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Editorial Note

Bilthoven, 4 July 2019

Dear colleague,

By mid-April 2019 we received the agreement from DG-SANTE on our work-programme for 2019 and 2020. Due to the late receipt of this agreement, we did not yet inform you about this work-programme in an earlier Newsletter. Therefore, the **work-programme of 2019-2020** is included in this Newsletter. The lay-out of the work-programme follows the task and duties described in Article 94(2) of Regulation (EU) 625/2017.

By mid-April we also sent the **annual technical report of the activities of EURL-*Salmonella* performed in 2018** to EC DG SANTE. For your information, the annual report (agreed upon by DG SANTE) is also included in this Newsletter.

By the end of May we organised our annual **EURL-*Salmonella* workshop**. This time it was situated in Amersfoort, the Netherlands. A total of 51 participants were present at the workshop. The presentations given at the workshop are available at our website since early June 2019, see: https://www.eurlsalmonella.eu/en/workshop-2019

In September we will organise the **Proficiency Test for detection of *Salmonella* in samples from the primary production stage**. The timetable of this study is included in this Newsletter. Also included is the timetable of the **Proficiency Test on typing of *Salmonella***, which will be organised in fall 2019. The study will contain an obligatory part on serotyping of *Salmonella*, and, if we will receive a sufficient number of applications, a voluntary part for molecular sub-typing to determine whether some strains concern common types (cluster analysis). The (molecular) method will be free of choice (PFGE and/or MLVA and/or WGS).

In earlier Newsletters you have been informed that an **amendment to EN ISO 6579-1:2017** is drafted, 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.
- To correct the concentration of $MgCl_2$ in MSRV agar. The final concentration $MgCl_2$ in MSRV agar should be 10.9 g/l. However, the composition given in ISO 6579-1:2017 results in a final concentration of 14.9 g/l $MgCl_2$. In Annex

B.4 (MSRV agar from individual ingredients) the concentrations of the ingredients of solution A, the base medium and complete medium will be corrected.

The DIS (Draft International Standard) voting of **ISO/DIS 6579-1:2017/Amd1** will take place from 08-07-2019 to 30-09-2019. Comments to the document can be sent to the National Standardisation bodies and/or to the EURL-*Salmonella*.

Reports published in the second quarter of 2019:

Mooijman, K.A. The 23rd EURL-*Salmonella* workshop – 29 and 30 May 2018, Uppsala, Sweden. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM Report no.: 2018-0024 (April 2019). http://www.rivm.nl/bibliotheek/rapporten/2018-0024.pdf

Pol-Hofstad, I.E. and Mooijman, K.A. EURL-*Salmonella* Proficiency Test Primary Production, 2018 - Detection of *Salmonella* in boot socks with chicken faeces. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM Report no.: 2019-0028 (June 2019); https://www.rivm.nl/bibliotheek/rapporten/2019-0028.pdf

Best wishes,
Kirsten Mooijman
Coordinator EURL-*Salmonella*

Contribution of the EURL-*Salmonella*

Timetable EURL-*Salmonella* Proficiency Test Primary Production Stage 2019

Week (2019)	Date	Subject
38	In week of 16 September	Mailing of the protocol, lab code, and the link to the electronic result form to the participants by email.
39	23 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	30 September	Performance of the study
43	25 October at the latest	Deadline for completing the electronic submission of results: **25 October** (23:59 h) After this deadline the electronic submission form will be closed.

Timetable EURL-*Salmonella* Proficiency Test Typing 2019

Week	Date	Topic
39	23 September	Emailing of the link to the registration form for the typing study. Please **register by 18 October** at the latest.
43	21-25 October	Emailing of the protocol 2019.
45	4-8 November	Shipment of the parcels to the participants as Biological Substance Category B (UN 3373). If you did not receive the parcel by **8 November**, please contact the EURL-*Salmonella*.
45	4-8 November	Sending the link for the web based test report on serotyping to the participants.
45	4-8 November	Sending the link for the web based test report on PFGE and/or MLVA and/or WGS cluster analysis to the participants in a separate email.
45	4-8 November	*Upon receipt*: Starting the identification of the strains, according to the usual practice of the laboratory.
50	13 December 2019 at the latest	Deadline for completing the electronic submission of **serotyping** results: **13 December 2019** After this deadline, the electronic submission form for serotyping results will be closed.
	31 January 2020 at the latest	Deadline for completing the electronic submission of **PFGE / MLVA / WGS cluster analysis** results: **31 January 2020**
	February 2020	Serotyping: Reporting of individual laboratory results and Interim Summary Report.
	April/May 2020	Pilot PFGE/MLVA/WGS cluster analysis: Reporting of individual laboratory results and Summary Report.
	Summer 2020	Final report.

EURL-*Salmonella* work-programme 2019-2020

INTRODUCTION

In this document the activities of the EURL-*Salmonella* are described for the years 2019-2020. 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 on 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 2019 and 2020:

ISO-WG3 'Method validation'. For part 6 of EN ISO 16140 on 'validation of confirmation methods', Wilma Jacobs is project leader and Kirsten Mooijman is co-project leader (both EURL-*Salmonella*).

The development of a procedure for validation of (proprietary) alternative confirmation and typing methods started in 2014. In fall 2018 voting on the pre-FDIS (pre-Final Draft International Standard) took place. The comments will be introduced in the next version of ISO 16140-6, after which the voting for the Final Draft International Standard (FDIS) is likely to be launched early 2019. If the result of voting on this last stage is positive, the final document may be published by mid-2019.

For parts 3-5 of EN ISO 16140, the EURL-*Salmonella* is member of ISO-WG3 and will comment on the draft versions of these documents. The FDIS voting of these 3 parts are also expected in the first half or during summer 2019. The outcome of the voting on all parts (3-6) will be discussed in one or more meetings of WG3 before the final documents can be prepared and published.

ISO - Ad hoc group 'Checklist to avoid ambiguity in drafting standards in food microbiology'. The project leader is Kirsten Mooijman and the co-project leader is Wilma Jacobs (both EURL-*Salmonella*).

In 2018, the first edition of guidance document for writing standards for ISO/TC34/SC9 and CEN/TC275/WG6 was published as an internal document for convenors and project leaders of SC9 and WG6. However, this guidance document is a 'dynamic' document and may need regular updating to keep the information up to date. For example, the current document already needs to be updated for the new ISO rules on definitions.

CEN/ISO - Amendment 1 to EN ISO 6579-1. The project leader is Kirsten Mooijman (EURL-*Salmonella*). 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. To determine if incubation of selective media at a broader temperature range is possible, experiments were performed by members of ISO and CEN in 2016 and 2017. The results of these experiments were presented at the annual meeting of ISO-SC9 and CEN-WG6 in 2018 and it was concluded that broadening of the temperature ranges for incubation of selective media was possible. It was suggested to include this information in an Amendment to EN ISO 6579-1 on Detection of *Salmonella*, additional to other necessary corrections to Annex D of EN ISO 6579-1 (detection of *S.* Typhi and Paratyphi), being: corrections to the composition of a culture medium and changing the status from normative to informative to prevent further confusion on the use of this Annex. The first draft version of this Amendment was launched for voting in fall 2018. After this, the comments will need to be introduced in a next version of the document after which a next voting round will take place. If comments are not too

substantial, it may be expected that the final version of Amd.1 of EN ISO 6579-1 is published by the end of 2020. If necessary, one or two meetings (physical and/or by teleconference) will be organised with the members of the group to discuss the comments.
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 in 2017. A subset of 172 strains (target and non-target) was used to verify the performance of the PCR procedures by the NRL-*Salmonella* in Germany and by the EURL-*Salmonella* in 2018. The first analysis of the results of this verification was performed in fall 2018 and some discrepancies were seen which will need further investigation. Depending on the final outcome of the verification, 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 2019). ISO-WG10 will then launch the New Work Item proposal (NWIP), including the draft document prepared by CEN-TAG3 (likely in 2019). The comments to the NWIP will need to be discussed in a meeting of WG10, after which the document needs to be updated and launched for the next voting round. An interlaboratory study to determine the performance characteristics of the three PCR protocols can only be organised when the final draft document of ISO/TS 6579-4 is available and this is likely not to be the case before 2020.

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'. TAG9 has prepared a draft protocol to evaluate pre-enrichment media. This protocol was distributed for comments to the members of ISO-SC9 and CEN-WG6 in fall 2018, after which it will be updated. Members of ISO and CEN are asked to use the (final draft) protocol by performing some experiments and to evaluate the suitability of the protocol. The EURL-*Salmonella* is planning to perform these experiments in 2019. The results found by the members of ISO and CEN after testing the protocol, as well as other activities of TAG9 will be discussed in meetings (physical and/or by teleconference).
ISO - WG4 'Revision of ISO/TS 22117 on organisation of Proficiency Testing'. The document is revised to become a full ISO standard (ISO 22117) and the final document is likely to be published in 2019.
ISO - WG25 'Whole-genome sequencing for typing and genomic characterization'. A first working draft document was sent to members of the working group in 2018. Also the Working Group EURLs on NGS (1.2) has been asked to comment on the first draft document. Next, draft versions are expected in 2019 and 2020 for further comments and discussion. Comments to the draft versions of the ISO document and the next steps will be discussed in meetings (physical and/or by teleconference).
ISO-Ad'hoc group 'General aspects'. This AHG was raised to discuss general aspects of EN ISO documents and to prepare proposals, e.g. for information in the introduction/scope of application of a standard and for the detection or enumeration limit of a method. Agreed amendments will be forwarded to the ISO AHG dealing with the guidance document for drafting standards, to update this guidance document as well.

The plenary meetings of both ISO/TC34/SC9 and CEN/TC275/WG6 will be organised in Italy in July 2019. The location and dates of the annual meetings of SC9 and WG6 in 2020 are not yet known, but it may be likely that the location will be outside Europe.
One representative of the EURL-*Salmonella* will participate in these meetings to present the progress with the activities for which EURL-*Salmonella* has the leadership.

Development of (alternative) methods

Several (proprietary) alternative methods have been developed for the detection of *Salmonella*. The application of these methods depends on its validation. Certificates of validated methods (following EN 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 until 2018. However, in 2018 first certificates have been issued for (pilot) validation studies following prEN ISO/DIS 16140-6 for an alternative confirmation method to confirm four different pathogens, including *Salmonella*. With these studies, also the applicability of (draft) EN ISO 16140-6 was shown. It may be expected that in the near future validation studies will follow for other alternative confirmation and typing methods, like for serotyping of *Salmonella*. The EURL-*Salmonella* will keep track on these alternative methods and when relevant, will also test these methods. Additionally, the EURL can advise NRLs on the protocol for internal validation.

Expected Output:

- FDIS voting of ISO 16140-6, followed by final publication.
- Publication of second edition of the Guidance document for drafting standards for microbiology of the food chain.
- One or more draft version(s) of Amd.1 of EN ISO 6579-1. Final publication (likely) by the end of 2020.
- Launch of NWIP vote by ISO-WG10 of (draft) ISO/TS 6579-4 and preparation of (final) draft ISO/TS 6579-4.
- Summary of results of experiments with protocol of CEN-TAG9.
- Reports annual meetings ISO/TC34/SC9 and CEN/TC275/WG6 2019 and 2020.
- Overview literature on new/alternative methods published in the EURL-*Salmonella* Newsletters.

Duration:

2019 and 2020, several ISO and CEN activities may continue after 2020.

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* has the lead of activity 7) and participates in the other activities. For each activity information is gathered and when possible guidance documents are drafted and discussed in the working group.
In 2018 a survey was conducted among all NRLs in the different networks to obtain a better view on the needs of the NRLs concerning NGS. Additional surveys were performed to obtain information on the bioinformatics used and to obtain information why some NRLs do not (yet) have plans to use NGS. The results of the surveys conducted by the EURLs will be compared with the results of the survey conducted by EFSA in 2016.
The information collected by the different EURLs will be presented and discussed in meetings of the working group in 2019 and 2020.
At a meeting in fall 2018 the working group agreed to try to build a reference collection of genomes (and potentially informative genes) of the different pathogens to be able to validate tools and pipelines. For building this reference collection, the results of Proficiency Tests for WGS may be used, with the permission of the NRLs.
For NGS trainings, also the possibility of joint EURL e-trainings will be considered. For this it will be investigated if presentations used in on-site trainings can be used for e-trainings as well.

The working group will meet twice a year.

Expected Output:
This concerns output of the whole working group:
- Summary of the results of the surveys amongst NRLs.
- Summary of the collection of information for the different activities.
- Investigation to start a reference collection of WGS data.
- Investigation to set up joint EURL e-trainings.

Duration:
Whole 2019 and 2020.

Sub-activity 1.3 *Proficiency Tests*

Objectives:
Evaluation of the performance of the NRLs-*Salmonella* for detection and typing of *Salmonella* by means of interlaboratory comparisons (Proficiency Tests).

Description:
Organisation of 3 Proficiency Tests (PTs) per year:
1. One PT on detection of *Salmonella* in samples from the primary production stage.
2. One PT on detection of *Salmonella* in food or animal feed samples.
3. One PT on typing of *Salmonella* (serotyping, molecular typing).
Additional to these 3 PTs, or in combination with one of the PTs described under 1. or 2., a PT will be organised for detection of *Salmonella* in bivalve molluscs in 2020. In 2019 it will be investigated what type of samples can be used for this study and who will be the NRLs-*Salmonella* responsible for analysis of bivalve molluscs.

For the set-up of the PTs on detection of *Salmonella*, EN ISO/TS 22117 ('Microbiology of food and animal feeding stuffs – Specific requirements and guidance for proficiency testing by interlaboratory comparison') will be followed. As soon as the revised version of EN ISO 22117 is available, it will be evaluated if the current set-up of the EURL-*Salmonella* PTs is still in line with the set-up of the amended EN ISO 22117. If necessary the set-up of the EURL-*Salmonella* PTs will be (slightly) amended.
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 PT. 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 PT.

The PTs for typing of *Salmonella* consist of an obligatory part on serotyping of *Salmonella* and on an optional part for molecular typing of *Salmonella*. For the serotyping part, the EURL-*Salmonella* will select different serovars from *Salmonella enterica* subsp. *enterica*, including serovars with public health significance, serovars with antigens similar to those of public health significant strains and serovars that caused typing problems in previous studies. Since the study of 2011, one additional *Salmonella* serovar from another subspecies than *Salmonella enterica* subsp. *enterica* is included in the study. Analysis of this additional strain is optional.
For the part on molecular typing, a new set-up is considered, starting with the PT in 2019. Up to and including 2018, PFGE typing was indicated to be used in the molecular typing part. However, for the PT in 2019 it is considered to organise a pilot study on cluster identification of a selected set of strains, and to give the NRLs the possibility to use other molecular methods as well, like Whole Genome Sequencing (WGS). The analytical method will be free of choice, but detailed information on the methodology will be requested, as well as uploading of data like raw reads (fastq files). Participation in this molecular part of the PT will be optional for the NRLs. Depending on the experiences and results, a similar or amended PT for molecular typing will be organised in 2020.

All samples for the PTs are blindly coded and send to the NRLs-*Salmonella* one week before the performance of the PT. 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-*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 PT. 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-*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 PTs is judged. If moderate performance is seen in three consecutive PTs, 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 27 EU Member States, also NRLs-*Salmonella* of other countries regularly participate in one or more Proficiency Tests, either for budget of the EURL-*Salmonella* or for own costs.
Participation in the PTs for budget of EURL-*Salmonella*:

- NRL-*Salmonella* of United Kingdom (if a transitional period for UK leaving the EU is agreed upon).
- NRLs-*Salmonella* of candidate countries Albania, Former Yugoslav Republic of Macedonia (FYROM) and Serbia.
- NRL-*Salmonella* of potential candidate country Bosnia and Herzegovina.

Participation in the PTs for own costs:
- NRLs-*Salmonella* of EFTA countries Iceland, Norway and Switzerland.
- NRL-*Salmonella* of candidate country Turkey.

Expected Output:
Organisation of 3 Proficiency Tests per year. Interim summaries (shortly after the study) and full reports (later) of the results of each PT.

Duration:
Preparation and testing of samples, organisation and reporting of the three PTs will be divided over each year. Some activities may continue in a following year (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- *Salmonella*. The workshops are generally organised in May and will last 1,5-2 days. For the location of the workshop it has become a 'tradition' to organise the workshop one year in the country where the EURL-*Salmonella* is situated (the Netherlands) and the other year in another EU Member State. In former years the workshop has been organised in France, Greece, Germany and Sweden with the help of the NRL in the relevant country. Changing the location is highly appreciated by the NRLs and gives the 'local' NRL the opportunity to present itself more pronounced.

For the workshop to be organised in 2019, the location will be in the Netherlands. For the location of the workshop to be organised in 2020 a suggestion has been made by the NRL-*Salmonella* of Hungary to organise it in their country. The feasibility of this location will be further explored.

The programme of each 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 Proficiency Tests organised by EURL-*Salmonella*;
- Experiences, problems, results in relation to monitoring surveys for *Salmonella*;
- *Salmonella* outbreaks;
- 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 27 EU Member States, also NRLs-*Salmonella* of other countries regularly participate in the workshops, either for budget of the EURL-*Salmonella* or for own costs.
Participation in the workshops for budget of EURL-*Salmonella*:
- NRL-*Salmonella* of United Kingdom (if a transitional period for UK leaving the EU is agreed upon).
- NRLs-*Salmonella* of candidate countries Albania, Former Yugoslav Republic of Macedonia (FYROM) and Serbia.
- NRL-*Salmonella* of potential candidate country Bosnia and Herzegovina.
- 2-3 guest speakers from different European countries.

Participation in the workshops for own costs:
- NRLs-*Salmonella* of EFTA countries Iceland, Norway and Switzerland.
- NRL-*Salmonella* of candidate country Turkey.

The NRLs-*Salmonella* responsible for analysing bivalve molluscs will be advised to participate in the extension of the annual workshop of the EURL-marine biotoxines. This extended workshop will focus on exchange of information related to microbiological aspects of production of bivalve molluscs. More details are given in sub-activity 2.3 (scientific advice and support of NRLs).

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:
Each workshop itself will last 1,5-2 days. Organisation and reporting will last several months (before and after the workshop).

Sub-activity 2.2 *Training courses*

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

Description:
The following training courses are foreseen in 2019 and 2020:
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 Proficiency Tests.
3. Joint EURLs training on molecular typing, organised in cooperation with the EURL-VTEC (ISS, Italy) and the EURL-*Listeria monocytogenes* (ANSES, France). In 2016, 2017 and 2018 these joint trainings focused on the use of the software package BioNumerics for PFGE profile analysis. However, for the training courses in 2019 and 2020 the focus may be more on Next Generation Sequencing (NGS) instead of PFGE, depending on the interest shown by the three networks. The choice between the two analytical platforms (for PFGE or NGS) will be made based on the responses on the call for training in the three networks. A 2-days' training session is foreseen, where the location will alternate between the three EURLs. The training session of 2019 is planned at the premises of EURL-*Listeria monocytogenes* (ANSES, France) and the one of 2020 at the EURL-VTEC (ISS, Italy). The next session in 2021 is forecasted to be organised at the premises of the EURL-*Salmonella* (RIVM, the Netherlands). Each EURL will make available didactic rooms equipped with at least 12 computer workstations, and each course will be attended by representatives of 4 NRLs for VTEC, 4 NRLs for *Listeria monocytogenes* and 4 NRLs for *Salmonella*. The training courses will be given by staff members of the three EURLs. Either the use of BioNumerics (PFGE) or of Bioinformatics (NGS) will be trained, depending on the needs of the network.

Additional to the NRLs of the 27 EU Member States, training is also offered to NRLs-*Salmonella* of other countries, either for budget of the EURL-*Salmonella* or for own costs.
Participation in the training courses for budget of EURL-*Salmonella*:
- NRL-*Salmonella* of United Kingdom (if a transitional period for UK leaving the EU is agreed upon).
- NRLs-*Salmonella* of candidate countries Albania, Former Yugoslav Republic of Macedonia (FYROM) and Serbia.
- NRL-*Salmonella* of potential candidate country Bosnia and Herzegovina.

