the highest inhibition activity among all extracts was obtained with the ratio of methanol:aquades (75:25) and extraction time around 72 hours against Escherichia coli and Salmonella typhimurium. The bioactive compounds of Gracilaria verrucosa tested by phytochemical analysisi consisted of flavonoid, alkaloid, and saponin. Those secondary metabolites may be approximated as antibactial substances. ISSN: 17551307

Busgang, A., Friedler, E., Gilboa, Y., Gross, A.
Quantitative microbial risk analysis for various bacterial exposure scenarios involving greywater reuse for irrigation
(2018) Water (Switzerland), 10 (4), art. no. 413, .
ABSTRACT: Greywater reuse can significantly reduce domestic water consumption. While the benefits are promising, risks are still under debate. Using a quantitative microbial risk-assessment model, we assessed the health risks associated with greywater reuse. The pathogens Salmonella enterica, Shigella spp., and Staphylococcus aureus were evaluated due to their possible prevalence in greywater and limited information regarding their potential risk with relation to greywater reuse for irrigation. Various exposure scenarios were investigated. Monte Carlo simulation was used and results were compared to the maximum "acceptable" limit of 10-6 disability-adjusted life years (DALY) set by the World Health Organization. Safe reuse was met for all worst-case exposure scenarios for Staphylococcus aureus, Salmonella enterica and Shigella spp. If their concentrations were kept below 10,000, 50 and 5 cfu/100 mL, respectively. For the best-practice (more realistic) scenarios, safe reuse was met for Staphylococcus aureus if its concentration was kept below 106 cfu/100 mL. Salmonella enterica met the safe reuse requirements if a maximum concentration of 500 cfu/100 mL was maintained and Shigella spp. if a maximum concentration was lower than 5 cfu/100 mL. Based on reported concentrations of these bacteria in greywater, proper treatment and disinfection are recommended. ISSN: 20734441

Chuah, L.-O., Shamila Syuhada, A.-K., Mohamad Suhaimi, I., Farah Hanim, T., Rusul, G.
Data on antibiogram and resistance genes harboured by Salmonella strains and their Pulsed-field gel electrophoresis clusters
(2018) Data in Brief, 17, pp. 698-702.
ABSTRACT: This article describes the Pulsed-field gel electrophoresis clustering of the predominant Salmonella strains (Salmonella ser. Albany, Salmonella ser. Brancaster, and Salmonella ser. Corvallis) isolated from poultry and processing environment in wet market and small-scale processing plant in Penang and Perlis, the northern states of Malaysia. Agar disk diffusion assay was performed to determine the phenotypic antibiotic resistance of these Salmonella strains. The most common antibiograms among the three predominant Salmonella serovars were reported. The presence of integrase genes and antibiotic resistance genes conferring to resistance against β-lactams, aminoglycosides, tetracyclines, quinolones, sulphonamides and chloramphenicol, was detected via PCR amplification. ISSN: 23523409

Nair, S., Farzan, A., O'Sullivan, T.L., Friendship, R.M.
Time course of salmonella shedding and antibody response in naturally infected pigs during grower-finisher stage
(2018) Canadian Journal of Veterinary Research, 82 (2), pp. 139-145.
ABSTRACT: A longitudinal trial was conducted to determine the course of Salmonella shedding and antibody response in naturally infected grower-finisher pigs. Ten-week-old pigs (n = 45) were transferred from a farm with history of salmonellosis and housed at a research facility. Weekly fecal samples (weeks 1 to 11) as well as tissue samples at slaughter were cultured for Salmonella. Serum samples were tested for presence of Salmonella antibody by enzyme-linked immunosorbent assay (ELISA). Data were analyzed using a multilevel mixed-effects logistic regression model. Over 10 wk, 91% and 9% of pigs shed Salmonella ≤ 4 and > 5 times, respectively. The estimated median of Salmonella shedding duration was 3 to 4 wk but some pigs shed Salmonella for up to 8 wk. Salmonella shedding increased 1 wk post-arrival but followed a decreasing pattern afterwards up to week 11 ($P < 0.05$). Salmonella isolates (n = 29), which were recovered from 18 pigs at different occasions, were S. Typhimurium (28%), S. Livingstone (21%), S. Infantis (14%), S. Montevideo (7%), S. Benfica (3%), S. Amsterdam (3%), S. Senftenberg (17%), and S. I:Rough-O (7%). Of 11 pigs from which the first and last isolates were serotyped, 10 pigs were reinfected with a different serotype. At slaughter, Salmonella was isolated from 7 pigs, of which 5 (71%) had not tested positive for at least 7 wk prior to slaughter. Antibody response peaked 4 wk after the peak of Salmonella infection; Salmonella shedding reduced as antibody response elevated ($P < 0.05$). These findings indicate that pigs may shed Salmonella into the mid-point of the grower-finisher stage and may be reinfected with different serotypes. ISSN: 08309000

Redemann, M.A., Brar, J., Niebuhr, S.E., Lucia, L.M., Acuff, G.R., Dickson, J.S., Singh, M.

Evaluation of thermal process lethality for non-pathogenic Escherichia coli as a surrogate for Salmonella in ground beef
(2018) LWT - Food Science and Technology, 90, pp. 290-296.
ABSTRACT: The United States Department of Agricultures' Food Safety and Inspection Service (USDA-FSIS) has developed thermal lethality guidelines for non-typhoidal Salmonella inactivation in ready-to-eat (RTE) beef and poultry, but additional means of thermal processing validation are limited. Thus, the objective of this study was to determine if non-pathogenic Escherichia coli could be used as a surrogate for Salmonella as means to validate thermal processing parameters per Appendix A. To develop thermal death time curves, ground beef at varying fat contents (5, 10, 20, 25, and 30%) was inoculated with either Salmonella or E. coli and heat treated. At 54, 57, 60, and 63 °C across all fat levels, the E. coli surrogates had significantly greater (P <.05) decimal-reduction values (D-values) than Salmonella. Beyond temperature 63 °C, regardless of fat, E. coli surrogates and Salmonella were inactivated at similar rates (P >.05). Greater reduction of E. coli surrogates in the ground beef post-lethality treatment suggest Salmonella inactivation at higher temperatures. The most appropriate use of the E. coli surrogates would be for predicting, ensuring, and validating thermal processing for Salmonella inactivation at lower temperatures. However, effects of meat product composition and processing facility variables need to be further assessed.
ISSN: 00236438

Belk, A.D., Arnold, A.N., Sawyer, J.E., Griffin, D.B., Taylor, T.M., Savell, J.W., Gehring, K.B.
Comparison of salmonella prevalence rates in bovine lymph nodes across feeding stages
(2018) Journal of Food Protection, 81 (4), pp. 549-553.
ABSTRACT: Peripheral lymph nodes (LNs) located in the fatty tissues of beef carcasses have been shown to harbor Salmonella and, thus, potentially contaminate ground beef. Salmonella prevalence within LNs is known to differ among feedlots. Two South Texas feeding operations (identified as locations A and B) known to harbor salmonellae in the feedlot environment, while historically producing cattle with opposing rates (one "high" and one "low") of Salmonella prevalence in LNs, were used in this study. To determine whether this difference was due to cattle source or factors associated with different stages of feeding, weanling steers of common and known origin were followed through normal feeding stages at both operations. Eighty Angus-sired beef steers were harvested at each of four feeding stages: 1, postweaning; 2, background or stocker; 3, 60 days on feed; and 4, 120 days on feed. Left and right subiliac and superficial cervical LNs (n= 304) were collected from each carcass, and similar node types were pooled by animal (n=152). Results showed a difference (P < 0.05) in prevalence of Salmonella in bovine lymph nodes between location A and location B and among feeding stages in location B. Salmonella was not isolated from any feeding stage 1 (postweaning) or location A LN samples. Within location B, there was an increase in Salmonella prevalence as cattle moved into later stages of feeding: at 22.2% (4 of 18), 77.8% (14 of 18), and 94.4% (17 of 18) for feeding stages 2, 3, and 4, respectively. Although the reasons for the differences seen between feeding operations and for increased Salmonella prevalence in LNs at later feeding stages remain unexplained, these results indicate that factors other than cattle source are likely influencing Salmonella prevalence in LNs. ISSN: 0362028X

Biasino, W., De Zutter, L., Mattheus, W., Bertrand, S., Uyttendaele, M., Van Damme, I.
Correlation between slaughter practices and the distribution of Salmonella and hygiene indicator bacteria on pig carcasses during slaughter
(2018) Food Microbiology, 70, pp. 192-199.
ABSTRACT: This study investigated the distribution of hygiene indicator bacteria and Salmonella on pig carcasses. Moreover, the relation between hygiene indicator counts and Salmonella presence as well as associations between specific slaughter practices and carcass contamination were determined for each carcass area. Seven Belgian pig slaughterhouses were visited three times to swab five randomly selected carcasses at nine different areas, after evisceration and trimming. Information about slaughter practices was collected using a questionaire. In all samples, the E. coli and Salmonella presence was analyzed and Enterobacteriaceae and total aerobic bacteria were quantified. Average total aerobic counts ranged from 3.1 (loin, pelvic duct, ham) to 4.4 log10 CFU/cm2 (foreleg). Median Enterobacteriaceae numbers varied between 0.4 (ham) an 1.8 log10 CFU/cm2 (foreleg). E. coli and Salmonella presence ranged from 15% (elbow) to 89% (foreleg) and 5% (elbow) to 38% (foreleg), respectively. Positive relations were found between hygiene indicator counts and Salmonella presence at the head, sternum, loin and throat. Several slaughter practices, such as splitting the head and incising tonsils, were associated with

higher levels of hygiene indicator bacteria and Salmonella. These findings can be used to educate slaughterhouse personnel and estimate the public health risk involved in consumption of different pork cuts. ISSN: 07400020

