NEWSLETTER

European Union Reference Laboratory for *Salmonella*

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European Union Reference Laboratory for *Salmonella*

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Contents

Editorial Note

Bilthoven, 25 June 2021

Dear colleague,

I hope that, like in the Netherlands, in your country the number of **COVID-19** cases are also decreasing and that we can all slowly go back to the 'old (or new) normal'. Hopefully it will also be possible to go on holidays soon, which we will all need very hard.

By the end of March 2021, we have submitted the **technical progress report on the activities of the EURL-*Salmonella* in 2019 and 2020** to DG SANTE. For your information, this report is also included in this Newsletter.

In March-May 2021, the evaluation of the cluster analysis results of the **PT on typing of *Salmonella* 2020** was performed. By the end of May 2021 the participants received their own results as well as the interim summary report containing the results of all participants. This interim summary report is also available at the EURL-*Salmonella* website:
https://www.eurlsalmonella.eu/documenten/interim-summary-report-eurl-salmonella-pt-cluster-analysis-2020

In March 2021 we organised the **PT on detection of *Salmonella* in liquid whole egg**. The results were analysed in April-May 2021 and by the end of May 2021 the participants received their own results as well as the interim summary report containing the results of all participants. This interim summary report is also available at the EURL-*Salmonella* website:
https://www.eurlsalmonella.eu/documenten/interim-summary-report-eurl-salmonella-pt-food-2021

Currently we are preparing the **PT on detection of *Salmonella* in samples from the primary production stage (PPS)**. This PT will be organised in September 2021, and the matrix of choice is boot socks with chicken faeces. The time table of this PT is included in the Newsletter.

The last PT planned for this year is the **PT on typing of *Salmonella***, which will be organised in November 2021. Like last year, the study will contain an obligatory part on serotyping of *Salmonella*, and a voluntary part on cluster analysis. The time table for this PT is also included in this Newsletter.

On 28 May 2021 we organised our **26th EURL-*Salmonella* workshop**, or 2nd online EURL-*Salmonella* workshop. Given the circumstances with the SARS-CoV-2 pandemic, it was unfortunately still not possible to meet physically. We still keep on hoping for better times next year. An advantage of an online workshop is the fact that it is possible to host more participants. Also in this years' workshop we could welcome 75 participants! Shortly after the workshop, the presentations were posted at the EURL-*Salmonella* website:
https://www.eurlsalmonella.eu/workshop-2021

From 22 April until 26 May 2021, the voting on **draft ISO/CD TS 6579-4 on identification of monophasic *Salmonella* Typhimurium** took place. This document was also sent to the NRLs-*Salmonella*. Comments were received from two members of ISO/TC 34/SC 9 (of which one is an NRL-*Salmonella*) and from in total eight NRLs-*Salmonella*! This is very helpful, and I would like to thank all NRLs very much for their contributions. The next steps will be to discuss the comments received on draft ISO/CD TS 6579-4 in ISO-WG 10 in the second half of 2021 and to prepare draft ISO/DTS 6579-4. This summer we also want to finalise the report of the method evaluation study (testing 172 strains by two

laboratories) for publication at EURL-*Salmonella* website. In (draft) ISO/TS 6579-4, reference is made to this report and by publication at our website it becomes more widely available. In fall 2021, we will start with the preparation for the organisation of an interlaboratory study (ILS) for determination of the performance characteristics (expected to be organised in 2022).

Reports published in the second quarter of 2021:

Pol-Hofstad, I.E. and Mooijman, K.A., 2021. Combined EURL-*Salmonella* Proficiency Test Primary Production and Food 2020 - Detection of *Salmonella* in hygiene swab samples. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM Report no.: 2020-0204. Final report May 2021. https://www.rivm.nl/bibliotheek/rapporten/2020-0204.pdf

Diddens, R.E. and Mooijman, K.A., 2021. EURL-*Salmonella* Proficiency Test Live Bivalve Molluscs 2020. Detection of *Salmonella* in mussels. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM Report no.: 2020-0203. Final report May 2021. https://www.rivm.nl/bibliotheek/rapporten/2020-0203.pdf

Mooijman, K.A. The 25th EURL-*Salmonella* workshop 17 and 18 September 2020, Online. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM Report no.: 2020-0202. Final Report June 2021. https://www.rivm.nl/bibliotheek/rapporten/2020-0202.pdf

I would like to finish this editorial note with a very nice poem we received from Serge Losch of the NRL-*Salmonella* at the State Veterinary Laboratory in Luxembourg, in which he announced his retirement per 1 May 2021.

When fall comes into your life,
You will cut with a knife
The string on which is suspended
The career that soon will be ended.

Many nice moments with you passed by
And, before I say good bye,
I want to thank you all for this
There is surely something I'll miss.

The memory will not vanish
May you go on with my best wish.
Retirement is like a reward
for hard work on the lab's board.

Stay healthy and enjoy the sun
That enlightens the meetings where you'll have fun
When the covid pandemic will have gone.

Have a nice summer!

Best wishes,
Kirsten Mooijman
Coordinator EURL-*Salmonella*

Contribution of the EURL-*Salmonella*

Timetable EURL- *Salmonella* Proficiency Test
Primary Production Stage 2021
Detection of *Salmonella* in bootsocks with chicken faeces

Week	Date	Subject
27	Week of 5 July	E-mailing the link to the registration form for the detection study. Please **register by 30 August** at the latest.
38	Week of 20 September	Shipment of the parcels to the participants as Biological Substance Category B (UN 3373).
38	Week of 20 September	E-mailing the link for the result form to the participants. E-mailing the protocol and instructions for the result form to the NRLs. Preparation of media by the NRLs.
39	Monday 27 September	Performance of the Proficiency Test.
43	29 October 2021 at the latest	Deadline for completing the result form: **29 October 2021** (23:59h CET). After this deadline the result form will be closed.
	December 2021	Interim summary report.

If you have questions or remarks about this Proficiency Test, or in case of problems, please contact:

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Timetable EURL- *Salmonella* Proficiency Test Typing 2021
Serotyping and
optional part MLVA and/or WGS Cluster Analysis

Week	Date	Subject
39	Week of 27 September	Emailing of the link to the registration form for the typing study. Please **register by 15 October 2021** at the latest.
43	Week of 25 October	Emailing of the protocol 2021.
45	Monday 8 November	Shipment of the parcels to the participants as Biological Substance Category B (UN 3373).
45	Week of 8 November	*Upon receipt*: Starting the identification of the strains, according to the usual practice of the laboratory. Sending the link for the result form on serotyping to the participants. Sending the link for the result form on MLVA and/or WGS Cluster Analysis to the participants in a separate email.
50	17 December 2021 at the latest	Deadline for completing the electronic submission of **Serotyping** results: **17 December 2021.** After this deadline, the result form for serotyping will be closed.
	28 January 2022 at the latest	Deadline for completing the electronic submission of **MLVA/WGS Cluster Analysis** results: **28 January 2022.**
	February 2022	Serotyping: Evaluation of individual laboratory results and Interim summary report.
	April/May 2022	MLVA/WGS Cluster Analysis: Evaluation of individual laboratory results and Interim summary report.

If you have questions or remarks about this Proficiency Test, or in case of problems, please contact:

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Submitted Technical progress report on activities of the European Union Reference Laboratory (EURL) for *Salmonella* 2019-2020

K.A. Mooijman, 18 March 2021
National Institute for Public Health and the Environment (RIVM)
Centre for Zoonoses and Environmental microbiology (Z&O)

Letter-report Z&O/2021-0024 Mo/km
RIVM project-number: E/114506/19 and E/114509/20

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Introduction

The work plan of the EURL-*Salmonella* for 2019-2020, was submitted to the European Commission in December 2018. This progress report details the activities of the EURL-*Salmonella* according to the agreed work plan for 2019-2020, 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.

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

1.1 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. In October 2019, CEN/TC275/WG6 has moved its activities to the new CEN/TC463 'Microbiology of the food chain'.

The annual meetings of both groups were organised in Milan, Italy from 8 to 12 July 2019 and as online meeting (due to the COVID-19 pandemic) from 2 to 5 June 2020.
The first meeting of CEN/TC463 was organised on 25 November 2019.

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 and updating of a guidance document for drafting ISO/CEN microbiological standard methods. Kirsten presented the progress of the relevant groups at both plenary meetings of ISO and CEN. Reports of the meetings have been drafted, coordinated by the EURL-*Salmonella*, by 5 EURLs in 2019 and by 6 EURLs in 2020 and sent to DG SANTE on respectively 2 August 2019 and 21 July 2020.

ISO-AHG Amendment for EN ISO 6579-1 'Detection of Salmonella'

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 in 2018, 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 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 (the outcome of the comparison study performed by 9 laboratories in 2017).

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 was prepared by the EURL-*Salmonella* and sent to the members of the expert group in February 2019. Next, the (draft) document was sent to ISO for launching the voting of the Draft International Standard (DIS). The DIS voting took place from 08-07-2019 until 30-09-2019. The DIS version of Amd.1 was also sent to the NRLs-*Salmonella* for information and comments. The outcome of the DIS voting was 100% positive with only a few editorial comments. As follow up to these editorial comments, the EURL-*Salmonella* updated Amd.1 and sent it to the expert group for a final check (22-10-2019 until 15-11-2019). No further comments were received and the final draft version of Amd.1 was sent to the secretariat of ISO early December 2019. Proof-checks of the final document were done in January and February 2020 after which the final document was published in March 2020 (EN ISO 6579-1:2017/Amd.1:2020). The NRLs-*Salmonella* were informed about the publication of this document in the EURL-*Salmonella* Newsletter Vol. 26, No. 1 of March 2020.

Information on (draft) EN ISO 6579-1/Amd.1, and especially on the results of the experiments performed to harmonise the incubation temperature at 34-38 °C, was presented at a symposium of the International Association for Food Protection (IAFP) in Nantes, France in April 2019, as well as on a seminar of Campden BRI (United Kingdom) in February 2020.

ISO-WG10 PCR identification of monophasic S. *Typhimurium (ISO/TS 6579-4)*

In May 2016, SC9 agreed to register the Preliminary Work Item of ISO 6579-4 to become a Technical Specification (TS). This document includes 3 PCR protocols for the identification of monophasic *Salmonella* Typhimurium:

- A probe-based multiplex real-time PCR protocol (Maurischat et al. 2015);
- An agarose gel-based multiplex PCR protocol (Tennant et al. 2010);
- An agarose gel-based single target PCR protocol (Maurischat et al. 2015)

The technical work for development of this document was initiated in Task Group (TAG) 3 of CEN/TC275/WG6. It was agreed that as soon as the technical work was finished, the work would be moved from CEN/TC275/WG6-TAG3 to ISO/TC34/SC9-WG10, after which ISO-WG10 would 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. 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 were further evaluated by additional testing in summer 2019. All testing results were summarised by EURL-*Salmonella* in a report, including the draft PCR protocols (draft ISO/TS 6579-4), by early December 2019 (included as annex to this progress report). The report was sent to the members of CEN/TC275/WG6-TAG3 (since fall 2019 changed to CEN/TC 463-WG1) in February 2020, asking the members for their agreement to move the work to ISO-WG10 and to launch the NWIP vote. This request was answered affirmative and the NWIP voting was launched on 18 May 2020 (deadline for voting 12 August 2020), together with a call for experts in ISO/TC34/SC9 and CEN/TC463. The outcome of the NWIP vote in ISO was: 18 approval, 0 disapproval and 17 abstention; and in CEN: 8 approval, 0 disapproval and 15 abstention. Additionally, only few comments were received for the draft document. As convenor of ISO-WG10, Kirsten Mooijman (EURL-*Salmonella*) drafted observations to the comments of the NWIP voting and prepared the first working draft (WD1) of ISO/TS 6579-4. These documents have been discussed in the first (online) meeting of ISO-WG10 on 16 November 2020. After this meeting, WD2 of ISO/TS 6579-4 was prepared and sent to the members of ISO-WG10 for further comments (deadline 28 January 2021). These comments will be taken into account in WD3 of ISO/TS 6579-4 and discussed at the second (online) meeting of ISO-WG10, planned on 15 March 2021.

ISO-AHG Template and guidance for drafting microbiological ISO/CEN standard methods

In 2014, it was decided to raise an Ad'hoc group for drafting a guidance document for the drafting of microbiological ISO/CEN standards. This document is intended to (further) harmonise the content and layout of standards for microbiology of the food chain. Kirsten Mooijman and Wilma Jacobs became respectively project leader and co-project leader of this group because of their extensive experiences with drafting ISO/CEN documents. The guidance document is an internal ISO/CEN document and is intended to help convenors and project leaders of ISO/TC34/SC9 and CEN/TC463 with the drafting of ISO/CEN documents.
In March 2018 the first edition of the guidance document was published. Immediately after publication it was discussed that the definitions of pathogens used in EN ISO documents for microbiology of the food chain had to be changed. These definitions needed to become 'standalone' definitions instead of being a reference to the procedure in the EN ISO document. A proposal for amended definitions was drafted and sent to the members of the Ad'hoc group in October 2018. An updated proposal was sent to the members of ISO and CEN early 2019 and discussed at the annual meeting in July 2019. In fall 2019, the EURL-*Salmonella* has prepared a next draft version of the guidance document, including the new information on definitions, as well as other items agreed upon the annual meeting. A second edition (ED2) of the guidance document was publishes in April 2020. The guidance document is a dynamic document and will need to be updated to any new agreements made in ISO and CEN. For that reason draft ED3 of this guidance document will be prepared early 2021 for further discussion in the Ad'hoc group and with the members of ISO/TC34/SC9 and CEN/TC463.