Participation in the training courses for own costs:
- NRLs-*Salmonella* of EFTA countries Iceland, Norway and Switzerland.
- NRL-*Salmonella* of candidate country Turkey.

Additional to the above mentioned training courses and together with the other EURLs of the working group on NGS (sub-activity 1.2), the possibilities for building an on-line e-training for NGS will be explored.

Expected Output:
The training courses intend to result in improved performance of the NRLs in the relevant work field and to build their capacity for new work fields like NGS. 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 2018, on initiative of the EURL *Listeria monocytogenes*, a working group of 5 EURLs was raised (EURLs for *Campylobacter*, Coagulase Positive Staphylococci, *Listeria monocytogenes*, *Salmonella* and VTEC) to develop a harmonised guidance document on outsourcing parts of proficiency tests organised by NRLs for national networks. NRLs in the 5 EURL/NRL networks expressed their need for such a guidance document. A document of the EURL *Listeria monocytogenes* on this topic was used as basis for preparing a draft joint document in fall 2018. Quite likely this joint guidance document will be finalised in 2019 after which it will be distributed to the NRLs of the relevant networks.

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.

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 are distributed over other EURLs. For that reason, investigations for *Salmonella, E. coli* and viruses in bivalve molluscs will become part of the activities of respectively EURL-*Salmonella,* EURL-*E. coli* and EURL-foodborne viruses. The microbiological monitoring of bivalve molluscs in production areas will become part of the activities of the EURL for marine biotoxines. This latter EURL is planning to organise a one day extension to their annual workshop to discuss issues related to microbiological monitoring of bivalve molluscs in 2019 and following years. The NRLs responsible for microbiological analysis of bivalve molluscs (*Salmonella, E. coli* and viruses) will be invited to participate in the extension of the annual workshop of the EURL-marine biotoxines. Additionally, one representative of each of the three microbiological EURLs (*Salmonella, E. coli* and foodborne viruses) will participate in this 'extended workshop' of the EURL-marine biotoxines. At the workshop these EURLs will present, amongst others, the plans and result of PTs organised for the relevant work field.

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.

- Joint guidance document on outsourcing parts of proficiency tests organised by NRLs for national networks.
- Confirmation of samples/isolates when applicable.
- Participation and giving one or more presentations in the 'extended workshops' of the EURL-marine biotoxines, related to microbiological monitoring of bivalve molluscs.

Duration:
Continuous activities in 2019 and 2020.

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).

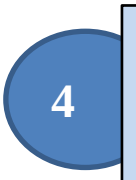
The EURL-*Salmonella* will participate in relevant (expert) working groups and scientific committees of DG SANTE and EFSA.

The EURL-*Salmonella* will assist DG SANTE, EFSA, NRLs, and (if relevant) ECDC in case of (international) *Salmonella* outbreaks. 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), and by helping with analysis and interpretation of the data.

The EURL-*Salmonella* is curator of *Salmonella* PFGE data which are 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 number of *Salmonella* PFGE data uploaded so far in the EFSA database is very limited and it is unsure if (historical) PFGE data will still be uploaded in the coming years, due to the fact that subtyping by PFGE is more and more replaced by WGS. In the coming year(s) the ECDC molecular database as well as the EFSA molecular database will be updated to be able to store WGS data. When needed (and when relevant), the EURL-*Salmonella* will still perform curation of *Salmonella* PFGE data when uploaded in the EFSA molecular database in 2019 and 2020.
The management of the joint EFSA-ECDC molecular typing database is performed by the joint EFSA-ECDC Steering Committee, in which members of EFSA, ECDC and the three EURLs (*Salmonella*, VTEC, *Listeria monocytogenes*) 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). During the meetings of the steering committee also the change from PFGE to WGS is discussed and information shared on the possible changes in the databases and treatment of data. In 2019 the chairmanship of the steering committee is in hands of EFSA so that it is expected that the meetings will be organised in Parma, Italy. In 2020, the chairmanship is in hands of ECDC so that it is expected that the meetings will be organised in Stockholm, Sweden.

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 meetings EFSA-ECDC steering committee.

Duration:
Continuous activities in 2019 and 2020.



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. Investigation to the possibility for setting up a reference collection of WGS data.

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 kept 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 Proficiency Tests 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.

The working group EURLs on NGS (sub-activity 1.2) agreed to try to build a reference collection of genomes (and potentially informative genes) of the different pathogens to be able to validate tools and pipelines. For building this reference collection, the results of Proficiency Tests for WGS may be used, with the permission of the NRLs. In 2019 and 2020 the feasibility for building a reference collection of genomes (and genes) of *Salmonella* serovars will be investigated.

Expected Output:
- Up to date information on reference strains and reference materials at the EURL-*Salmonella* website. This work 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 part of sub-activity 4.1 is merged with sub-activity 2.3.

- Information on the feasibility to build a reference collection of genomes (and genes) of *Salmonella* serovars. This work is also part of sub-activity 1.2 (EURLs working group on NGS) so that for the planning and output of this part of sub-activity 4.1, reference is made to sub-activity 1.2.

Duration:
Continuous activities in 2019 and 2020.

Technical report on activities of the EURL-*Salmonella* 2018

K.A. Mooijman
28 March 2019

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

Letter-report 039/2019 Z&O Mo/km
RIVM project-number: E/114506/18

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Introduction

The work plan of the EURL-*Salmonella* for the year under review, 2018, was submitted to the European Commission in November 2017. This report details the activities of the EURL-*Salmonella* according to the agreed work plan for 2018, following the order of the work plan. The activities are based on the responsibilities and tasks described in Article 94 of Regulation (EU) 2017/625 for European Reference Laboratories.

Activity 1 - To ensure availability and use of high quality methods and to ensure high quality performance by NRLs

Sub-activity 1.1 Analytical methods

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 Lausanne, Switzerland from 17 to 22 June 2018.

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 the relevant groups at the plenary meeting of SC9 and of WG6. A consolidated report of 8 EURLs (coordinated by the EURL-*Salmonella*) on the meetings was sent to DG-SANTE on 19 July 2018.

CEN Mandate M/381 (CEN lead)

All 15 EN ISO standards under the CEN Mandate were published in 2017, including performance characteristics. The final report was sent to EC DG-SANTE in September 2017.
For the validation study of each individual standard under the CEN Mandate M/381, manuscripts have been drafted in 2018, which were published in a special issue of the International Journal of Food Microbiology (including the validation of EN ISO 6579-1 for detection of *Salmonella*) in January 2019.

ISO Ad hoc group 'Harmonisation of selective incubation temperatures (35 °C vs 37 °C)'

In 2017 experiments were performed by the members of ISO/TC34/SC9 to compare incubation of selective media for detection of *Salmonella* at 35°C and at 37 °C. In total 9 laboratories, representing 6 countries participated. A total of 855 test results were analysed by Kirsten Mooijman (EURL-*Salmonella*), Daniele Sohier (Germany) and Maryse Rannou (France). The results were presented at the meeting of SC9 in June 2018. The members of SC9 agreed with the conclusions of the Ad hoc group:

- The overall results showed similar sensitivity results: 97.5% for incubation of the selective media at 37 °C and 98.3% for incubation at 35 °C.
- The data interpretation in relation to the deviating results fulfilled both proposed 'amended' acceptability limits.
- The reported amount of background flora after incubation of the selective media at 35 °C or at 37 °C was comparable.

These results indicate that comparable results are obtained when incubating selective media for detection of *Salmonella* at 35 °C and at 37 °C. It can therefore be concluded that for incubation of these selective media also a temperature range of 34-38 °C can be used (like earlier agreed for the incubation of non-selective media).

It was agreed that the information on extension of the temperature range for incubation of selective media will be added to an amendment to be drafted for EN ISO 6579-1.

The comparison study was also presented at the International symposium for *Salmonella* and salmonellosis (I3S) in St. Malo, France, September 2018.

Amendment for EN ISO 6579-1 'Detection of* Salmonella' *(CEN lead)

Early 2018, a written consultation took place among the members of CEN/TC275/WG6 and ISO/TC34/SC9 to ask for agreement to publish a correction or amendment of EN ISO 6579-1 (Detection of *Salmonella*), because of mistakes detected in the document after publication. During the consultation it was also possible to indicate other mistakes. The members voted positive and after the annual meeting of ISO and CEN it was agreed to draft an amendment to EN ISO 6579-1:2017, instead of a correction, 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 <u>not</u> 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 <u>10</u> 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 (see above).

The EURL-*Salmonella* drafted the first draft Amd.1 of EN ISO 6579-1:2017 in fall 2018 and a voting took place among the members of ISO/TC34/SC9 from 08-11-2018 until 14-12-2018. The outcome was positive with some comments. A next version of draft Amd.1 is expected to be sent around for voting in spring 2019.

PCR identification of monophasic* S. Typhimurium *(ISO/TS 6579-4)

In May 2016, SC9 agreed to register the Preliminary Work Item (PWI) of ISO 6579-4 to become a Technical Specification (TS). As soon as the technical work is finished, the work will be moved from CEN-TAG3 to ISO-WG10, after which ISO-WG10 will launch the New Work Item Proposal (NWIP).
In 2017, after a call among the NRLs-*Salmonella*, the EURL-*Salmonella* received approximately 400 strains (target and non-target) for testing the three PCR protocols for identification of monophasic *Salmonella* Typhimurium. The identity of all strains has been verified by the EURL and the information was communicated to the relevant NRLs early 2018. In fall 2017, The EURL-*Salmonella* has made a selection of 172 out of these 400 test strains (target and non-target strains) to test the three PCR protocols of draft ISO/TS 6579-4. In 2018, the NRL-*Salmonella* in Germany (Burkhard Malorny, project leader in TAG3) and the EURL-*Salmonella* tested all strains with the three PCR protocols. A first comparison of results was done in December 2018 and showed a few discrepancies in results between the NRL-*Salmonella* in Germany and the EURL-*Salmonella*. These results will be further evaluated (e.g. additional testing) in 2019. The final results will be used to update the draft protocols (if necessary) and to decide on a further selection of strains for use in the validation study to set performance characteristics of the three PCR protocols.

ISO-WG3 Method validation

EURL-*Salmonella* (Wilma Jacobs and Kirsten Mooijman) is member of the ISO working group on validation of microbiological methods and follow (and comment on) the drafting of parts 3-5 of EN ISO 16140. Wilma Jacobs is project group leader of the drafting of EN ISO 16140-6 'Protocol for the validation of alternative (proprietary) methods for microbiological confirmation and typing procedures'. In December 2017 the official ISO/DIS (Draft International Standard) 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. The comments were discussed at a meeting of ISO-WG3 in May 2018. Next a pre-FDIS (Final Draft International Standard) was distributed among the members of WG3 as well as among the members of ISO/TC34/SC9 for voting and to give a final option for (small) technical comments. The voting took place from the end of September 2018 until the end of November 2018. The outcome and comments from this voting stage were discussed at a meeting of ISO-WG3 in December 2018. The next step is to launch the voting of the Final Draft International Standard (FDIS). This last voting step before publication of the EN ISO document is expected in spring/summer 2019.

Information on draft EN ISO 16140-6 and its application in the laboratory was presented at a workshop of MicroVal validation (Wilma Jacobs and Kirsten Mooijman) in Wageningen, the Netherlands in October 2018.

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. Since 2014, several draft versions of the guidance document have been prepared. The guidance document is an internal document and is intended to help convenors and project leaders of SC9 and WG6 with the drafting of ISO/CEN documents.
In March 2018 the first edition of the guidance document was published. However, immediately after publication it was discussed that the definitions of pathogens used in EN ISO documents for microbiology of the food chain are not correct. A proposal for amended definitions was drafted and sent to the members of the Ad hoc group in October 2018. An updated proposal will be sent to the

members of CEN/TC275/WG6 and ISO/TC34/SC9 early 2019. After agreement, the information on definitions will be updated in the guidance document and in time the definitions will also be updated in the EN ISO documents. The guidance document is a dynamic document and will need to be updated to any new agreements made in ISO and CEN.

ISO-WG 25 Whole-genome sequencing for typing and genomic characterisation

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 (NWIP) and members of ISO/SC9 could comment on it until May 2018. The comments were discussed at a meeting of WG25 in November 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-TAG 9 Improvement of the 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. As it turned out to be complicated to come to an optimal pre-enrichment medium, it was decided to draft a protocol to evaluate the performance of pre-enrichment media instead. A first proposal of this protocol was discussed at a meeting of TAG9 in April 2018. In July 2018 the protocol was sent to the members of CEN/TC275/WG6 and ISO/TC34/SC9 for comments. After this the protocol was updated in fall 2018 and members of WG6 and SC9 were requested to test the protocol early 2019. In the protocol, information is 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*. The first experiments will be done with *Salmonella* only.
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.

Development of (alternative) methods

Developments in (alternative) methods occur especially in the field of Whole Genome Sequencing (WGS). At the Dutch National Institute for Public Health and the Environment (RIVM), where the EURL-*Salmonella* is situated, more effort is put into in-house sequencing since 2018. The EURL makes use of the knowledge obtained from these activities. WGS is used for sub-typing strains, to investigate similarities in case of outbreaks. It can also be used as alternative (molecular) method for serotyping of *Salmonella*. In 2017, the EURL-*Salmonella* helped with setting up an interlaboratory study for the use of WGS for serotyping of *Salmonella* organised as part of EFSA project ENGAGE (also see annual report EURL-*Salmonella* 2017; Mooijman, 2018a). The EURL advised on the number and type of strains (target and non-target), and on the analysis of the results, following the set-up of ISO/DIS 16140-6:2017. In the interlaboratory study 10 laboratories participated analysing the sequence data of in total 18 isolates. 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. The 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 of this study was drafted and was published as part of the final report of ENGAGE in June 2018 (Hendriksen et al., 2018).

Deliverables

- 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.
- 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. International Journal of Food Microbiology, 288 (2019), 3-12.
- A consolidated report of 8 EURLs (coordinated by the EURL-*Salmonella*) of the meetings of ISO/TC34/SC9 and CEN/TC275/WG6 held in Lausanne, Switzerland on 17-22 June 2018, was sent to DG-SANTE on 19 July 2018. A summary of relevant *Salmonella* items was published in the EURL-*Salmonella* Newsletter Vol. 24 No. 3, September 2018. https://www.eurlsalmonella.eu/media/1511
- Draft EN ISO 6579-1:2017/Amd.1. Amendment 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. Document ISO/TC34/SC9 N 2265, voting period 08-11-2018 until 14-12-2018.
- pre-FDIS 16140-6 Microbiology of the food chain - Method validation — Part 6: Protocol for the validation of alternative (proprietary) methods for microbiological confirmation and typing procedures. Document ISO/TC 34/SC 9/WG 3 N 474, voting period 28-09-2018 until 23-11-2018.
- Microbiology of the food chain - Template and guidance for drafting ISO/CEN standard methods. Document ISO/TC 034/SC 09 N 2188. 24-05-2018.
- Presentation on 'Detection and serotyping of *Salmonella* (EN ISO 6579-1 and CEN ISO/TR 6579-3)' by Kirsten Mooijman at a symposium on 'new standards in the laboratory', organised by the Dutch Foundation Food industry Microbiological Methods (FIMM) in Wageningen, the Netherlands on 25 January 2018.
- Presentation on 'General rules for pooling and compositing samples (EN ISO 6887-1:2017)' by Kirsten Mooijman at a Workshop on Rules for Microbiological Sampling and Testing, organised by the Food Safety Authority of Ireland, Dublin, Ireland on 19 April 2018.
- Presentation on 'ISO 6579-1:2017 What's new since ISO 6579:2002' by Kirsten Mooijman at a *Salmonella* and *Listeria* seminar organised by Campden BRI, Chipping Campden, UK on 24 April 2018.
- Presentation on 'Can we harmonise incubation temperatures of 35-37°C for detection of *Salmonella* spp.?' by Kirsten Mooijman. International Symposium for *Salmonella* and salmonellosis (I3S), St. Malo, France, 24-26 September 2018.
- Presentation on 'Accreditation and MicroVal certification' by Kirsten Mooijman and on 'Introduction to (draft) ISO 16140-6' by Wilma Jacobs. Workshop MicroVal on alternative microbiological confirmation methods, Wageningen, the Netherlands, 29 October 2018.

Missions

Meetings ISO/TC34/SC9 – WG3 on validation of microbiological methods (including draft ISO 16140-6 on validation of confirmation/typing methods)
23-25 May 2018: Helsinki, Finland
Participant: Wilma Jacobs (project leader drafting ISO 16140-6 and member WG3)
3-6 December 2018: Brussels, Belgium
Participant: Wilma Jacobs

Annual meetings of CEN/TC275/WG6 and ISO/TC34/SC9 (Microbiology of the food chain)
17-22 June 2018: Lausanne, Switzerland
Participant: Kirsten Mooijman

Meeting CEN/TC275/WG6 TAG9 on improvement of the pre-enrichment
27 February 2018: Teleconference
17-18 April 2018: Ayr, United Kingdom
Participant: Kirsten Mooijman

Sub-activity 1.2 EURLs working group on NGS

In 2017 a working group (WG) of 8 EURLs was raised on Next Generation Sequencing (NGS), with the aim to promote the use of NGS across the EURLs' networks, to build capacity towards the use of NGS within the EU and ensure liaison with the work of EFSA and ECDC on the Whole Genome Sequencing (WGS) mandate sent by the Commission.
One of the first actions of this WG was to get a more precise knowledge of the status of the capacity towards the use of NGS at the NRLs of the different networks. For this purpose, the WG prepared a survey which was sent to the 8 NRL networks by the end of March 2018. The NRLs were requested to participate and fill in the questions related to the status of using NGS in the NRL. Each of the EURLs composing the WG sent out the same survey to their NRL network. This could result in the fact that some organisations received more than one request to fill in the questionnaire if this organisation was at the same time NRL for more than one hazard. Still it was requested to complete the relevant survey for each NRL.
EURL-*Salmonella* sent the survey to 54 NRLs-*Salmonella* in 36 countries. By the end of April 2018, completed surveys of 23 NRLs-Salmonella in 20 countries were received, a response rate at the level of NRLs of 42.6%, and at the level of countries of 55.5%. Eight NRLs reported that they do not (yet) perform any NGS/WGS activities. The other 15 NRLs reported different NGS activities. A summary of the survey results were presented at the workshop of the EURL-*Salmonella* in May 2018 (Mooijman, 2018b).
To obtain more information on the Bioinformatics used by the NRLs, a second (short) survey was sent to the (15) NRLs-*Salmonella* which indicated to perform NGS/WGS activities in the first survey. This survey was sent in August 2018. Ten NRLs-*Salmonella* replied to this second survey.
Additionally, a third (short) survey was sent to the (8) NRLs-*Salmonella* which indicated not to perform NGS/WGS activities in the first survey. This survey was sent in November 2018 and concerned a combined survey by the 8 EURLs of the WG NGS, organised by the EURL-antimicrobial resistance (AR). Five NRLs-*Salmonella* replied to this third survey.
The results of the three surveys held under the 8 EURL/NRL networks will be summarised in one report by the EURLs WG NGS in 2019.

During summer 2018, an inventory was made among the 8 EURLs (initiated by the EURL-AR) to obtain information on conducted and planned PTs on NGS organised by the EURLs. By the end of August this was discussed in a teleconference. The information of the 8 EURLs was summarised by EURL-AR and sent to the members of the EURLs WG NGS in February 2019.

Deliverables

- Questionnaire on the support expected from the EURL on the application of Next Generation Sequencing technology, including WGS of bacterial/viral/parasites and metagenomics by the EU NRLs, March 2018.
- Summary results of the survey on NGS, NRLs-*Salmonella*. Presented at EURL-*Salmonella* workshop May 2018 (Mooijman, 2018b).
- Second survey of the bioinformatics used to analyse NGS data, August 2018.
- Third survey to NRLs not performing NGS/WGS activities, November 2018.