López-Gálvez, F., Gil, M.I., Allende, A.
Impact of relative humidity, inoculum carrier and size, and native microbiota on Salmonella ser. Typhimurium survival in baby lettuce
(2018) Food Microbiology, 70, pp. 155-161.
ABSTRACT: The effects of relative humidity (RH), fluctuating climate conditions, inoculum size and carrier on the survival of Salmonella enterica serovar Typhimurium on baby lettuce in environmental test chambers were studied. Buffered peptone water (BPW), distilled water (DW), and irrigation water (IW) were compared as inoculum carriers. Additionally, survival of Salmonella in suspensions prepared using filtered and unfiltered IW was assessed. Salmonella Typhimurium survived better on baby lettuce plants at high RH independently of the inoculum size. When lettuce plants were grown under fluctuating environmental conditions, Salmonella survival was similar under both RH conditions. Regarding the inoculum carrier, the inoculated microorganism survived better on lettuce plants when BPW was used as carrier both at high and low RH. Survival rate of Salmonella in IW was affected by the presence of native microbiota. Native microbiota present in IW did not affect survival of Salmonella or the levels of mesophilic bacteria on the baby lettuce leaves. The information obtained in the present study contributes to the knowledge on the effect of environmental conditions on pathogenic bacteria survival on growing edible plants. These results are useful when selecting the methodology to carry out experimental studies on the survival of microbial pathogens under different pre-harvest conditions. ISSN: 07400020

Keller, S.E., Anderson, N.M., Wang, C., Burbick, S.J., Hildebrandt, I.M., Gonsalves, L.J., Suehr, Q.J., Farakos, S.M.S.
Survival of Salmonella during production of partially sprouted pumpkin, sunflower, and chia seeds dried for direct consumption
(2018) Journal of Food Protection, 81 (4), pp. 520-527.
ABSTRACT: Ready-to-eat foods based on dried partially sprouted seeds have been associated with foodborne salmonellosis. Whereas research has focused on the potential for Salmonella initially present in or on seeds to grow and survive during fresh sprout production, little is known about the potential for growth and survival of Salmonella associated with seeds that have been partially sprouted and dried. The goal of this study was to determine the growth of Salmonella during soaking for partial germination of pumpkin, sunflower, and chia seeds and subsequent survival during drying and storage. Pumpkin, sunflower, and chia seeds were inoculated with a four-serotype Salmonella cocktail by the dry transfer method and were soaked in sterile water at 25 or 37°C for 24 h. During the soaking period, Salmonella exhibited growth rates of 0.37 ± 0.26, 0.27 ± 0.12, and 0.45 ± 0.19 log CFU/h at 25°C and 0.94 ± 0.44, 1.04 ± 0.84, and 0.73 ± 0.36 log CFU/h at 37°C for chia, pumpkin, and sunflower seeds, respectively. Soaked seeds were drained and dried at 25, 51, and 60°C. Drying resulted in >5 log CFU/g loss at both 51 and 60°C and ~3 log CFU/g loss at 25°C on partially sprouted pumpkin and sunflower seeds. There was no decrease in Salmonella during drying of chia seeds at 25°C, and only drying at 60°C provided losses >5 log CFU/g. Dried seeds were stored at 37 and 45°C at 15 and 76% relative humidity (RH) levels. The combination of temperature and RH exerted a stronger effect than either factor alone, such that rates at which Salmonella decreased generally followed this order: 37°C at 15% RH <45°C at 15% RH < 37°C at 76% RH < 45°C at 76% RH for all seeds tested. Rates differed based on seed type, with chia seeds and chia seed powder having the smallest rate of Salmonella decrease, followed by sunflower and pumpkin seeds. Drying at higher temperatures (50 and 61°C) or storing at elevated temperature and humidity (45°C and 76% RH) resulted in significantly different rates of Salmonella decrease. ISSN: 0362028X

Murray, K., Tremblay, C., Rghei, A., Warriner, K.
Challenges and options for enhancing Salmonella control in partially cooked breaded poultry products
(2018) Current Opinion in Food Science, 20, pp. 44-50.
ABSTRACT: There has been an increased incidence of foodborne illness cases associated with partially cooked breaded poultry contaminated with hypervirulent Salmonella Enteritidis. The increased incidence have been primarily caused by undercooking and hence initiatives to date have focused on ensuring labeling clearly states that the product is raw. A more effective approach is to reduce carriage of Salmonella associated with breaded poultry products. Interventions that have included biological, chemical and

physical interventions have demonstrated potential. Physical treatments based on high pressure processing are effective but commercially unfeasible. A more promising approach is inclusion of essential oils or organic acids in product formulations that sensitizes Salmonella to thermal inactivation during the browning and cooking step along with reducing persistence during frozen storage. ISSN: 22147993

Alikhan, N.-F., Zhou, Z., Sergeant, M.J., Achtman, M.
A genomic overview of the population structure of Salmonella
(2018) PLoS Genetics, 14 (4), art. no. e1007261, .
ABSTRACT: For many decades, Salmonella enterica has been subdivided by serological properties into serovars or further subdivided for epidemiological tracing by a variety of diagnostic tests with higher resolution. Recently, it has been proposed that so-called eBurst groups (eBGs) based on the alleles of seven housekeeping genes (legacy multilocus sequence typing [MLST]) corresponded to natural populations and could replace serotyping. However, this approach lacks the resolution needed for epidemiological tracing and the existence of natural populations had not been independently validated by independent criteria. Here, we describe EnteroBase, a web-based platform that assembles draft genomes from Illumina short reads in the public domain or that are uploaded by users. EnteroBase implements legacy MLST as well as ribosomal gene MLST (rMLST), core genome MLST (cgMLST), and whole genome MLST (wgMLST) and currently contains over 100,000 assembled genomes from Salmonella. It also provides graphical tools for visual interrogation of these genotypes and those based on core single nucleotide polymorphisms (SNPs). eBGs based on legacy MLST are largely consistent with eBGs based on rMLST, thus demonstrating that these correspond to natural populations. rMLST also facilitated the selection of representative genotypes for SNP analyses of the entire breadth of diversity within Salmonella. In contrast, cgMLST provides the resolution needed for epidemiological investigations. These observations show that genomic genotyping, with the assistance of EnteroBase, can be applied at all levels of diversity within the Salmonella genus.
ISSN: 15537390

Panzenhagen, P.H.N., Cabral, C.C., Suffys, P.N., Franco, R.M., Rodrigues, D.P., Conte-Junior, C.A.
Comparative genome analysis and characterization of the Salmonella Typhimurium strain CCRJ_26 isolated from swine carcasses using whole-genome sequencing approach
(2018) Letters in Applied Microbiology, 66 (4), pp. 352-359.
ABSTRACT: Abstract: Salmonella pathogenicity relies on virulence factors many of which are clustered within the Salmonella pathogenicity islands. Salmonella also harbours mobile genetic elements such as virulence plasmids, prophage-like elements and antimicrobial resistance genes which can contribute to increase its pathogenicity. Here, we have genetically characterized a selected S. Typhimurium strain (CCRJ_26) from our previous study with Multiple Drugs Resistant profile and high-frequency PFGE clonal profile which apparently persists in the pork production centre of Rio de Janeiro State, Brazil. By whole-genome sequencing, we described the strain's genome virulent content and characterized the repertoire of bacterial plasmids, antibiotic resistance genes and prophage-like elements. Here, we have shown evidence that strain CCRJ_26 genome possible represent a virulence-associated phenotype which may be potentially virulent in human infection. Significance and Impact of the Study: Whole-genome sequencing technologies are still costly and remain underexplored for applied microbiology in Brazil. Hence, this genomic description of S. Typhimurium strain CCRJ_26 will provide help in future molecular epidemiological studies. The analysis described here reveals a quick and useful pipeline for bacterial virulence characterization using whole-genome sequencing approach.
ISSN: 02668254

Wambui, J., Lamuka, P., Karuri, E., Matofari, J., Njage, P.M.K.
Microbial contamination level profiles attributed to contamination of beef carcasses, personnel, and equipment: Case of small and medium enterprise slaughterhouses
(2018) Journal of Food Protection, 81 (4), pp. 684-691.
ABSTRACT: The microbial contamination level profiles (MCLPs) attributed to contamination of beef carcasses, personnel, and equipment in five Kenyan small and medium enterprise slaughterhouses were determined. Aerobic plate counts, Enterobacteriaceae, Staphylococcus, and Salmonella were used to determine contamination at four different slaughter stages, namely, dehiding, evisceration, splitting, and dispatch. Microbiological criteria of the four microorganisms were used to score contamination levels (CLs) as poor (0), poor to average (1), average (2), or good (3). MCLPs were further assigned to carcasses, personnel, and equipment at each stage by summing up the CL scores. The CL score attributed to aerobic plate count contamination was 2 or 3 for carcasses but 0 for

personnel and equipment in almost all slaughterhouses. A score of 0 on carcasses was mostly attributed to Enterobacteriaceae at evisceration and to Salmonella at dehiding and evisceration. In addition, a score of 0 was mostly attributed to Staphylococcus contamination of personnel at dehiding. A score of 3 was attributed mostly to Enterobacteriaceae on hands at splitting, whereas a score of 2 was mostly attributed to the clothes at dehiding and evisceration. A CL score of 3 was mostly attributed to Enterobacteriaceae and Salmonella contamination of equipment at dehiding and splitting, respectively. Although CLs attributed to contamination of carcasses, personnel, and equipment ranged from 0 to 3, the maximum MCLP score of 9 was only attained in carcasses from two slaughterhouses at dehiding and from one slaughterhouse at dispatch. There is, therefore, a lot of room for small and medium enterprise slaughterhouses to improve their food safety objectives by improving food safety management systems at the points characterized by low CL scores. ISSN: 0362028X

Kawasaki, S., Hosotani, Y., Noviyanti, F., Koseki, S., Inatsu, Y.
Growth delay analysis of heat-injured Salmonella Enteritidis in ground beef by real-time PCR
(2018) LWT - Food Science and Technology, 90, pp. 499-504.
ABSTRACT: We aimed to estimate the bacterial injury level from ground beef samples that underwent various heat exposure treatments. The growth delay time (GDT) in recovery medium was estimated by real-time PCR monitoring assay. Samples of Salmonella Enteritidis in PBS and ground beef were exposed to heat stress in water bath at 52.5–62.5 °C for 0–60 min. Heat-treated samples were transferred to fresh trypticase soy broth, and S. Enteritidis growth recovery was monitored by real-time PCR. Sampling was conducted every 2 h, and total DNA was extracted. The S. Enteritidis cell number was estimated by real-time PCR, and growth recovery curve was constructed from the DNA copy number of the Salmonella invA gene. Growth recovery curve was used for kinetic analysis of GDT. Injured bacteria level in ground beef samples after heat exposure shown differences compared to PBS, where ground beef samples had lower variations in GDT than did PBS samples. Relationship between GDT and heat exposure time was observed where the slope of GDT increased as heat exposure time extended. Recovery of heat-treated S. Enteritidis in near sub-lethal conditions in PBS and ground beef samples, which could not be evaluated by traditional culture methods, was successfully monitored by real-time PCR. ISSN: 00236438