ISO-WG3 Method validation

Wilma Jacobs of the EURL-*Salmonella* is member of the ISO working group on validation of microbiological methods and followed (and commented on) the drafting of parts 3-5 of EN ISO 16140 ('Microbiology of the food chain - Method validation'). Additionally, Wilma was 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 spring 2019 pre-Final Draft International Standard (pre-FDIS) versions of parts 3, 4, 5, and 6 of EN ISO 16140 were distributed to the members of ISO/TC34/SC9 for comments. In June 2019 the FDIS voting was launched of parts 4-6 (deadline of voting 19 August 2019).
EN ISO 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) was finalised and published in December 2019.
In the FDIS versions of part 4 ('Protocol for method validation in a single laboratory') and of part 5 ('Protocol for factorial interlaboratory validation for non-proprietary methods') several technical errors were detected and the EURL-*Salmonella* gave a substantial input to improve the documents after which the second FDIS voting was launched in January 2020. Both documents were finalised and published in July 2020.
For part 3 ('Protocol for the verification of reference methods and validated alternative methods in a single laboratory'), two more pre-FDIS versions were prepared and discussed in ISO-WG3 in summer and December 2019. The draft FDIS version was sent to the secretariat of ISO/TC34/SC9 in December 2019. However, the ISO Central secretariat indicated that the structure of part 3 needed to be changed (not changing the technical content). Therefore, a new FDIS version had to be drafted. The draft version of FDIS 16140-3 was tested for its practical use at the laboratory of the EURL-*Salmonella* after which several suggestions for improvement were made. In October 2020, the FDIS voting of ISO 16140-3 was launched (deadline of voting 27 November 2020). The document was finalised and published in January 2021.

In 2020, WG3 started with the following (new) activities:

- Development of Amendment 1 of EN ISO 16140-2. This Amendment will include revision of the qualitative method study data evaluation. Wilma Jacobs actively follows the development of this Amendment.
- Drafting of part 7 of ISO 16140. This part will contain information on validation of identification methods. Several draft versions of this document were prepared to which Wilma also commented.
- A subgroup to review questions and documents concerning validation of methods from working groups of ISO/TC34/SC9 and CEN/TC463. Wilma is also member of this subgroup.
- Development of a calculation tool in Excel, to facilitate the use of EN ISO 16140-3. Wilma was involved in this, especially by giving input on the correct interpretations of the technical microbiological use of the International Standard. The tool will be made available on the ISO website in March 2021.

In July 2019, ISO/TC34/SC9 decided to start the revision of EN ISO 17468 (' Microbiology of the food chain – Technical requirements and guidance on establishment or revision of a standardized reference method'). For this activity, Bertrand Lombard (ANSES, France) and Wilma Jacobs (EURL-*Salmonella*) have become the project leaders. The revision of the document started early 2020 and will include (amongst others): Inclusion of information on EN ISO 16140-4 (in-house validation), EN ISO 16140-6 (validation of confirmation and typing methods) and EN ISO 11133 (performance testing of culture media) and addition of informative annexes with examples of (recently) developed ISO methods. Additionally, the content will be extended for situations where it is not possible to compare a new ISO method with a former reference method. A first draft revision of ISO 17468 (first draft Committee Draft – draft-CD) was sent to the members of WG3 for comments in July 2020. The comments and progress with the documents was discussed at the (online) meeting of WG3 in October 2020. A second draft CD version is prepared in January 2021 for further discussion in WG3.

ISO-AHG Validation status of ISO standards

Wilma Jacobs of the EURL-*Salmonella* is member of this Ad'hoc group which started its activities in 2020 with making an inventory on whether EN ISO documents of the Food chain contain performance characteristics or not. Additionally, if performance characteristics are included, it was checked whether these are valid for a broad range of foods or only for a limited number of food categories. The ISO-AHG is drafting a table to show for each EN ISO document of the Food chain what performance characteristics are published in the EN ISO document and which are still missing. In case a validation has not been performed for a broad range of foods, the next step is to check if additional data can be used from validation studies of proprietary methods (performed by MicroVal and Afnor). Wilma Jacobs is doing this for the validation data of EN ISO 6579-1 on detection of *Salmonella*.
The Ad'hoc group met several times in 2020 (all teleconferences): in January, March, May, October and December.

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

Early 2018, this working group launched the first draft document 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 Committee Draft (CD) version of ISO(/CD) 23418 was launched in May 2019 (deadline voting 7 July 2019). The working group EURLs NGS discussed the document at their meeting in June 2019 and decided to advice ISO-WG25 to make ISO 23418 a guidance document, especially because of the amount of (normative) metadata included in the ISO document. The EURL-*Salmonella* is member of this ISO working group and participated in the teleconference discussing the comments in August 2019. At this meeting it was agreed that additional comments (especially concerning the metadata) could be sent within one month. The EURL-*Salmonella* used this option by sending substantial comments. The majority of comments were introduced in a next draft version of ISO 23418. Although the document will remain a full ISO and not a guidance document, the information on metadata has become informative instead of normative throughout the document. The draft DIS version of the document was discussed at the meeting of WG25 in March 2020. After some last amendments, the DIS voting was launched on 18 September 2020 (deadline 11 December 2020). The result of the DIS voting was 100% positive in ISO as well as in CEN, but still with a substantial amount of comments (approx. 30 pages). These comments are expected to be discussed at a next meeting of ISO-WG25 in spring 2021.

CEN-TAG 9 Improvement of the pre-enrichment

The CEN Task group, TAG9, was originally 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, uniform pre-enrichment medium, it was decided to draft a protocol to evaluate the performance of pre-enrichment media instead. Several versions of the protocol were discussed with the members of CEN-TAG9 and finalised in fall 2018 and tested by some members of CEN and ISO 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, *Cronobacter, Enterobacteriaceae*, *Listeria, Salmonella* and STEC. The first experiments were done with *Salmonella* only. A draft report of testing of the protocol was sent to the members of CEN-TAG9 in December 2019.
TAG9 was 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.

Early 2020, the group leader of TAG9 has moved to another job and could no longer lead this activity. This resulted in the fact that no progress was made with the documents in 2020. By the end of 2020, CEN/TC463 made a call among its members to reactivate the work in 2021.

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 nowadays an important technique 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 2020, the typing department of RIVM performed a validation study for the use of WGS for serotyping of *Salmonella*. The EURL-*Salmonella* closely cooperates with this department for (sero)typing of *Salmonella* and was asked to advice on the set-up of the validation study. For the validation study, the demands for accreditation of medical laboratories (EN ISO 15189:2015) as well as the demands for validation of typing methods for microbiology of the food chain (EN ISO 16140-6:2019) were followed. The in-house pipeline 'Juno' is used for de-novo assembly, including trimming and QC algorithms. The *in silico Salmonella* serotyper is based on SeqSero2 (Zhang et al, 2019). Approximately 500 different *Salmonella* strains (inclusivity study), comprising 176 different serovars, were tested as well as 100 non-*Salmonella* strains (exclusivity study). The results fulfilled the criteria of both EN ISO documents. *Salmonella* serovars which cannot be fully determined with the *Salmonella* serotyper pipeline are confirmed additionally with serological, biochemical or molecular tests guided by the White Kauffman Le Minor scheme (Grimont and Weill 2007).

Deliverables
2019

Microbiology of the food chain - Template and guidance for drafting ISO/CEN standard methods. Edition 1 - Document ISO/TC34/SC9 N2188; 24-05-2018. Draft Edition 2 – December 2019

2020

Presentation given by Kirsten Mooijman on the content of EN ISO 6579-1/Amd.1 at a seminar of Campden BRI, Chipping Campden, United Kingdom in February 2020.
Publication of EN ISO 6579-1:2017/Amd.1 'Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* — Part 1: Detection of *Salmonella* spp. - Amendment 1 Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSRV and SC' in March 2020.
Microbiology of the food chain - Template and guidance for drafting ISO/CEN standard methods. Edition 2 - Document ISO/TC34/SC9 N2500 published 16 April 2020.
A consolidated report of 6 EURLs (coordinated by the EURL-*Salmonella*) of the meetings of ISO/TC34/SC9 and CEN/463 held online on 2-5 June 2020, was sent to DG SANTE on 21 July 2020. A summary of relevant *Salmonella* items was presented at the workshop in September 2020 (sub-activity 2.1).
ISO/CEN voting of new work item proposal 'ISO/TS NP 6579-4 - Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 4: Identification of monophasic *Salmonella* Typhimurium (1,4,[5],12:i:-) by PCR'. Voting period 18-05-2020 until 16-08-2020. Second working draft (WD2) of ISO/TS 6579-4 sent to ISO-WG10 in December 2020.

Meetings (teleconferences and missions)
2019

Annual meetings of CEN/TC275/WG6 and ISO/TC34/SC9 (Microbiology of the food chain)
8-12 July 2019: Milan, Italy
Participant: Kirsten Mooijman

Meeting ISO/TC34/SC9 – WG3 Validation of microbiological methods (including draft ISO 16140-6 on validation of confirmation/typing methods)
18-19 June 2019: Bremen, Germany
Participant: Wilma Jacobs (project leader drafting ISO 16140-6 and member WG3)

Meetings ISO/TC34/SC9 – WG25 Whole Genome Sequencing
6 August 2019: Teleconference
29 October 2019: Teleconference
Participants: Kirsten Mooijman, Angela van Hoek

For budget IAFP:
Symposium of the International Association for Food Protection (IAFP). Participation in and giving a presentation on international harmonisation of the incubation temperature at 35-37 °C.
24-26 April 2019: Nantes, France
Participant: Kirsten Mooijman

2020

Annual meetings of CEN/TC463 and ISO/TC34/SC9 (Microbiology of the food chain)
2-5 June 2020: Teleconference
Participant: Kirsten Mooijman

Meeting ISO/TC34/SC9 – WG10 Identification of monophasic *Salmonella* Typhimurium
16 November 2020: Teleconference
Participants: Kirsten Mooijman (convenor), Robin Diddens

Meetings ISO/TC34/SC9 – WG3 Validation of microbiological methods
12-13 February 2020: Dauphin Island, USA
15-16 October 2020: Teleconference
Participant: Wilma Jacobs

Meetings ISO/TC34/SC9 - AHG Validation status of ISO standards
8 January 2020: Teleconference
25 March 2020: Teleconference
25 May 2020: Teleconference
5 October 2020: Teleconference
17 December 2020: Teleconference
Participant: Wilma Jacobs

Meeting ISO/TC34/SC9 – WG25 Whole Genome Sequencing
12 March 2020: Teleconference
Participants: Kirsten Mooijman, Angela van Hoek

For budget Campden BRI:
STEC & *Salmonella* Seminar of Campden BRI, Chipping Campden, United Kingdom.
Presentation on the content of EN ISO 6579-1/Amd.1.
27 February 2020: Chipping Campden, United Kingdom
Participant: Kirsten Mooijman

1.2 Sub-activity 1.2 EURLs working group 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, a survey held amongst the 8 NRL networks in 2018, followed by 2 more small surveys to collect additional information. A summary of the survey results were presented at the workshop of the EURL-*Salmonella* in May 2018 (Mooijman, 2018).

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. The information of the 8 EURLs was summarised by EURL-AR and sent to the members of the EURLs WG NGS in February 2019.
In July 2019 a progress report on the activities of the EURLs working group on WGS has been prepared and submitted to DG SANTE.
The group has prepared/ is preparing several (harmonised) documents and are meant to provide guidance to the laboratories in the area of application of NGS. Each EURL is responsible for drafting, and keeping up to date, one or more guidance documents. The responsible EURL publishes the relevant guidance document(s) at its website and the other EURLs will provide a link to these documents at their website. If a document is updated, it should be published at the same place and under the same name as the original document so that the link at the websites of the other EURLs is maintained.
By the end of 2020/early 2021 the following guidance documents have been published:

- Reference Whole Genome Sequencing collection – curated by EURL-*Salmonella*.
- Bioinformatics tools for basic analysis of Next Generation Sequencing data – curated by EURL-*E. coli.*
- Guidance document for cluster analysis of whole genome sequence data – curated by EURL-*Campylobacter*.
- Inventory of training supports – curated by EURL-Coagulase-Positive Staphylococci.
- Survey on the use of NGS across the NRLs networks – curated by EURL-*E. coli.*

These documents can be approached through the NGS page on https://www.eurlsalmonella.eu/publications/analytical-methods - Next Generation Sequencing (NGS).