- Minutes meetings EURLs WG NGS, June and November 2018.

Missions
Meetings EURLs working group NGS
11 June 2018: Brussels, Belgium
Participants: Kirsten Mooijman, Indra Bergval
29 November 2018: Brussels, Belgium
Participants: Kirsten Mooijman, Angela van Hoek

Sub-activity 1.3 Interlaboratory comparison studies
In 2018, three interlaboratory comparison studies were organised by the EURL-*Salmonella*, two studies on detection of *Salmonella* and one study on (sero)typing of *Salmonella*. For the studies on detection of *Salmonella,* the directions of CEN ISO/TS 22117:2010 for the number of samples of qualitative Proficiency Tests are followed. This indicates the use of 18 samples per participant, consisting of six replicates of three different levels of the target strain: blank, low level and high level samples.
For the reporting of the results of the interlaboratory comparison studies by the NRLs-*Salmonella*, electronic result forms are used. In fall 2018, a new software programme was used for drafting these forms. The advantages of this new software were that the editing of the forms can be done by the EURL staff, and that it is no longer needed to consult the IT department. The electronic result form drafted with the new software has the same functionalities as the former forms, but it is more user friendly.

Follow-up interlaboratory comparison study on detection of *Salmonella* organised in 2017
In October 2017, a combined interlaboratory comparison study for Food and Primary Production Stage (PPS) 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 as well as 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. 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 and a follow-up study was organised in March 2018. In this follow-up study the NRL performed well. The results were presented at the EURL-*Salmonella* workshop in May 2018. More details on the study can be found in the (interim) summary report and in the full report (Pol-Hofstad and Mooijman, 2017 and 2018a).

Follow-up 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, 11 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 selected in close cooperation with Statens Serum Institute (SSI) in Denmark. SSI organised Proficiency Tests for the same subject for laboratories analysing human samples of the Food and Waterborne (FWD) network of ECDC.
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 were analysed in spring 2018 and reported to the participants in July 2018. The results of all laboratories for both typing methods (serotyping and PFGE) were presented at the EURL-*Salmonella* workshop in May 2018.
More details on the typing study of 2017 can be found in the (interim) summary reports and full report (Jacobs-Reitsma et al., 2018a,b,c).

Interlaboratory comparison studies on detection of *Salmonella* organised in 2018

In February/March 2018, an interlaboratory comparison study on the detection of *Salmonella* in animal feed samples was organised.
In this study, 35 NRLs for *Salmonella* participated: 30 NRLs from the (28) EU Member States and 5 NRLs from third countries (member countries of the EFTA, (potential) EU candidate Member States and one non-European country).
Each NRL had to analyse a total of 20 samples: 18 samples of 25 g chicken feed artificially contaminated with three different levels (blank, low and high level) of *Salmonella* Mbandaka and 2 control samples. The inoculation levels were: 8 cfu/sample and 91 cfu/sample.
The prescribed method was EN ISO 6579-1:2017 for analysing animal feed samples, including selective enrichment in Muller Kauffmann Tetrathionate broth with novobiocin (MKTTn) and on Modified Semi-solid Rappaport Vassiliadis (MSRV) agar or in Rappaport Vassiliadis with Soya (RVS) broth. All NRLs reported their results before the end of March 2018, after which the analysis of the results was performed. In April 2018, the participants received information on their results as well as an interim summary report including the results of all participants.
The study showed an unexpected high number of negative results for the artificially contaminated chicken feed samples. Therefore, it was decided not to set criteria for these samples, but only to compare the number of positive samples found per laboratory with the mean number of positive samples found by all participants. Overall, the laboratories found *Salmonella* in 52% of the high level and in only 5% of the low-level contaminated samples. The MPN (Most Probable Number) analysis of the chicken feed samples showed a very low level of *Salmonella* even in the high-contaminated samples at the day of performance. The high-contaminated samples could have been evaluated as low-contaminated samples, as the sensitivity rate was approximately 50%, indicating a final level in the feed samples close to the detection limit. The number of positive samples found by all participants was evenly distributed across both the high- and low-level contaminated samples. This indicates that the detection of *Salmonella* in the chicken feed was influenced evenly over all samples. These results were unexpected when compared to the results of the pre-tests, for which the same type of chicken feed and *Salmonella* Mbandaka strain were used. In the batch of chicken feed used for the interlaboratory comparison study, a reduction of almost 2 log cfu of *Salmonella* Mbandaka occurred. This reduction explains the high

number of negative samples in the interlaboratory comparison study. The cause of this reduction remained unclear, but was most likely due to the presence of inhibitory substances in the batch of chicken feed used in the main study.
Due to these problems with the chicken feed samples it was not possible to evaluate the NRLs' performance for *Salmonella* in this study.
More details can be found in the interim summary report and full report (Kuijpers and Mooijman, 2018 and 2019).

In October 2018, an interlaboratory comparison study on detection of *Salmonella* in PPS samples was organised. The matrix under analysis concerned artificially contaminated boot sock samples with chicken faeces. In this study 36 NRLs 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 non-European country).
Each NRL analysed in total 20 samples: 18 boot sock samples with chicken faeces artificially contaminated with three different levels (blank, low and high level) of *Salmonella* Infantis and 2 control samples. The inoculation levels were: 10 cfu/sample and 53 cfu/sample.
The prescribed method was EN ISO 6579-1:2017 for analysing PPS samples, including selective enrichment on Modified Semi-solid Rappaport Vassiliadis (MSRV) agar. All NRLs reported their results before the end of October 2018, after which the analysis of the results was performed. In December 2018, the participants received information on their performance as well as an interim summary report including the results of all participants. In total, 35 laboratories fulfilled the criteria of good performance for the prescribed method. One laboratory was facing some problems with the contaminated boot sock samples, scoring five of the six low-level samples negative for *Salmonella* and one of the six high-level samples negative. Most likely, this was caused by the high temperature this parcel experienced during a long transport time, which negatively affected the concentration of *Salmonella* in the boot sock samples with chicken faeces. Due to the poor temperature conditions in the parcel during the seven days of transport, the quality of the samples could not be guaranteed and therefore the results of this laboratory were not evaluated.
More details on the study can be found in the (interim) summary report (Pol-Hofstad and Mooijman, 2018b). The full report is under preparation.

Interlaboratory comparison study on typing of *Salmonella* organised in 2018

In November 2018 the 23rd interlaboratory comparison study on typing of *Salmonella* spp. was organised. This study contained a compulsory part on serotyping and an optional part on PFGE typing.
In total 36 NRLs for *Salmonella* participated: 29 NRLs from the (28) EU Member States and 7 NRLs from third countries (EU (potential) candidate MS, EFTA countries and one non-EU country).
All (36) 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.
Twelve NRLs participated in the PFGE part of the study. For this, 11 different *Salmonella* strains had to be analysed. The NRLs reported the results of the serotyping before mid-December 2018 and the PFGE results by the end of December 2018/early January 2019.
The analysis of the serotyping results was performed in January/February 2019 after which the NRLs received their own results and an interim summary report, including the results of all laboratories in February 2019. Two participants did not meet the level of good performance at the first stage of the study and a follow-up study for these laboratories will be organised in spring 2019.
The results of the study on PFGE typing are under analysis.

More details on the serotyping part of this typing study can be found in the (interim) summary report (Jacobs-Reitsma et al., 2019).

Deliverables

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- Jacobs-Reitsma, W.F., Verbruggen, A.J. and Mooijman, K.A. Interim Summary Report EURL-*Salmonella* Proficiency Test Serotyping 2018. (February 2019). https://www.eurlsalmonella.eu/documenten/interim-summary-report-eurl-salmonella-pt-serotyping-2018
- Kuijpers A.F.A. and Mooijman, K.A. EURL-*Salmonella* 8th interlaboratory comparison study Food 2016 - Detection of *Salmonella* in minced chicken meat. RIVM report 2017-0081 (February 2018). https://www.rivm.nl/bibliotheek/rapporten/2017-0081.pdf
- Kuijpers, A.F.A. and Mooijman, K.A. Interim summary report EURL-*Salmonella.* Interlaboratory Comparison study FEED IV (2018) Detection of *Salmonella* in chicken feed. National Institute for Public Health and the Environment, Bilthoven, The Netherlands. RIVM Letter Report 50/2018 Z&O ku/ak. (April 2018). https://www.eurlsalmonella.eu/media/561
- Kuijpers, A.F.A. and Mooijman, K.A. 4th EURL-*Salmonella* interlaboratory comparison study Animal Feed 2018 Detection of *Salmonella* in chicken feed. National Institute for Public Health and the Environment, Bilthoven, The Netherlands. RIVM Report 2018-0023 (February 2019). https://www.rivm.nl/bibliotheek/rapporten/2018-0023.pdf
- 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 2017-0083 (May 2018). https://www.rivm.nl/bibliotheek/rapporten/2017-0083.pdf
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Activity 2 - To provide scientific and technical assistance to NRLs

Sub-activity 2.1 Workshop

On 29 and 30 May 2018, the annual workshop was organised in Uppsala, Sweden. The workshop was organised with the kind help of the NRL-*Salmonella* of Sweden. A total of 47 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*
2 guest speakers
1 participant from EFSA
1 participant from DG-SANTE
Members of NRLs from one EU Member State, and two (potential) candidate countries, were unable to come to the workshop due to lack of staff or lack of funding.

Presentations were given on the following subjects:

- Results of the interlaboratory comparison studies as organised by the EURL-*Salmonella* since the previous workshop (May 2017);
- Proposals for new interlaboratory comparison studies;
- No decrease of human *Salmonella* Enteritidis despite *Salmonella* control programs in poultry in the European Union, 2013-2016;
- Information on the activities of the EURL-*Campylobacter*;
- Multi country outbreak of *Salmonella* Agona infections linked to infant formula;
- Investigating *Salmonella* in the cattle production in France;
- Birds, cats, humans and host adaptation in *Salmonella* Typhimurium;
- Update on activities in ISO and CEN;
- Pilot validation study for confirmation of *Salmonella* following ISO/DIS 16140-6;
- EFSA's molecular typing activities for food-borne pathogens;
- EURL working group on Whole Genome Sequencing (WGS);
- 5 NRLs presented their activities for being NRL-*Salmonella;*
- Work-program 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 to 43 workshop participants; 38 completed forms were returned, a response rate of 88%. 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 finalised in March 2019 and the final report is likely to be published in spring 2019. All presentations were placed on the EURL-*Salmonella* website on 1 June 2018: https://www.eurlsalmonella.eu/workshop-2018

Deliverables

- 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 2017-0080 (June 2018). https://www.rivm.nl/bibliotheek/rapporten/2017-0080.pdf

- Presentations of the workshop 2018 published at the EURL-*Salmonella* website (June 2018). https://www.eurlsalmonella.eu/workshop-2018
- Mooijman, K.A. The 23rd EURL-*Salmonella* workshop – 29 and 30 May 2018, Uppsala, Sweden. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM Report 2018-0024 (final draft March 2019).

Missions
Three staff members of the EURL-*Salmonella* also travelled to Uppsala, Sweden for organisation, coordination, participation and giving presentations at the annual workshop, 29-30 May 2018.

Sub-activity 2.2 Training courses
On 26 and 27 June 2018, 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-*Salmonella*, Bilthoven, the Netherlands. 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. At the end of the training course, the NRLs-*Salmonella* were requested to complete an evaluation form. The results of this evaluation are summarised in Annex 1, showing that the NRL-*Salmonella* participants were satisfied with the training course.

Deliverables
- Evaluation of the training course by NRL-*Salmonella* participants (Annex 1).
- Summary report of the 'Third joint training course on the use of BioNumerics software to analyse PFGE data of Shiga-toxin producing *Escherichia coli, Salmonella* and *Listeria monocytogenes*, 26-27 June 2018'. This summary report, including the program of the training course and the list of participants, was prepared by the EURL-*Salmonella,* and sent to all participants on 18 July 2018, and can also be found in Annex 2 of this report (without the list of participants).

Sub-activity 2.3 Scientific advice and support of NRLs
In 2018, several questions were received from NRLs-*Salmonella*, and other institutes inside and outside the EU on the following subjects (list not exhaustive):
- Explanation and interpretation of information in EN ISO 6579-1:2017 (and its annexes) for detection (including confirmation) of *Salmonella*.
- Advise on preparation of samples.
- Problems with MSRV agar.
- Validation and verification of (alternative) methods on detection and/or serotyping of *Salmonella*.
- Advise on, and supply of *Salmonella* strains for validation and/or quality control of (alternative) methods.
- Application of (validated) alternative methods.
- Serotyping of *Salmonella* and advice on media/antisera for phase inversion.
- Differentiation of *Salmonella* vaccine strains from wild strains;
- Use of EN ISO 6579-1:2017 in relation to (not yet updated) EU legislation.
- Serological tests for detection of *Salmonella*.
- Information on serotyping of different *Salmonella* serovars.
- Information on (availability of) reference materials.

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.

In 2018, the EURL-*Salmonella* performed (confirmation of) serotyping of several *Salmonella* isolates for NRLs-*Salmonella* of the following countries:

- Albania (20 isolates).
- Germany (2 isolates).
- Greece (13 isolates, including 2 possible new serovars).
- Ireland (2 isolates).
- Slovenia (8 isolates).
- United Kingdom, Northern Ireland (1 isolate, possibly a new serovar).

In 2018, two NRLs-*Salmonella* requested the EURL-*Salmonella* to approach the NRL network to collect information on *Salmonella* serovars related to local/national outbreaks. This concerned requests from:

- NRL-*Salmonella* in Belgium in relation to an outbreak with *Salmonella* Typhimurium at schools in Belgium (June 2018).
- NRL-*Salmonella* in Norway in relation to an outbreak with monophasic *Salmonella* Typhimurium in horses in Norway (October 2018).

In June 2018, the EURL-*Salmonella* informed the NRLs-*Salmonella* about the possibility to comment on the draft proposal 'Revision of Regulation (EC) 20173/2005'. In relation to this, the EURL replied to some questions of NRLs. For example why no reference is made to CEN ISO/TR 6579-3:2014 for serotyping of *Salmonella* (an ISO Technical Report (TR) concerns a guidance document and does not have the same legal status in all countries).

In July 2018, the EURL-*Salmonella* informed the NRLs-*Salmonella* about the publication of two EFSA reports related to the use of WGS for food safety.
In December 2018, the EURL-*Salmonella* informed the NRLs-*Salmonella* about a training course organised by the Joint Research Centre (JRC) on the implementation of the EN ISO/IEC 17043:2010 for the organisation of proficiency tests, in February 2019.

In October 2018, delegates from the Cantacuzino National Medico Military Institute for Research and Development, Bucharest, Romania visited the RIVM to discuss cooperation between the institutes. Kirsten Mooijman presented the role and activities of the EURL-*Salmonella* and the Dutch NRL-*Salmonella*.

In 2018, a joint EURLs working group was raised on initiative of EURL-*Listeria monocytogenes*, to draft a guidance document for the organisation of Proficiency Tests by NRLs for national networks, including partial outsourcing. The working group exists of 5 EURLs (*Listeria monocytogenes,* coagulase positive staphylococci, STEC, *Campylobacter* and *Salmonella*) and discussed draft versions of the guidance document in teleconferences in October and December 2018.

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 2018, volume 24 no 1 of the newsletter was published, which included information on interlaboratory comparison studies organised in fall 2017, and a call to the NRLs to complete the survey on the use of NGS.
- In June 2018, volume 24 no 2 of the newsletter was published including the timetables of the interlaboratory comparison study for detection of *Salmonella* in samples from the Primary Production Stage (October 2018) and of the interlaboratory comparison study on typing of *Salmonella* (November 2018). Additionally, the EURL-*Salmonella* work programme of 2018 was

included, as well as the technical report on activities of the EURL-*Salmonella* performed in 2017.
- In September 2018, volume 24 no 3 of the newsletter was published, which included again the time table of the interlaboratory comparison study on typing of *Salmonella* (November 2018), and a summary was included of '*Salmonella*-related items' as discussed at the annual meetings of ISO/TC34/SC9 and CEN/TC275/WG6 in June 2018. In addition, information was given by the NRL-*Salmonella* of Finland on troubleshooting of Modified semi-solid Rappaport Vassiliadis (MSRV) agar.
- In December 2018, volume 24 no 4 of the newsletter was published, which included the time table of the combined Food-Feed EURL-*Salmonella* Proficiency Test on detection of *Salmonella* in flaxseed (March 2019).

Other relevant information is also published through the website: www.eurlsalmonella.eu. Two staff members of the EURL regularly keep the information on the website up to date.
The EURL-*Salmonella* website is hosted by the RIVM, and a change was made to new software for management of all RIVM-websites in 2018. In July 2018, the content of the EURL-*Salmonella* website was migrated to the new version of the website. This resulted in a new lay-out of the website and in the fact that old documents (approx. > 5 years) became approachable through a link to the archive of the website. The url of the website did not change, and the NRLs-*Salmonella* were informed about the change of the website in the Newsletters of June and September 2018.

Deliverables
- Draft guidance document for the organisation of Proficiency Tests by NRLs for national networks (joint EURLs document).
- Four EURL-*Salmonella* Newsletters Vol. 24 No. 1-4, published at the EURL-*Salmonella* website: https://www.eurlsalmonella.eu/publications/newsletters
- New version EURL-*Salmonella* website: https://www.eurlsalmonella.eu/

Activity 3 - To provide scientific and technical assistance to the European Commission and other organisations

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

In 2018, several questions were received from EC DG-SANTE, the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) on the following subjects (list not exhaustive):

- Advice DG-SANTE on *Salmonella* methods in the amendment of Regulation (EC) 2073/2005.
- Participation in an interview on data gathering study in the food chain area (on behalf of DG-SANTE).
- Participation in an interview to evaluate the ECDC laboratory network of Food and Waterborne Diseases (FWD) for 7 priority diseases.
- Request for information (ECDC) on ESBL producing *Salmonella* Kentucky.
- Inform DG-SANTE on *Salmonella* testing in spent irrigation water.
- Advice EFSA on application of draft ISO 16140-6 for validation of alternative (proprietary) methods for microbiological confirmation and typing procedures.
- Inform DG-SANTE on *Salmonella* vaccination in poultry.

In 2018, the EURL-*Salmonella* assisted DG-SANTE and EFSA with several multi-country *Salmonella* outbreaks, by approaching the NRL-*Salmonella* network for information and by helping with analysis of sequence data.

- May-June 2018: call to the NRLs-*Salmonella* for molecular data of *Salmonella* Bareilly found in food or animals in the period 2016-2018, and cluster analysis of sequence data.
- July-August 2018: call to the NRLs-*Salmonella* for sequence data of *Salmonella* Agona found in food and processing environment, and cluster analysis of sequence data.
- October-November 2018: call to the NRLs-*Salmonella* for sequence data of *Salmonella* Mikawasima found in food or animals in the period 2017-2018, and cluster analysis of sequence data.
- December 2018: call to the NRLs-*Salmonella* for sequence data of *Salmonella* Coeln found in food or in animals in 2018. Further analysis is performed in 2019.

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 2018, the steering committee organised two physical meetings (in March and October 2018) in Stockholm, Sweden.

The EFSA pilot database for the collection of molecular data was activated in December 2014, but for *Salmonella* little activities were employed so far. Initially this was likely caused by the fact that agreement on and signature of the collaboration agreement by all parties lasted until April 2016. Also, 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. Additionally, the molecular (sub)typing method of choice changed from PFGE to WGS. However, it was not yet possible to upload WGS data to the database in 2018. 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.

Due to the planned departure of the United Kingdom from the European Union, the EURL-bivalve molluscs ceased to exist on 31-12-2018 and its activities had to be distributed over other EURLs. In June 2018, a meeting was organised by DG-SANTE to discuss with the EURLs for bivalve molluscs, foodborne viruses, *E. coli,*

Salmonella and biotoxines the distribution of activities of EURL-bivalve molluscs after 01-01-2019.