De Cesare, A., Doménech, E., Comin, D., Meluzzi, A., Manfreda, G.
Impact of Cooking Procedures and Storage Practices at Home on Consumer Exposure to Listeria Monocytogenes and Salmonella Due to the Consumption of Pork Meat
(2018) Risk Analysis, 38 (4), pp. 638-652.
ABSTRACT: The objective of this research was to analyze the impact of different cooking procedures (i.e., gas hob and traditional static oven) and levels of cooking (i.e., rare, medium, and well-done) on inactivation of Listeria monocytogenes and Salmonella in pork loin chops. Moreover, the consumer's exposure to both microorganisms after simulation of meat leftover storage at home was assessed. The results showed that well-done cooking in a static oven was the only treatment able to inactivate the tested pathogens. The other cooking combinations allowed to reach in the product temperatures always ≥73.6 °C, decreasing both pathogens between 6 log10 cfu/g and 7 log10 cfu/g. However, according to simulation results, the few cells surviving cooking treatments can multiply during storage by consumers up to 1 log10 cfu/g, with probabilities of 0.059 (gas hob) and 0.035 (static oven) for L. monocytogenes and 0.049 (gas hob) and 0.031 (static oven) for Salmonella. The key factors affecting consumer exposure in relation to storage practices were probability of pathogen occurrence after cooking, doneness degree, time of storage, and time of storage at room temperature. The results of this study can be combined with prevalence data and dose–response models in risk assessment models and included in guidelines for consumers on practices to be followed to manage cooking of pork meat at home. ISSN: 02724332

Chang, H.-S., Kim, D.-H., Jeong, D., Kang, I.-B., Kim, H.-S., Kim, H., Song, K.-Y., Seo, K.-H.
Fates of Salmonella Enteritidis and Cronobacter sakazakii in various multiple-strain yogurts and kefir during cold storage
(2018) Journal of Food Safety, 38 (2), art. no. e12429, .
ABSTRACT: Many multiple-strain fermented milks such as yogurt and kefir have been developed and consumed to improve their nutritional and functional benefits. Since these fermented milks can be a vehicle of foodborne illness, we investigated the fates of Salmonella Enteritidis and Cronobacter sakazakii in four fermented milks: multiple-lactic

acid bacteria (multi-LAB) yogurt, multiple-LAB-Bifidobacterium (multi-LAB-BIF) yogurt, pH 4.5 kefir (mild kefir), and pH 3.6 kefir (strong kefir). Each was inoculated with 5.6 and 5.8 log cfu/ml of S. Enteritidis and C. sakazakii, respectively, and stored at 4°C for 5 days. Strong and mild kefirs exhibited more potent antimicrobial activities than multi-LAB and multi-LAB-BIF yogurts, inactivating all viable pathogenic bacteria within 1 and 5 days, respectively. Despite having lower pH values than mild kefir (pH 4.49), multi-LAB (pH 4.25) and multi-LAB-BIF (pH 4.38) yogurts failed to clear viable S. Enteritidis cells in 5 days (> 5 log cfu/ml cells survived). Practical applications: Yogurt is one of the most popular fermented milk, and has been implicated in several human foodborne outbreaks. Kefir is unique fermented milk containing multiple strains of lactic acid bacteria and yeast. In this study, we investigated the fates of Salmonella Enteritidis and Cronobacter sakazakii in these fermented milks. Two types of commercial yogurt failed to clear viable S. Enteritidis cells in 5 days, whereas kefir successfully killed all viable S. Enteritidis and C. sakazakii cells in 5 and 4 days, respectively, even with significantly higher pH values suggesting that pH might not be a suitable indicator to ensure the microbiological safety of yogurts and kefir, and that pathogens face other antimicrobial hurdles than low pH and high acidity. Considering the potent self-clearance effects of kefir against foodborne pathogens, novel yogurt products containing strains with potent antimicrobial activity such as kefir microorganisms could be newly developed in the future. ISSN: 01496085

Eriksson, H., Söderlund, R., Ernholm, L., Melin, L., Jansson, D.S.
Diagnostics, epidemiological observations and genomic subtyping in an outbreak of pullorum disease in non-commercial chickens
(2018) Veterinary Microbiology, 217, pp. 47-52.
ABSTRACT: Salmonella Gallinarum biovar Pullorum (S. Pullorum) is a poultry pathogen associated with significant economic losses and animal suffering. Strict eradication programmes have eliminated S. Pullorum from the commercial poultry sector in most regions, but occasional outbreaks still occur in the non-commercial population. In 2012, pullorum disease was diagnosed in a non-commercial flock in Sweden. Epidemiological, post-mortem and bacteriological investigations identified three more infected flocks. To study the epidemiological relationships between the flocks and evaluate different subtyping methods for S. Pullorum, 13 isolates from the four infected flocks were investigated by pulsed-field gel electrophoresis (PFGE) and multi-locus variable number tandem repeat analysis (MLVA). Four isolates collected from two non-commercial flocks in 2001 were also included. Six representative isolates from 2012 were further analysed by high-throughput sequencing. To differentiate between biovars Gallinarum and Pullorum, ten regions of difference (RODs) were investigated by in silico PCR. Three different PFGE-types were observed from the four epidemiologically linked farms in 2012 and MLVA further discriminated the isolates. SNP typing based on high-throughput sequencing clustered the four farms from the 2012 outbreak in two pairs. The PFGE, MLVA and high-throughput sequencing results suggested at least two different sources of infection or a common genetically mixed source in 2012. High-throughput sequencing is useful both for subtyping of S. Pullorum and may also be used for differentiating between the two biovars Pullorum and Gallinarum. ISSN: 03781135

Goeman, V.R., Tinkler, S.H., Hammac, G.K., Ruple, A.
Evaluation of environmental sampling methods for detection of Salmonella enterica in a large animal veterinary hospital
(2018) Canadian Veterinary Journal, 59 (4), pp. 408-412.
ABSTRACT: Environmental surveillance for Salmonella enterica can be used for early detection of contamination; thus routine sampling is an integral component of infection control programs in hospital environments. At the Purdue University Veterinary Teaching Hospital (PUVTH), the technique regularly employed in the large animal hospital for sample collection uses sterile gauze sponges for environmental sampling, which has proven labor-intensive and time-consuming. Alternative sampling methods use Swiffer brand electrostatic wipes for environmental sample collection, which are reportedly effective and efficient. It was hypothesized that use of Swiffer wipes for sample collection would be more efficient and less costly than the use of gauze sponges. A head-to-head comparison between the 2 sampling methods was conducted in the PUVTH large animal hospital and relative agreement, cost-effectiveness, and sampling efficiency were compared. There was fair agreement in culture results between the 2 sampling methods, but Swiffer wipes required less time and less physical effort to collect samples and were more cost-effective. ISSN: 00085286

Aydin, M., Carter-Conger, J., Gao, N., Gilmore, D.F., Ricke, S.C., Ahn, S.

Molecular identification of common Salmonella serovars using multiplex DNA sensor-based suspension array
(2018) Analytical and Bioanalytical Chemistry, 410 (10), pp. 2637-2646.
ABSTRACT: Salmonella is one of major foodborne pathogens and the leading cause of foodborne illness-related hospitalizations and deaths. It is critical to develop a sensitive and rapid detection assay that can identify Salmonella to ensure food safety. In this study, a DNA sensor-based suspension array system of high multiplexing ability was developed to identify eight Salmonella serovars commonly associated with foodborne outbreaks to the serotype level. Each DNA sensor was prepared by activating pre-encoded microspheres with oligonucleotide probes that are targeting virulence genes and serovar-specific regions. The mixture of 12 different types of DNA sensors were loaded into a 96-well microplate and used as a 12-plex DNA sensor array platform. DNA isolated from Salmonella was amplified by multiplex polymerase chain reaction (mPCR), and the presence of Salmonella was determined by reading fluorescent signals from hybridization between probes on DNA sensors and fluorescently labeled target DNA using the Bio-Plex® system. The developed multiplex array was able to detect synthetic DNA at the concentration as low as 100 fM and various Salmonella serovars as low as 100 CFU/mL within 1 h post-PCR. Sensitivity of this assay was further improved to 1 CFU/mL with 6-h enrichment. The array system also correctly and specifically identified serotype of tested Salmonella strains without any cross-reactivity with other common foodborne pathogens. Our results indicate the developed DNA sensor suspension array can be a rapid and reliable high-throughput method for simultaneous detection and molecular identification of common Salmonella serotypes.
ISSN: 16182642

Cameron-Veas, K., Fraile, L., Napp, S., Garrido, V., Grilló, M.J., Migura-Garcia, L.
Multidrug resistant Salmonella enterica isolated from conventional pig farms using antimicrobial agents in preventative medicine programmes
(2018) Veterinary Journal, 234, pp. 36-42.
ABSTRACT: A longitudinal study was conducted to investigate the presence of multidrug antimicrobial resistance (multi-AR) in Salmonella enterica in pigs reared under conventional preventative medicine programmes in Spain and the possible association of multi-AR with ceftiofur or tulathromycin treatment during the pre-weaning period. Groups of 7-day-old piglets were treated by intramuscular injection with ceftiofur on four farms (n = 40 piglets per farm) and with tulathromycin on another four farms (n = 40 piglets per farm). A control group of untreated piglets (n = 30 per farm) was present on each farm. Faecal swabs were collected for S. enterica culture prior to treatment, at 2, 7 and 180 days post-treatment, and at slaughter. Minimal inhibitory concentrations of 14 antimicrobial agents, pulsed-field gel electrophoresis and detection of resistance genes representing five families of antimicrobial agents were performed. Plasmids carrying cephalosporin resistant (CR) genes were characterised. Sixty-six S. enterica isolates were recovered from five of eight farms. Forty-seven isolates were multi-AR and four contained blaCTX-M genes harboured in conjugative plasmids of the IncI1 family; three of these isolates were recovered before treatment with ceftiofur. The most frequent AR genes detected were tet(A) (51/66, 77%), sul1 (17/66, 26%); tet(B) (15/66, 23%) and qnrB (10/66, 15%). A direct relation between the use of ceftiofur in these conditions and the occurrence of CR S. enterica was not established. However, multi-AR was common, especially for ampicillin, streptomycin, sulphonamides and tetracycline. These antibiotics are used frequently in veterinary medicine in Spain and, therefore, should be used sparingly to minimise the spread of multi-AR. ISSN: 10900233