The following documents are expected to be published early 2021:

- Overview of conducted and planned PTs – curated by EURL-Antimicrobial Resistance.
- NGS laboratory procedures – curated by EURL-Parasites
- Guidance document for NGS-Benchmarking – curated by EURL-*Listeria monocytogenes*.

Upon an initiative of the EURL-*E. coli*, on behalf of the joint working group EURLs NGS, a proposal for organising a joint NGS workshop was sent to the Med-Vet-Net Association by the end of 2019. This workshop aimed to inform Member States about the benefits of introducing NGS for typing food-borne pathogens and to start a general discussion on the infrastructure needed to enable the competent authorities to take measures based on evidences from the application of NGS, including the need for a legal framework. The workshop was originally planned to be organised in Rome on 10 March 2020, but due to the COVID-19 pandemic it was postponed to 25 September 2020 and turned into a webconference. The NRLs of the 8 EURLs networks were informed about the workshop at several times (December 2019 and April and July 2020). The conference was very well received (over 500 participants) and the working group is considering to organise a second conference, to give a follow-up to the questions from the first conference, in fall 2021.

Deliverables

Progress report EURLs Working group on WGS, 10 July 2019.
Minutes meetings EURLs WG NGS of June and December 2019 and of June and December 2020.
Flyer Met-Vet-Net workshop 'Science meets Policy' conference: Modern technologies to enable response to crises: Next Generation Sequencing to tackle food-borne diseases in the EU (25 September 2020), sent to NRLs-*Salmonella* for the first time in December 2019, followed by information on postponement and changing the conference into a webconference, in April and July 2020.
Preparation of guidance document Inter-EURLs WG on NGS 'Reference WGS collection' in fall 2020 and publication at the EURL-*Salmonella* website early 2021.
Adding links to the other guidance documents of the Inter-EURLs WG on NGS at the NGS page of the EURL-*Salmonella* website: https://www.eurlsalmonella.eu/publications/analytical-methods - Next Generation Sequencing (NGS).

Meetings (teleconferences and missions)

Meetings EURLs working group NGS
11 June 2019: Brussels, Belgium
Participant: Angela van Hoek
13 December 2019: Brussels, Belgium

Participants: Kirsten Mooijman, Angela van Hoek
16 June 2020: Teleconference
Participant: Angela van Hoek
15 December 2020: Teleconference
Participants: Kirsten Mooijman, Angela van Hoek

Conference "Science meets Policy" Modern technologies to enable response to crises: Next Generation Sequencing to tackle food-borne diseases in the EU
25 September 2020: Webconference
Participants: Angela van Hoek and Robin Diddens

1.3 Sub-activity 1.3 Proficiency Tests

PTs organised in 2019
In 2019, three Proficiency Tests (PTs) 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 EN ISO 22117:2019 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: negative, low level and high level samples. However, this number of samples is not a 'normative' number and to make the EURL-*Salmonella* studies less 'predictive', the number of samples of the second detection study organised in 2019 was amended to 4 negative, 6 low level and 4 high level samples.

To learn more about 'General requirements for proficiency testing according to EN ISO/IEC 17043 (2010)', one staff member of the EURL-*Salmonella* participated in a training course on this subject organised by the EC-Joint Research Centre (JRC) in Geel, Belgium in February 2019.

Follow-up Proficiency Test on typing of *Salmonella* organised in 2018
In November 2018 the 23rd Proficiency Test 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 36 NRLs for *Salmonella* participated: 29 NRLs from the 28 EU Member States (MSs) and 7 NRLs from third countries (EU (potential) candidate MSs, members of the European Free Trade Association (EFTA) 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. Three participants did not meet the level of good performance at the first stage of the study and a follow-up study for these laboratories was organised in March/April 2019. Two of these laboratories met the level of good performance in the follow-up study, but one laboratory did not. This latter laboratory (from a third country) participated for the first time in a PT for typing of *Salmonella* and has some problems in obtaining all relevant, and of good quality, antisera. In May 2019 a staff member of this NRL-*Salmonella* visited the EURL-*Salmonella* for a training on serotyping (see sub-activity 2.2).

The results of the study on PFGE typing were analysed in Spring 2019 and reported to the participants in April 2019. The results of all laboratories for both typing methods (serotyping and PFGE) were presented at the EURL-*Salmonella* workshop in May 2019.
More details on the typing study of 2018 can be found in the (interim) summary reports and in the full report (Jacobs-Reitsma et al., 2019a,b, 2020a).

Proficiency Tests on detection of *Salmonella* organised in 2019
In March 2019, a Proficiency Test on the detection of *Salmonella* in a food/feed matrix was organised. The matrix under analysis was flaxseed. Flaxseed is used as a food product as well as an ingredient of animal feed. Therefore, NRLs-*Salmonella* which analyse food (products), as well as NRLs-*Salmonella* which analyse animal feed were invited to participate in this PT.
In total 42 NRLs-*Salmonella* participated in this study: 37 NRLs from the 28 EU MSs and 5 NRLs from third countries (EU candidate MSs and potential EU candidate MSs and EFTA member countries). Of the 42 participants, 27 were NRLs-*Salmonella* for food & animal feed, 9 NRLs-*Salmonella* for food and 6 NRLs-*Salmonella* for animal feed.
Each NRL analysed a total of 20 samples: 18 samples of each 25 g flaxseed with different levels (6 negative, 6 low and 6 high level samples) of *Salmonella* Typhimurium and 2 control samples. The inoculation levels were: 10 cfu/sample and 105 cfu/sample.
The prescribed method was EN ISO 6579-1:2017 for analysing food samples and 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 by mid-April 2019 after which the analysis of the results was performed. Early May 2019, the participants received information on their results as well as an interim summary report including the results of all participants.
Forty-one laboratories fulfilled the criteria of good performance.
One laboratory scored a moderate performance. This laboratory made a mistake when entering the results of the two control samples and switched these results on the result form. This was confirmed by their raw data and no further actions were considered necessary for this laboratory.
More details can be found in the interim summary report and full report (Diddens and Mooijman, 2019a, b).

In October 2019, a Proficiency Test on detection of *Salmonella* in samples from the primary production stage (PPS) was organised. The matrix under analysis concerned artificially contaminated chicken faeces. In this study 35 NRLs participated: 29 NRLs from the 28 EU MSs and 6 NRLs from third countries (EFTA member countries, EU (potential) candidate MSs and one non-European country).
Each NRL analysed a total of 16 samples: 14 samples of each 25 g chicken faeces with different levels (4 negative, 6 low and 4 high level samples) of *Salmonella* Typhimurium and 2 control samples. The inoculation levels were: 16 cfu/sample and 30 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 2019, after which the analysis of the results was performed. In December 2019, the participants received information on their performance as well as an interim summary report including the results of all participants. In total, 34 laboratories fulfilled the criteria of good performance for the prescribed method. One laboratory scored a moderate performance because it switched the results of the positive and negative control samples.
More details on the study can be found in the (interim) summary report and full report (Pol-Hofstad and Mooijman, 2019b, 2020a).

Proficiency Test on typing of *Salmonella* organised in 2019

In November 2019 the 24th Proficiency Test on typing of *Salmonella* strains was organised. This study contained a compulsory part on serotyping and a new, voluntary part for molecular sub-typing to determine whether some strains concern common types (cluster analysis). The (molecular) methods used for this part were free of choice (PFGE, and/or MLVA, and/or WGS).
In total 35 NRLs for *Salmonella* participated: 29 NRLs from the 28 EU MSs and 6 NRLs from third countries (EU (potential) candidate MSs, and EFTA countries).
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.
Twenty NRLs participated in the new part on cluster analysis. For this, 10 different *Salmonella* strains had to be analysed.
The NRLs reported the results of the serotyping before mid-December 2019. The deadline for reporting the results of the cluster analysis was set at 31 January 2020.
The analysis of the serotyping results was performed in January/February 2020 after which the NRLs received their own results and an interim summary report, including the results of all laboratories in February 2020. Two participants did not meet the level of good performance in this study. Both concerned NRLs from non-EU MSs and are not obliged to participate in a follow-up study. In one NRL the unsatisfactory performance was caused by one mistake in the serotyping of one of the top-5 *Salmonella* serovars. This NRL immediately performed an internal investigation to the cause of the mistake and could pinpoint the problem to be caused by lack of staff at the date of the PT. Re-analysis of the 'problem-strain' by another staff member showed good results. The second NRL was facing several problems with the serotyping of a number of strains in the PT, mainly due to lack of sufficient, relevant and of good quality, antisera. Similar problems were seen for this laboratory in the PT for typing of *Salmonella* in 2018. The EURL-*Salmonella* suggested to organise a training at the laboratory of this NRL, but due to the COVID-19 pandemic it has not yet been possible to organise such training.
The results of the study on cluster analysis were analysed in spring 2020 and reported to the participants in June 2020. In this pilot PT, participants were free to use their own definition of cluster analysis and 'closely related strains', which resulted, especially with WGS-derived data, in quite some differences in cluster identification. It was expected that two technical duplicates of the same strain would be reported of being part of the same cluster. However, this strain showed far more variability than expected, probably due to the biological variable nature of the strain. To obtain more information on the genetic stability of different strains some additional experiments were performed in summer and fall 2020. For this, the same strains as used for the PT were stored and sequenced after different times of storage. Additionally, the influence of repeated sub-culturing of the same strain on the WGS profile was tested. The results of these additional tests will be published in the full report of this PT (under preparation) and were used for the set-up of the PT on cluster analysis of *Salmonella* organised in 2020.
The results of all laboratories for both typing methods (serotyping and cluster analysis) were presented at the EURL-*Salmonella* workshop in September 2020.
More details on the typing study of 2019 can be found in the (interim) summary reports (Jacobs-Reitsma et al., 2020b,c). The full report is under preparation.

PTs organised in 2020

In 2020, also three PTs were organised by the EURL-*Salmonella*, of which again two studies on detection of *Salmonella* and one study on (sero)typing of *Salmonella*.

Proficiency Tests on detection of *Salmonella* organised in 2020

In March 2020, a Proficiency Test on the detection of *Salmonella* in Live Bivalve Molluscs (LBM) was organised for the NRLs-Salmonella. The matrix under analysis was mussels. Due to measurements taken against the COVID-19 pandemic, not all NRLs were able to participate in March. To give these laboratories the opportunity to participate in this PT, a second round was organised in August 2020.

In total 23 NRLs-*Salmonella* participated in this PT: 20 NRLs from 20 EU MSs and 3 NRLs from third countries (EU candidate MSs and EFTA countries).

The set-up of this study differed from other EURL-*Salmonella* PTs for detection of *Salmonella,* because it was considered important that the preparation of the mussels was part of the study. For that reason, it was not possible to spike the samples at the laboratory of the EURL, as for doing so the mussels had to be opened. Therefore a set-up was chosen in which the NRLs had to spike the mussel samples themselves with blindly coded reference materials. Each NRL received a package with 2 kg mussels and 4 vials of customised reference materials. Following a protocol, each NRL had to prepare 4 samples of each 25 g mussel flesh and intravalvular fluid. Next, the laboratories had to spike each sample with 100 µl of the reference material with the same number as the sample. Three reference materials contained *Salmonella* Typhimurium and one reference material did not contain *Salmonella* (negative sample). The inoculation levels of *Salmonella* Typhimurium in the mussel samples (tested at the EURL-*Salmonella* at the start of each round of the PT) were 13 cfu/mussel sample in March and 12 cfu/mussel sample in August 2020. The NRLs also had to test two control samples in the PT: a procedure control (only Buffered Peptone Water) and a positive control with *Salmonella*.

The prescribed method was EN ISO 6579-1:2017 for analysing food samples and animal feed samples, including selective enrichment in MKTTn broth and on MSRV agar or in RVS broth.

Twenty-one laboratories fulfilled the criteria of good performance. One laboratory scored a moderate performance as this NRL mixed up the control samples and one NRL scored an unsatisfactory performance in the first round of the PT in March 2020, because this NRL reported a false positive result for a *Salmonella*-negative mussel sample. This NRL participated in a follow-up study organised in August 2020, at the same time as the second round of the PT, but with a different set of samples. In the follow-up study this NRL scored a good performance.

Immediate after both rounds of the PT, each NRL received its own results (in April and September 2020), indicating if the results of the laboratory fulfilled the criteria of good performance or not. The results of all participants in this PT were presented at the EURL-*Salmonella* workshop in September 2020 and an interim summary report was sent to the NRLs in October 2020 (Diddens and Mooijman, 2020). The full report is under preparation.

In October 2020, a combined Proficiency Test for Primary Production stage (PPS) and Food was organised. The matrix under analysis concerned artificially contaminated hygiene swabs. In this study both NRLs-*Salmonella* for analysis of food samples as well as for analysis of samples from the primary production stage were invited to participate. In total 67 NRLs for *Salmonella* participated in the study, of which 37 NRLs PPS and 30 NRLs Food, originating from 34 countries: 27 EU MSs plus the United Kingdom, 5 third countries within Europe (EU (potential) candidate countries and EFTA countries) and one non-European country.