In December 2018, DG-SANTE organised a meeting for the directors of the EURLs in the field of animal health and food and feed safety. The EURLs were updated on the Official Control Regulation (EC) 2017/625, on the financial aspects of the EURLs and on the new EURLs for plant health. The head of EURL-*Salmonella* also participated in this meeting.

Deliverables

- Collection of information and cluster analysis of sequence data possibly related to multi-country outbreaks with *Salmonella* Bareilly, *Salmonella* Agona, *Salmonella* Mikawasima and *Salmonella* Coeln.
- Contribution to Rapid Outbreak Assessment of *Salmonella* Agona infections possibly linked to ready-to-eat food.
  http://www.efsa.europa.eu/en/supporting/pub/en-1465

Missions

Meetings EFSA-ECDC steering committee
14 March 2018: Stockholm, Sweden
24-25 October 2018; Stockholm, Sweden
Participant: Kirsten Mooijman

Meetings, for which travel costs were for budget DG-SANTE:

Meeting on the distribution of tasks from EURL-bivalve molluscs
29 June 2018: Brussels, Belgium
Participant EURL-*Salmonella*: Kirsten Mooijman

Meeting directors of the EURLs in the field of animal health and food and feed safety.
7 December 2018: Brussels, Belgium
Participant EURL-*Salmonella*: Kirsten Mooijman

Activity 4 - Reagents and reference collections

Sub-activity 4.1 Reference strains and reference materials

Information on the *Salmonella* serovar names and antigenic 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 (Grimont and Weill, 2007). A link to this scheme is available at the website of the EURL-*Salmonella*: https://www.eurlsalmonella.eu/publications/eurl-manual.
In 2018, the EURL-*Salmonella* contacted the WHO collaborating Centre twice (February and May 2018) to ask for a possible update of the White-Kauffmann-Le Minor scheme. However, the WHO collaborating Centre could only indicate that they hoped to publish the updated scheme by the end of 2018. As this turned out not to be feasible, the EURL-*Salmonella* will again contact the WHO collaborating centre on this subject in 2019.

The EURL-*Salmonella* stores an 'in-house' collection of *Salmonella* strains which have been collected from different projects performed at the RIVM. New/interesting strains are regularly added to this collection. The collection is mainly used for 'in-house' use, e.g. for use in Proficiency Tests and testing/verification of methods. Occasionally, strains are provided to NRLs when needed for specific tests.

From 1986 until 2003, the RIVM and its affiliated foundation, developed and produced microbiological reference materials (RMs). Some of the reference materials have been certified (CRMs) and moved to the Institute for Reference Materials and Methods (IRMM) of the Joint Research Centre (JRC) in Geel, Belgium. The RIVM and EURL-*Salmonella* no longer produce reference materials, but the knowledge on production and use of (C)RMs is still available. At the EURL-*Salmonella* website information is given on (C)RMs and links are given to producers of (C)RMs: https://www.eurlsalmonella.eu/publications/eurl-manual

This sub-activity is considered to be part of the sub-activity for keeping information at the EURL-*Salmonella* website up to date, so that output of this sub-activity is merged with sub-activity 2.3

Abbreviations

CEN	European Committee for Standardization
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
MPN	Most Probable Number
MS	Member State
MSRV	Modified Semi-solid Rappaport Vassiliadis
NGS	Next Generation Sequencing
NRL	National Reference Laboratory
PPS	Primary Production Stage
RIVM	National Institute for Public Health and the Environment
RVS	Rappaport Vassiliadis broth with Soya
SSI	Statens Serum Institute
STEC	Shiga toxin-producing *Escherichia coli*
TAG	Technical Advisory Group
TC	Technical Committee
TR	Technical Report
TS	Technical Specification
WG	Working Group
WGS	Whole Genome Sequencing

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Annex 1 Evaluation joint training course organized by 3 EURLs on the use of BioNumerics (26 and 27 June 2018)

Evaluation Joint Training Course of 3 EURLs on the use of BioNumerics Software to analyse PFGE data of STEC, *Salmonella* and *Listeria monocytogenes*
Dates: 26 and 27 June 2018.
Location: EURL-*Salmonella*, Bilthoven, the Netherlands.

Only NRLs-*Salmonella* participating in the training completed the evaluation form for EURL-*Salmonella*

Total number of participants training course (of which NRL-*Salmonella***)**	11 (4)
Number of participants (NRLs-*Salmonella***) completing evaluation**	4 NRLs-*Salmonella*
Participating countries (NRLs-*Salmonella*)	France, Portugal, Slovenia, United Kingdom
What did you expect to learn from this training (on forehand)?	- Implement knowledge on BioNumerics software and consolidation of cluster analysis. - Utilisation of software BioNumerics and strains comparisons. - An overview of PFGE and how to use BioNumerics software. - Details on image analysis.
Were the trainers able to fulfil your expectations	
Yes	4
No	-
Was the time sufficient for your training?	
Yes	3
No too short	1; would have needed 0.5-1 days more.
Can you please describe (in short) what you have learned during the training?	- I had the possibility to consolidate my knowledge on cluster analysis. - How to upload images to BioNumerics, process these images, and how to compare these results. - Solved the dilemma's regarding image analysis, clarified the EFSA-ECDC database facts, clarified questions related to cluster investigation inquiries.
Is what you have learned during the training applicable in your laboratory?	
Yes	4
No	-
Comments	- We do not currently use this software or PFGE, but we are

	looking to use PFGE/WGS in the future.
Overall, did the training fulfil your expectations?	
Yes	4
No	-
Any other comments?	- Thank you for excellent organization and useful workshop.

Annex 2 Summary report third joint training course on the use of BioNumerics software to analyse PFGE data of shiga-toxin producing *Escherichia coli, Salmonella* and *Listeria monocytogenes*

Third joint training course on the use of BioNumerics software to analyse PFGE data of shiga-toxin producing *Escherichia coli, Salmonella* and *Listeria monocytogenes*

26-27 June 2018

National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

Summary report

In 2012, the EC DG SANTE decided to organize the collection of molecular typing data for isolates of *Listeria monocytogenes*, *Salmonella* and Shiga-toxin producing *Escherichia coli* (STEC) from food, animal feed and samples from the primary production stage, to improve the surveillance and trace-back of food-borne infections as well as the preparedness to face foodborne outbreaks at the European level. The strategy of this molecular surveillance system is described in the DG SANTE document 'Vision paper on the development of data bases for molecular testing of foodborne pathogens in view of outbreak preparedness', available at the url:
https://ec.europa.eu/food/sites/food/files/safety/docs/biosafety-crisis-vision-paper_en.pdf

According to the DG SANTE mandate, the responsibility of data collection was assigned to the European Food Safety Authority (EFSA), with the scientific and technical support of the relevant European Union Reference Laboratories (EURLs), which have been assigned the role of curators of the submitted data. The data collection has started during 2017.
The bulk of molecular typing data on food/animal isolates is primarily produced by the networks of National Reference Laboratories (NRLs) operating with the relevant EURLs. Therefore, among the initiatives to provide training to the respective NRL networks, the EURLs for *Listeria monocytogenes*, *Salmonella* and STEC decided to organize joint training courses on the use of the software BioNumerics, which offers an integrated platform for the analysis of PFGE fingerprints and allows the storage of gel images and epidemiological metadata in a single database.

The first edition of the training course took place in July 2016 at the laboratory for food safety located at Maisons-Alfort of the French Agency for Food,

Environmental and Occupational Health & Safety (ANSES), located at Maisons-Alfort (Paris, France), hosting the EURL for *Listeria monocytogenes*.
The second edition was held on 3-4 July 2017 at the IT-training room of the Istituto Superiore di Sanità in Rome, Italy, hosting the EURL-STEC.
The third edition was held on 26-27 June 2018 at the IT-training room of the National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands, hosting the EURL for *Salmonella*.

The objectives of the course are to make the participants able:
- to visually evaluate the quality of PFGE gel images;
- to know the technical flaws resulting in poor-quality and not-analyzable pictures;
- to use the BioNumerics software package for PFGE profile analysis, through hands-on exercises on the acquisition and normalization of .tiff files;
- to build up a database of PFGE profiles;
- to perform band assignment and profile analysis;
- to perform cluster analysis, in order to identify the level of similarity between the profiles.

Representatives of the three EURLs gave presentations and guided the trainees through the application of the standard operating procedures (SOPs) for PFGE analysis published by EFSA in 2014:
- *Listeria* SOP: http://onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2014.EN-702/pdf
- *Salmonella* SOP: http://onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2014.EN-703/pdf
- STEC SOP: http://onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2014.EN-704/pdf

The third training edition, hosted at the EURL-*Salmonella* in Bilthoven on 26-27 June 2018, was attended by a total of eleven participants. Regrettably, the twelfth applicant had to withdraw on short notice, due to unexpected personal circumstances.

The participants were divided in three groups according to their preparedness in PFGE images analysis, assigned computers organized in as many rows and assigned two trainers per group among the representatives from the three EURLs. The course consisted of interactive oral presentations, followed by extended hands-on training sessions regarding the subjects discussed.
Each participant was placed in front of a workstation equipped with the software BioNumerics version 7.6 for the practical sessions. The BioNumerics temporary licenses were provided by Applied Maths (Sint-Martens-Latem, Belgium).
The presentations and the exercises were managed by staff from the three EURLs. An invited talk was given by Dr. Valentina Rizzi from EFSA, who described the state of play of the data collection in the EFSA-ECDC joint database for the molecular-typing based surveillance of foodborne infections.

The Course was opened by **Dr. Kirsten Mooijman**, director of the EURL-*Salmonella*, who welcomed all participants to the training course and briefly introduced its aims and program.
Dr. Valeria Michelacci, from EURL-STEC, presented three examples of case studies demonstrating the relevance of PFGE analysis for the molecular typing of STEC. In detail, she showed how PFGE analysis can be crucial to identify the sources of infection and recall the concerned products from the market, implement hygiene measures when animal contact is involved or rule out the involvement of suspected food products differentiating isolates which could be undistinguishable when typed with other molecular methods. **Dr. Benjamin Felix,** from EURL-*Listeria monocytogenes*, presented details of a current and still ongoing investigation of a multi-country outbreak investigation on *L. monocytogenes*, showing the value of using combined information from both

WGS and PFGE data. Responsive collaboration within the EURL-NRLs network was shown to be of great importance as well. **Dr. Wilma Jacobs,** from EURL-*Salmonella*, gave an overview on the interlaboratory comparison studies as organized for the network of NRLs on serotyping and PFGE typing of *Salmonella*.
Dr. Antonella Maugliani, from EURL-STEC, then gave a presentation illustrating all the phases of PFGE typing, from DNA preparation to image acquisition, with particular attention to the possible problems encountered, giving a sort of 'trouble shooting' and introducing the criteria to be adopted to perform a preliminary visual evaluation of the gel image before importing it in BioNumerics software for the analysis.
Dr. Jacobs and **Dr. Maugliani** jointly presented the steps to perform when starting using BioNumerics software for PFGE interpretation, starting from databases creation and import of digital images of the gel in TIFF format.
In the following presentation, **Dr. Ludivine Bonanno,** from EURL-*Listeria*, and **Dr. Maugliani** carefully explained the process of PGFE images analysis, describing how to set the reference system, how to normalize the gel and to assign bands for producing the final fingerprints.

These presentations were followed by a practical session managed by **Dr. Maugliani** and **Dr. Michelacci**. The session consisted of examples of visual evaluation of PFGE images and exercises on the same topic carried out by the participants.
After the lunch break, the practical work continued, where all participants were able to experience the use of BioNumerics for database creation, experiments set up and PFGE image import and analysis.

The second day of the training was opened by **Dr. Felix**, who introduced the cluster analysis, with particular focus on the purposes, the algorithms and the parameters to be applied for such analysis. He finally gave an overview of the different protocols which are available for the exchange of files across databases.
The last presentation was given by **Dr. Valentina Rizzi** from EFSA, who described the state of play of the EFSA-ECDC joint database for molecular surveillance of foodborne pathogens.
The rest of the morning was completely devoted to a practical session mainly focused on cluster analysis, during which the participants had the possibility to go in depth in profiles analysis and comparison with the concrete help of the trainers from the three EURLs to answer all the technical questions arising explaining in depth the functionalities of the BioNumerics software to serve the purpose of PFGE molecular typing of foodborne pathogens.
The training was concluded at lunch time, and all participants received a signed certificate of attendance.

PROGRAM

Tuesday 26 June

9.00 **Registration**
9.15 Welcome, housekeeping, and general overview on the training course (K. Mooijman)
9.30 Case studies on PFGE typing: *Listeria*; *Salmonella*, VTEC, (The three EURLs)
10.30 Molecular typing by PFGE: gel production and staining, image acquisition, analysis and self-evaluation (A. Maugliani)
11.00 **Coffee break**
11.30 The BioNumerics Software: database creation, import of TIFF files, setting up experiments and image analyses (A. Maugliani, W. Jacobs)
12.00 Profiles Analysis and Interpretation (L. Bonanno, A. Maugliani)
12:30 **HANDS-ON EXERCISES:** Visual evaluation of PFGE gel images fingerprint data analysis (all)

13.00 **LUNCH** (at RIVM, offered by the EURL-*Salmonella*)
14.00 **PRACTICAL SESSION**
Description of the dataset
Start of exercises
17.00 End of the first day

Wednesday 27 June
9.00 Introduction to cluster analysis: purpose and parameters and Global Database Management (e.g. re-normalisation, re-mapping) (B. Felix)
9.30 Molecular surveillance of foodborne infections in the EU: EFSA-ECDC Joint Database. State of play (V. Rizzi - EFSA)
10.00 **Coffee break**
10.30 **PRACTICAL SESSION**
12.45 Concluding remarks
13.00 **LUNCH**

----- End of training course ------

From the Literature

Salmonella-related Literature from Scopus: April – June 2019

Yang, L., Zhang, X., Liu, Y., Li, H., Qiu, S., Li, P., Song, H.
CSESA: An R package to predict Salmonella enterica serotype based on newly incorporated spacer pairs of CRISPR
(2019) BMC Bioinformatics, 20 (1), art. no. 215, .
ABSTRACT: Background: Salmonella enterica is a major cause of bacterial food-borne disease worldwide. Immunological serotyping is the most commonly used typing method to characterize S. enterica isolates, but is time-consuming and requires expensive reagents. Here, we developed an R package CSESA (CRISPR-based Salmonella enterica Serotype Analyzer) to predict the serotype based on the CRISPR loci of S. enterica. Results: CSESA has implemented the CRISPR typing method CLSPT and extended its coverage on diverse S. enterica serotypes. This package takes CRISPR sequences or the genome sequences as input and provides users with the predicted serotypes. CSESA has shown excellent performance with currently available sequences of S. enterica. Conclusions: CSESA is a convenient and useful tool for the prediction of S. enterica serotypes. The application of CSESA package can improve the efficiency of serotyping for S. enterica and reduce the burden of manpower resources. CSESA is freely available from CRAN at https://cran.r-project.org/web/packages/CSESA/. ISSN: 14712105

Hellgren, J., Hästö, L.S., Wikstrom, C., Fernström, L.-L., Hansson, I.
Occurrence of Salmonella, Campylobacter, Clostridium and Enterobacteriaceae in raw meat-based diets for dogs
(2019) Veterinary Record, 184 (14), .
ABSTRACT: The practice of feeding raw meat-based diets (RMBD) to dogs has increased in popularity in recent years. However, RMBD are based on offal that has not undergone any type of treatment to reduce the microbial content, so there is a risk of potential pathogenic microorganisms being present. Frozen samples from 60 RMBD packs produced by 10 different manufacturers were analysed for their content of bacteria belonging to the family Enterobacteriaceae, for Clostridium perfringens and for the presence of Salmonella and Campylobacter. Enterobacteriaceae were detected in all 60 samples and in 31 samples exceeded a level of 5000 bacteria/g, which is the threshold for satisfactory microbial hygiene according to EU regulations. In two samples, the amount of C. perfringens exceeded 5000 bacteria/g, which is the maximum level of anaerobic bacteria permitted by Swedish national guidelines. Salmonella species were found in four (7 per cent) and Campylobacter species in three (5 per cent) samples. These results show that it is critical to maintain good hygiene when storing, handling and feeding RMBD, in order to limit the potential health risks to animals and humans, especially young and immunocompromised individuals. ISSN: 00424900

Richardson, K.E., Cox, N.A., Cosby, D.E., Berrang, M.E., Holcombe, N.L., Weller, C.E.
Dry and heat stress affects H 2 S production of Salmonella on selective plating media
(2019) Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes, 54 (4), pp. 313-316.
ABSTRACT: The pH of Salmonella pre-enrichment media can become acidic (pH 4.0–5.0) when feeds/ingredients are incubated for 24 h. Salmonella in feed that have been stressed by heat and desiccation exhibit different pH tolerances than non-stressed cultures. Acidic conditions can result in cell injury/death and affect biochemical pathways. In this study, eight serotypes of Salmonella were grown in sterile meat and bone meal that was subjected to desiccation and heat stress. Cultures of non-stressed and stressed isolates were subsequently exposed to acidic pH from 4.0 to 7.0 in 0.5 pH increments (3 replicates/pH increment) in citrate buffer. At 6 and 24 h, serial dilutions were plated in duplicate on XLT-4 (xylose lysine tergitol-4) agar. Four serotypes showed an impaired ability to decarboxylate lysine on XLT-4. This inability to decarboxylate lysine was dependent on isolate, stress status, and incubation time. When the isolates' ability to decarboxylate lysine was examined using biochemical tests, cultures were found to be able to decarboxylate lysine with the exception of S. Infantis. This suggests that XLT-4 contains a biochemical stressor(s) which affects the rate of decarboxylation by these Salmonella. These results suggest that acidic conditions may influence the detection and confirmation of Salmonella in feed. ISSN: 03601234

von Hertwig, A.M., Amorim Neto, D.P., de Almeida, E.A., Casas, M.R.T., Nascimento, M.D.S.D.
Genetic diversity, antimicrobial resistance and virulence profile of Salmonella isolated from the peanut supply chain
(2019) International Journal of Food Microbiology, 294, pp. 50-54.
ABSTRACT: Thirty-Eight Salmonella isolates recovered from different stages of the peanut supply chain in three Brazilian States (São Paulo, Minas Gerais and Bahia) were subtyped by pulsed-field gel electrophoresis (PFGE) and characterized by phenotypic and genotypic tests for antimicrobial resistance and virulence genes. The isolates were distributed into seven PFGE pulsotypes. All the isolates were resistant to sulfonamide. However, only one isolate from a production site in Minas Gerais had resistance to two types of antimicrobials (sulfonamide and ampicillin). Furthermore, the isolates had intermediary resistance to kanamycin (16/38), streptomycin (14/38) and ceftazidime (12/38). Four isolates had the antimicrobial resistance gene related to phenicols (floR) and 37 related to aminoglycosides (strA). The bla shv gene related to β-lactams was detected in isolates recovered from all the production regions. Six virulence genes (invA, sefA, sivH, mgtC, ssaQ and agfA) were observed in all isolates. The sopE gene was detected in 24 isolates, avrA in 12. The gtgB, ipfA and rck genes were not detected. The results showed that the pulsotype 1 was restricted to Minas Gerais whereas the pulsotype 7 was present in São Paulo and Bahia. In addition, most of the isolates were not multidrug resistant. ISSN: 01681605

LeLièvre, V., Besnard, A., Schlusselhuber, M., Desmasures, N., Dalmasso, M.
Phages for biocontrol in foods: What opportunities for Salmonella sp. control along the dairy food chain?
(2019) Food Microbiology, 78, pp. 89-98.
ABSTRACT: Controlling the presence of pathogenic bacteria, such as Salmonella sp., in dairy products production is a burning issue since contamination with Salmonella can occur at any stage of the production chain. The use of Salmonella-phages applied as control agents has gained considerable interest. Nonetheless, Salmonella-phage applications specifically intended for ensuring the safety of dairy products are scarce. This review identifies recent advances in the use of Salmonella-phages that are or could be applied along the dairy food chain, in a farm-to-fork approach. Salmonella-phages can be promising tools to reduce the shedding of Salmonella in cattle, and to reduce and control Salmonella occurrence in postharvest food (such as food additives), and in food processing facilities (such as biosanitizing agents). These control measures, combined with existing methods and other biocontrol agents, constitute new opportunities to reduce Salmonella occurrence along the dairy food production, and consequently to alleviate the risk of Salmonella contamination in dairy products. ISSN: 07400020