Medrano-Félix, J.A., Chaidez, C., Mena, K.D., Soto-Galindo, M.S., Castro-del Campo, N.
Characterization of biofilm formation by Salmonella enterica at the air-liquid interface in aquatic environments
(2018) Environmental Monitoring and Assessment, 190 (4), art. no. 221, .
ABSTRACT: Survival of bacterial pathogens in different environments is due, in part, to their ability to form biofilms. Four wild-type Salmonella enterica strains, two Oranienburg and two Saintpaul isolated from river water and animal feces, were tested for biofilm formation at the air-liquid interface under stressful conditions (pH and salinity treatments such as pH 3, NaCl 4.5 w/v; pH 7, NaCl 4.5 w/v; pH 10, NaCl 4.5 w/v; pH 3, Nacl 0.5 w/v; pH 7, NaCl 0.5 w/v; and pH 10, NaCl 0.5 w/v); Salmonella Typhimurium DT104 was used as a control strain. Salmonella Oranienburg and Saintpaul from feces were moderately hydrophobic and motile, while S. Saintpaul from water and the control strain S. Typhimurium showed high hydrophobicity, which helped them form more resistant biofilms than S. Oranienburg. Under stressful conditions, all strains experienced difficulties in forming biofilms. Salmonella Saintpaul and Typhimurium expressed the red dry and rough

(RDAR) morphotype and were able to form biofilm at air-liquid interface, contrarily to Oranienburg that showed incomplete rough morphology. This study contributes to the knowledge of biofilm formation as a survival strategy for Salmonella in aquatic environments. ISSN: 01676369

Mil-Homens, D., Barahona, S., Moreira, R.N., Silva, I.J., Pinto, S.N., Fialho, A.M., Arraiano, C.M.
Stress response protein BolA influences fitness and promotes Salmonella enterica serovar Typhimurium virulence
(2018) Applied and Environmental Microbiology, 84 (8), art. no. e02850-17, .
ABSTRACT: The intracellular pathogen Salmonella enterica serovar Typhimurium has emerged as a major cause of foodborne illness, representing a severe clinical and economic concern worldwide. The capacity of this pathogen to efficiently infect and survive inside the host depends on its ability to synchronize a complex network of virulence mechanisms. Therefore, the identification of new virulence determinants has become of paramount importance in the search of new targets for drug development. BolA-like proteins are widely conserved in all kingdoms of life. In Escherichia coli, this transcription factor has a critical regulatory role in several mechanisms that are tightly related to bacterial virulence. Therefore, in the present work we used the well-established infection model Galleria mellonella to evaluate the role of BolA protein in S. Typhimurium virulence. We have shown that BolA is an important player in S. Typhimurium pathogenesis. Specifically, the absence of BolA leads to a defective virulence capacity that is most likely related to the remarkable effect of this protein on S. Typhimurium evasion of the cellular response. Furthermore, it was demonstrated that BolA has a critical role in bacterial survival under harsh conditions since BolA conferred protection against acidic and oxidative stress. Hence, we provide evidence that BolA is a determining factor in the ability of Salmonella to survive and overcome host defense mechanisms, and this is an important step in progress to an understanding of the pathways underlying bacterial virulence. ISSN: 00992240

Sundström, K.
Cost of Illness for Five Major Foodborne Illnesses and Sequelae in Sweden
(2018) Applied Health Economics and Health Policy, 16 (2), pp. 243-257.
ABSTRACT: Objectives: The main objective of this study was to derive cost estimates of five major foodborne illnesses (campylobacteriosis, salmonellosis, enterohemorrhagic Escherichia coli (EHEC), yersiniosis and shigellosis) in Sweden. These estimates provide a necessary contribution to perform future cost-benefit analyses aimed at reducing the burden of foodborne disease. A secondary aim was to obtain estimates of the true number of cases that occur in the community, thus providing necessary ground for calculating costs. Methods: The true number of cases for each foodborne illness was simulated by multiplying the reported number of cases by sequential multipliers, one for each potential source of information loss about a case. This assessment of the true number of cases was then used to estimate the number of cases of sequelae for each illness. An incidence-based analysis was then used to calculate direct medical and non-medical costs, as well as indirect costs. Data for estimating the true number of cases for each illness were primarily based on an expert panel, while the derivation of costs mainly utilized national registries, databases and published literature. Results: The estimated number of cases was between 7- and 11-fold higher than the reported number of cases, indicating the importance of taking information loss into account when calculating costs. By far the most common pathogen of the five was campylobacter, with an estimated 101,719 (90% credibility interval [CI] 59,640–158,025) human cases occurring annually. For salmonella, 19,678 (90% CI 8394–40,456) cases were estimated to occur each year, while the other three pathogens were less common, with a yearly incidence of approximately 2500–5500 cases each. The total cost for the five pathogens (including sequelae) amounted to €142 million annually. Campylobacter was the most costly pathogen, representing 69% of the total costs. Salmonellosis and EHEC constituted 18 and 9% of these costs, respectively, while yersiniosis and shigellosis represented approximately 2% each. Costs for sequelae were significant and accounted for approximately 50% of the total costs. Conclusions: Our simulations indicated that campylobacter infection was more common and more costly than salmonella, EHEC, yersinia and shigella combined. Estimated costs for all illnesses were highly influenced by (1) considering potential information losses about cases in the population (which increased costs 7- to 11-fold), and (2) taking account of post-infection sequelae (which doubled the costs). ISSN: 11755652

Jongman, M., Korsten, L.

Irrigation water quality and microbial safety of leafy greens in different vegetable production systems: A review
(2018) Food Reviews International, 34 (4), pp. 308-328.
ABSTRACT: Access to large sources of quality water for irrigation is fundamental to the hygienic cultivation of fresh produce. However, due to factors such as contamination of water bodies, access to clean uncontaminated water is fast becoming an ever increasing global challenge. The unavailability of quality source water increases the risk of contamination of fresh produce with human pathogenic microorganisms, which may compromise public health. Over the past few years, there has been a decline in the microbiological quality of surface water and other sources used for irrigation. This is mainly due to upstream fecal contamination. Therefore, the assessment and subsequent suitability of alternative water sources for irrigation such as roof-harvested rainwater should be considered. Contrasting views regarding the quality of roof-harvested rainwater (RHRW) have been published. Pathogens such as Salmonella and Campylobacter species and Listeria monocytogenes have been reported in RHRW. Leafy green vegetables such as cabbage, spinach, and lettuce are produced across a wide range of farming systems from regulated formal (commercial farms) to informal (small-scale and homestead gardens) setups. This review will discuss global water challenges associated with irrigation water, microbial quality of source water for irrigation, crop contamination, and pathogen detection and characterization methodologies. ISSN: 87559129

Methner, U.
Immunisation of chickens with live Salmonella vaccines – Role of booster vaccination
(2018) Vaccine, 36 (21), pp. 2973-2977.
ABSTRACT: It is accepted that booster vaccinations of chickens with live Salmonella vaccines are essential part of vaccinations schemes to induce an effective adaptive immune response. As manufacturer of registered live Salmonella vaccines recommend different times of booster the question raises whether the duration between the first and second immunisation might influence the protective effect against Salmonella exposure. Chickens were immunised with a live Salmonella Enteritidis vaccine on day 1 of age followed by a booster vaccination at different intervals (day 28, 35 or 42 of age) to study the effects on the colonisation and invasion of the Salmonella vaccine strain, the humoral immune response and the efficacy against infection with Salmonella Enteritidis on day 56 of age. Immunisation of all groups resulted in a very effective adaptive immune response and a high degree of protection against severe Salmonella exposure, however, the time of booster had only an unverifiable influence on either the colonisation of the vaccine strain, the development of the humoral immune response or the colonisation of the Salmonella challenge strain. Therefore, the first oral immunisation of the chicks on day 1 of age seems to be of special importance and prerequisite for the development of the effective immune response. A booster immunisation should be carried out, however, the time of booster may vary between week 3 and week 7 of age of the chickens without adversely impact on the efficacy of the adaptive immune response or the protective effects. ISSN: 0264410X

Mba-Jonas, A., Culpepper, W., Hill, T., Cantu, V., Loera, J., Borders, J., Saathoff-Huber, L., Nsubuga, J., Zambrana, I., Dalton, S., Williams, I., Neil, K.P.
A Multistate Outbreak of Human Salmonella Agona Infections Associated with Consumption of Fresh, Whole Papayas Imported from Mexico - United States, 2011
(2018) Clinical Infectious Diseases, 66 (11), pp. 1756-1761.
ABSTRACT: Background Nontyphoidal Salmonella causes ~1 million food-borne infections annually in the United States. We began investigating a multistate outbreak of Salmonella serotype Agona infections in April 2011. Methods A case was defined as infection with the outbreak strain of Salmonella Agona occurring between 1 January and 25 August 2011. We developed hypotheses through iterative interviews. Product distribution analyses and traceback investigations were conducted. The Food and Drug Administration (FDA) tested papayas from Mexico for Salmonella. Results We identified 106 case patients from 25 states. Their median age was 21 years (range, 1-91). Thirty-nine of 61 case patients (64%) reported Hispanic/Latino ethnicity; 11 of 65 (17%) travelled to Mexico before illness. Thirty-two of 56 case patients (57%) reported papaya consumption. Distribution analyses revealed that three firms, including Distributor A, distributed papaya to geographic areas that aligned with both the location and timing of illnesses. Traceback of papayas purchased by ill persons in four states identified Distributor A as the common supplier. FDA testing isolated the outbreak strain from a papaya sample collected at distributor A and from another sample collected at the US-Mexico border, destined for distributor A. FDA isolated Salmonella species from 62 of 388 papaya import samples (16%). The investigation led to a recall of fresh, whole papayas from Distributor A and an FDA import alert for all papayas from Mexico. Conclusions This is the first reported