Each NRL analysed in total 16 samples: 14 hygiene swabs and 2 control samples. The hygiene swabs were all artificially contaminated with background flora (a mixture of *Escherichia coli* and *Citrobacter freundii* of approx. 10^8 cfu/swab) and in addition 6 swabs were artificially contaminated with a low level of *Salmonella* Typhimurium (inoculation level 7 cfu/sample) and 4 swabs with a high level of *S.* Typhimurium (inoculation level 47 cfu/sample).

The prescribed method for analyses was EN ISO 6579-1:2017, following the relevant procedure for the relevant NRL. Thus if the swabs were analysed by an

NRL for PPS, MSRV agar should be used as the only selective enrichment medium. If the swabs were analysed by an NRL for food, two selective enrichment media had to be used: MKTTn broth and MSRV agar or RVS broth. All NRLs reported the results by November 2020. In December 2020, the participants received information on their performance as well as an interim summary report including the results of all participants. Sixty-three laboratories fulfilled the criteria of good performance. The results of two laboratories were scored moderate due to incorrect reporting of their results of the positive control sample.

More details on the study can be found in the interim summary report (Pol-Hofstad and Mooijman, 2020b). The full report is under preparation.

Proficiency Test on typing of *Salmonella* organised in 2020

In November 2020 the 25th Proficiency Test on typing of *Salmonella* strains was organised. This study contained a compulsory part on serotyping and a voluntary part for molecular sub-typing to determine whether some strains concern common types (cluster analysis). The (molecular) methods used for this part were free of choice (PFGE, and/or MLVA, and/or WGS).
A total of 37 laboratories participated in this PT. These included 29 NRLs-*Salmonella* in the 27 EU MSs plus the United Kingdom, 2 NRLs of EU-candidate countries, 3 NRLs of EFTA countries, and 3 additional participants (including EFSA) to compare with their WGS-based results.
All (37) laboratories 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.
Twenty-one NRLs participated in the part on cluster analysis. For this, 10 different *Salmonella* strains had to be analysed. In this second pilot PT on cluster analysis, more guidance was given on the definition of cluster analysis than in the first pilot PT. The NRLs were informed that this study mimicked an outbreak investigation of monophasic *Salmonella* Typhimurium of a given MLVA and WGS type.
Due to the COVID-19 pandemic, some laboratories were facing problems to perform all analysis before the originally set deadline, so that the deadline for reporting the serotyping results was postponed to January 2021.
The serotyping results have been analysed and reported to the NRLs in the first quarter of 2021. The evaluation of the part on cluster analysis will be reported separately.
More details on the serotyping part of the study of 2020 can be found in the interim summary report (Jacobs-Reitsma et al., 2021).

Deliverables

2019

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2020

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2. Activity 2 – To provide scientific and technical assistance to NRLs

2.1 Sub-activity 2.1 Workshop

2019

On 28 and 29 May 2019, the annual workshop was organised in Amersfoort, the Netherlands.
A total of 51 participants were present at the workshop:
38 participants from 27 EU-MS
3 participants from the EFTA countries
4 participants from EU candidate MSs or potential EU candidate MSs
3 participants from EURL-*Salmonella*
2 guest speakers
1 participant from EFSA
A delegate of one NRL from a EU Member State, was unable to come to the workshop due to lack of staff.

Presentations were given on the following subjects:

- Results of the Proficiency Tests organised by the EURL-*Salmonella* since the previous workshop (May 2018);
- Proposals for new Proficiency Tests;
- Stalled *Salmonella* situation in EU & assessment EU reduction targets;
- *Salmonella* Agona in animal feed in Germany;
- *Salmonella* in fresh edible leaves;
- *Salmonella* in bivalve molluscs;
- Detection and differentiation of *Salmonella* spp., *S.* Typhimurium, and *S.* Enteritidis by multiplex real-time PCR;
- Multi-country outbreak of *Salmonella* Bareilly confirmed with WGS in the Czech Republic;
- Multi-country cluster of *Salmonella* Coeln in 2018: involvement of EURL/NRL-*Salmonella* network;
- Update on activities in ISO and CEN;
- WGS based typing of *Salmonella* spp. and molecular analyses;
- Update on joint ECDC-EFSA typing database; outcome of EFSA-ECDC working group on 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 47 workshop participants; 41 completed forms were returned, a response rate of 87%. 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 August 2019 and the final version was published in December 2019. All presentations were placed on the EURL-*Salmonella* website early June 2019: https://www.eurlsalmonella.eu/node/691

2020

Due to the COVID-19 pandemic, the workshop of 2020 was postponed from 28-29 May to 17-18 September 2020 and was changed into an online meeting. The advantage of an online meeting was the fact that it was possible to host more participants than in a physical meeting. In total 75 persons subscribed for participation in this workshop:

52 participants from 27 EU-MS
3 participants from 3 EFTA countries
9 participants from 5 EU candidate MSs or potential EU candidate MSs
5 participants from EURL-*Salmonella*
2 guest speakers
1 participant from EC DG SANTE
3 participants from EFSA
A delegate of one NRL from a EU Member State, was unable to participate in the workshop due to technical problems.

Presentations were given on the following subjects:

- Results of the Proficiency Tests organised by the EURL-*Salmonella* since the previous workshop (May 2019);
- Proposals for new Proficiency Tests;
- Cluster analysis of WGS-data;
- European Commission mandate on 'One-Health' system for the collection and analysis of WGS data from food/animal isolates;
- *Salmonella* Enteritidis outbreak in a hotel school in Belgium;
- WGS comparison of multidrug resistant *Salmonella* Infantis isolates from broilers and humans in the Netherlands;
- Development and testing of draft ISO/TS 6579-4: Identification of monophasic *Salmonella* Typhimurium;
- Comparison of *Salmonella* Typhimurium and monophasic variants from farms in East Anglia;
- Verification of methods following EN ISO 16140-3; Theory and practice;
- Work-program of the EURL-*Salmonella* for the coming year.

Immediately after the workshop an online evaluation form about the workshop was distributed and the participants were requested to complete this form anonymously. The response rate was 53%, which was lower than in former years (2019: 87%) and probably due to the fact that the evaluation form was not physically handed over to the participants, but had to be completed online. Still, a response rate of 53% is fine, especially as the respondents were satisfied with the workshop and considered the scientific programme as interesting.

More details on the presentations, discussion and evaluation of the workshop will be summarised in the report of the workshop, which is under preparation. The presentations, which the presenters allowed the EURL to make public, were placed on the EURL-*Salmonella* website one week after the EURL-*Salmonella* workshop in September 2020: https://www.eurlsalmonella.eu/workshops

Deliverables

- 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 (April 2019). https://www.rivm.nl/bibliotheek/rapporten/2018-0024.pdf
- Presentations of the workshop 2019 published at the EURL-*Salmonella* website (June 2019). https://www.eurlsalmonella.eu/node/691
- Mooijman, K.A. The 24th EURL-*Salmonella* workshop – 28 and 29 May 2019, Amersfoort, the Netherlands. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM Report 2019-0135 (December 2019). https://www.rivm.nl/bibliotheek/rapporten/2019-0135.pdf
- Presentations of the workshop 2020 published at the EURL-*Salmonella* website (September 2020). https://www.eurlsalmonella.eu/workshops

2.2 Sub-activity 2.2 Training courses

In May 2019, a one day training on serotyping was organised for a delegate of an NRL-*Salmonella* from a (potential) candidate country.

On 17 and 18 October 2019, a joint training course was organised by three EURLs (*Listeria monocytogenes*, *Salmonella* and STEC) on the use of bioinformatic tools for analysis of WGS typing data at the premises of the EURL-*Listeria monocytogenes*, Maisons-Alfort, France.
Of each EURL network, 4 NRLs participated, resulting in a total of 12 participants. From EURL-*Salmonella*, two staff members (Angela van Hoek and Robin Diddens) were part of the group of trainers at this course. The former joint trainings (2016-2018) concerned the use of BioNumerics for analysis of PFGE data of the three pathogens. In the first quarter of 2019, the NRLs of the 3 networks were requested to indicate their preferred subject for the 2019 training course. The majority of NRLs indicated a preference for a basic course on Bioinformatics tools for Whole Genome Sequencing data analysis. At this 1,5 days' training course an introduction was given by the trainers of the 3 EURLs on:

- Sequencing platform & output data;
- Quality check and basic analytical tools;
- WGS data analysis (cg/wgMLST, wgSNPs, k-mer approach);
- Bioinformatics analysis of NGS data: approaches and opportunities.

After the introduction, three tools were presented (BioNumerics, ARIES and SeqSphere) for analysing WGS data, followed by hands-on training by the participants with these three tools. The program of the training course can be found in Annex 1.

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 2, showing that the NRL-*Salmonella* participants were satisfied with the training course.

It was planned to organise a similar joint EURLs training course on the use of bioinformatic tools for analysis of WGS typing data in 2020. However, due to the COVID-19 pandemic it was not possible to organise a physical training. An online training was briefly considered, but it was thought to be too complicated for the participants with hardly or no prior experience with WGS data analysis to change this into an online training and for that reason this joint EURLs training has been postponed to 2021.

Early 2020 requests were received for trainings on serotyping, preparation of (stable) samples for microbiological PTs and MLVA typing. Unfortunately, none of these trainings could be organised due to the COVID-19 pandemic. Also these trainings are postponed to 2021/2022 and will be organised as soon as physical training courses are feasible again.

Deliverables

- Program training course on the use of bioinformatic tools for analysis of WGS typing data (Annex 1).
- Evaluation of the training course on the use of bioinformatic tools for analysis of WGS typing data, by NRL-*Salmonella* participants (Annex 2).

Missions

Training course WGS typing
17-18 October 2019: Maisons-Alfort, France
Participants: Angela van Hoek, Robin Diddens

2.3 Sub-activity 2.3 Scientific advice and support of NRLs

In 2019 and 2020, 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) and Amendment 1 of EN ISO 6579-1, for detection (including confirmation) of *Salmonella*.
- Explanation and interpretation of information in CEN ISO/TR 6579-3:2014 for serotyping of *Salmonella.*
- Information on the development of (draft) ISO/TS 6579-4 for identification of monophasic *Salmonella* Typhimurium.
- Advise on sampling at the primary production stage.
- Advise on preparation of samples and pooling of samples.
- Detection of *Salmonella* in different matrices, like live bivalve molluscs, chocolate, cocoa, cannabis, saffron, gelatin.
- Detection of *Salmonella* Gallinarum (biovars gallinarum and pullorum).
- Detection of *Salmonella* in reptile meat.
- Calculation of MPN.
- Validation and verification of (alternative) methods on detection, confirmation and/or serotyping of *Salmonella*.
- Application of (validated) alternative methods for detection and/or serotyping of *Salmonella*.
- Information on serotyping of different *Salmonella* serovars and advice on media/antisera for phase inversion.
- Advice on how to distinguish different *Salmonella* serovars or how to distinguish different biovars from one serovar.
- Information on the White-Kauffmann-Le Minor scheme and its supplements.
- Differentiation of *Salmonella* vaccine strains from wild strains;
- Identification of monophasic *Salmonella* Typhimurium.
- Information on control strain *Salmonella* Braenderup for PFGE typing.
- Advise on organisation of Proficiency Tests for qualitative microbiological (detection) methods.

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 Proficiency 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 2019 and 2020, the EURL-*Salmonella* performed (confirmation of) (sero)typing of several *Salmonella* isolates for NRLs-*Salmonella* of the following countries:

- Bulgaria (1 isolate).
- Denmark (1 isolate).
- Greece (8 isolates, including 2 possible new serovars).
- Ireland (3 isolates).
- Italy (2 isolates; for confirmation of MLVA reference strains).
- Lithuania (3 isolates, including 1 for WGS analysis).
- Slovak Republic (1 isolate).
- Switzerland (5 isolates).

In 2019, the EURL-*Salmonella* obtained 5 (frozen) samples from an NRL-*Salmonella* of an EU Member State for second opinion analysis. All samples were tested negative for *Salmonella*.

Three NRLs for *Salmonella* of EU MSs asked the EURL for sets of reference strains for MLVA typing of *Salmonella* Typhimurium and *Salmonella* Enteritidis. As the ownership of these sets of strains lies at other organisations (SSI, Denmark for the set of *Salmonella* Typhimurium strains and PHE, United Kingdom for the set of

Salmonella Enteritidis strains), the EURL discussed with SSI and PHE about the options for further distribution to the NRLs-*Salmonella*. Material Transfer Agreements (MTA's) were set up and agreed upon with (and signed by) SSI and PHE, as well as with the requesting NRLs. Next, the EURL-*Salmonella* cultured and checked the sets of strains before sending them to the NRLs.