Cevallos-Almeida, M., Martin, L., Houdayer, C., Rose, V., Guionnet, J.-M., Paboeuf, F., Denis, M., Kerouanton, A.
Experimental infection of pigs by Salmonella Derby, S. Typhimurium and monophasic variant of S. Typhimurium: Comparison of colonization and serology
(2019) Veterinary Microbiology, 231, pp. 147-153.
ABSTRACT: Salmonella serovars Derby, Typhimurium and the monophasic variant of Salmonella Typhimurium are the most frequently isolated serovars in pigs in France. To compare the excretion patterns, seroconversion to Salmonella and contamination of the organs of pigs inoculated with strains of all three serovars, we conducted an experimental trial with 28 SPF piglets. Four were used as a negative control, while the other 24 were divided equally into three groups. Each group was inoculated at 7 weeks of age with a different strain: S. Derby (SDb), S. Typhimurium (ST), and the monophasic variant of S. Typhimurium (mST). Fecal and blood samples were collected twice a week up until necropsy, on 21 days post-inoculation (DPI) for half of each group and 49 DPI for the remaining piglets. During necropsy, the tonsils, mesenteric lymph nodes and various intestinal contents were collected from each pig. Salmonella bacteria were quantified in CFU/g by a bacteriological method, and levels of Salmonella antibodies were measured using an ELISA Kit. Piglets inoculated with mST continuously excreted Salmonella in their feces throughout the trial. For each of the other serovars, one piglet was Salmonella-negative on one DPI. The quantity of Salmonella excreted was statistically different between the group inoculated with ST and mST ($p < 0.05$), but no differences were found between the other serovars. The tonsils, cecum and jejunum were the most contaminated organs in all groups. Seroconversion for all the piglets was completed by different DPI: 28 for ST, 31 for mST and 38 for SDb. No major differences were found in terms of excretion and colonization among the studied serovars. ISSN: 03781135

Pulido-Landínez, M.
Food safety - Salmonella update in broilers
(2019) Animal Feed Science and Technology, 250, pp. 53-58.
ABSTRACT: The increase in the presence of Salmonella in the processing plants is alarming. This problem represents challenges both at the processing level and for the whole integration. Chickens are the most important input in the processing plant, so the increase of Salmonella in the plant directly suggests what is happening in the primary production. All the interventions in breeder farms, hatchery, feed mills, and broiler farms that are related to the control of Salmonella will play a crucial role in the fulfillment of the processing plants objectives. The vertical integration model for chicken meat production, should act as one of the main tools for reducing the presence of bacteria that is related to foodborne diseases. Salmonella is one of the main food safety challenges for modern poultry production. Controls and interventions established in each vertical integration step will contribute to the reduction of the unwanted presence of Salmonella in the final product. ISSN: 03778401

Buehler, A.J., Wiedmann, M., Kassaify, Z., Cheng, R.A.
Evaluation of inva diversity among salmonella species suggests why some commercially available rapid detection kits may fail to detect multiple salmonella subspecies and species
(2019) Journal of Food Protection, 82 (4), pp. 710-717.
ABSTRACT: invA is a common molecular target for Salmonella-specific detection methods and is recommended by the U.S. Food and Drug Administration Bacteriological Analytical Manual as a target for PCR confirmation of putative Salmonella isolates. Novel assays designed for the rapid detection of foodborne pathogens are often validated according to guidelines provided by validation schemes, such as the AOAC International or the International Organization for Standardization. However, these validation guidelines allow for flexibility in the validation study experimental design, which may inflate the assay's ability to detect foodborne pathogens, especially for foodborne pathogens such as Salmonella, exhibiting tremendous species diversity with.2,600 confirmed serovars. This study was conducted to (i) describe the sequence diversity of invA, across a diverse set of Salmonella serovars and (ii) evaluate the ability of two commercially available, AOAC International–validated rapid detection assays to detect a diverse collection of Salmonella spp. strains. In silico analyses identified 362 of 2,058 nucleotide sites that were variable among invA sequences from a diverse collection, representing 86 unique serovars spanning all species and subspecies. Not surprisingly, the majority of variable sites (308 of 2,058) occurred in non–Salmonella enterica subsp. enterica strains, including Salmonella bongori and the other S. enterica subspecies. In vitro testing showed that both rapid detection assays, examined here, failed to detect all Salmonella strains at 1 log above the limit of detection, with assay A failing to detect S. enterica subsp. salamae, and assay B failing to detect S. bongori. Both strains were eventually detected at 100,000 times the limit of detection. Taken together, our study highlights the need to include non–subsp. S. enterica strains in the development and validation of rapid detection methods to limit false-negative test results. ISSN: 0362028X

Chekabab, S.M., Rehman, M.A., Yin, X., Carrillo, C., Mondor, M., Diarra, M.S.
Growth of salmonella enterica serovars typhimurium and enteritidis in iron-poor media and in meat: Role of catecholate and hydroxamate siderophore transporters
(2019) Journal of Food Protection, 82 (4), pp. 548-560.
ABSTRACT: Enteritidis and Typhimurium are among the top Salmonella enterica serovars implicated in human salmonellosis worldwide. This study examined the individual and combined roles of catecholate-iron and hydroxamate-iron transporters in the survival in meat of Salmonella Enteritidis and Typhimurium. Catecholate–iron-III (Fe3þ) and hydroxamate-Fe3þ transporter genes fepA, iroN, and fhuACDB were deleted in isolates of these serovars to generate single, double, and triple mutants. Growth rate in high-and low-iron media was compared among mutants, complements, and their wild-type parents. Susceptibility to 14 antibiotics, the ability to produce and utilize siderophores, and survival on cooked chicken breast were evaluated. In iron-poor liquid media, differences were observed between the growth characteristics of mutant Salmonella Enteritidis and Typhimurium. The double ΔiroNΔfepA and the triple ΔfhuΔiroNΔfepA mutants of Salmonella Enteritidis exhibited prolonged lag phases (λ ¼ 9.72 and 9.53 h) and a slow growth rate (μmax ¼ 0.35 and 0.25 h1) similar to that of its ΔtonB mutant (λ ¼ 10.12 h and μmax ¼ 0.30 h1). In Salmonella Typhimurium, double ΔiroNΔfepA and triple ΔfhuΔiroNΔfepA mutations induced a similar growth pattern as its ΔtonB mutant. Double deletions of fepA and iroN reduced the siderophore production and the use of enterobactin as an iron source. In the ΔiroNΔfepA mutant, but not in ΔfhuΔiroNΔfepA, the ferrichrome or deferrioxamine promoted growth for both serovars, confirming the specific role of the

FhuACDB system in the uptake and transport of hydroxamate Fe3þ. Survival of the mutants was also evaluated in a meat assay, and no difference in survival was observed among the mutants compared with wild type. This study showed differences between serovars in the importance of catecholate-iron and hydroxamate-iron uptake on Salmonella growth in iron-restricted media. Data also confirmed that both Salmonella Enteritidis and Typhimurium are well equipped to survive on cooked chicken meat, offering a rich iron condition. ISSN: 0362028X

Monte, D.F., Lincopan, N., Fedorka-Cray, P.J., Landgraf, M.
Current insights on high priority antibiotic-resistant Salmonella enterica in food and foodstuffs: a review
(2019) Current Opinion in Food Science, 26, pp. 35-46.
ABSTRACT: Multi-drug resistant Salmonella enterica remains one of the most pressing global concerns. The use of antimicrobials in food chain has contributed to the selection of resistant strains and their dissemination through vectors contributes to persistence in the environment. Further, the emergence of international clones could be favored by the versatility of Salmonella through host adaptability, ubiquity, and persistence along the food chain. Mobile genetic elements are likely a key vector, which propagates resistant clones. In this regard, Salmonella isolates displaying resistance, especially to the last resort antibiotics raises a public health concern as treatment options become limited. This review aims to provide insights on our current understanding of the antibiotic-resistant S. enterica and of their persistence along food chain. ISSN: 22147993

Magossi, G., Bai, J., Cernicchiaro, N., Jones, C., Porter, E., Trinetta, V.
Seasonal Presence of Salmonella spp., Salmonella Typhimurium and Its Monophasic Variant Serotype i 4,[5],12:i:-, in Selected United States Swine Feed Mills
(2019) Foodborne Pathogens and Disease, 16 (4), pp. 276-281.
ABSTRACT: This study evaluated the seasonal prevalence and distribution of Salmonella spp., Salmonella enterica serovar Typhimurium (ST) and its monophasic variant 4,[5],12:i:- (STM), in selected swine feed mills across the United States. Eleven facilities were selected for this study and 12 sites were sampled within each mill during fall 2016, early spring 2017, and summer 2017. Samples were evaluated following the USDA-FSIS guidelines for Salmonella isolation and culture positive samples were analyzed by polymerase chain reaction (PCR). A multiplex real-time PCR was used to differentiate ST and STM from other serotypes. Associations between season, mill, and sample site with Salmonella presence were investigated using generalized linear mixed models. Both season ($p < 0.007$) and mill ($p < 0.005$) were significantly associated with Salmonella spp. presence. Fall months were associated with a higher Salmonella prevalence (13.2%) compared with early spring and summer. A total of five isolates, among the 383 samples were serotyped as ST and STM. These two serotypes showed a similar seasonal presence throughout the study, being found during fall and summer seasons. These findings demonstrated the seasonal presence of Salmonella spp. in feed mills and the role of these environments as potential pathogen entry route into the human food chain.
ISSN: 15353141

Khan, S.B., Khan, M.A., Ahmad, I., ur Rehman, T., Ullah, S., Dad, R., Sultan, A., Memon, A.M.
Phentotypic, gentotypic antimicrobial resistance and pathogenicity of Salmonella enterica serovars Typimurium and Enteriditis in poultry and poultry products
(2019) Microbial Pathogenesis, 129, pp. 118-124.
ABSTRACT: For detection and isolation of Salmonella enterica, 650 meat and tissue samples were processed using Rappaport-Vassiliadis Enrichment broth and Salmonella Chromogenic agar followed by confirmation through specific antisera and polymerase chain reaction (PCR) targeting their Specific Serovar Genomic Regions (SSGRS). Isolates were tested for 15 antibiotics (CRO, AMX, GEN, STR, TET, CHL, CLR, LVX, OFX, GAT, CIP, SXT, AMP, LIN and AZM) according to the disc diffusion method and antimicrobial resistant genes (tet(A), tet(B), tet(C), strA/strB, aadA, aac(3)IV), aadB, sul1, sul2 and sul3, blaCMY-2, blaTEM and blaSHV) using PCR. The overall prevalence of Salmonella enterica was 12%, being higher in markets (15%) as compared to poultry farms (37.2%). The MPN of all positive meat and tissue samples was found 3.6 MPN/gram (0.17–18). A total of 234 isolates were obtained, serovar Typimurium (139) and Enteridits (95) were the most prevalent. Antimicrobial resistance patterns were different in different serovars according to origin of Salmonella isolates. The overall isolates were highly resistant for LIN (93.1%, 218/234) followed by AMX (80%, 187/234), AMP (74.3%, 174/234), TET (64.5%, 151/234) and STR (64.5%, 151/234). Overall, the most common ARG was bla TEM (76%,

178/234), followed by bla SHV (71.7%, 168/234), tet(A) (64%, 151/234) and tet(B) (64%, 150/234), while the least ARG was aadB (7.2%, 17/234). Both Typimurium and Enteridits were tested in the Balb/C mice for pathogenicity. Both Typimurium and Enteridits were found to cause successful colonization, 100% morbidity but Enteriditis were found to cause 33% mortality. ISSN: 08824010

Eady, M., Setia, G., Park, B.
Detection of Salmonella from chicken rinsate with visible/near-infrared hyperspectral microscope imaging compared against RT-PCR
(2019) Talanta, 195, pp. 313-319.
ABSTRACT: Salmonella is an organism of importance to the poultry industry with increasingly stringent government regulatory standards. Real-time polymerase chain reaction (RT-PCR) and plating procedures on nutrient enriched growth media have been the standard detection methods of Salmonella from broiler chicken carcasses for years. These methods are proven, but offer disadvantages in the amount of time or reoccurring sample cost. Here, we propose the use of a hyperspectral microscope imaging system (HMI) for comparison to standard detection methods. Broiler chicken carcasses were rinsed and plated on Salmonella selective agar. Colonies from plates were picked and RT-PCR was used as a confirmation test to verify plating results, while HMI was collected from the same colonies. Spectral signatures of cells were extracted between 450 and 800 nm from HMI collected with 100x objective. A quadratic discriminant analysis (QDA) was used to classify cells as either Salmonella positive or negative (n = 341). Spectra preprocessing minimized the influence of cellular shape on the spectra, increasing the initial classification accuracy of 81.8–98.5%, yielding a sensitivity of 1.0, and a specificity of 0.963. Results showed the potential as an initial investigation of HMI as a microbial confirmation tool, compared to RT-PCR. ISSN: 00399140

Xu, J., Tang, J., Jin, Y., Song, J., Yang, R., Sablani, S.S., Zhu, M.-J.
High temperature water activity as a key factor influencing survival of Salmonella Enteritidis PT30 in thermal processing
(2019) Food Control, 98, pp. 520-528.
ABSTRACT: Salmonella in low-moisture foods has enhanced thermal tolerance and is difficult to control. The objective of this research was to study relationship between thermal tolerance of Salmonella Enteritidis PT30 and water activity (aw) of food matrices measured at elevated temperatures during thermal processing. Three different foods were selected for this study. They were wheat flour (WF), almond flour (AF) and whey protein (WP), representing carbohydrate-, fat-, and protein-rich food systems, respectively. Pre-equilibrated powders were inoculated independently with S. Enteritidis PT30 and conditioned to aw of 0.25, 0.45, 0.60, and 0.80 at room temperature (~20 °C). Aluminum thermal death time test cells (TDT cells) and newly designed thermal aw cells (TAC, with controlled aw) were heated at 80 °C to determine D-values (the time needed to active 90% of target bacteria) of S. Enteritidis PT30 in the three powders. Water activities of powders in the TDT cells at 80 °C were calculated to be between 0.41 and 0.89, while in the TAC were controlled to 0.32, 0.50, 0.63, and 0.81, respectively. Results showed that D80°C-values of S. Enteritidis PT30 decreased exponentially with increasing aw of foods at the treatment temperature 80 °C regardless of the food matrices and the testing methods. Thus, it is critical to understand how aw of a food matrix changes with temperature when selecting appropriate treatment conditions for thermal control of Salmonella in low-moisture foods. ISSN: 09567135

Yang, Y., Geveke, D.J., Brunkhorst, C.D., Sites, J.E., Geveke, N.J., Tilman, E.D.
Optimization of the radio frequency power, time and cooling water temperature for pasteurization of Salmonella Typhimurium in shell eggs
(2019) Journal of Food Engineering, 247, pp. 130-135.
ABSTRACT: Radio frequency (RF) power, treatment time and cooling water temperature affect inactivation of Salmonella Typhimurium in shell eggs and internal quality. Eggs were processed using 40.68 MHz RF at 30–45 W, 2.5–8 min and 30–38 °C, followed by hot water (HW) treatment at 56.7 °C for 15 min. Five conditions achieved >5 log reduction of Salmonella without observable quality change. Analyses of the longest (8 min at 30 W and 30 °C) and shortest (4.5 min at 35 W and 38 °C) treatments indicated that combined RF/HW treatments significantly ($P < 0.05$) preserved quality better than HW pasteurization (56.7 °C for 60 min). No significant ($P \geq 0.05$) difference in egg quality was observed between the longest and shortest treatment, except that the shortest resulted in greater albumen turbidity. As the longest treatment required 78% more time and 47% more energy than the shortest, industry may wish to use the shortest RF treatment time. ISSN: 02608774

Cetin, E., Serbetcioglu, T., Temelli, S., Eyigor, A.
Nontyphoid Salmonella carriage, serovar profile and antimicrobial resistance phenotypes in slaughter cattle
(2019) Journal of Food Safety, 39 (2), art. no. e12603, .
ABSTRACT: Current nontyphoid Salmonella (NTS) carriage in 200 apparently healthy slaughter cattle by ISO 6579 standard bacteriology (ISO) was 1% (2/200) in carcass and fecal content, and 2% (4/200) in mesenterial lymph nodes. There was no isolation from liver, kidney, spleen, and gallbladder, with an overall prevalence of 4% (8/200). Real-time PCR was in substantial agreement to ISO in confirming Salmonella-suspect isolates (Relative Trueness: 93.33%). Predominant serovar was S. Enteritidis (50%) followed by S. Typhimurium (37.5%), and S. Albany (12.5%). Five and three of eight NTS isolates were susceptible (62.5%) and resistant (37.5%) to 18 antimicrobials, respectively. Only three S. Enteritidis isolates (37.5%) showed multidrug resistance to 2–3 of 7 antimicrobials (amikacin, cefotaxime, cefoxitin, gentamicin, norfloxacin, pefloxacin, and tobramycin). S. Enteritidis predominance over S. Typhimurium, first detection of S. Albany in cattle in Turkey, and sole resistance in mesenterial lymph node S. Enteritidis isolates highlights study findings. Practical applications: Contaminated carcass and related material, for example, fecal content and mesenterial lymph nodes of apparently healthy slaughter cattle carrying nontyphoid Salmonella serovars still pose significant health risk to public in Turkey, where bovine meat covers the highest annual red meat consumption quota with high demand to edible offal. In this study, current predominance of S. Enteritidis, particularly in mesenterial lymph nodes, and the MDR pattern identified; the presence of S. Typhimurium as the second dominant and pansusceptible serovar; detection of S. Albany for the first time in cattle fecal content are new epidemiological findings. This data could be used in revising both bovine meat and offal's actual NTS status, and the control and prevention programs in our country and in the neighboring countries of interest.
ISSN: 01496085

Yap, M., Chau, M.L., Hartantyo, S.H.P., Oh, J.Q., Aung, K.T., Gutiérrez, R.A., Ng, L.C.
Microbial quality and safety of sushi prepared with gloved or bare hands: Food handlers' impact on retail food hygiene and safety
(2019) Journal of Food Protection, 82 (4), pp. 615-622.
ABSTRACT: Food handlers play an important role in retail food hygiene and safety. This study was conducted to better understand the impact of food handlers on the microbiological quality and safety of sushi and ingredients handled by gloved and bare hands. At retail premises, food handlers were asked to prepare a batch of sushi with raw fish followed by a batch of sushi with cooked ingredients. Food (sushi and ready-to-eat ingredients), hand, and glove samples were collected for analysis of overall microorganisms (standard plate counts) and targeted foodborne bacteria: Staphylococcus aureus, Bacillus cereus, Escherichia coli, Salmonella, and Listeria monocytogenes. Results suggested that cross-contamination was more prevalent at premises where bare hands were used to prepare sushi. When bare hands were used, significantly higher standard plate counts were obtained from samples of cooked rice (2.3 to 4.9 log CFU/g) and sushi (2.8 to 6.9 log CFU/g) and the prevalence of S. aureus in samples was higher on food (21.7%, 28 of 129 samples) and hands (30%, 18 of 60 samples) (P, 0.05). Glove changing in combination with hand washing minimized cross-contamination during sushi preparation as indicated by the lower prevalence of S. aureus (0%, 0 of 28 samples) and total targeted foodborne bacteria (3.6%, 1 of 28 samples) on the gloves of food handlers who changed gloves and washed their hands compared with those handlers who did not don new gloves. Repeated use of dishcloths could be a cause of cross-contamination, and the prevalence of total targeted foodborne bacteria was significantly higher on hands dried with dishcloths (64.7%, 11 of 17 samples) than on hands dried with paper towels (12.5%, 1 of 8 samples) (P, 0.05). The prevalences of B. cereus, L. monocytogenes, and Salmonella in the 356 food samples were 5.1% (18 samples), 0.8% (3 samples), and 0%, respectively. Improvements to hand washing, hand drying, and glove changing practices are needed to lower the occurrence of cross-contamination during sushi preparation. ISSN: 0362028X