Salmonella outbreak in the United States linked to fresh, whole papayas. The outbreak highlights important issues regarding the safety of imported produce. ISSN: 10584838

Yang, Y., Tellez, G., Latorre, J.D., Ray, P.M., Hernandez, X., Hargis, B.M., Ricke, S.C., Kwon, Y.M.
Salmonella excludes Salmonella in poultry: Confirming an old paradigm using conventional and barcode-tagging approaches
(2018) Frontiers in Veterinary Science, 5 (MAY), art. no. 101, .
ABSTRACT: Salmonella is one of the major foodborne bacterial pathogens, and the consumption of contaminated chicken meats isa primary route of Salmonella transmission into human food chains. However, the mechanism of Salmonella transmission within the chicken flock is not fully understood, including competition among Salmonella strains during chicken infection. The purpose of the present study was to evaluate the competitive exclusion (CE) between different or same Salmonella species consecutively challenged through the oral route. Two different approaches were used to evaluate the CE effect, including tracking Salmonella colonization by wild-type strains with difference in natural antibiotic resistance or DNA barcode-tagged isogenic strains. When day-of-hatch chicks were administered by wild-type S. Typhimurium (ST) on day 1, followed by infection on day 2 by S. Enteritidis (SE) or vice versa, most of the birds were colonized only by the first strains administered (82% by ST or 83% by SE). When similar experiments were performed using two different isogenic barcode-tagged SE strains, Illumina sequencing analysis of the barcode region showed that the first barcode-tagged strains administered were dominant strains, ranging from 92 to 99% of the Salmonella recovered from ceca. These results provide quantitative evidence supporting the CE theory that oral administration of Salmonella will produce predominant inhibition over the subsequent colonization of ceca by the following administration one day later by different or same Salmonella species. We also showed that the use of barcode-tagged isogenic strains in combination with deep profiling of barcodes by Illumina sequencing can serve as a quantitative method for studying complex dynamics of Salmonella infection, transmission and colonization in poultry. ISSN: 22971769

Chousalkar, K., Gast, R., Martelli, F., Pande, V.
Review of egg-related salmonellosis and reduction strategies in United States, Australia, United Kingdom and New Zealand
(2018) Critical Reviews in Microbiology, 44 (3), pp. 290-303.
ABSTRACT: Globally, Salmonella enterica subsp. enterica is one of the most commonly reported causes of foodborne illness in humans. Contaminated food products of animal origin, particularly egg and egg products are frequently implicated in outbreaks of human salmonellosis. Salmonella enteritidis is frequently involved in egg and egg products-associated foodborne outbreaks in the USA and UK. However, in Australia and New Zealand, human infections caused by this serovar occur as a result of infection acquired while overseas travel, with Salmonella typhimurium being a predominant cause of local foodborne outbreaks. In this paper, an overview of Salmonella epidemiology on laying farms, egg-related Salmonella outbreaks in humans, and regulatory practises to control Salmonella across USA, UK, Australia and New Zealand is provided. Considering the estimated production of eggs in the USA, UK, Australia and New Zealand in 2015, the risk of foodborne illness in general is quite low for humans consuming eggs. Salmonella diagnostics, reporting and surveillance systems have improved over the years and will continue to improve in the years to come. However, given the number of different emerging Salmonella serovars a regular review of Salmonella control strategies from farm to fork is required. ISSN: 1040841X

Rönnqvist, M., Välttilä, V., Ranta, J., Tuominen, P.
Salmonella risk to consumers via pork is related to the Salmonella prevalence in pig feed
(2018) Food Microbiology, 71, pp. 93-97.
ABSTRACT: Pigs are an important source of human infections with Salmonella, one of the most common causes of sporadic gastrointestinal infections and foodborne outbreaks in the European region. Feed has been estimated to be a significant source of Salmonella in piggeries in countries of a low Salmonella prevalence. To estimate Salmonella risk to consumers via the pork production chain, including feed production, a quantitative risk assessment model was constructed. The Salmonella prevalence in feeds and in animals was estimated to be generally low in Finland, but the relative importance of feed as a source of Salmonella in pigs was estimated as potentially high. Discontinuation of the present strict Salmonella control could increase the risk of Salmonella in slaughter pigs and consequent infections in consumers. The increased use of low risk and controlled feed

ingredients could result in a consistently lower residual contamination in pigs and help the tracing and control of the sources of infections. ISSN: 07400020

Eeckhaut, V., Haesebrouck, F., Ducatelle, R., Van Immerseel, F.
Oral vaccination with a live Salmonella Enteritidis/Typhimurium bivalent vaccine in layers induces cross-protection against caecal and internal organ colonization by a Salmonella Infantis strain
(2018) Veterinary Microbiology, 218, pp. 7-12.
ABSTRACT: Salmonella is an important zoonotic agent, and poultry products remain one of the main sources of infection for humans. Salmonella Infantis is an emerging serotype in poultry worldwide, reflected by an increased prevalence in poultry flocks, on broiler meat and in human foodborne illness cases. In the current study, the efficacy of oral administration of a live monovalent Salmonella Enteritidis and a live bivalent Salmonella Enteritidis/Typhimurium vaccine, against a Salmonella Enteritidis and Infantis infection, was determined. Oral administration of the live vaccines to day-old chickens caused a decrease in caecal colonization by Salmonella Enteritidis, but not Infantis, at day 7, when challenged at day 2. Vaccination with the bivalent vaccine at day 1 resulted in a decreased spleen colonization by both Salmonella Infantis and Enteritidis. Twice (at day 1 and week 6) and thrice vaccination (at day 1, week 6 and 16) of laying hens with the bivalent vaccine resulted in a decreased caecal colonization by Salmonella Enteritidis and Infantis, and significantly lower oviduct colonization levels by Salmonella Enteritidis. These data show cross-protection against Salmonella Infantis by oral administration of live vaccine strains belonging to other serogroups. ISSN: 03781135

Bai, J., Trinetta, V., Shi, X., Noll, L.W., Magossi, G., Zheng, W., Porter, E.P., Cernicchiaro, N., Renter, D.G., Nagaraja, T.G.
A multiplex real-time PCR assay, based on invA and pagC genes, for the detection and quantification of Salmonella enterica from cattle lymph nodes
(2018) Journal of Microbiological Methods, 148, pp. 110-116.
ABSTRACT: Cattle lymph nodes can harbor Salmonella and potentially contaminate beef products. We have developed and validated a new real-time PCR (qPCR) assay for the detection and quantification of Salmonella enterica in cattle lymph nodes. The assay targets both the invA and pagC genes, the most conserved molecular targets in Salmonella enterica. An 18S rRNA gene assay that amplifies from cattle and other animal species was also included as an internal control. Available DNA sequences for invA, pagC and 18S rRNA genes were used for primer and probe selections. Three Salmonella serotypes, S. Typhimurium, S. Anatum, and S. Montevideo, were used to assess the assay's analytical sensitivity. Correlation coefficients of standard curves generated for each target and for all three serotypes were >99% and qPCR amplification efficiencies were between 93% and 110%. Assay sensitivity was also determined using standard curve data generated from Salmonella-negative cattle lymph nodes spiked with 10-fold dilutions of the three Salmonella serotypes. Assay specificity was determined using Salmonella culture method, and qPCR testing on 36 Salmonella strains representing 33 serotypes, 38 Salmonella strains of unknown serotypes, 252 E. coli strains representing 40 serogroups, and 31 other bacterial strains representing 18 different species. A collection of 647 cattle lymph node samples from steers procured from the Midwest region of the US were tested by the qPCR, and compared to culture-method of detection. Salmonella prevalence by qPCR for pre-enriched and enriched lymph nodes was 19.8% (128/647) and 94.9% (614/647), respectively. A majority of qPCR positive pre-enriched samples (105/128) were at concentrations between 104 and 105 CFU/mL. Culture method detected Salmonella in 7.7% (50/647) and 80.7% (522/647) of pre- and post-enriched samples, respectively; 96.0% (48/50) of pre-enriched and 99.4% (519/522) of post-enriched culture-positive samples were also positive by qPCR. More samples tested positive by qPCR than by culture method, indicating that the real-time PCR assay was more sensitive. Our data indicate that this triplex qPCR can be used to accurately detect and quantify Salmonella enterica strains from cattle lymph node samples. The assay may serve as a useful tool to monitor the prevalence of Salmonella in beef production systems. ISSN: 01677012

Sternberg Lewerin, S.
Theoretical value of pre-trade testing for Salmonella in Swedish cattle herds
(2018) Food Microbiology, 71, pp. 68-72.
ABSTRACT: The Swedish Salmonella control programme includes mandatory action if Salmonella is detected in a herd. The aim of this study was to assess the relative value of different strategies for pre-movement testing of cattle. Three fictitious herds were included: dairy, beef and specialised calf-fattening. The yearly risks of introducing Salmonella with and without individual serological or bulk milk testing were assessed as

well as the effects of sourcing animals from low-prevalence areas or reducing the number of source herds. The initial risk was highest for the calf-fattening herd and lowest for the beef herd. For the beef and dairy herds, the yearly risk of Salmonella introduction was reduced by about 75% with individual testing. Sourcing animals from low-prevalence areas reduced the risk by >99%. For the calf-fattening herd, the yearly risk was reduced by almost 50% by individual testing or sourcing animals from a maximum of five herds. The method was useful for illustrating effects of risk mitigation when introducing animals into a herd. Sourcing animals from low-risk areas (or herds) is more effective than single testing of individual animals or bulk milk. A comprehensive approach to reduce the risk of introducing Salmonella from source herds is justified. ISSN: 07400020