In October 2019, the EURL-*Salmonella* approached the network of NRLs-*Salmonella* as well as the former NRLs for live bivalve molluscs to collect contact details of the organisations acting as NRL-*Salmonella* for live bivalve molluscs per 01-01-2019 (after the EURL for monitoring bacteriological and viral contamination of bivalve molluscs ceased to exist). By December 2019, replies were received of approx. 30 NRLs-*Salmonella* in the EU Member States, EU (potential) candidate countries and EFTA countries.
Related to this activity, the EURL-*Salmonella* published a document on its website on *Salmonella* detection in shellfish in January 2020. In this document information is given on transport and receipt of shellfish samples, on selection of shellfish for analysis and on preparation of shellfish samples for *Salmonella* analysis. The document is available at the EURL-*Salmonella* website: https://www.eurlsalmonella.eu/documenten/salmonella-detection-in-bivalve-molluscs

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 existed of 5 EURLs (*Listeria monocytogenes,* coagulase positive staphylococci, STEC, *Campylobacter* and *Salmonella*) and discussed draft versions of the guidance document in teleconferences in February and April 2019. In March 2019 a first version of this guidance document was sent to the NRLs of the 5 networks for comments. After incorporation of the comments, the second (and final) version of the guidance document was sent to the NRLs in July 2019 and published at the EURL-*Salmonella* website: https://www.eurlsalmonella.eu/documenten/eurls-guidance-document-nrl-pts

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 and information on activities of the EURL and/or NRLs.

Newsletters 2019 (volume 25)

- In March 2019, volume 25 no 1 of the newsletter was published, which included information on the Proficiency Tests organised in fall 2018, and information on the publication of Regulation (EU) 2019/229 (amending Regulation (EC) No 2073/2005).
- In June 2019, volume 25 no 2 of the newsletter was published including the timetables of the Proficiency Tests for detection of *Salmonella* in samples from the Primary Production Stage (September/October 2019) and of the Proficiency Test for typing of *Salmonella* (November 2019). Additionally, the EURL-*Salmonella* work programme of 2019-2020 was included, as well as the technical report on activities of the EURL-*Salmonella* performed in 2018. Furthermore, the NRLs were informed about the content of the (draft) amendment to EN ISO 6579-1:2017 and the possibility to comment on the DIS version of the document from 08-07-2019 to 30-09-2019.
- In September 2019, volume 25 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 the results of the ISO/DIS voting of ISO 6579-1:2017/DAmd 1:2019.
- In December 2019, volume 25 no 4 of the newsletter was published, which included the tentative timetable of the EURL-*Salmonella* Proficiency Test Live Bivalve Molluscs 2020, on detection of *Salmonella* in mussels (March 2020).

Additionally, information was given on the Med-Vet-Net workshop on WGS (originally planned in March 2020).

Newsletters 2020 (volume 26)

- In March 2020, volume 26 no 1 of the newsletter was published, which included information on the Proficiency Tests organised in fall 2019, the planned PT on live bivalve molluscs and information on postponement of the EURL-*Salmonella* workshop and of the conference on NGS due to the COVID-19 pandemic.
- In June 2020, volume 26 no 2 of the newsletter was published including the timetables of the Proficiency Tests for detection of *Salmonella* in hygiene swabs (PT PPS-FOOD, September/October 2020) and of the Proficiency Test for typing of *Salmonella* (November 2020). Additionally, the updated agenda for the (online) conference on NGS was included. Furthermore, the NRLs were informed about the fact that the workshop was changed to an online meeting, and on publication of the New Work Item Proposal (NWIP) of ISO/TS 6579-4 (identification of monophasic *Salmonella* Typhimurium).
- In September 2020, volume 26 no 3 of the newsletter was published, which included again the time tables of the PTs PPS-FOOD 2020 and Typing 2020. Additionally, information was given on the PT on live bivalve molluscs (which was organised in 2 rounds) and on the workshop organised in September.
- In December 2020, volume 26 no 4 of the newsletter was published, which included the timetable of the EURL-*Salmonella* Proficiency Test FOOD 2021, on detection of *Salmonella* in liquid whole egg (March 2021). Additionally, some first results were given of the combined PT PPS-FOOD and on the progress with the PT on typing of *Salmonella*. Furthermore, a link to the presentations given at the conference on NGS, organised by the inter-EURLs WG on NGS (with support of the Med-Vet-Net association) was published.

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. In 2020 the website was reviewed thoroughly and at many pages text and links have been updated. Improvement of the website still continues in 2021.

Deliverables

- Guidance document for the organisation of Proficiency Tests by NRLs for national networks (joint EURLs document); Version 1, March 2019 and Version 2, July 2019. https://www.eurlsalmonella.eu/documenten/eurls-guidance-document-nrl-pts
- Four EURL-*Salmonella* Newsletters Vol. 25 No. 1-4 (2019), published at the EURL-*Salmonella* website: https://www.eurlsalmonella.eu/publications/newsletters
- Four EURL-*Salmonella* Newsletters Vol. 26 No. 1-4 (2020), published at the EURL-*Salmonella* website: https://www.eurlsalmonella.eu/publications/newsletters
- Document on *Salmonella* detection in matrix shellfish; EURL-*Salmonella* 10-01-2020. https://www.eurlsalmonella.eu/documenten/salmonella-detection-in-bivalve-molluscs

Meetings (teleconferences and missions)

Meetings joint EURLs working group on PTs
February 2019: Teleconference
April 2019: Teleconference
Participant: Kirsten Mooijman

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

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

In 2019 and 2020, 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):

- Request for clarification (DG SANTE) on *Salmonella* methods used in a third country.
- Participation in an interview for a study on the EU summary reports on zoonoses and food-borne outbreaks, and on antimicrobial resistance (on behalf of EFSA).
- Completion of a questionnaire (of DG SANTE) on accreditation.
- Request for help (ECDC) with sequencing of ESBL producing *Salmonella* Kentucky isolated from food.
- Give information (to EFSA) on detection of *Salmonella* in dried products.
- Request for information(DG SANTE) on how the EURL manage with requests from laboratories inside and outside EU and on participation of official laboratories in PTs.
- Request for information (ECDC) on the organisation of PTs (by the EURL) on WGS.
- Request for information (EFSA) on the use of MPN for analysing *Salmonella* in samples from the primary production stage of poultry.
- Request for information (Joint Research Centre) on the costs for detection and serotyping of *Salmonella*.
- Request for information (DG SANTE) on the precision of methods for detection and serotyping of *Salmonella* in samples from the primary production stage of poultry.
- Request for information (DG SANTE) on the effect of the COVID-19 pandemic on the activities of the NRLs and official laboratories.

In 2019 and 2020, 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.

- December 2018: call to the NRLs-*Salmonella* for sequence data (or isolates to be sequenced at EURL) of *Salmonella* Coeln found in food or in animals in 2018 (UI-526). Cluster analysis (cgMLST) of sequence data performed in 2019. Commented on Joint ECDC-EFSA Notification Summary - JNS (August 2019).
- February 2019: request (EFSA) for information on *Salmonella* Poona (UI-537; joint ECDC–EFSA Rapid Outbreak Assessment – ROA, March 2019).
- May-September 2019: call to the NRLs-*Salmonella* for sequence data (or isolates to be sequenced at the EURL) of monophasic *Salmonella* Typhimurium 3-13-11-00-211 found in food items in the period 2018-2019 (UI-483). MLVA typing and sequencing of some isolates; cluster analysis (cgMLST) of sequence data. Commented on JNS (August 2019).
- July-October 2019: call to some NRLs-*Salmonella* for sequence data (or isolates to be sequenced at the EURL) of *Salmonella* Bredeney found in a food product (UI-572). Sequencing of some isolates and cluster analysis (cgMLST) of sequence data.
- August 2019-Early 2021: call to some NRLs-*Salmonella* for sequence data (or isolates to be sequenced at the EURL) of *Salmonella* Enteritidis found in food products (UI-584 and UI-656). Collection of sequence data and uploading to the EFSA database, and cluster analysis (cgMLST) of sequence data.
- September-October 2019: call to some NRLs-*Salmonella* for sequence data (or isolates to be sequenced at the EURL) of *Salmonella* Muenchen found in a

food product (UI-591). Sequencing of some isolates and cluster analysis (cgMLST) of sequence data.
- November 2019 – Early 2020: call to the NRLs-*Salmonella* for sequence data (or isolates to be sequenced at the EURL) of *Salmonella* Enteritidis (MLVA types 2-9-7-3-2; 2-9-6-3-2; 2-9-10-3-2; 2-10-8-3-2; or 2-11-8-3-2) found in poultry production in 2018 and 2019 (UI-367). Cluster analysis (cgMLST) of sequence data. Commented on draft ROA (January 2020).
- November 2019 – Early 2020: call to the NRLs-*Salmonella* for sequence data (or isolates to be sequenced at the EURL) of *Salmonella* Enteritidis (MLVA types 2-11-7-3-2; 3-10-5-4-1; 2-10-7-3-2; or 3-11-5-4-1) found in food products, or in samples from the primary production stage in 2018 and 2019 (UI-601).
- November 2019 – Early 2020: call to the NRLs-*Salmonella* for sequence data (or isolates to be sequenced at the EURL) of *Salmonella* Mikawasima found in food products or in samples from the primary production stage in 2019 (UI-606). Cluster analysis (cgMLST) of sequence data.
- March 2020: call to some NRLs-*Salmonella* for sequence data (or isolates to be sequenced at the EURL) of *Salmonella* Dublin (UI-627) and *Salmonella* Enteritidis (UI-620).
- March-July 2020: call to some NRLs-*Salmonella* for sequence data (or isolates to be sequenced at the EURL) of *Salmonella* Agona (UI-611).
- May-October 2020: call to the NRLs-*Salmonella* for sequence data (or isolates to be sequenced at the EURL) of *Salmonella* Typhimurium from (any) food products, animal feed or animals in the first quarter of 2020 (UI-636); followed by a more specific request for sequence data (or isolates) of *Salmonella* Typhimurium and/or *Salmonella* Anatum found in nuts or products derived from nuts in the period September 2019 until September 2020. Sequencing of some isolates and cluster analysis (cgMLST) of sequence data.
- July-December 2020: Collection of sequence data and uploading to the EFSA database of *Salmonella* Enteritidis closely related to UI-644.

In February 2020, ECDC and EFSA asked the EURL-*Salmonella* to start a monitoring on the incidence of *Salmonella* Mikawasima in food (products), animals, animal feed or the environment. The aim of this monitoring was to follow cases throughout the year, as there seems to be a yearly trend with peaks in human cases across EU/EEA member states in autumn each year. By monitoring the events during the year, EFSA and ECDC could be prepared to react more rapidly when outbreaks are reported. The NRLs-*Salmonella* were asked to report any findings of *Salmonella* Mikawasima in food (products), animals, animal feed or the environment throughout 2020 in a digital reporting form to the EURL-*Salmonella*. The link to this form was (also) made available through the EURL-*Salmonella* website. When one or more isolates were reported, the EURL-*Salmonella* contacted the reporting NRL for possible sharing of the WGS data or for sharing the isolate(s) so that the EURL could perform the sequencing. Sequence data and (if available and allowed) metadata were shared with EFSA by the EURL. From mid-March 2020 until January 2021, 6 NRLs-*Salmonella* from 5 EU MS and from one EFTA country reported in total 33 *Salmonella* Mikawasima strains isolated from food or animals in 2020.

One or two members 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 2019, the steering committee organised two physical meetings (in February and October 2019) in Brussels, Belgium and Parma, Italy. In 2020, the steering committee organised one physical meeting (February) in Stockholm and two teleconferences (November and December).

In February 2019, ECDC organised its 9th Food-and Waterborne Diseases and Zoonoses Network meeting in Stockholm, Sweden. The EURLs of the EFSA-ECDC steering committee were invited to participate in this meeting.

The EFSA pilot database for the collection of molecular data was activated in December 2014 for collection of PFGE data (*Salmonella*, *Listeria monocytogenes* and STEC) and MLVA data (*S.* Typhimurium and *S.* Enteritidis). However, for *Salmonella* little activities have been employed. 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 needed to agree for its own country which molecular typing data are suitable for uploading in the database and who in the MS was allowed and able to do so. Additionally, the molecular (sub)typing method of choice changed from PFGE and MLVA to WGS. In 2019, it was not yet possible to upload WGS data to the database. In December 2019, ECDC and EFSA received a mandate from the EC to set up two interoperable systems for the collection and sharing of WGS data. In the first quarter of 2020, EFSA set-up a temporary solution for collection of WGS data. The development of this One Health WGS data collection system has been presented and discussed by EFSA in teleconferences with the EURL-*Salmonella*. Additionally, the information was shared with the NRLs-*Salmonella* at the EURL-*Salmonella* workshops.

In May 2019, delegates from the Food Safety and Inspection Service (FSIS), USA and in November 2019, delegates from the Food and Drug Administration (FDA), USA visited the RIVM. Kirsten Mooijman, Angela van Hoek and Irene Pol presented the role and activities of the EURL-*Salmonella* and the Dutch NRL-*Salmonella* at these visits.