Mayton, H.M., Marcus, I.M., Walker, S.L.
Escherichia coli O157:H7 and Salmonella Typhimurium adhesion to spinach leaf surfaces: Sensitivity to water chemistry and nutrient availability
(2019) Food Microbiology, 78, pp. 134-142.
ABSTRACT: This study investigated the effects of solution chemistry and growth conditions on bacterial deposition on spinach leaf surfaces using a parallel plate flow cell. Two food safety pathogens of concern and two non-pathogen bacterial surrogates (environmental E.

coli isolates) were grown in ideal (LB media) and nutrient-restricted (M9 media) conditions. Bacterial attachment was quantified as mass transfer rate coefficients for cells suspended in 10 mM KCl, CaCl2 and artificial groundwater, and cell and leaf surfaces were extensively characterized (zeta potential, hydrophobicity, extracellular polymer (EPS) composition). Between the pathogens, E. coli O157:H7 attachment was greater than that of Salmonella Typhimurium, attributed to measurable variability in cell surface charge and hydrophobicity. When grown in M9 media, both pathogens were significantly more adhesive to spinach surfaces (p < 0.01) than when grown in LB media. Surrogates did not follow this trend and showed minimal changes in adhesion kinetics and surface properties between growth conditions. EPS sugar/protein ratios were reduced in some of the highest attachment scenarios, suggesting that changes in EPS composition in favor of proteins may play a role. These results show the importance of growth conditions and solution complexities in understanding mechanisms of aqueous bacterial adhesion to food surfaces. ISSN: 07400020

Okuno, K., Xu, J., Isogai, E., Nakamura, S.
Salmonella Typhimurium is Attracted to Egg Yolk and Repelled by Albumen
(2019) Current Microbiology, 76 (4), pp. 393-397.
ABSTRACT: Salmonella Typhimurium is the causative agent of non-typhoidal, foodborne salmonellosis. Contamination of hen eggs by the bacterium is a common source of S. Typhimurium infection. S. Typhimurium is peritrichous, and flagellum-dependent motility and chemotaxis are believed to facilitate egg contamination despite the presence of many antimicrobial egg components. We performed motility and chemotaxis assays to demonstrate that S. Typhimurium cells are attracted to egg yolks and are repelled by albumen. The bacterial flagellar motor shows bidirectional rotation, and counterclockwise-biased rotation allows cells to swim smoothly. A rotation assay for a single flagellum showed that, in comparison with thin albumen, the thick albumen more strongly affected the directional bias of the flagellar rotation, resulting in a remarkable suppression of the migration distance. Nevertheless, the S. Typhimurium cells retained positive chemotaxis toward the yolk in the presence of the albumens, suggesting that motility facilitates the growth of S. Typhimurium and survival in eggs. ISSN: 03438651

Jacobs, R., Lesaffre, E., Teunis, P.F.M., Höhle, M., van de Kassteele, J.
Identifying the source of food-borne disease outbreaks: An application of Bayesian variable selection
(2019) Statistical Methods in Medical Research, 28 (4), pp. 1126-1140.
ABSTRACT: Early identification of contaminated food products is crucial in reducing health burdens of food-borne disease outbreaks. Analytic case-control studies are primarily used in this identification stage by comparing exposures in cases and controls using logistic regression. Standard epidemiological analysis practice is not formally defined and the combination of currently applied methods is subject to issues such as response misclassification, missing values, multiple testing problems and small sample estimation problems resulting in biased and possibly misleading results. In this paper, we develop a formal Bayesian variable selection method to account for misclassified responses and missing covariates, which are common complications in food-borne outbreak investigations. We illustrate the implementation and performance of our method on a Salmonella Thompson outbreak in the Netherlands in 2012. Our method is shown to perform better than the standard logistic regression approach with respect to earlier identification of contaminated food products. It also allows relatively easy implementation of otherwise complex methodological issues. ISSN: 09622802

Gurtler, J.B., Fan, X., Jin, T., Niemira, B.A.
Influence of antimicrobial agents on the thermal sensitivity of foodborne pathogens: A review
(2019) Journal of Food Protection, 82 (4), pp. 628-644.
ABSTRACT: Consumers are driving food production toward the use of more organic antimicrobial agents such as essential oils (EOs) by demanding more natural and clean-label food products. Due to the strong aromatic and flavor properties of EOs, their use is often precluded, or limited to concentrations below the flavor threshold. However, adding EOs at concentrations this low often renders their biocidal activity ineffective. An opportunity exists for low concentrations of EO antimicrobial agents to be combined with mild heating (e.g., 42 to 558C) for short treatment times to use the hurdle concept for additive or synergistic effects on foodborne pathogens such as Escherichia coli O157:H7, Salmonella, or Listeria monocytogenes; norovirus; and surrogate organisms. In some cases, especially with fruit juices, this intervention combination is described as antimicrobial-assisted pasteurization. Used below the organoleptic quality threshold, EOs,

which otherwise would have little effect on the inactivation of foodborne pathogens, are effective antimicrobial agents when used in conjunction with mild thermal processes. Thermal processes combined with antimicrobial agents can be used for processing liquids, eggs, juices, drinks, and fresh produce. This review highlights research literature where antimicrobial agents and mild heating have been combined to increase the inactivation of foodborne pathogen populations. Commodities and testing substrates reviewed include buffers and nutrient broths, juices, liquid egg, mangoes, cut lettuce, cut and shredded cabbage, shredded carrot, baby spinach leaves, and salsa. Opportunities exist for the application of this hurdle technology to a whole array of food products, which could benefit from pathogen reduction or elimination, and to prevention of aqueous cross-contamination and/or internalization during the washing of fresh produce. ISSN: 0362028X

Asses, N., Farhat, W., Hamdi, M., Bouallagui, H.
Large scale composting of poultry slaughterhouse processing waste: Microbial removal and agricultural biofertilizer application
(2019) Process Safety and Environmental Protection, pp. 128-136.
ABSTRACT: This work investigated the potential of composting treatment for hygienization and material recycling from poultry slaughtering by-products and wastes in the context of management aspects and the agricultural value of the final product. A large scale composting cycle of 300 m 3 was performed for 90 days, in which poultry slaughterhouse waste (59.65%) was mixed with sewage sludge (4.83%), agricultural waste, cardboard (4.83%), and wood dust (19.35%) and activated compost (11.29%). During the composting progress, physical chemical parameters, FTIR analysis and biological indicators reflecting stability of the compost were analysed for the assessment of the product quality. The composted mixture showed a high microbial activity with a succession of microbial populations depending on the temperature reached during different degradation phases. The thermophilic phase lasted 20 days with temperature exceeding 65 °C allowing pathogens reduction. Fecal coliforms, Streptococci and Escherichia coli were reduced and remained less than values recommended by international guideline. Whilst Salmonella has disappeared at the end of the process. The Composting poultry slaughterhouse waste allowed obtaining hygienic compost with sufficient agronomic quality characterized by a relatively high organic matter content (49.12%), a C/N ratio (13.92%), an alkaline pH (7.7) and a high level of nutrients. The germination indexes reached 91% in the end of process that proved to be good maturity indicators. The compost application in peat amended at ratios equal to 8 t.ha −1 , improved the growth of stem length (63.8%), leaf length (57.9%), fresh biomass (65.1%) and dry biomass (66.6%) of maize plants showing that the final slaughterhouse compost presented high level of maturity and it was not phytotoxic. ISSN: 09575820

Kogut, M.H.
The effect of microbiome modulation on the intestinal health of poultry
(2019) Animal Feed Science and Technology, 250, pp. 32-40.
ABSTRACT: The chicken gastrointestinal (GI) tract is home to a complex microbial community that underlines the links between diet and health. The GI tract is rich in microbial biodiversity, playing home to ≥500 phylotypes or ~1 million bacterial genes, which equates to 40–50 times the number in the chicken genome. Manipulating the microbiota would serve as promising therapeutic paradigm; albeit not a new concept for the poultry industry as evidenced by competitive exclusion where newly hatched chickens could be protected against colonization by Salmonella enteritidis by dosing a suspension of gut contents derived from healthy adult chickens. This concept of adding beneficial bacteria to the intestine has led to the development of probiotics and prebiotics. Unlike the host genome, which is rarely manipulated by xenobiotic intervention, the microbiome is readily changeable by diet, ingestion of antibiotics, infection by pathogens and other host- and environmental-dependent events. The plasticity of the microbiome has been implicated in numerous disease conditions, and an unfavorable alteration of the commensal structure of gut microbiota is referred to as dysbiosis; this includes a reduction in the number of tolerogenic bacteria and an over-growth of potentially pathogenic bacteria (pathobionts) that can penetrate the intestinal epithelium and induce diseases in certain genetic or environmental contexts. This review highlights the plasticity of the avian microbiome that allows defined interventions as a means of enhancing poultry health and productivity. The ability to intentionally manipulate the microbiota by providing nutrients, modulating host immunity, inhibiting/preventing pathogen intestinal colonization, or improving intestinal barrier function has led to a number of novel methods to prevent disease, but also led to improved body weight, feed conversion, and carcass yield. ISSN: 03778401

Dev Kumar, G., Ravishankar, S.
Ozonized water with plant antimicrobials: An effective method to inactivate Salmonella enterica on iceberg lettuce in the produce wash water
(2019) Environmental Research, pp. 213-217.
ABSTRACT: Post-harvest washing of produce is performed to remove physical debris and to lower microbial load. The use of ozone in combination with plant-based antimicrobials was evaluated as an alternative to conventional sanitizers such as chlorine. Plant based antimicrobials that were evaluated in combination with ozone included oregano oil, carvacrol, Quillaja saponin and olive extract. Ozone was dispersed in phosphate buffered saline (PBS), following which individual antimicrobials or their combinations were added. Iceberg lettuce leaves (10 g portions) inoculated with Salmonella enterica serotype Newport (6.5 ± 1 log CFU/g) were added to the wash suspension. The leaves were tested for reduction in S. Newport population after 60, 90 and 120 min of treatment. Exposure to ozonized water for 120 min resulted in a 2.1 log CFU/g ($p < 0.05$) reduction in S. Newport population. The addition of 0.1% oregano oil to ozonized water resulted in 3 log CFU/g reduction after 120 min but a 4.1 log CFU/g reduction after 60 min, indicating that the antioxidant property of oregano oil might have diminished ozone activity and resuscitated injured S. Newport cells. The addition of 5% olive extract to ozonized water resulted in 4.2 log CFU/g reduction of S. Newport after 120 min ($p < 0.05$) of treatment. While 5% olive extract did not confer protection to S. Newport cells from ozone, 1% olive extract resulted in higher S. Newport survival after 120 min treatment than the 60 min treatment. The use of carvacrol (0.1%, 0.3% and 0.5%) in ozonized water reduced the pathogen population to below the limit of detection (10 CFU/g) ($p < 0.05$) which was in excess of 6 log CFU/g. These results indicate that the efficacy of ozone is compounded by the addition of certain plant-based antimicrobials when used at optimum concentrations. Ozone combined with plant antimicrobials could serve as an effective alternative to sanitizers currently used for washing and processing of produce. ISSN: 00139351

Elobeid, T., Savvaidis, I., Ganji, V.
Impact of food safety training on the knowledge, practice, and attitudes of food handlers working in fast-food restaurants
(2019) British Food Journal, 121 (4), pp. 937-949.
ABSTRACT: Purpose: In many developing countries, the main source of food related illness is the fast foods restaurants. Health inspections of fast-food restaurants may not be sufficient to ensure and enforce the food safety regulations. The purpose of this paper is to investigate the food safety knowledge, attitudes and practices (KAP) of fast food handlers in Qatar. Design/methodology/approach: Data were collected from 102 fast-food handlers through a structured survey. The questionnaire comprised questions on food safety KAP. The association between scores for KAP among the food handlers was measured with Spearman's rank correlation. Findings: A significant direct association was found throughout the different criteria of food safety KAP. In total, 90 percent of fast food handlers had undergone formal training on food safety. Although fast food handlers thought they had overall good knowledge on food safety (93.9 percent), results showed that they had a poor knowledge on proper cleaning of equipment, cross-contamination, foodborne diseases, food danger zone and correct procedures for thawing of frozen food. Only (34.7 percent) of the food handlers correctly identified Salmonella as a food pathogen. Originality/value: Based on the current findings, the authors believe that continuous food safety and hygiene training should be implemented in all food service operations especially in fast-food restaurants in Qatar to ensure that all food handlers have the knowledge and the skill to provide safe food. ISSN: 0007070X

Rakov, A.V., Mastriani, E., Liu, S.-L., Schifferli, D.M.
Association of Salmonella virulence factor alleles with intestinal and invasive serovars
(2019) BMC Genomics, 20 (1), art. no. 429, .
ABSTRACT: Background: The role of Salmonella virulence factor (VF) allelic variation in modulating pathogenesis or host specificity has only been demonstrated in a few cases, mostly through serendipitous findings. Virulence factor (VF) alleles from Salmonella enterica subsp. enterica genomes were compared to identify potential associations with the host-adapted invasive serovars Typhi, Dublin, Choleraesuis, and Gallinarum, and with the broad host-range intestinal serovars Typhimurium, Enteritidis, and Newport. Results: Through a bioinformatics analysis of 500 Salmonella genomes, we have identified allelic variants of 70 VFs, many of which are associated with either one of the four host-adapted invasive Salmonella serovars or one of the three broad host-range intestinal serovars. In addition, associations between specific VF alleles and intra-serovar clusters, sequence types (STs) and/or host-adapted FimH adhesins were identified. Moreover, new allelic VF associations with non-typhoidal S. Enteritidis and S. Typhimurium (NTS) or invasive NTS

(iNTS) were detected. Conclusions: By analogy to the previously shown association of specific FimH adhesin alleles with optimal binding by host adapted Salmonella serovars, lineages or strains, we predict that some of the identified association of other VF alleles with host-adapted serovars, lineages or strains will reflect specific contributions to host adaptation and/or pathogenesis. The identification of these allelic associations will support investigations of the biological impact of VF alleles and better characterize the role of allelic variation in Salmonella pathogenesis. Most relevant functional experiments will test the potential causal contribution of the detected FimH-associated VF variants in host adapted virulence. ISSN: 14712164

Kerr, D.E., Shen, G., Lienau, A.H., Deng, T., Kaur, M., Immermann, A.L., Feldsine, P.T., John, L., Chen, Y., Brodsky, M., Ziemer, W.
Comparative validation study to demonstrate the equivalence of an alternate next-day enrichment protocol for the transia® plate Salmonella gold method to culture methods for the detection of Salmonella in selected foods and environmental surfaces
(2019) Journal of AOAC International, 102 (3), pp. 828-841.
ABSTRACT: TRANSIA® PLATE Salmonella Gold is an ELISA that was validated by Association Française de Normalisation (AFNOR) in 2001 and as a Performance Tested MethodSM (PTM) by AOAC in 2006 (PTM No. 010602) as a two-step enrichment protocol requiring 48 h. A simple next-day enrichment protocol using modified Enterohemorrhagic Escherichia coli media was developed for the TRANSIA PLATE Salmonella Gold to improve the time-to-results and laboratory work flow. We tested 128 Salmonella strains, representing all serotypes from A though Z and 51-66. TRANSIA PLATE Salmonella Gold detected all 128 of these strains. None of the 50 non-Salmonella strains were detected by TRANSIA PLATE Salmonella Gold. Performance of TRANSIA PLATE Salmonella Gold using the new enrichment protocol was compared with U.S. Department of Agriculture Microbiology Laboratory Guidebook reference culture procedure for the detection of Salmonella in ready-to-eat poultry, ready-to-eat beef, and chicken carcass rinsate. In addition, TRANSIA PLATE Salmonella Gold performance was compared with U.S. Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM) for the detection of Salmonella from raw spinach, raw almonds, raw pasta, and environmental surfaces (stainless steel, rubber, and plastic). There was no statistically significant difference in the numbers of positive results TRANSIA PLATE Salmonella Gold protocol compared with the appropriate U.S. Department of Agriculture Food Safety and Inspection Service or FDA-BAM reference methods for any of these matrixes. Robustness testing demonstrated that the introduction of small changes in the normal assay parameters had no impact on the method performance. This new enrichment protocol has been approved as a Third Level modification to Performance Tested Method 010602. ISSN: 10603271

Kerr, D.E., Shen, G., Lienau, A.H., Kaur, M., Immermann, A.L., Feldsine, P.T., John, L., Chen, Y., Brodsky, M., Ziemer, W.
Comparative validation study to demonstrate the equivalence of an alternate next-day enrichment protocol for ViP ® gold for Salmonella method to culture methods for the detection of Salmonella in selected foods and environmental surfaces
(2019) Journal of AOAC International, 102 (3), pp. 815-827.
ABSTRACT: Background: VIP ® Gold for Salmonella is a lateral flow immunodetection device that was validated by AOAC in 1999 as Official Method of Analysis 999.09. It was improved upon in 2009 by introducing gold colloid as the detection method. Objective: A simple next-day enrichment protocol using modified enterohemorrhagic Escherichia coli media was developed for the VIP Gold for Salmonella to improve the time-to-results and laboratory work flow. Methods: We tested 128 Salmonella strains, representing all serotypes from A to Z and 51 to 66 as well as 50 non-Salmonella strains for inclusivity/exclusivity. Performance of the VIP using the new enrichment protocol was compared with the U.S. Department of Agriculture (USDA) Microbiology Laboratory Guidebook reference culture procedure for the detection of Salmonella in ready-to-eat poultry, roast beef, and chicken carcass rinsate. VIP performance was also compared with the U.S. Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM) for the detection of Salmonella from raw spinach, raw almonds, raw pasta, and environmental surfaces (stainless steel, rubber, and plastic). Results: The VIP detected all 128 of Salmonella strains and none of the 50 non-Salmonella strains. There was no statistically significant difference in the numbers of positive results with VIP Gold for Salmonella protocol compared with appropriate USDA-Food Safety and Inspection Service or FDA-BAM reference methods for any of these matrixes. Conclusions: This new enrichment protocol has met all the requirements to be approved as a Performance Tested Method SM . Highlights: The new enrichment protocol will improve the time-to-results and allow quicker decisions about the contamination of food products. ISSN: 10603271

Prestes, F.S., Pereira, A.A.M., Silva, A.C.M., Pena, P.O., Nascimento, M.S.
Effects of peanut drying and blanching on Salmonella spp.
(2019) Food Research International, 119, pp. 411-416.
ABSTRACT: In order to evaluate the behavior of Salmonella during peanut drying and blanching, a study was conducted with Runner type peanuts. Samples of raw in-shell or unblanched peanuts were inoculated by spraying with a pool of five Salmonella serotypes isolated from the peanut supply chain (Miami, Muenster, Yoruba, Javiana and Glostrup). The in-shell peanuts were submitted to drying at 35 and 40 °C up to 18 h. After this time, the Salmonella counts went down ca. 2.0 log MPN/g at 35 and 40 °C. According to the Weibull model the time needed to achieve Salmonella 3-log reduction (T 3d) and 5-log reduction (T 5d) on the in-shell peanuts would be ca. 49 and 117 h at 35 °C and 35 and 79 h at 40 °C, respectively. The results showed that there was no statistical difference (p >.05) between either of the temperatures employed in the process. The blanching process was performed in two steps: pre-roasting (step 1) and skin removal (step 2). Reduction of up to 2.1 log MPN/g was observed after blanching at 100 °C/15 min plus 15 s of air impact. The skin removal process did not result in recontamination of the final sample. The Weibull model predicted 3- and 5-log reductions of Salmonella in 37.0 and 68.9 min for blanching at 95 °C, and in 39.1 and 114.9 min at 100 °C. The results demonstrated that drying and blanching processes did not generate large reductions of Salmonella in the peanut samples. Thus, the product resulting from these steps may be a possible source of cross-contamination for the processing plant and the final product. ISSN: 09639969