Mughini-Gras, L., Franz, E., van Pelt, W.
New paradigms for Salmonella source attribution based on microbial subtyping
(2018) Food Microbiology, 71, pp. 60-67.
ABSTRACT: Microbial subtyping is the most common approach for Salmonella source attribution. Typically, attributions are computed using frequency-matching models like the Dutch and Danish models based on phenotyping data (serotyping, phage-typing, and antimicrobial resistance profiling). Herewith, we critically review three major paradigms facing Salmonella source attribution today: (i) the use of genotyping data, particularly Multi-Locus Variable Number of Tandem Repeats Analysis (MLVA), which is replacing traditional Salmonella phenotyping beyond serotyping; (ii) the integration of case-control data into source attribution to improve risk factor identification/characterization; (iii) the investigation of non-food sources, as attributions tend to focus on foods of animal origin only. Population genetics models or simplified MLVA schemes may provide feasible options for source attribution, although there is a strong need to explore novel modelling options as we move towards whole-genome sequencing as the standard. Classical case-control studies are enhanced by incorporating source attribution results, as individuals acquiring salmonellosis from different sources have different associated risk factors. Thus, the more such analyses are performed the better Salmonella epidemiology will be understood. Reparametrizing current models allows for inclusion of sources like reptiles, the study of which improves our understanding of Salmonella epidemiology beyond food to tackle the pathogen in a more holistic way. ISSN: 07400020

Besser, J.M.
Salmonella epidemiology: A whirlwind of change
(2018) Food Microbiology, 71, pp. 55-59.
ABSTRACT: The field of infectious disease epidemiology for Salmonella and other enteric pathogens is undergoing some of the most profound changes since the time of Kauffman and White. Rapid advances in “big data” technologies such as genomics and metagenomics are making it possible to monitor and control salmonellosis in new and exciting ways. Epidemiological methods are becoming increasingly robust through the routine use of standardized hypothesis-generating questionnaires, iterative open-ended interviewing, informational trace-backs and new modeling techniques for describing the attribution of disease to food sources. In addition, Salmonella epidemiology is facing important challenges and new opportunities due to the rapid adoption of culture independent diagnostic test panels by clinical laboratories. Where is this unprecedented wave of change taking us? This chapter will examine emerging trends in Salmonella epidemiology, and take a peek into the not-so-distant future. ISSN: 07400020

Mooijman, K.A.
The new ISO 6579-1: A real horizontal standard for detection of Salmonella, at last!
(2018) Food Microbiology, 71, pp. 2-7.
ABSTRACT: Up to 2016, three international standard methods existed for the detection of Salmonella spp. in food, animal feed and samples from the primary production stage: ISO 6785:2001 for milk and milk products, ISO 6579:2002 for (other) food and animal feed and Annex D of ISO 6579:2007 for samples from the primary production stage. In 2009, an ISO/CEN working group started with the revision of ISO 6579:2002 with two main aims: combining the three aforementioned standards in one document and improving the information in ISO 6579:2002. Additionally it was decided to split ISO 6579 into three parts, where part 1 describes the detection, part 2 the enumeration by mini-MPN (published in 2012) and part 3 the serotyping of Salmonella (published in 2014). This paper describes the experiments and choices made for improving the part on detection of Salmonella (ISO 6579-1). The final voting stage on (draft) ISO 6579-1 was finished by the end of December 2016, with a positive outcome. Finally, a real horizontal standard became available for detection of Salmonella in food, animal feed, environmental samples in the

area of food production and food handling and in samples from the primary production stage in 2017. ISSN: 07400020

Sánchez-Rodríguez, J.A., Navas, L., Vinuesa, F.M., Castells, C., Martínez, M.A., López, A., Lindez, B., Cabrera-Vique, C.
New insights on the risk factors associated with the presence of Salmonella on pig carcasses. Lessons from small slaughterhouses
(2018) Food Control, 87, pp. 46-52.
ABSTRACT: This study analyses the prevalence of Salmonella on the surface of pig carcasses and identifies the serotypes present, in order to determine risk factors associated with the slaughter process. We analysed 393 samples, using the abrasive pad method, from pig carcasses obtained from three small slaughterhouses in southern Spain. Of the 393 samples, 45 (11.4%; 95% CI: 8.3–14.6%) were contaminated with Salmonella, with the main serotypes being S. Typhimurium (35.6%), S. Derby (31.1%) and S. Rissen (11.1%). In addition to this analysis, we examined 13 risk factors that have been described in previous research. The polishing stage, the use of pressurised water at the intermediate cleaning stage and the turbidity of the scalding water were the main risk factors found to be associated with the presence of Salmonella. Scalding the carcass using water with a turbidity exceeding 1000 NTU increases the risk of contamination by Salmonella (1001–2000 NTU, OR = 3.24, 95%CI: 1.12–9.35; >2000 NTU, OR = 6.26, 95%CI: 1.42–27.46), while the omission of the polishing stage (OR = 0.10; 95%CI: 0.01–0.87) and not using pressurised water in an intermediate cleaning process (OR = 0.09, 95%CI: 0.01–0.48) decrease the risk of contamination by this pathogen. ISSN: 09567135

Shippy, D.C., Bearson, B.L., Holman, D.B., Brunelle, B.W., Allen, H.K., Bearson, S.M.D.
Porcine Response to a Multidrug-Resistant Salmonella enterica serovar i 4,[5],12:i:- Outbreak Isolate
(2018) Foodborne Pathogens and Disease, 15 (5), pp. 253-261.
ABSTRACT: Salmonella enterica serovar I 4,[5],12:i:- has emerged as a common nontyphoidal Salmonella serovar to cause human foodborne illness. An interesting trait of serovar I 4,[5],12:i:- is that it only expresses the fliC gene for bacterial motility (i.e., monophasic), while most Salmonella strains alternately express two flagellin genes (fliC and fljB). The goal of this study was to characterize the porcine response following inoculation with a multidrug-resistant (MDR) serovar I 4,[5],12:i:- isolate associated with a multistate pork outbreak to determine if the increased prevalence of serovar I 4,[5],12:i:- in swine is due to enhanced pathogenicity. Pigs were inoculated and subsequently evaluated for the ability of the isolate to colonize intestinal tissues, cause clinical symptoms, induce an immune response, and alter the fecal microbiota over a 7-day period. Pigs exhibited a significant increase in rectal temperature (fever) ($p < 0.01$) and fecal moisture content (diarrhea) ($p < 0.05$) at 2 days postinoculation (d.p.i.) compared with preinoculation (day 0). Serum analyses revealed significantly increased interferon-gamma (IFN-γ) levels at 2 ($p \leq 0.0001$) and 3 ($p < 0.01$) d.p.i. compared with day 0, and antibodies against Salmonella lipopolysaccharide (LPS) were present in all pigs by 7 d.p.i. Serovar I 4,[5],12:i:- colonized porcine intestinal tissues and was shed in the feces throughout the 7-day study. Analysis of the 16S rRNA gene sequences demonstrated that the fecal microbiota was significantly altered following MDR serovar I 4,[5],12:i:- inoculation, with the largest shift observed between 0 and 7 d.p.i. Our data indicate that the pork outbreak-associated MDR serovar I 4,[5],12:i:- isolate induced transient clinical disease in swine and perturbed the gastrointestinal microbial community. The porcine response to MDR serovar I 4,[5],12:i:- is similar to previous studies with virulent biphasic Salmonella enterica serovar Typhimurium, suggesting that the absence of fljB does not substantially alter acute colonization or pathogenesis in pigs. ISSN: 15353141

Simon, S., Trost, E., Bender, J., Fuchs, S., Malorny, B., Rabsch, W., Prager, R., Tietze, E., Flieger, A.
Evaluation of WGS based approaches for investigating a food-borne outbreak caused by Salmonella enterica serovar Derby in Germany
(2018) Food Microbiology, 71, pp. 46-54.
ABSTRACT: In Germany salmonellosis still represents the 2nd most common bacterial foodborne disease. The majority of infections are caused by Salmonella (S.) Typhimurium and S. Enteritidis followed by a variety of other broad host-range serovars. Salmonella Derby is one of the five top-ranked serovars isolated from humans and it represents one of the most prevalent serovars in pigs, thus bearing the potential risk for transmission to humans upon consumption of pig meat and products thereof. From November 2013 to January 2014 S. Derby caused a large outbreak that affected 145 primarily elderly people.

Epidemiological investigations identified raw pork sausage as the probable source of infection, which was confirmed by microbiological evidence. During the outbreak isolates from patients, food specimen and asymptomatic carriers were investigated by conventional typing methods. However, the quantity and quality of available microbiological and epidemiological data made this outbreak highly suitable for retrospective investigation by Whole Genome Sequencing (WGS) and subsequent evaluation of different bioinformatics approaches for cluster definition. Overall the WGS-based methods confirmed the results of the conventional typing but were of significant higher discriminatory power. That was particularly beneficial for strains with incomplete epidemiological data. For our data set both, single nucleotide polymorphism (SNP)- and core genome multilocus sequence typing (cgMLST)-based methods proved to be appropriate tools for cluster definition.
ISSN: 07400020

Kanagarajah, S., Waldram, A., Dolan, G., Jenkins, C., Ashton, P.M., Carrion Martin, A.I., Davies, R., Frost, A., Dallman, T.J., De Pinna, E.M., Hawker, J.I., Grant, K.A., Elson, R.
Whole genome sequencing reveals an outbreak of Salmonella Enteritidis associated with reptile feeder mice in the United Kingdom, 2012-2015
(2018) Food Microbiology, 71, pp. 32-38.
ABSTRACT: Analysis of whole genome sequencing data uncovered a previously undetected outbreak of Salmonella Enteritidis that had been on-going for four years. Cases were resident in all countries of the United Kingdom and 40% of the cases were aged less than 11 years old. Initial investigations revealed that 30% of cases reported exposure to pet snakes. A case-control study was designed to test the hypothesis that exposure to reptiles or their feed were risk factors. A robust case-definition, based on the single nucleotide polymorphism (SNP) profile, increased the power of the analytical study. Following univariable and multivariable analysis, exposure to snakes was the only variable independently associated with infection (Odds ratio 810 95% CI (85–7715) $p < 0.001$). Isolates of S. Enteritidis belonging to the outbreak profile were recovered from reptile feeder mice sampled at the retail and wholesale level. Control measures included improved public health messaging at point of sale, press releases and engagement with public health and veterinary counterparts across Europe. Mice destined to be fed to reptiles are not regarded as pet food and are not routinely tested for pathogenic bacteria. Routine microbiological testing to ensure feeder mice are free from Salmonella is recommended.
ISSN: 07400020