In December 2019 and in October 2020, DG SANTE organised meetings for the directors of the EURLs and EURCs in the field of animal health, animal welfare, plant health and food and feed safety. In 2019, 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. In 2020, the EURLs were updated on information in the OCR on accreditation of laboratory methods, exchanges of views on organisation of annual workshops in 2021 (face-to-face vs remote), on difficulties encountered for implementing EURLs/EURCs tasks due to the COVID-19 pandemic, on financial aspects of EURLs/EURCs and on the status of laboratories in United Kingdom and Northern Ireland after Brexit.
The head of the EURL-*Salmonella* also participated in these meetings.

Deliverables

- Collection of information, MLVA typing and/or sequencing of isolates and cluster analysis of sequence data (cgMLST) possibly related to multi-country outbreaks with *Salmonella* Coeln, *Salmonella* Poona, monophasic *Salmonella* Typhimurium, *Salmonella* Bredeney, *Salmonella* Muenchen, *Salmonella* Enteritidis (different MLVA/WGS types), *Salmonella* Mikawasima, *Salmonella* Dublin, *Salmonella* Agona, *Salmonella* Typhimurium and *Salmonella* Anatum.
- Collection of sequence data and/or sequencing of isolates and cluster analysis of sequence data (cgMLST) from the monitoring of *Salmonella* Mikawasima.
- Contribution to relevant joint ECDC-EFSA Rapid Outbreak Assessments and/or joint ECDC-EFSA Notification Summaries.
- Co-author of a publication on *Salmonella* Enteritidis outbreak: Roan Pijnacker, Timothy J Dallman, Aloys S L Tijsma, Gillian Hawkins, Lesley Larkin, Saara M Kotila, Giusi Amore, Ettore Amato, Pamina M Suzuki, Sarah Denayer, Sofieke Klamer, Judit Pászti, Jacquelyn McCormick, Hassan Hartman, Gareth J Hughes, Lin C T Brandal, Derek Brown, Joël Mossong, Cecilia Jernberg, Luise Müller, Daniel Palm, Ettore Severi, Joannna

  Garvey, Kirsten Mooijman, Ingrid H M Friesema, Coen van der Weijden,

Menno van der Voort, Valentina Rizzi, Eelco Franz, on behalf of the International Outbreak Investigation Team, 2019. An international outbreak of *Salmonella enterica* serotype Enteritidis linked to eggs from Poland: a microbiological and epidemiological study. The Lancet Infectious Diseases Vol 19, Issue, pp 778-786, 7 July 2019. https://www.thelancet.com/journals/laninf/article/PIIS1473-3099(19)30047-7/fulltext

Meetings (teleconferences and missions)

2019

Meetings EFSA-ECDC steering committee
20 February 2019: Brussels, Belgium
7-8 October 2019: Parma, Italy
Participant: Kirsten Mooijman

Upon invitation (and for budget) of ECDC, participation in 9th Food-and Waterborne Diseases and Zoonoses Network meeting
7-8 February 2019: Stockholm, Sweden
Participant: Kirsten Mooijman

Meeting of directors of the EURLs in the field of animal health and food and feed safety.
12 December 2019: Brussels, Belgium
Participant EURL-*Salmonella*: Kirsten Mooijman

2020

Meetings EFSA and EURL-*Salmonella,* discussion of *Salmonella* outbreaks
14 February 2020: Teleconference
3 March 2020: Teleconference
Participants: Kirsten Mooijman and Angela van Hoek

Meetings EFSA-ECDC steering committee
25-26 February 2020: Stockholm, Sweden
Participant: Kirsten Mooijman
17 November 2020: Teleconference
1 December 2020: Teleconference
Participants: Kirsten Mooijman and Angela van Hoek

Meetings EFSA and EURL-*Salmonella* on One Health WGS data collection system
16 March 2020: Teleconference
25 August 2020: Teleconference
Participants: Kirsten Mooijman and Angela van Hoek

Meeting directors of the EURLs and EURCs in the field of animal health, animal welfare, plant health and food and feed safety
28 October 2020: Teleconference
Participant EURL-*Salmonella*: Kirsten Mooijman

4. Activity 4 – Reagents and reference collections

4.1 Sub-activity 4.1 Reference strains and reference materials

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/analytical-methods - Serotyping of *Salmonella*

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.

In 2019, the EURL-*Salmonella* signed Material Transfer Agreements (MTA's) with Public Health England (PHE), United Kingdom and with Statens Serum Institute (SSI), Denmark, about providing NRLs-*Salmonella* with sets of reference strains for MLVA typing. For this purpose, a set of 33 different strains of *S.* Typhimurium (ownership at SSI) and a set of 16 different strains of *S.* Enteritidis (ownership at PHE) have been stored at, and controlled by, the EURL-*Salmonella.*

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.
In 2020, more detailed information on (microbiological) reference materials has been added to the EURL-*Salmonella* website. Also a list of culture collections and producers of microbiological (certified) reference materials have been added (lists not exhaustive). Details can be found at the page 'Reference materials' at the following link:
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

CD	Committee Draft
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
EU	European Union
EURC	European Reference Centre
EURL	European Union Reference Laboratory
FDIS	Final Draft International Standard
ISO	International Standardization Organization
JNS	Joint (EDCD-EFSA) Notification Summary
LBM	Live Bivalve Molluscs
MKTTn	Mueller Kauffmann Tetrathionate novobiocin broth
MLVA	Multi-Locus Variable number tandem repeat Analysis
MPN	Most Probable Number
MS	Member State
MSRV	Modified Semi-solid Rappaport Vassiliadis
NGS	Next Generation Sequencing
NRL	National Reference Laboratory
NWIP	New Work Item Proposal
OCR	Official Control Regulation
PHE	Public Health England
PFGE	Pulsed Field Gel Electrophoresis
PPS	Primary Production Stage
PT	Proficiency Test
RIVM	National Institute for Public Health and the Environment
ROA	Rapid Outbreak Assessments
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
UI	Urgent Inquiry
WD	Working Draft
WG	Working Group
WGS	Whole Genome Sequencing
XLD	Xylose Lysine Deoxycholate (agar)

References

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Annex 1 Program training course on the use of bioinformatic tools for analysis of WGS typing data of shiga-toxin producing *Escherichia coli, Salmonella* and *Listeria monocytogenes*

Fourth Joint Training Course
Analysis of molecular typing data
Basic course on Bioinformatics tools for Whole Genome Sequencing data analysis

17-18 October 2019

French agency for food, environmental and occupational health & safety (ANSES)
14 rue Pierre et Marie Curie
94701 Maisons-Alfort

Organized for the 3 networks of National Reference Laboratories for STEC, *Listeria monocytogenes* & *Salmonella* by:

- **The EU Reference Laboratory for STEC (ISS:** Antonella Maugliani, Valeria Michelacci)
- **The EU Reference Laboratory for *L. monocytogenes* (ANSES-Laboratory for Food Safety:** Benjamin Felix, Maroua Sayeb**)**
- **The EU Reference Laboratory for *Salmonella*** (**RIVM**: Angela van Hoek, Robin Diddens)

Funded by the European Commission – DG SANTE

Thursday 17 October

9.00 **Registration**
9.15 Welcome and general overview on the training course (B. Lombard)
9.30 Introduction to WGS
1. Sequencing platform & output data (Maroua Sayeb)
2. Quality check and basic analytical tools (Valeria Michelacci)
3. WGS data analysis (cg/wgMLST, wgSNPs, k-mer approach) (Angela van Hoek)

11.00 **Coffee break**
11.30 Bioinformatics analysis of NGS data: approaches and opportunities (Antonella Maugliani)
11.45 *Lm* opportunity to use WGS - cluster analysis pipeline from front line method to WGS typing (Benjamin Felix)
12:15 General hints on the use of BioNumerics (EURL-Lm)
12.30 **LUNCH** (at ANSES canteen)
13.30 **Hands on exercises – BioNumerics pipeline applied on *Listeria* WGS typing**
16.00 General hints on the use of ARIES (EURL-VTEC)
16.15 **Hands on exercises – Use of ARIES for STEC WGS typing**
17.30 End of the first day

Friday 18 October

9.00 **Hands on exercises – Use of ARIES for STEC WGS typing**
10.15 General hints on the use of SeqSphere (EURL- *Salmonella*)
10.30 Hands on exercises – WGS serotyping & SeqSphere pipeline applied on Salmonella WGS typing
13.00 **LUNCH** (at ANSES canteen)
14.00 **Closing**

Annex 1 Evaluation joint training course organised by 3 EURLs on analysis of WGS typing data (17 and 18 October 2019)

Evaluation Joint Training Course of 3 EURLs on analysis of WGS typing data of STEC, *Salmonella* and *Listeria monocytogenes*
Dates: 17 and 18 October 2019.
Location: EURL-*Listeria monocytogenes*, Maisons Alfort, France.

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

Total number of participants training course (of which NRL-*Salmonella***)**	12 (4)
Number of participants (NRLs-*Salmonella***) completing evaluation**	4 NRLs-*Salmonella*
Participating countries (NRLs-*Salmonella*)	France, Germany, Slovenia, Spain
What did you expect to learn from this training (on forehand)?	- How to interpret raw WGS data and particularly the use of BioNumerics software. - See different options for data analysis. - Get an insight, talk to people dealing with similar issues while analysing outbreaks with WGS. - To get an insight to the entire WGS analysis pipeline from raw reads to specific analysis (e.g. resistance, serotyping, …); - Get an insight in different tools to be able to analyse sequence data.
Were the trainers able to fulfil your expectations	
Yes	4
No	-
Remarks	The presentations of the EURLs for *Salmonella* and STEC were really appreciated. The presentation of EURL-*Listeria* on BioNumerics was too fast and without support.
Was the time sufficient for your training?	
Yes	2
No too short	2; would have needed 0.5-2 days more.

Can you please describe (in short) what you have learned during the training?	- To better analyse our raw data and particularly to check the quality of our data. - I learned what I expected to learn (see earlier). - Basic principles and applications of all three tools for WGS data analysis. - The database used is very important when analysing data.
Is what you have learned during the training applicable in your laboratory?	
Yes	4
No	-
Remarks	- Really interesting.
Overall, did the training fulfil your expectations?	
Yes	4
No	-
Any other comments?	- The hands-on exercises (step by step) were very nice and helped for the comprehension. - Thank you very much for giving me the opportunity to participate in this training. - Enjoyed it a lot! - Thank you for the organisation and great two days! - The organisation of the training was great, thank you very much!

From the Literature

Salmonella-related Literature from Scopus: April – June 2021

Yamawaki, R.A., Rubio, M.D.S., Alves, L.B.R., de Almeida, A.M., Ferreira, T.S., Berchieri Junior, A., Penha Filho, R.A.C.
Evaluation of transfer of maternal immunity to the offspring of broiler breeders vaccinated with a candidate recombinant vaccine against Salmonella Enteritidis
(2021) Vaccine, 39 (17), pp. 2408-2415.
ABSTRACT: Salmonella Enteritidis (SE) is a major cause of foodborne diseases in humans being frequently related to the consumption of poultry products. Therefore, guaranteeing early immunity to chicks is an important tool to prevent the colonization and infection by this pathogen. The present study evaluated the effectiveness of a candidate recombinant vaccine against SE. Thirty female and five male broiler breeders that were ten weeks-old were divided into 3 groups: unvaccinated (UV), vaccinated with recombinant vaccine candidate (VAC) and vaccinated with commercial bacterin (BAC). Samples of serum and embryonated egg were collected at seven and twelve weeks after the booster dose to quantify the transfer rate of IgY to egg yolks and offspring. Subsequently, forty day-old offspring were divided into two groups (UV and VAC) and challenged on the following day with 107 CFU/chick of SE. Samples of serum, intestine, liver, and cecal content were harvested. Throughout the experiment period, significantly higher levels of IgY were observed in the egg yolk and also in the serum of broiler breeders and offspring of the VAC group in comparison to the UV group. In addition, increased transfer rates of IgY were observed in the VAC group when compared to the BAC group. Furthermore, higher villus-crypt ratios were found out in duodenum, jejunum and ileum at four days post-infection in the offspring from the VAC group. A high challenge dose of SE (107 CFU per chick) was used and despite the stronger humoral immune response provoked by the candidate vaccine, there were no statistical differences in the recovery of viable SE cells from the offspring cecal contents. Therefore, the effect of vaccination to improve intestinal quality may affect the development of the chickens and consequently increase the resistance to lower SE challenge doses. ISSN: 0264410X