Merino, L., Procura, F., Trejo, F.M., Bueno, D.J., Golowczyc, M.A.
Biofilm formation by Salmonella sp. in the poultry industry: Detection, control and eradication strategies
(2019) Food Research International, 119, pp. 530-540.
ABSTRACT: Salmonella represents an important global public health problem and it is an emerging zoonotic bacterial threat in the poultry industry. Diverse registered human cases of salmonellosis shown poultry origins. Various control measures have been employed both at the farming and processing levels to address it. This review focuses on traditional and new detection techniques of biofilm formation by Salmonella spp. and different approaches that can be used to prevent and/or control biofilm formation by these bacteria. A number of methodologies based on different approximations have been recently employed to detect and evaluate bacteria attached to surfaces, including real-time polymerase chain reaction (PCR), confocal laser scanning microscopy and Optical Coherence Tomography. Due to persistence of Salmonella biofilm in food processing environments after cleaning and sanitation, control and eradication strategies in poultry industry should be constantly studied. In this sense, the use of several alternatives to control Salmonella biofilm formation, such as lactic acid bacteria, phagetherapy, extracts from aromatic plants, quorum sensing inhibitors, bacteriocins and nanomaterials, have been successfully tested and will be reviewed. ISSN: 09639969

Liu, D., Cui, Y., Walcott, R., Díaz-Pérez, J., Tishchenko, V., Chen, J.
Transmission of human enteric pathogens from artificially-inoculated flowers to vegetable sprouts/seedlings developed via contaminated seeds
(2019) Food Control, 99, pp. 21-27.
ABSTRACT: Seeds contaminated with bacterial pathogens were found to be the primary cause of sprout-associated outbreaks of human gastrointestinal infections. This study was undertaken to determine if cells of selected Salmonella enterica and enterohemorrhagic Escherichia coli (EHEC) strains, artificially inoculated onto the flowers of vegetable plants, will result in contamination of sprouts/seedlings that develop from seeds produced by the inoculated flowers. Pistils of alfalfa, fenugreek, lettuce, and tomato flowers were inoculated with cells of selected S. enterica or EHEC strains. A total of 906, 715, 1236, and 1276 mature seeds, produced by lettuce, tomato, alfalfa or fenugreek flowers inoculated with Salmonella were collected as 48, 94, 109, and 116 composite samples (367 in total), respectively. Correspondingly, 934, 640, 1827 and 1027 seeds, produced by the four respective types of flowers after inoculation with E. coli were divided into 42, 81, 162, and 107 composite samples (392 in total), respectively. Seeds in each composite sample were surface-decontaminated with NaOCl solution and germinated at 25 °C in the dark for 5 days. Subsequently, pathogen populations on the sprouts/seedlings developed from each composite seed sample were determined by the plate count assay. The overall Salmonella recovery rate from vegetable sprouts/seedlings developed from the 367 composite seed samples was 2.7%, while none of the sprouts/seedlings grown from the 392 composite seed samples with prior E. coli inoculation tested positive for the pathogen. One of the 94

tomato seedling samples contained 4 CFU of Salmonella and five additional samples tested positive by enrichment (6.4%). Two out of 109 (1.8%) alfalfa and 116 (1.7%) fenugreek composite samples tested positive for Salmonella by enrichment. However, none of the lettuce seedlings tested positive for Salmonella or E. coli even after enrichment. This study suggests that under controlled environmental conditions, human pathogens inoculated onto flowers of vegetable plants can result in the contamination of sprouts/seedlings via seeds produced by the inoculated flowers. However, the frequency of sprout/seedling contamination was low and could be affected by characteristics of the pathogens and plant species tested. ISSN: 09567135

Oliveira, G.R., Oliveira, W.K., Andrade, C., Melo, A.D.B., Luciano, F.B., Macedo, R.E.F., Costa, L.B.
Natural antimicrobials for control of Salmonella Enteritidis in feed and in vitro model of the chicken digestive process
(2019) Journal of Animal Physiology and Animal Nutrition, 103 (3), pp. 756-765.
ABSTRACT: This study evaluated the antimicrobial effect of essential oils (EO) and organic acids (OA) against Salmonella Enteritidis in chicken feed and during an in vitro model that mimics the chicken digestive process. The minimal inhibitory concentration (MIC) of allyl isothiocyanate (AITC), carvacrol (CV), propionic acid (PROP) and caproic acid (CAP) were individually determined. Then, based on the MICs of each compound, combinations of EOs and/or OAs were tested to evaluate their synergic antimicrobial effect. The synergic effect of AITC and CAP was the most efficient against the bacterial strain tested. Commercial feed was inoculated with a 5-strain cocktail of S. Enteritidis and treated with different doses of AITC + CAP to evaluate their effect on the growth/survival of the pathogen. In addition, the simulated digestion model was used to access the antimicrobial effect of AITC + CAP added to the feed towards S. Enteritidis and Lactobacillus plantarum. Synergistic effect was found between AITC (0.065 mM) and CAP (17.5 mM) against S. Enteritidis in chicken feed, where S. Enteritidis was reduced to undetectable levels (<1.00 log CFU/g). AITC (1.95 mM) + CAP (45 mM) also decreased ($p < 0.05$) the population of S. Enteritidis in the simulated digestion, while the growth of L. plantarum was not affected. Therefore, the addition of AITC + CAP in feed might be a potential natural antimicrobial able to prevent economic losses caused for Salmonella in chicken. ISSN: 09312439

Dar, M.A., Urwat, U., Ahmad, S.M., Ahmad, R., Kashoo, Z.A., Dar, T.A., Bhat, S.A., Mumtaz, P.T., Shabir, N., Shah, R.A., Heidari, M.
Gene expression and antibody response in chicken against Salmonella Typhimurium challenge
(2019) Poultry Science, 98 (5), pp. 2008-2013.
ABSTRACT: Salmonella enterica serovar Typhimurium (S. Typhimurium) is a primary avian pathogen responsible for severe intestinal pathology in younger chickens and economic losses to poultry industry. Furthermore, S. Typhimurium is also able to cause infection in humans, characterized by acute gastrointestinal disease. A study was conducted to investigate antibody response and expression kinetics of interferon gamma (IFNγ), interleukin (IL-12, and IL-18) genes in broiler chicken at 0, 1, 3, 5, 7, 9, 11, 13, and 15 D post infection following experimental infection of S. Typhimurium. Immunological studies showed higher titres of IgG and IgM in the infected group as compared to the age-matched un-infected control group. The Real-Time PCR-based gene expression analysis revealed significant increase of IFNγ, IL-12, and IL-18 mRNA levels in the infected group as compared to their respective controls ($P < 0.05$). The present study shall help in understanding the immune responses in birds, thus allowing development of more effective vaccines and vaccination strategies. ISSN: 00325791

Dangel, A., Berger, A., Messelhäußer, U., Konrad, R., Hörmansdorfer, S., Ackermann, N., Sing, A.
Genetic diversity and delineation of Salmonella Agona outbreak strains by next generation sequencing, Bavaria, Germany, 1993 to 2018
(2019) Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin, 24 (18), .
ABSTRACT: BackgroundIn 2017, a food-borne Salmonella Agona outbreak caused by infant milk products from a French supplier occurred in Europe. Simultaneously, S. Agona was detected in animal feed samples in Bavaria.AimUsing next generation sequencing (NGS) and three data analysis methods, this study's objectives were to verify clonality of the Bavarian feed strains, rule out their connection to the outbreak, explore the genetic diversity of Bavarian S. Agona isolates from 1993 to 2018 and compare the analysis approaches employed, for practicality and ability to delineate outbreaks caused by the genetically monomorphic Agona serovar.MethodsIn this observational retrospective study,

three 2017 Bavarian feed isolates were compared to a French outbreak isolate and 48 S. Agona isolates from our strain collections. The later included human, food, feed, veterinary and environmental isolates, of which 28 were epidemiologically outbreak related. All isolates were subjected to NGS and analysed by: (i) a publicly available species-specific core genome multilocus sequence typing (cgMLST) scheme, (ii) single nucleotide polymorphism phylogeny and (iii) an in-house serovar-specific cgMLST scheme. Using additional international S. Agona outbreak NGS data, the cluster resolution capacity of the two cgMLST schemes was assessed.ResultsWe could prove clonality of the feed isolates and exclude their relation to the French outbreak. All approaches confirmed former Bavarian epidemiological clusters.ConclusionEven for S. Agona, species-level cgMLST can produce reasonable resolution, being standardisable by public health laboratories. For single samples or homogeneous sample sets, higher resolution by serovar-specific cgMLST or SNP genotyping can facilitate outbreak investigations. ISSN: 15607917

Casanova-Higes, A., Marín-Alcalá, C.M., Andrés-Barranco, S., Cebollada-Solanas, A., Alvarez, J., Mainar-Jaime, R.C.
Weaned piglets: Another factor to be considered for the control of Salmonella infection in breeding pig farms
(2019) Veterinary Research, 50 (1), art. no. 45, .
ABSTRACT: Field studies on Salmonella infection in suckling piglets are scarce due to the intrinsic difficulties of collecting proper samples (i.e. tonsils or mesenteric lymph nodes), and most of them rely on the analysis of rectal swabs that limit their accuracy. We used 495 slaughtered 4-weeks-old male piglets intended for human consumption from 5 Salmonella-seropositive breeding farms to collect gastrointestinal packages and perform a thorough detection of Salmonella on mesenteric lymph nodes and intestinal content. The overall prevalence of both infection and shedding was high (≈ 36%) indicating that piglets played an active role in Salmonella maintenance in the farms. Major serotypes found in piglets included 4,[5],12:i: (35.4%), Rissen (17.1%), Derby (10.9%) and Bovismorbificans (10.3%). In most of the infected animals (72.8%) the same serotype was found in mesenteric lymph nodes and feces. Significant higher ELISA OD% values were found in meat juice samples from non-infected piglets compared to infected ones (median OD% of 12.0 and 17.3, respectively; P = 0.002) suggesting some protective effect of sow's colostrum. Salmonella was also isolated from feces from weaned sows contemporary of the slaughtered piglets, and 89% of the serotypes identified in sows were also detected in piglets. Pulsed field gel electrophoresis analyses showed that 75% of the piglet isolates that were compared to those of sows were related to them, suggesting the circulation of Salmonella strains between sows and piglets. It appears that improving piglet colostrum intake along with the reduction of the shedding in sows may favor the control of Salmonella infection in breeding farms. ISSN: 09284249

Gand, M., Mattheus, W., Saltykova, A., Roosens, N., Dierick, K., Marchal, K., De Keersmaecker, S.C.J., Bertrand, S.
Development of a real-time PCR method for the genoserotyping of Salmonella Paratyphi B variant Java
(2019) Applied Microbiology and Biotechnology, 103 (12), pp. 4987-4996.
ABSTRACT: Discriminating between d-tartrate fermenting and non-fermenting strains of Salmonella enterica subsp. enterica serotype Paratyphi B is of major importance as these two variants have different pathogenic profiles. While d-tartrate non-fermenting S. Paratyphi B isolates are the causative agent of typhoid-like fever, d-tartrate fermenting isolates (also called variant Java) of the same serotype trigger the less dangerous gastroenteritis. The determination of S. Paratyphi B variants requires a time-consuming process and complex biochemical tests. Therefore, a quadruplex real-time PCR method, based on the allelic discrimination of molecular markers selected from the scientific literature and from whole genome sequencing data produced in-house, was developed in this study, to be applied to Salmonella isolates. This method was validated with the analysis of 178 S. Paratyphi B (d-tartrate fermenting and non-fermenting) and other serotypes reaching an accuracy, compared with the classical methods, of 98% for serotyping by slide agglutination and 100% for replacement of the biochemical test. The developed real-time PCR permits to save time and to obtain an accurate identification of a S. Paratyphi B serotype and its d-tartrate fermenting profile, which is needed in routine laboratories for fast and efficient diagnostics. ISSN: 01757598

Vinueza-Burgos, C., Baquero, M., Medina, J., De Zutter, L.
Occurrence, genotypes and antimicrobial susceptibility of Salmonella collected from the broiler production chain within an integrated poultry company
(2019) International Journal of Food Microbiology, 299, pp. 1-7.

ABSTRACT: Salmonella is a common foodborne pathogen in the poultry production systems. Its presence in this food industry is determined by the fact that it can survive and pass throughout the different steps in the poultry production. In this study we aimed to study the occurrence, genotypes and antimicrobial resistance of Salmonella collected from the broiler production chain within an integrated poultry company. Three hundred fourteen samples were collected in the feeding plant, farms and the slaughterhouse. Samples were cultured for Salmonella isolation according to the ISO6579/Amd 1. Isolates were further typed by Kauffmann-White scheme and pulse field gel electrophoresis (PFGE). Antimicrobial resistance to 11 antimicrobials was studied by disk diffusion tests and sequencing of ESBL genes. From the collected samples 70 (22%) were found to be Salmonella positive. The lowest Salmonella rates were found in feed samples while in farm and slaughterhouse samples Salmonella presence ranged from 5% to 88%. S. Infantis was the most common serotype (94%, 66/70). PFGE demonstrated that isolates belonged to 11 genotypes. Some genotypes were continuously identified throughout the production chain. 97% of the isolates showed resistance to at least one antimicrobial. Moreover, all S. Infantis isolates and one auto-agglutinable isolate showed resistance to at least 6 antimicrobials. 30 and 8 isolates were positive to bla CTX-M-65 and bla CTX-M-14 genes respectively. No bla KPC resistance genes were identified in any isolate. This study highlights the predominance of S. Infantis in the integrated poultry company. Genotypes showed that cross-contamination between stages of poultry production can occur, stressing the importance of implementing good hygiene practices in every level of the production. Moreover, multidrug resistance patterns and the presence of important ESBL genes have public health implications that need to be deeply discussed with a one health approach. ISSN: 01681605

Lytou, A.E., Tzortzinis, K., Skandamis, P.N., Nychas, G.-J.E., Panagou, E.Z.
Investigating the influence of organic acid marinades, storage temperature and time on the survival/inactivation interface of Salmonella on chicken breast fillets
(2019) International Journal of Food Microbiology, 299, pp. 47-57.
ABSTRACT: The aim of this work was to study the influence of lemon and vinegar marinades on Salmonella inoculated on chicken fillets and stored under different storage temperatures for nine days in the presence of indigenous microbiota. In addition to this, model development for the determination of the inactivation boundaries and the prediction of pathogens response was attempted. The different acid concentrations in the marinades, the type of acid, the storage temperature as well as the duration of storage impacted the levels of pathogens and background flora. The higher tested concentrations (2% and 4% v/v for acetic and citric acid) were more effective against Salmonella and spoilage microorganisms than the lower ones (0.5 and 1% v/v for acetic and citric acid), while the intermediate concentrations (1, 1.5 and 2, 3% v/v for acetic and citric acid, respectively) presented differentiations of particular interest to the microbial responses to acidic stress. The aforementioned parameters also differentiated Salmonella serovars persistence and spoilage microorganisms dominance. Regarding model development, the probability of inactivation of Salmonella was satisfactorily predicted particularly in the case of acetic acid marination while in model validation, the majority of the vinegar marinated samples were correctly classified, whereas, in case of lemon marination, a higher number of misclassifications was observed, indicating a partial weakness of the model to predict the pathogens response at interface concentrations. ISSN: 01681605

Rouzeau-Szynalski, K., Barretto, C., Fournier, C., Moine, D., Gimonet, J., Baert, L.
Whole genome sequencing used in an industrial context reveals a Salmonella laboratory cross-contamination
(2019) International Journal of Food Microbiology, 298, pp. 39-43.
ABSTRACT: In 2013, during a routine laboratory analysis performed on food samples, one finished product from a European factory was tested positive for Salmonella Hadar. At the same period, one environmental isolate in the same laboratory was serotyped Salmonella Hadar. Prior to this event, the laboratory performed a proficiency testing involving a sample spiked with NCTC 9877 Salmonella Hadar. The concomitance of Salmonella Hadar detection led to the suspicion of a laboratory cross-contamination between the Salmonella Hadar isolate used in the laboratory proficiency testing and the Salmonella Hadar isolate found on the finished product by the same laboratory. Since the classical phenotypic serotyping method is able to attribute a serotype to Salmonella isolates with a common antigenic formula, but cannot differentiate strains of the same serotype within the subspecies, whole genome sequencing was used to test the laboratory cross-contamination hypothesis. Additionally, 12 Salmonella Hadar from public databases, available until the time of the event, were included in the whole genome sequencing analysis to better understand the genomic diversity of this serotype in Europe. The outcome of the analysis

showed a maximum of ten single nucleotide polymorphisms (SNPs) between the isolates coming from the laboratory and the finished product, and thus confirmed the laboratory cross-contamination. These results combined with all additional investigations done at the factory, allowed to release finished product batches produced and thus circumvented unnecessary food waste and economic losses for the factory. ISSN: 01681605

McLauchlin, J., Aird, H., Andrews, N., Chattaway, M., de Pinna, E., Elviss, N., Jørgensen, F., Larkin, L., Willis, C.
Public health risks associated with Salmonella contamination of imported edible betel leaves: Analysis of results from England, 2011–2017
(2019) International Journal of Food Microbiology, 298, pp. 1-10.
ABSTRACT: Fresh betel leaves (Piper betle L.), imported into the UK are a traditional ready-to-eat food consumed by Asian populations. We report here the consolidation of routinely collected data to model the public health risks from consumption of this food. Amongst 2110 samples collected at Border Inspection, wholesale, catering or retail, Salmonella was detected in 488 (23%) of samples tested between 2011 and 2017 and was the most commonly Salmonella-contaminated ready-to-eat food examined by Public Health England during this period. Using data from multiple samples (usually 5) tested per consignment sampled at Border Inspection, contamination levels were calculated by most probable number: seasonal, temporal and country specific differences were detected. Quantitative contamination data was used to estimate the levels present at retail, and a β-Poisson dose response model the probability of illness was calculated. Using data for products imported from India, the probability of acquiring infection following a single exposure (comprising of a single leaf) was estimated to be between 0.00003 (January–March) and 0.0001 (July–September). Using British Asian population data for individuals over 30 years of age in England in 2011, two estimates of consumption were modelled as 2.1 and 12.8 million servings per annum. Results from the model estimated 160 cases (range 102 to 242) and 960 cases (range 612 to 1456) per year in England for the two consumption estimates and equated to 34 (range 22 to 51) and 204 (range 130 to 310) salmonellosis cases per year reported to national surveillance. Salmonella from 475 of the contaminated samples were further characterised which showed a heterogeneous population structure with 46 S. enterica subsp. Enterica serovars, together with S. enterica subs diarizonae and salamae identified. Isolates from individual consignments were diverse and close genetic relationships between independent isolates were very rare except from within an individual consignment. There were no outbreaks detected as associated with betel leaf consumption. However analysis by whole genome sequencing of the 2014–17 data identified two cases where the clinical isolate had <5 single nucleotide polymorphism differences to isolates from betel leaves which is indicative of a likely epidemiological link and common source of contamination. Due to the diversity of the Salmonella contaminating this product, associations between salmonellosis cases and betel leaf consumption will appear sporadic and unlikely to be detected by current surveillance strategies based on outbreak detection. ISSN: 01681605