Waldram, A., Dolan, G., Ashton, P.M., Jenkins, C., Dallman, T.J.
Epidemiological analysis of Salmonella clusters identified by whole genome sequencing, England and Wales 2014
(2018) Food Microbiology, 71, pp. 39-45.
ABSTRACT: The unprecedented level of bacterial strain discrimination provided by whole genome sequencing (WGS) presents new challenges with respect to the utility and interpretation of the data. Whole genome sequences from 1445 isolates of Salmonella belonging to the most commonly identified serotypes in England and Wales isolated between April and August 2014 were analysed. Single linkage single nucleotide polymorphism thresholds at the 10, 5 and 0 level were explored for evidence of epidemiological links between clustered cases. Analysis of the WGS data organised 566 of the 1445 isolates into 32 clusters of five or more. A statistically significant epidemiological link was identified for 17 clusters. The clusters were associated with foreign travel (n = 8), consumption of Chinese takeaways (n = 4), chicken eaten at home (n = 2), and one each of the following; eating out, contact with another case in the home and contact with reptiles. In the same time frame, one cluster was detected using traditional outbreak detection methods. WGS can be used for the highly specific and highly sensitive detection of biologically related isolates when epidemiological links are obscured. Improvements in the collection of detailed, standardised exposure information would enhance cluster investigations. ISSN: 07400020

Chlebicz, A., Śliżewska, K.
Campylobacteriosis, salmonellosis, yersiniosis, and listeriosis as zoonotic foodborne pathogens: A review
(2018) International Journal of Environmental Research and Public Health, 15 (5), art. no. 863, .
ABSTRACT: Zoonoses are diseases transmitted from animals to humans, posing a great threat to the health and life of people all over the world. According to WHO estimations, 600 million cases of diseases caused by contaminated food were noted in 2010, including almost 350 million caused by pathogenic bacteria. Campylobacter, Salmonella, as well as

Yersinia enterocolitica and Listeria monocytogenes may dwell in livestock (poultry, cattle, and swine) but are also found in wild animals, pets, fish, and rodents. Animals, often being asymptomatic carriers of pathogens, excrete them with faeces, thus delivering them to the environment. Therefore, pathogens may invade new individuals, as well as reside on vegetables and fruits. Pathogenic bacteria also penetrate food production areas and may remain there in the form of a biofilm covering the surfaces of machines and equipment. A common occurrence of microbes in food products, as well as their improper or careless processing, leads to common poisonings. Symptoms of foodborne infections may be mild, sometimes flu-like, but they also may be accompanied by severe complications, some even fatal. The aim of the paper is to summarize and provide information on campylobacteriosis, salmonellosis, yersiniosis, and listeriosis and the aetiological factors of those diseases, along with the general characteristics of pathogens, virulence factors, and reservoirs. ISSN: 16617827

Ju, X., Li, J., Zhu, M., Lu, Z., Lv, F., Zhu, X., Bie, X.
Effect of the luxS gene on biofilm formation and antibiotic resistance by Salmonella serovar Dublin
(2018) Food Research International, 107, pp. 385-393.
ABSTRACT: Biofilms are communities of bacterial cells that serve to protect them from external adverse influences and enhance bacterial resistance to antibiotics and sanitizers. Here, we studied the regulatory effects of glucose and sodium chloride on biofilm formation in Salmonella serovar Dublin (S. Dublin). To analyze expression levels of the quorum sensing gene luxS, we created a luxS knockout mutant. Also, antimicrobial resistance, hydrophobicity and autoinducer-2 (AI-2) activity of both the wild-type (WT) and the mutant strain were investigated. Our results revealed that glucose was not essential for S. Dublin biofilm formation but had an inhibitory effect on biofilm formation when the concentration was over 0.1%. NaCl was found to be indispensable in forming biofilm, and it also exerted an inhibitory effect at high concentrations (>1.0%). Both the WT and the mutant strains displayed significant MIC growth after biofilm formation. An increase of up to 32,768 times in the resistance of S. Dublin in biofilm phonotype against antibiotic (ampicillin) compared to its planktonic phonotype was observed. However, S. Dublin luxS knockout mutant only showed slight differences compared to the WT strain in the antimicrobial tests although it displayed better biofilm-forming capacity than the WT strain. The mutant strain also exhibited higher hydrophobicity than the WT strain, which was a feature related to biofilm formation. The production of the quorum sensing autoinducer-2 (AI-2) was significantly lower in the mutant strain than in the WT strain since the LuxS enzyme, encoded by the luxS gene, plays an essential role in AI-2 synthesis. However, the limited biofilm-forming ability in the WT strain indicated AI-2 was not directly related to S. Dublin biofilm formation. Furthermore, gene expression analysis of the WT and mutant strains revealed upregulation of genes related to biofilm stress response and enhanced resistance in the luxS mutant strain, which may provide evidence for the regulatory role of the luxS gene in biofilm formation. ISSN: 09639969

McWhorter, A.R., Chousalkar, K.K.
A long-term efficacy trial of a live, attenuated Salmonella Typhimurium vaccine in layer hens
(2018) Frontiers in Microbiology, 9 (JUN), art. no. 1380, .
ABSTRACT: Salmonella remains one of the most common causes of bacterial foodborne gastrointestinal disease in humans. Raw eggs or food items containing undercooked eggs are frequently identified as the source of Salmonella. Salmonella Typhimurium contamination of table eggs most commonly occurs when they are laid in a contaminated environment. Several control strategies, including vaccination, are widely used to mitigate the total Salmonella load. It is unclear, however, whether live attenuated Salmonella vaccines are efficacious over the life span of a layer hen. Live attenuated Salmonella vaccines have been favored due to their ability to illicit a strong humoral immune response. The lifespan of a layer hen ranges between 60 and 80 weeks and the long term efficacy of attenuated vaccine strains has not been investigated. In this study, commercial brown layer chicks were vaccinated at day old, 6 weeks of age, and again at 10 weeks of age with the Bioproperties Vaxsafe™ STM1 aroA mutant vaccine. Birds were challenged at 18 weeks of age with Salmonella Typhimurium DT9 (MLVA 03 15 08 11 550). Feces and eggs were monitored for S. Typhimurium for 40 weeks post-infection. Birds produced a strong immune response following the final dose which was administered intramuscularly. The serum antibody response to S. Typhimurium DT9 infection did not differ between challenged groups. Fecal shedding and egg contamination was highly variable and did not differ significantly between vaccinated and unvaccinated birds that had been challenged with S. Typhimurium DT9. Total bacterial load in feces was quantified using qPCR. No

significant difference was detected between unvaccinated and vaccinated birds after challenge. ISSN: 1664302X

Møller, F.T., Mølbak, K., Ethelberg, S.
Analysis of consumer food purchase data used for outbreak investigations, a review (2018) Eurosurveillance, 23 (24), art. no. 1700503, 9 p.
ABSTRACT: Background: Investigations of food-borne outbreaks are frequently unsuccessful and new investigation methods should be welcomed. Aim: Describe the use of consumer purchase datasets in outbreak investigations and consider methodological and practical difficulties. Methods: We reviewed published papers describing the use of consumer purchase datasets, where electronic data on the foods that case-patients had purchased before onset of symptoms were obtained and analysed as part of outbreak investigations. Results: For the period 2006–17, scientific articles were found describing 20 outbreak investigations. Most outbreaks involved salmonella or Shiga toxin-producing Escherichia coli and were performed in eight different countries. The consumer purchase datasets were most frequently used to generate hypotheses about the outbreak vehicle where case-interviews had not been fruitful. Secondly, they were used to aid trace-back investigation, where a vehicle was already suspected. A number of methodological as well as (in some countries) legal and practical impediments exist. Conclusions: Several of the outbreaks were unlikely to have been solved without the use of consumer purchase datasets. The method is potentially powerful and with future improved access to big data purchase information, may become a widely applicable tool for outbreak investigations, enabling investigators to quickly find hypotheses and at the same time estimate odds ratios or relative risks hereof. We suggest using the term 'consumer purchase data' to refer to the approach in the future. ISSN: 1025496X

Pearce, M.E., Alikhan, N.-F., Dallman, T.J., Zhou, Z., Grant, K., Maiden, M.C.J.
Comparative analysis of core genome MLST and SNP typing within a European Salmonella serovar Enteritidis outbreak
(2018) International Journal of Food Microbiology, 274, pp. 1-11.
ABSTRACT: Multi-country outbreaks of foodborne bacterial disease present challenges in their detection, tracking, and notification. As food is increasingly distributed across borders, such outbreaks are becoming more common. This increases the need for high-resolution, accessible, and replicable isolate typing schemes. Here we evaluate a core genome multilocus typing (cgMLST) scheme for the high-resolution reproducible typing of Salmonella enterica (S. enterica) isolates, by its application to a large European outbreak of S. enterica serovar Enteritidis. This outbreak had been extensively characterised using single nucleotide polymorphism (SNP)-based approaches. The cgMLST analysis was congruent with the original SNP-based analysis, the epidemiological data, and whole genome MLST (wgMLST) analysis. Combination of the cgMLST and epidemiological data confirmed that the genetic diversity among the isolates predated the outbreak, and was likely present at the infection source. There was consequently no link between country of isolation and genetic diversity, but the cgMLST clusters were congruent with date of isolation. Furthermore, comparison with publicly available Enteritidis isolate data demonstrated that the cgMLST scheme presented is highly scalable, enabling outbreaks to be contextualised within the Salmonella genus. The cgMLST scheme is therefore shown to be a standardised and scalable typing method, which allows Salmonella outbreaks to be analysed and compared across laboratories and jurisdictions. ISSN: 01681605