Avidov, R., Varma, V.S., Saadi, I., Hanan, A., Lublin, A., Saldinger, S.S., Chen, Y., Laor, Y.
Factors Influencing the Persistence of Salmonella Infantis in Broiler Litter During Composting and Stabilization Processes and Following Soil Incorporation
(2021) Frontiers in Sustainable Food Systems, 5, art. no. 645721, .
ABSTRACT: Broiler litter (BL), a by-product of broiler meat production, is frequently contaminated with Salmonella and other zoonotic pathogens. To ensure the safety of crop production chains and limit pathogen spread in the environment, a pre-treatment is desired before further agricultural utilization. The objective of this study was to characterize the effect of physico-chemical properties on Salmonella persistence in BL during composting and stabilization and following soil incorporation, toward optimization of the inactivation process. Thirty-six combinations of temperature (30, 40, 50, and 60°C), water content (40, 55, and 70%; w/w), and initial pH (6, 7, and 8.5) were employed in static lab vessels to study the persistence of Salmonella enterica serovar Infantis (S. Infantis; a multidrug-resistant strain) during incubation of artificially-inoculated BL. The effect of aeration was investigated in a composting simulator, with controlled heating and flow conditions. Temperature was found to be the main factor significantly influencing Salmonella decay rates, while water content and initial pH had a secondary level of influence with significant effects mainly at 30 and 40°C. Controlled simulations showed faster decay of Salmonella under anaerobic conditions at mesophilic temperatures (<45°C) and no effect of NH3 emissions. Re-wetting the BL at mesophilic temperatures resulted in Salmonella burst, and led to a higher tolerance of the pathogen at increased temperatures. Based on the decay rates measured under all temperature, water content, and pH conditions, it was estimated that the time required to achieve a 7 log10 reduction in Salmonella concentration, ranges between 13.7–27.2, 6.5–15.6, 1.2–4.7, and 1.3–1.5 days for 30, 40, 50, and 60°C, respectively. Inactivation of BL indigenous microbial population by autoclaving or addition of antibiotics to which the S. Infantis is resistant, resulted in augmentation of Salmonella multiplication. This suggests the presence of

microbial antagonists in the BL, which inhibit the growth of the pathogen. Finally, Salmonella persisted over 90 days at 30°C in a Vertisol soil amended with inoculated BL, presumably due to reduced antagonistic activity compared to the BL alone. These findings are valuable for risk assessments and the formulation of guidelines for safe utilization of BL in agriculture. ISSN: 2571581X

Olsen, J.V., Christensen, T., Jensen, J.D.
Pig Farmers' Perceptions of Economic Incentives to Control Salmonella Prevalence at Herd Level
(2021) Frontiers in Veterinary Science, 8, art. no. 647697, .
ABSTRACT: This paper investigates how perceived costs and benefits of Salmonella control among Danish pig farmers affect the farmers' choice of action toward reducing the prevalence of Salmonella in their herds. Based on data from an online questionnaire involving 163 Danish pig farmers, we find a considerable uncertainty among pig farmers about the perceived effects of the Salmonella reducing actions. The results indicate large variations in the perceived costs of implementing different types of Salmonella reducing actions (management-, hygiene- and feed-related). For some cases, farmers associate net benefits and positive productivity effects with implementation of the actions while studies by the industry indicate net costs to the farmers. Differences among farmers support the idea of an outcome-based Salmonella penalty scheme but the large uncertainties about costs and effects of actions toward Salmonella control might hamper the effectiveness of such a penalty scheme as a regulatory instrument to affect farmer behavior.
ISSN: 22971769

Martínez-Puchol, S., Riveros, M., Ruidias, K., Granda, A., Ruiz-Roldán, L., Zapata-Cachay, C., Ochoa, T.J., Pons, M.J., Ruiz, J.
Dissemination of a multidrug resistant CTX-M-65 producer Salmonella enterica serovar Infantis clone between marketed chicken meat and children
(2021) International Journal of Food Microbiology, 344, art. no. 109109, .
ABSTRACT: The objective of the present study was to characterize Salmonella enterica serovar Infantis isolated from chicken meat determining their clonal relationships with S. Infantis isolated from children with diarrhea. Fifteen meat-recovered S. Infantis were analyzed. Susceptibility levels to 14 antibacterial agents, the presence of ESBL and that of inducible plasmid-mediated AmpC (i-pAmpC) were determined by phenotypical methods. The presence of ESBL and pAmpC was confirmed by PCR, and detected ESBL-encoding genes were sequenced and their transferability tested by conjugation. The presence of gyrA mutations as well as Class 1 integrons was determined by PCR. Clonal relationships were established by REP-PCR and RAPD. In addition, 25 clinical isolates of S. Infantis were included in clonality studies. All meat-recovered S. Infantis were MDR, showing resistance to ampicillin, nitrofurans and quinolones, while none was resistant to azithromycin, ceftazidime or imipenem. ESBL (blaCTX-M-65) and i-pAmpC (blaDHA) were detected in 2 and 5 isolates respectively (in one case concomitantly), with blaCTX-M-65 being transferable through conjugation. In addition, 1 isolate presented a blaSHV gene. All isolates presented D87Y at GyrA, nalidixic acid active efflux pump and a Class 1 integron of ~1000 bp (aadA1). Clonal analysis showed that all isolates were related. Further they were identical to MDR blaCTX-M-65-producing S. Infantis isolates causing children diarrhea in Lima. The dissemination of MDR blaCTX-M-65-producing S. Infantis between marketed meat and children highlights a public health problem which needs be controlled at livestock level. ISSN: 01681605

Milkievicz, T., Badia, V., Souza, V.B., Longhi, D.A., Galvão, A.C., Robazza, W.D.S.
Modeling Salmonella spp. inactivation in chicken meat subjected to isothermal and non-isothermal temperature profiles
(2021) International Journal of Food Microbiology, 344, art. no. 109110, .
ABSTRACT: Salmonella genus has foodborne pathogen species commonly involved in many outbreaks related to the consumption of chicken meat. Many studies have aimed to model bacterial inactivation as a function of the temperature. Due to the large heterogeneity of the results, a unified description of Salmonella spp. inactivation behavior is hard to establish. In the current study, by evaluating the root mean square errors, mean absolute deviation, and Akaike and Bayesian information criteria, the double Weibull model was considered the most accurate primary model to fit 61 datasets of Salmonella inactivation in chicken meat. Results can be interpreted as if the bacterial population is divided into two subpopulations consisting of one more resistant (2.3% of the total population) and one more sensitive to thermal stress (97.7% of the total population). The thermal sensitivity of the bacteria depends on the fat content of the chicken meat. From an adapted version of the Bigelow secondary model including both temperature and fat content, 90% of the

Salmonella population can be inactivated after heating at 60 °C of chicken breast, thigh muscles, wings, and skin during approximately 2.5, 5.0, 9.5, and 57.4 min, respectively. The resulting model was applied to four different non-isothermal temperature profiles regarding Salmonella growth in chicken meat. Model performance for the non-isothermal profiles was evaluated by the acceptable prediction zone concept. Results showed that >80% of the predictions fell in the acceptable prediction zone when the temperature changes smoothly at temperature rates lower than 20 °C/min. Results obtained can be used in risk assessment models regarding contamination with Salmonella spp. in chicken parts with different fat contents. ISSN: 01681605

Avila-Novoa, M.-G., Guerrero-Medina, P.-J., Navarrete-Sahagún, V., Gómez-Olmos, I., Velázquez-Suárez, N.-Y., De la Cruz-Color, L., Gutiérrez-Lomelí, M.
Biofilm formation by multidrug-resistant serotypes of salmonella isolated from fresh products: Effects of nutritional and environmental conditions
(2021) Applied Sciences (Switzerland), 11 (8), art. no. 3581, .
ABSTRACT: Salmonella serotypes can develop biofilms in fresh food products. This study focused on determining the antimicrobial resistance profile and the effects of different growth media and environmental conditions on biofilm formation by multidrug-resistant serotypes of Salmonella. All 49.4% of the Salmonella strains (five serotypes) were multidrug resistant. Assessment of the ability to form biofilms using the crystal violet staining method revealed that 95.6% of the strains of Salmonella were strong biofilm producers in 96-well polystyrene microtiter plates. Overall, 59.3% of the Salmonella strains showed the rdar (red dry and rough colony) morphotype, 2.1% pdar (pink dry and rough colony), 27.4% bdar (brown dry and rough colony) and 10.9% saw (smooth and -species biofilms of Salmonella serotypes showed a mean cell density of 8.78 log10 CFU/cm2 ± 0.053 in TSBS (1/20 diluted TSB (tryptic soy broth) + 1% strawberry residues) and 8.43 log10 CFU/cm2 ± 0.050 in TSBA (1/20 diluted TSB + 1% avocado residues) on polypropylene type B (PP) (p < 0.05). In addition, epifluorescence microscopy and scanning electron microscopy (SEM) enabled visualizing the bacteria and extracellular polymeric substances of biofilms on PP. Salmonella form biofilms depending on the serotype of the strains and the environmental conditions. Mono-species biofilms formed by Salmonella serotypes respond to nutrient limitation with the use of simplified culture media such as TSBA and TSBS.
ISSN: 20763417

Nurjayadi, M., Efrianti, U.R., Azizah, N., Kurniadewi, F., Saamia, V., Wiranatha, M., Nastassya, L., El-Enshasy, H.A.
Detection of Salmonella typhimurium on artificially contaminated milk by real time PCR using STM4497 and fljB primers
(2021) AIP Conference Proceedings, 2331, art. no. 040028, .
ABSTRACT: Detection of food-borne bacterial pathogens was developed to overcome the limitations. The aim of this research was to develop Salmonella typhimurium detection by Real Time Polymerase Chain Reaction (RT-PCR) using two pairs of primers. The ability of primer pairs to detect S. typhimurium is seen from cycle threshold or Ct. Artificially contaminated milk sample with 24 ng each microliter can be detected with fljB (flagellin gene) primers on Ct 12,933 and with STM4497 (hypothetical protein code) primers on Ct 13,665. The specificity test of both primers showed that melting temperature (Tm) of fljB was 80,5 degree Celsius, and STM449 was 81,6 degree Celsius. FljB and STM4497 primers gave an average detection limit respectively of 11,75 Colony Forming Unit (CFU) each milliliter and 6,8 CFU each milliliter. The time needed throughout the detection process of S. typhimurium with fljB and STM4497 primers is faster than conventional methods. Based on the results it can be concluded that primers fljB and STM449 S. typhimurium can be applied to detection and quantification of S. typhimurium in milk samples.
ISSN: 0094243X, ISBN: 9780735440753

Ahmed, M.F.E., El-Wahab, A.A., Kriewitz, J.-P., Hankel, J., Chuppava, B., Ratert, C., Taube, V., Visscher, C., Kamphues, J.
Mitigating the spread and translocation of salmonella enteritidis in experimentally infected broilers under the influence of different flooring housing systems and feed particle sizes
(2021) Microorganisms, 9 (4), art. no. 874, .
ABSTRACT: This study aimed to evaluate the influences of different flooring designs and feed particle sizes on the spread of Salmonella (S.) in broiler chickens. Birds (n = 480) were allocated to four different housing systems (fully littered with and without floor heating, partially and fully slatted flooring with sand bath) and two dietary treatments (finely and coarsely ground diets) in 24 boxes. Two broilers per box were experimentally infected with S. Enteritidis (8.00 log10 CFU/bird) at d 17. Salmonella prevalence in caecal

contents and the liver was highest in broilers housed on fully slatted floor until d 36/37 (88.1% and 91.5%, respectively), and lowest in litter flooring (caecal content 64.4%) and litter flooring with floor heating (liver 61.7%). In turn, broilers on littered flooring expressed the lowest Salmonella counts in caecal content at d 36/37 (2.21 ± 1.75 log10 CFU/g), partial slatted flooring the highest (3.76 ± 1.46 log10 CFU/g). The mean Salmonella count in the caecal content was significantly lower for birds fed a coarsely ground diet (0.96 and 1.94 log10 CFU/g) than a finely ground diet (5.07 and 3.34 log10 CFU/g) at d 23 and d 36/37, respectively (p < 0.0001). Slatted flooring with a sand bath did not show advantages in terms of Salmonella reduction, whereas the coarsely ground diet markedly reduced the spread of Salmonella. ISSN: 20762607

Bonifait, L., Thépault, A., Baugé, L., Rouxel, S., Le Gall, F., Chemaly, M.
Occurrence of salmonella in the cattle production in france
(2021) Microorganisms, 9 (4), art. no. 872, .
ABSTRACT: Salmonella is among the most common foodborne pathogens worldwide, and can lead to acute gastroenteritis. Along with poultry, cattle production is recognized as an important source of human infection. Salmonella transmission from cattle to humans can occur through the environment, or through close contact with sick animals or their derived products. This study aimed to investigate the intestinal carriage of Salmonella spp. within French cattle production. A total of 959 cattle intestinal samples, from one of the largest French slaughterhouses, were analyzed. Isolated strains were genotyped by pulsed field gel electrophoresis (PFGE), and a sub-selection was taken by whole genome sequencing (WGS). Twenty-nine samples were positive for Salmonella spp., yielding an esti-mated prevalence of 3% in cattle production. Eight different Salmonella serotypes were found: Mon-tevideo was the most prevalent (34%), followed by Mbandaka (24%) and Anatum (14%). PFGE gen-otyping allowed the clustering of Salmonella isolates according to their serotype. Within the clusters, some isolates presented 100% similarity. To investigate potential epidemiological links between them, WGS and core genome multilocus sequence typing (cgMLST) were used, revealing identical profiles between isolates originating from different areas and/or different animal breeds. This in-vestigation provides new insights on Salmonella serotype epidemiology in cattle production in France. ISSN: 20762607