Wynants, E., Frooninckx, L., Van Miert, S., Geeraerd, A., Claes, J., Van Campenhout, L.
Risks related to the presence of Salmonella sp. during rearing of mealworms (Tenebrio molitor) for food or feed: Survival in the substrate and transmission to the larvae
(2019) Food Control, 100, pp. 227-234.
ABSTRACT: During rearing of insects for food and feed, their microbial safety is of utmost importance, but little is known on the transmission of food pathogens from the substrate to the insects. The aim of this study was to investigate whether transmission of Salmonella sp. to mealworms (Tenebrio molitor) can occur, in case mealworms are fed with contaminated wheat bran as substrate. Three consecutive contamination levels of a mixed culture of three Salmonella enterica strains in wheat bran were studied, being 7, 4, and 2 log cfu/g. At each of these contamination levels, Salmonella sp. remained present in the bran during the experimental period of seven days when larvae were absent. This indicates that Salmonella sp. can survive for at least seven days when wheat bran is stored, as is done in industrial rearing facilities. When larvae were present, however, the survival of Salmonella sp. in larvae and bran depended on the contamination level. When bran was contaminated with 7 log cfu/g Salmonella sp., the bacterium was still present after seven days in both larvae and bran, with average numbers of 3.7–4.1 log cfu/g, respectively. At a contamination level of the bran of 4 log cfu/g, Salmonella sp. counts decreased until <1.5 log cfu/g and <1.0 log cfu/g on average in bran and larvae, respectively. However, the pathogen was still detected in most larvae and bran samples after seven days, as was shown using presence/absence testing. At a contamination level of 2 log cfu/g, presence/absence testing revealed Salmonella sp. to remain present in some bran samples

after seven days, but surprisingly was not detected in the larval samples. Apparently, when present at a low level in the substrate, Salmonella sp. is not retained by the larvae during the seven day period, likely either because of competitive exclusion by the endogenous larval microbiota and/or because of antibacterial activity of the larvae. ISSN: 09567135

Ebel, E.D., Williams, M.S., Tameru, B.
Relatedness of Salmonella contamination frequency on chicken carcasses and parts when processed in the same establishment
(2019) Food Control, 100, pp. 198-203.
ABSTRACT: Understanding how changes in the prevalence of Salmonella-positive chicken carcasses affects the prevalence of Salmonella-positive chicken parts samples across slaughter establishments is necessary to model the risk of foodborne illness. When developing new Salmonella performance standards for chicken parts—that were implemented following existing chicken carcass performance standards—FSIS made assumptions regarding the correlation between these two forms of chicken products marketed to consumers. These assumptions were necessary because data were not available at the time to measure this correlation. Using recent FSIS sampling data and covariate information concerning antimicrobials applied during chicken processing, regression analysis suggests a slight negative relationship between the prevalence of Salmonella-positive chicken parts in slaughter establishment and the use of peracetic acid on carcasses, as well as a large positive relationship between prevalence of Salmonella-positive chicken parts and carcasses. Nevertheless, using a repeated random sub-sampling cross-validation approach, the regression model has very limited predictive value. In lieu of a predictive model, estimation of the correlation between prevalence of Salmonella-positive chicken carcass and parts samples is useful for understanding the public health value of performance standards and other interventions applied to these chicken products. After adjusting the observed correlation of the sampling evidence to account for the underlying beta and binomial distribution errors inherent in observed results, the estimated correlation of prevalence of Salmonella-positive chicken carcass and parts is 0.54. This implies a moderate degree of relatedness but may still understate the true correlation because of limitations in the available data. ISSN: 09567135

Pulford, C.V., Wenner, N., Redway, M.L., Rodwell, E.V., Webster, H.J., Escudero, R., Kröger, C., Canals, R., Rowe, W., Lopez, J., Hall, N., Rowley, P.D., Timofte, D., Harrison, R.A., Baker, K.S., Hinton, J.C.D.
The diversity, evolution and ecology of Salmonella in venomous snakes
(2019) PLoS neglected tropical diseases, 13 (6), p. e0007169.
ABSTRACT: BACKGROUND: Reptile-associated Salmonella bacteria are a major, but often neglected cause of both gastrointestinal and bloodstream infection in humans globally. The diversity of Salmonella enterica has not yet been determined in venomous snakes, however other ectothermic animals have been reported to carry a broad range of Salmonella bacteria. We investigated the prevalence and diversity of Salmonella in a collection of venomous snakes and non-venomous reptiles. METHODOLOGY/PRINCIPLE FINDINGS: We used a combination of selective enrichment techniques to establish a unique dataset of reptilian isolates to study Salmonella enterica species-level evolution and ecology and used whole-genome sequencing to investigate the relatedness of phylogenetic groups. We observed that 91% of venomous snakes carried Salmonella, and found that a diverse range of serovars (n = 58) were carried by reptiles. The Salmonella serovars belonged to four of the six Salmonella enterica subspecies: diarizonae, enterica, houtanae and salamae. Subspecies enterica isolates were distributed among two distinct phylogenetic clusters, previously described as clade A (52%) and clade B (48%). We identified metabolic differences between S. diarizonae, S. enterica clade A and clade B involving growth on lactose, tartaric acid, dulcitol, myo-inositol and allantoin. SIGNIFICANCE: We present the first whole genome-based comparative study of the Salmonella bacteria that colonise venomous and non-venomous reptiles and shed new light on Salmonella evolution. Venomous snakes examined in this study carried a broad range of Salmonella, including serovars which have been associated with disease in humans such as S. Enteritidis. The findings raise the possibility that venomous snakes could be a reservoir for Salmonella serovars associated with human salmonellosis. ISSN: 19352735

Erickson, M.C., Liao, J.-Y.
Variation in recovery of Salmonella strains extracted from leafy greens
(2019) LWT, 107, pp. 185-190.
ABSTRACT: This study investigated the degree of extraction from leafy green tissue (green leafy lettuce, spinach, red cabbage) artificially inoculated with five individual strains of

Salmonella at 3–4 log CFU. Inclusion of salt at levels of 0.50 M–0.75 M in the extraction solution improved recovery of S. Enteritidis Benson (P < 0.05) but salt in the range of 0.09–0.75 M had no effect on S. Enteritidis ME18, S. Newport, S. Oranienburg, and S. Typhimurium. Salmonella strain had a significant effect on extraction efficiency from lettuce tissue (P < 0.05) with Newport (68.7%) and Oranienburg (18.7%) exhibiting the highest and lowest recoveries, respectively. Recoveries were statistically higher in spinach than in green leafy lettuce or red cabbage (P < 0.05); however, S. Enteritidis ME18, S. Newport, and S. Typhimurium exhibited the same order of extractability in all three tissue types. Repeated extraction of spinach tissue continued to recover Salmonella in each extraction step. After extracting lettuce tissue multiple times, Salmonella was not detected in 25-g samples subjected to enrichment culture when 100 cells were initially applied. Continued detection of Salmonella occurred in extracted tissue when 400 cells of the pathogen were initially applied to the tissue. ISSN: 00236438

Brar, P.K., Danyluk, M.D.
Validation of Enterococcus faecium as a surrogate for Salmonella under different processing conditions for peanuts and pecans
(2019) Food Microbiology, 80, pp. 9-17.
ABSTRACT: Food Safety and Modernization Act (FSMA) Preventive Control rules require nut processors validate thermal processes to ensure a desirable log reduction of Salmonella is achieved. Due to the complex nature of nut and nut products, processes and equipment, it is difficult to use one validation study for all and may requires individual equipment be validated at the plant level. In plant validation studies, pathogens such as Salmonella cannot be used due to the risk of contamination, thus the suitability of a non-pathogenic organism, Enterococcus faecium as a surrogate for Salmonella was evaluated for peanut and pecan thermal processing. Stagnant and forced dry air heating conditions, (120 °C (20, 30, 40 min), 130 °C (10, 20, 30 min), 140 °C (10, 20, 30 min)) were evaluated for unblanched peanut kernels. Oil heating conditions (116 °C, 121 °C, and 127 °C for 0.5, 1.0, 1.5, 2.0, 2.5 min) were evaluated for pecan kernels. Inshell pecans are conditioned in hot or cold water to facilitate the shelling process. Water heating conditions (75 °C (20, 40, 80, 120 s), 80 °C (20, 40, 80, 120 s), 85 °C (20, 40, 80, 120 s), 90 °C (20, 40, 60, 80 s), and 95 °C (20, 40, 60, 80 s)) were evaluated for inshell pecans. Under conditions, except forced air treatment, E. faecium reductions (Log N/N 0) were either not significantly different (P > 0.05) or significantly lower than Salmonella (P < 0.05), making it a suitable surrogate for the processes evaluated. ISSN: 07400020

Chang, C.H., Teng, P.Y., Lee, T.T., Yu, B.
The effects of the supplementation of multi-strain probiotics on intestinal microbiota, metabolites and inflammation of young SPF chickens challenged with Salmonella enterica subsp. enterica
(2019) Animal Science Journal, 90 (6), pp. 737-746.
ABSTRACT: This study assessed the effect of probiotics on cecal microbiota, cecal short-chain fatty acids (SCFAs), and the gene expression of cytokines in young specific-pathogen-free (SPF) chickens infected with S. enterica subsp. enterica. One-day-old SPF chickens (n = 105) were randomly assigned to one of the three treatment groups: control (Cont) group, Salmonella-infected (Sal) group, and a Salmonella-infected group treated with multi-strain probiotics (ProSal group). All chickens except those in the Cont group were challenged orally with 1 × 108 cfu/ml of Salmonella 4 days after hatching. Chickens in the Sal group exhibited more abundance of Proteobacteria than those in the Cont and ProSal groups. At the genus level, chickens in ProSal group exhibited increased numbers of Lactobacillus and Oscillospira compared with those in the other groups. Chickens in the ProSal group exhibited a significant increase of cecal SCFAs compared with chickens in the Sal group. Chickens in the ProSal group exhibited increased gene expression of anti-inflammatory cytokines, IL-10 and TGF-β4, and decreased expression of the proinflammatory cytokine, IFN-γ, in the cecal tonsil compared with those in the Sal group. The results of this study indicated that the administration of probiotics can modulate microbiota, SCFAs, and immunomodulatory activity in SPF chickens. ISSN: 13443941

Marus, J.R., Magee, M.J., Manikonda, K., Nichols, M.C.
Outbreaks of Salmonella enterica infections linked to animal contact: Demographic and outbreak characteristics and comparison to foodborne outbreaks—United States, 2009–2014
(2019) Zoonoses and Public Health, 66 (4), pp. 370-376.
ABSTRACT: In the United States, multistate Salmonella outbreaks are most commonly linked to a food source; however, contact with live animals can also result in outbreaks of human illness. To characterize Salmonella outbreaks linked to animal contact and examine

differences compared to foodborne outbreaks, we analysed data reported to the Centers for Disease Control and Prevention through the National Outbreak Reporting System (NORS) from 2009 to 2014 with a primary mode of transmission listed as “animal contact” or “food.” Four hundred and eighty-four outbreaks with animal contact or foodborne transmission were reported through NORS; of these outbreaks, 99 (20.5%) resulted from Salmonella transmission through animal contact and 385 (79.5%) resulted from foodborne transmission, which resulted in 3,604 (19.8%) and 13,568 (80.2%) illnesses, respectively. A higher proportion of illnesses among children aged <1 year and children aged 1–4 years were linked to animal contact outbreaks compared to foodborne outbreaks (15.2% vs. 1.4%, $p < 0.01$ and 24.5% vs. 5.6%, $p < 0.01$, respectively). Illnesses resulting in hospitalizations (OR: 1.81, 95% CI: 1.62, 2.02) were more likely to be associated with animal contact compared to food. Animal contact outbreaks reported to NORS were more likely to be multistate compared to foodborne outbreaks (OR: 5.43, 95% CI: 3.37, 8.76) and had a longer median duration (99.0 days vs. 9.0 days, $p < 0.01$). Characterizing the differences between outbreaks of illness linked to animal contact and outbreaks linked to food provides useful information to investigators to improve public health response. ISSN: 18631959

Kilonzo-Nthenge, A., Liu, S.
Antimicrobial efficacy of household sanitizers against artificially inoculated Salmonella on ready-to-eat spinach (Spinacia oleracea)
(2019) Journal fur Verbraucherschutz und Lebensmittelsicherheit, 14 (2), pp. 105-112.
ABSTRACT: Due to health concerns regarding the microbiological safety of fresh produce, consumers frequently wash fresh produce before consumption. Household sanitizers including tap water, vinegar (5.0, 1.5, 1.0%), baking soda, commercial wash, and bleach solutions were evaluated for their effectiveness in reducing counts of Salmonella enterica on spinach leaves. Treatments were carried out at 23 °C for 2 min. An online survey was also conducted in different parts of the United States to collect information on consumers' practices of washing produce with common household sanitizers. A significantly higher ($p < 0.05$) bacteria reduction (1.95–2.19 log CFU/g) was achieved using chlorine solution (200 ppm) when compared with other treatments (0.01–1.64 log CFU/g). Running tap water physically reduced bacteria on spinach by 1.52–1.62 log CFU/g. Vinegar solutions at 5, 1.5, and 1.0% promoted reductions of 1.56–1.64, 1.01–1.12, and 0.9–1.02 log CFU/g, respectively. Notably, none of the household sanitizers were capable of entirely removing Salmonella on spinach leaves. With exclusion of chlorinated water and 5% vinegar solution, Salmonella was present in all spend solutions. Our survey showed that three washing solutions were most commonly used by consumers; rubbing produce under running tap water (54.3%), holding produce under running tap water (53.5%), and soaking produce in water (32.9%). Bleached water was the least applied household sanitizer (0.59%). Based on the results of the present work, household sanitizers do not guarantee the complete inactivation of pathogenic bacteria on leafy produce, particularly Salmonella on spinach. ISSN: 16615751

Gurtler, J.B., Keller, S.E., Kornacki, J.L., Annous, B.A., Jin, T., Fan, X.
Challenges in Recovering Foodborne Pathogens from Low-Water-Activity Foods
(2019) Journal of food protection, 82 (6), pp. 988-996.
ABSTRACT: There are numerous obstacles to the detection of foodborne pathogens in foods that exhibit a low water activity (aw). These obstacles include the presence of antimicrobial compounds, particulates, PCR inhibitors, and fatty matrices. New approaches should be sought to increase the sensitivity of pathogen testing in low-aw foods and to overcome the effects of various inhibitors and antimicrobials. The U.S. Food and Drug Administration and other laboratories are working toward this goal. This review will address these issues while delineating specific inhibitors and antimicrobials that impede testing of low-aw foods. A review of relevant rapid and conventional testing methodologies for Salmonella in low-aw foods will also be discussed. ISSN: 19449097

Han, J.-Y., Song, W.-J., Kang, D.-H.
Optimization of broth recovery for repair of heat-injured Salmonella enterica serovar Typhimurium and Escherichia coli O157:H7
(2019) Journal of Applied Microbiology, 126 (6), pp. 1923-1930.
ABSTRACT: Aims: The purpose of this research was to determine optimum conditions for broth recovery of heat-injured Salmonella Typhimurium and Escherichia coli O157:H7. Methods and Results: Exposure to 55°C for 15 and 25 min, respectively, induced cellular injury to those pathogens. Comparison was made with the commonly used overlay method using selective medium for recovering sublethally injured cells of S. Typhimurium. For E. coli O157:H7, phenol red agar base with 1% sorbitol was used. After cell suspensions

were heated at 55°C for selected time intervals, microbes were 10-fold diluted with brain heart infusion (BHI), tryptic soy broth (TSB) and TSB with 0·6% yeast extract (TSBYE) and incubated at 37°C for up to 3 h. At hourly intervals, diluents were plated onto selective medium for recovery. Simultaneously, diluents were plated onto tryptic soy agar (TSA) for recovery of sublethally injured cells. For overlays, diluents were plated onto TSA and overlaid with selective agar after a resuscitation interval. Broth recovery conditions for S. Typhimurium and E. coli O157:H7 were determined to be 1 h in any of the following broth media: BHI, TSB or TSBYE. When liquid resuscitation was applied to sublethally injured cells in food samples (milk), 1 h was also sufficient time for recovery. Conclusions: The broth recovery method is a convenient alternative to conventional recovery methods. Significance and Impact of the Study: Cells sublethally injured by control interventions might not grow on selective medium because they have no resistance to several selective compounds. However, injured cells can recuperate and multiply under conditions sufficient for recovery. To repair and detect heat-injured cells, the overlay method is commonly used but this method has some limitations. This study confirms the effectiveness of liquid resuscitation method on recovery of injured cells. The broth recovery can replace the overlay method due to greater convenience and timesaving. ISSN: 13645072

Huang, J., Luo, Y., Zhou, B., Zheng, J., Nou, X.
Growth and survival of Salmonella enterica and Listeria monocytogenes on fresh-cut produce and their juice extracts: Impacts and interactions of food matrices and temperature abuse conditions
(2019) Food Control, 100, pp. 300-304.
ABSTRACT: Storage temperature and nutrient availability are major factors impacting pathogen growth and thus food safety risks. This study evaluated the survival and growth of Salmonella enterica, and Listeria monocytogenes in relation to temperature abuse variations, and food matrices. Fresh-cut cantaloupe, honeydew, watermelon, pineapple, and radish contaminated with S. enterica and L. monocytogenes were subjected to cold (4 °C), chronic temperature abuse at 8 and 12 °C, and acute temperature abuse (35 °C for 2 h followed by 4 °C for the remainder 7-day storage). Pathogen growth potential in the juice extracts from each product was further compared to that on the respective cut produce. Under chronic temperature abuse, three different pathogen growth patterns emerged on five test products: both S. enterica and L. monocytogenes grew significantly on cut cantaloupe, honeydew and watermelon at 8 and 12 °C; but only survived on cut radish, and even declined in population on cut pineapple under the same conditions. Specifically, S. enterica populations reached up to 5.28 log CFU/g and L. monocytogenes up to 7.77 log CFU/g after 7 days at 12 °C. During cold storage at 4 °C, significantly different growth patterns were also observed between S. enterica and L. monocytogenes on cut melons, where S. enterica populations remained unchanged during the 7-day storage while L. monocytogenes grew continuously. In the juice extracts, S. enterica and L. monocytogenes reached maximum population density in melon juices, but failed to grow in pineapple juice, similar to the growth patterns on cut melon and pineapple. Distinctly different growth patterns, however, were shown in S. enterica and L. monocytogenes on cut radish and in radish juice; exhibiting no growth on cut radish, but maximum growth in radish juice. The disparity in pathogen growth observed on cut pineapple and radish versus on melon in this study supports commodity specific risk-based food safety policies pertaining to temperature control for food safety. ISSN: 09567135

Li, M., Havelaar, A.H., Hoffmann, S., Hald, T., Kirk, M.D., Torgerson, P.R., Devleesschauwer, B.
Global disease burden of pathogens in animal source foods, 2010
(2019) PLoS ONE, 14 (6), art. no. e0216545, .
ABSTRACT: Animal source foods (ASF) such as dairy, eggs, fish and meat are an important source of high-quality nutrients. Lack of ASF in diets can result in developmental disorders including stunting, anemia, poor cognitive and motor development. ASF are more effective in preventing stunting than other foods and promoting ASF consumption in low- and middle-income countries could help improve health, particularly among pregnant women and young children. Production and consumption of ASF are, however, also associated with potential food safety risks. Strengthening of food control systems, informed by quantitative assessments of the disease burden associated with ASF is necessary to meet global nutrition goals. We present the human disease burden associated with 13 pathogens (bacteria and parasites) in ASF, based on an analysis of global burden of foodborne disease (FBD) estimates of the WHO Foodborne Disease Burden Epidemiology Reference Group (FERG). The FBD burden of these pathogens was combined with estimates of the proportion of disease transmitted by eight main groups of ASF. Uncertainty in all estimates was accounted for by Monte Carlo simulation. In 2010, the global burden of ASF was 168

(95% uncertainty interval (UI 137–219) Disability Adjusted Life Years (DALYs) per 100,000 population, which is approximately 35% of the estimated total burden of FBD. Main pathogens contributing to this burden included non-typhoidal Salmonella enterica, Taenia solium, and Campylobacter spp. The proportion of FBD burden associated with ASF varied considerably between subregions and between countries within subregions. Likewise, the contribution of different pathogens and ASF groups varied strongly between subregions. Pathogens with a localized distribution included T. solium and fishborne trematodes. Pathogens with a global distribution included non-typhoidal S. enterica, Campylobacter spp., Toxoplasma gondii, and Mycobacterium bovis. Control methods exist for many hazards associated with ASF, and their implementation is linked to economic development and effective food safety systems. ISSN: 19326203