Bonardi, S., Bolzoni, L., Brindani, F., Scaltriti, E., Cavallini, P., Giuseppe, C., Pongolini, S.
Salmonella Detection and Counting on Pig Carcasses and Cutting Lines in Italian Slaughterhouses
(2018) Foodborne Pathogens and Disease, 15 (6), pp. 339-345.
ABSTRACT: During 2014-2015, 300 pig carcasses before chilling and 85 food contact surfaces (FCSs) at cutting lines were tested for Salmonella in three slaughterhouses (namely A, B, and C) of northern Italy. In slaughterhouses A and B, four carcass sites of 100 cm2 each (from both the exterior and interior side) were swabbed with a single sponge. In abattoir C, four 100 cm2 sites of the exterior and the interior sides were swabbed with two independent sponges. The population average prevalence of Salmonella-positive carcasses (which takes into account the structure of the study design, with multiple samples collected in a single day) in slaughterhouses A and B was 12.3%, while in slaughterhouse C it was 11.2%. Presence of Salmonella on exterior and interior sides of carcasses showed a low level of concordance (only 3/12 of the contaminated carcasses were positive on both sides). No significant difference was found for FCSs contamination in the three slaughterhouses, with a population average prevalence of Salmonella-positive

FCSs of 19.9%. In addition, we found that the clustering due to the day of sampling account for more than 36% and 60% of the overall prevalence variation on carcasses and FCSs, respectively. Eight serovars were identified, with Salmonella Derby as the most common type. The counting of Salmonella on carcasses showed large variability. It was low (<0.0075 most probable number [MPN]/cm2) in 46.6% of the carcasses and as high as 2.7 MPN/cm2 in 4.7%. Specifically, we found that counts on carcasses fit with "heavy tailed" distributions (lognormal and Weibull with a small shape parameter), suggesting not negligible probability of episodes of high Salmonella contamination. The mean values of contamination obtained with the two distributions ranged from 0.235 to 0.435 MPN/cm2. ISSN: 15353141

Gast, R.K., Guard, J., Guraya, R., Locatelli, A.
Multiplication in egg yolk and survival in egg albumen of genetically and phenotypically characterized salmonella enteritidis strains
(2018) Journal of Food Protection, 81 (6), pp. 876-880.
ABSTRACT: Prompt refrigeration of eggs to prevent the multiplication of Salmonella Enteritidis to high levels during storage is an important practice for reducing the risk of egg-transmitted human illness. The efficacy of egg refrigeration for achieving this goal depends on the interaction among the location of contamination, the ability of contaminant strains to survive or multiply, and the rate at which growth-restricting temperatures are attained. The present study assessed the significance of several characterized genetic and phenotypic properties for the capabilities of 10 Salmonella Enteritidis isolates to multiply rapidly in egg yolk and survive for several days in egg albumen during unrefrigerated (25°C) storage. The growth of small numbers of each Salmonella Enteritidis strain (approximately 101 CFU/mL) inoculated into egg yolk samples was determined after 6 and 24 h of incubation. The survival of larger numbers of Salmonella Enteritidis (approximately 105 CFU/mL) inoculated into albumen samples was determined at 24 and 96 h of incubation. In yolk, the inoculated Salmonella Enteritidis strains multiplied to mean levels of approximately 102.6 CFU/mL after 6 h of incubation and 108.3 CFU/mL after 24 h. In albumen, mean levels of approximately 104.6 CFU/mL Salmonella Enteritidis were maintained through 96 h. The concentrations of the various Salmonella strains after incubation in either yolk or albumen were distributed over relatively narrow ranges of values. Significant (P < 0.01) differences observed among individual strains suggested that maintenance of the fimbrial gene sefD may have positive genetic selection value by improving fitness to grow inside egg yolk, whereas the antibiotic resistance gene blaTEM-1 tet(A) appeared to have negative genetic selection value by decreasing fitness to survive in egg albumen. ISSN: 0362028X

Vrbova, L., Sivanantharajah, S., Walton, R., Whitfield, Y., Lee, C., Picard, I., Chapinal, N., Gaulin, C., Tschetter, L., Tataryn, J.
Outbreak of Salmonella Typhimurium associated with feeder rodents
(2018) Zoonoses and Public Health, 65 (4), pp. 386-394.
ABSTRACT: In December 2012, an increase in human Salmonella Typhimurium cases was identified in the province of Ontario, Canada launching an outbreak investigation. The outbreak spanned 3 years (2012–2014), with 134 cases reported from five Canadian provinces. There was a substantial burden of illness among children: 45% of cases were children 12 years old or under, and 23% of cases were under 5 years old. Epidemiologic, traceback and laboratory findings linked this outbreak to feeder rodents (used to feed snakes) supplied by a network of rodent breeders in Ontario. Cases likely acquired their illness through either direct or indirect contact with feeder rodents. This investigation not only contributes to the weight of evidence on the risk that feeder rodents pose, but also underscores the importance of investigating indirect animal contact and associated risks, especially for high-risk individuals. © 2018 Her Majesty the Queen in Right of Canada Zoonoses Public Health ISSN: 18631959

Bai, J., Trinetta, V., Shi, X., Noll, L.W., Magossi, G., Zheng, W., Porter, E.P., Cernicchiaro, N., Renter, D.G., Nagaraja, T.G.
Comparison data of a two-target real-time PCR assay with and without an internal control in detecting Salmonella enterica from cattle lymph nodes
(2018) Data in Brief, 18, pp. 1819-1824.
ABSTRACT: A real-time PCR (qPCR) assay targeting on invA and pagC genes was developed and validated for the detection and quantification of Salmonella enterica strains (Bai et al., 2018) [1]. A host gene, normally an endogenous housekeeping gene (Beer-Davidson et al., 2018; Poon et al., 2004) [2,3], or an irrelevant exogenous gene (Cheng et al., 2015; Sedlak et al., 2014) [4,5] has been widely used as an internal control to monitor nucleic acid extraction efficiencies and potential PCR inhibitions in PCR-based detection

assays. An endogenous internal control designed based on the 18S rRNA gene was used in the above-mentioned qPCR assay. This 18S rRNA internal control amplifies the target gene in multiple species including bovine, swine, ovine, caprine and cervine. Data was generated by the duplex qPCR assay on 138 enriched cattle lymph node samples without the internal control, and compared with data on the same samples tested by the triplex qPCR assay that has the 18S rRNA gene as internal control. Threshold cycle (Ct) data for the duplex and the triplex qPCR on the 138 samples were similar, and are presented in this brief report. ISSN: 23523409

Abhisingha, M., Dumnil, J., Pitaksutheepong, C.
Selection of Potential Probiotic Lactobacillus with Inhibitory Activity Against Salmonella and Fecal Coliform Bacteria
(2018) Probiotics and Antimicrobial Proteins, 10 (2), pp. 218-227.
ABSTRACT: Three hundred and sixty presumptive lactic acid bacteria (LAB) isolated from pregnant sows, newborn, suckling, and weaned piglets were preliminarily screened for anti-Salmonella activity. Fifty-eight isolates consisting of Lactobacillus reuteri (n = 32), Lactobacillus salivarius (n = 10), Lactobacillus mucosae (n = 8), Lactobacillus johnsonii (n = 5), and Lactobacillus crispatus (n = 3) were selected and further characterized for probiotic properties including production of antimicrobial substances, acid and bile tolerance, and cell adherence to Caco-2 cells. Eight isolates including Lact. johnsonii LJ202 and Lact. reuteri LR108 were identified as potential probiotics. LJ202 was selected for further use in co-culture studies of two-bacterial and multiple-bacterial species to examine its inhibitory activity against Salmonella enterica serovar Enteritidis DMST7106 (SE7106). Co-culture of LJ202 and SE7106 showed that LJ202 could completely inhibit the growth of SE7106 in 10 h of co-culture. In co-culture of multiple-bacterial species, culturable fecal bacteria from pig feces were used as representative of multiple-bacterial species. The study was performed to examine whether interactions among multiple-bacterial species would influence antagonistic activity of LJ202 against SE7106 and fecal coliform bacteria. Co-culture of SE7106 with different combinations of fecal bacteria and probiotic (LJ202 and LR108) or non-probiotic (Lact. mucosae LM303) strains revealed that the growth of SE7106 was completely inhibited either in the presence or in the absence of probiotic strains. Intriguingly, LJ202 exhibited notable inhibitory activity against fecal coliform bacteria while LR108 did not. Taken together, the results of co-culture studies suggested that LJ202 is a good probiotic candidate for further study its inhibitory effects against pathogen infections in pigs. ISSN: 18671306

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Salmonella heidelberg resistant to ceftiofur and disinfectants routinely used in poultry
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ABSTRACT: Bacteria of the genus Salmonella may infect humans and domestic animals, causing a serious public health problem worldwide. Nowadays, Salmonella enterica serovar Heidelberg (SH) is among the top three serovars isolated from people with salmonellosis and it is present in the poultry production chain. Moreover, it seems to be more invasive than other serotypes that cause enteritis. The overall status of the antimicrobial resistant of Brazilian strains of SH is still unknow. The bacterium may use similar mechanisms of resistance to antibotics, as well as disinfectants such as the efflux system and enzymatic degradation of chemical compounds. Thus, the objective of this study was to identify the Minimal Inhibitory Concentration (MIC) for ceftiofur of SH isolated from different materials of poultry origin, as well as to verify the relation between antibiotic resistance and disinfectant resistance. In addition, the screening efflux system was performed, using ethidium bromide to determine the presence of this mechanism of resistance. MIC results indicated high levels of SH resistance to ceftiofur, indicating the need for alternative drugs to treat salmonellosis. The concentration of ceftiofur needed to eliminate SH resistant isolates were 32 times higher than the therapeutic dose. Regarding disinfectants, most of the disinfectants tested were efficient to eliminate SH, however one isolate was resistant to glutaraldehyde-quaternary ammonia. All isolates were negatives in the screening efflux system, which suggest a different mechanism of resistance. It is possible to conclude that SH shows a real threat to poultry production, and caution should be taken when choosing the right antibiotic and disinfectant against this serovar. ISSN: 1676546X