Orlíková, H.
A cross-border outbreak of salmonella bareilly cases confirmed by whole genome sequencing, Czech Republic and Slovakia, 2017 to 2018
(2021) Eurosurveillance, 26 (14), art. no. 2000131, .
ABSTRACT: In August 2017, an increased incidence of Salmonella Bareilly was detected in the Czech Republic. An investigation was conducted with Slovakia to confirm the outbreak and identify the source. Probable outbreak cases were defined as cases with laboratory-confirmed S. Bareilly reported in either of the national surveillance systems, and/or the Czech and Slovak National Reference Laboratory databases from July 2017. Confirmed cases had the pulsed-field gel electrophoresis (PFGE) outbreak pulsotype or up to 5 alleles difference from outbreak cluster members by core genome multilocus sequence typing (cgMLST). PFGE and whole genome sequencing were used for isolate comparison. The same trawling questionnaire was used in both countries. By the end of October 2018, 325 cases were identified. Among 88 human S. Bareilly isolates analysed by PFGE, 82 (93%) shared an identical pulsotype; cgMLST of 17 S. Bareilly human isolates showed 1-2 allele difference. The trawling questionnaire excluded consumption of unusual or imported foods. In September 2018, an isolate closely related to the outbreak isolates was identified in a powdered egg product. A spray dryer was recognised as the contamination source and the production plant was closed. Using molecular typing methods, we detected a diffuse cross-border outbreak caused by S. Bareilly. ISSN: 1025496X

Johansen, T.B., Brandal, L.T., MacDonald, E., Naseer, U., Stefanoff, P., Røed, M.H., Berglund, T.M., Johannessen, G.S., Bergsjø, B., Vold, L., Lange, H.
Exotic dried fruits caused Salmonella Agbeni outbreak with severe clinical presentation, Norway, December 2018 to March 2019
(2021) Eurosurveillance, 26 (14), art. no. 2000221, .

ABSTRACT: We describe an outbreak of Salmonella Agbeni sequence type (ST)2009 infections in Norway. Between 31 December 2018 and 16 March 2019, 56 cases (33 female and 23 male; median age: 50 years, range: 2-91) were reported, of which 21 were hospitalised. Cases were defined as people living in Norway, with laboratory-confirmed infection with S. Agbeni ST2009 and cluster type (CT)2489, reported between 31 December 2018 and 30 March 2019. We conducted a case-control study, with three controls per case (matched by age, sex and municipality), using the Norwegian National Registry. Cases were more likely to have consumed a commercial mix of dried exotic fruits than controls (cases = 8, controls = 31; odds ratio: 50; 95% confidence interval: 3-2,437). The outbreak strain was confirmed by whole genome sequencing (WGS) and was isolated from the fruit mix consumed by cases, resulting in withdrawal from the market on 6 March 2019.The fruit mix consisted of fruits from different countries and continents. It was packed in Italy and distributed to several European countries, including Norway. However, no other countries reported cases. This outbreak highlights that dried fruits could represent a risk in terms of food-borne infections, which is of particular concern in ready-to-eat products. ISSN: 1025496X

Buytaers, F.E., Saltykova, A., Mattheus, W., Verhaegen, B., Roosens, N.H.C., Vanneste, K., Laisnez, V., Hammami, N., Pochet, B., Cantaert, V., Marchal, K., Denayer, S., De Keersmaecker, S.C.J.

Application of a strain-level shotgun metagenomics approach on food samples: resolution of the source of a Salmonella food-borne outbreak

(2021) Microbial genomics, 7 (4), .

ABSTRACT: Food-borne outbreak investigation currently relies on the time-consuming and challenging bacterial isolation from food, to be able to link food-derived strains to more easily obtained isolates from infected people. When no food isolate can be obtained, the source of the outbreak cannot be unambiguously determined. Shotgun metagenomics approaches applied to the food samples could circumvent this need for isolation from the suspected source, but require downstream strain-level data analysis to be able to accurately link to the human isolate. Until now, this approach has not yet been applied outside research settings to analyse real food-borne outbreak samples. In September 2019, a Salmonella outbreak occurred in a hotel school in Bruges, Belgium, affecting over 200 students and teachers. Following standard procedures, the Belgian National Reference Center for human salmonellosis and the National Reference Laboratory for Salmonella in food and feed used conventional analysis based on isolation, serotyping and MLVA (multilocus variable number tandem repeat analysis) comparison, followed by whole-genome sequencing, to confirm the source of the contamination over 2 weeks after receipt of the sample, which was freshly prepared tartar sauce in a meal cooked at the school. Our team used this outbreak as a case study to deliver a proof of concept for a short-read strain-level shotgun metagenomics approach for source tracking. We received two suspect food samples: the full meal and some freshly made tartar sauce served with this meal, requiring the use of raw eggs. After analysis, we could prove, without isolation, that Salmonella was present in both samples, and we obtained an inferred genome of a Salmonella enterica subsp. enterica serovar Enteritidis that could be linked back to the human isolates of the outbreak in a phylogenetic tree. These metagenomics-derived outbreak strains were separated from sporadic cases as well as from another outbreak circulating in Europe at the same time period. This is, to our knowledge, the first Salmonella food-borne outbreak investigation uniquely linking the food source using a metagenomics approach and this in a fast time frame. ISSN: 20575858

Steinbrunner, P., Marks, B.P., Ryser, E.T., Suehr, Q.J., Jeong, S.

Fate of salmonella and enterococcus faecium during pilot-scale spray drying of soy protein isolate

(2021) Journal of Food Protection, 84 (4), pp. 674-679.

ABSTRACT: Outbreaks and recalls associated with microbial contamination of powdered foods have raised concern for the safety of the spray-drying process and its products. However, little research on the fate of bacteria during the spray-drying process has been done, leaving much unknown about the risks of contamination in spray dryers. Therefore, quantifying the contamination levels of Salmonella and Enterococcus faecium (as a surrogate) in various locations within a pilot-scale spray dryer can help illustrate the distribution of bacterial contamination, including in the final product. A 10% (w/w)

dispersion of water and soy protein isolate was mixed with tryptic soy broth containing yeast extract inoculated with Salmonella enterica serovar Enteritidis phage type 30 (PT30) or E. faecium strain NRRL B-2354. This dispersion was spray dried using a pilot-scale tall-form cocurrent spray dryer at an inlet air temperature of 180, 200, or 2208C. After drying, samples of powder from eight locations within the system were collected or surface swabbed, plated, and enumerated. Spray drying achieved 2.40 to 4.15 and 2.33 to 2.83 log reductions in the concentrations of Salmonella and E. faecium, respectively, in the final powder product accumulated in the dryer's collectors. Salmonella and E. faecium were found in various concentrations in all locations within the spray dryer after a complete drying cycle. Differences in inlet air temperature between 180 and 2208C had no significant effect on the inactivation levels. As a surrogate, E. faecium was more resistant to spray drying than Salmonella. Overall, spray drying is capable of significant bacterial reduction in the final powder product, which can be combined with other hurdle technologies. However, adequate cleaning and sanitization procedures must be taken into consideration to prevent cross-contamination. ISSN: 0362028X

Channaiah, L.H., Michael, M., Acuff, J.C., Vega, D., Lopez, K., Phebus, R.K., Thippareddi, H., Milliken, G.
Validation of simulated commercial manufacturing of flour tortillas to control Salmonella contamination
(2021) Journal of Food Safety, 41 (2), art. no. e12879, .
ABSTRACT: This study validated a typical batch commercial flour tortilla cooking process against Salmonella contamination. Tortilla dough prepared from flour inoculated with a 7-serovar Salmonella cocktail was pressed in a dough press (preset at 200 °F [93.3°C]) for 3 s, then heated on a griddle (preheated to 221.1°C [430 °F]) for 30, 45 or 60 s on each side, followed by ~8 min of ambient air cooling. The maximum internal temperature of tortillas during cooking was >97°C for all cooking times. The Salmonella counts in tortillas decreased >6-log at all cooking times; however, tortillas cooked for 30 and 45 s had an undercooked appearance. The water activity and pH of cooked tortillas after 60 s of heating on both sides followed by cooling were 0.934 and 6.62, respectively. The D-values of the Salmonella cocktail in raw tortilla dough were 22.2, 13.4, and 4.6 min at 55, 58, and 61°C, respectively, and the z-value was 8.9°C. ISSN: 01496085

La Tela, I., Peruzy, M.F., D'Alessio, N., Di Nocera, F., Casalinuovo, F., Carullo, M.R., Cardinale, D., Cristiano, D., Capuano, F.
Serotyping and evaluation of antimicrobial resistance of salmonella strains detected in wildlife and natural environments in southern Italy
(2021) Antibiotics, 10 (4), art. no. 353, .
ABSTRACT: Wild animals are potential vectors of antibiotic-resistant bacteria in the environment. The present study aimed to investigate the occurrence of antimicrobial resistance among Salmonella serovars isolated from wildlife and the environment in Italy. A total of 164 Salmonella isolates were analyzed, and six different subspecies and 64 serovars were detected. High proportions of Salmonella isolates proved resistant to streptomycin (34.1%), followed by trimethoprim-sulfamethoxazole (23.2%), tetracycline (17.7%), ciprofloxacin (14.63%) and ampicillin (11.59%). By source, the lowest level of resistance was observed in Salmonella serovars isolated from a water environment, while antimicrobial resistance was frequent in strains collected from shellfish, reptiles and birds. Multidrug-resistant strains were recovered from seafood (n = 11), mammals (n = 3) and water (n = 1). Three S. Typhimurium monophasic variant strains showed asimultaneous resistance to ampicillin, streptomycin, tetracycline and trimethoprim-sulfamethoxazole, which represents a recognized alert resistance profile for this serovar. These data indicate the environmental dissemination of resistant strains due to anthropogenic activities, which, in southern Italy, probably have a higher impact on marine ecosystems than on terrestrial ones. Moreover, as most of the animals considered in the present study are usually consumed by humans, the presence of resistant bacteria in them is a matter of great concern. ISSN: 20796382

Sher, A.A., Mustafa, B.E., Grady, S.C., Gardiner, J.C., Saeed, A.M.
Outbreaks of foodborne Salmonella enteritidis in the United States between 1990 and 2015: An analysis of epidemiological and spatial-temporal trends
(2021) International Journal of Infectious Diseases, 105, pp. 54-61.
ABSTRACT: Objectives: To evaluate the role of eggs and other food vehicles as risk factors associated with Salmonella enteritidis (SE) outbreaks in order to address the endemicity of SE infections in the USA. Methods: We retrieved and analyzed data relating to all SE outbreaks reported to the Centers for Disease Control and Prevention (CDC) between 1990 and 2015. We then used descriptive and analytical statistical methods, including negative

binomial regression models for the estimation of rate-ratios, to analyze the data. Results: Analyses showed that egg-based dishes were the most common food vehicle associated with outbreaks of SE in the USA (273 cases [24%]); this was followed by several other food items, including meat (130 cases [11%]), vegetables (96 cases [8%]), chicken items (95 cases [8%]), dairy products (55 cases [5%]), and bakery items (8 cases [1%]). Compared to egg-based dishes, other food items such as meat (exp( ) = 0.51, 95% CI 0.37, 0.69), chicken (exp( ) = 0.42, 95% CI 0.30, 0.58), vegetables (exp( ) = 0.41, 95% CI 0.29, 0.55), and dairy items (exp( ) = 0.27, 95% CI 0.18, 0.40) were significantly associated with outbreaks of SE in the USA. Of 1144 SE outbreaks, 402 (35%) occurred in the Northeast region of the USA, followed by the South (253 [22%]), West (250 [22%]), and Midwestern regions (239 [21%]). Conclusions: Epidemiological and spatiotemporal trends analyses demonstrated that a significant proportions of Salmonella enteritidis outbreaks in the USA are attributed to food vehicles other than eggs. Our findings can be used to plan effective strategies to mitigate the increasing occurrence of foodborne SE outbreaks. ISSN: 12019712

Zeng, H., De Reu, K., Gabriël, S., Mattheus, W., De Zutter, L., Rasschaert, G.
Salmonella prevalence and persistence in industrialized poultry slaughterhouses
(2021) Poultry Science, 100 (4), art. no. 100991, .
ABSTRACT: Salmonella contamination sources and transmission routes were studied in 5 Belgian poultry slaughterhouses. Samples from the slaughter and cutting line after cleaning and disinfection were collected, as well as neck skin samples and thighs during slaughter of the first flock. In total, 680 swab and water samples were taken from the slaughter line before slaughter. In all slaughterhouses, Salmonella was notwithstanding cleaning and disinfection still isolated from the slaughter line before start of activities. The prevalence of Salmonella in the plucking area was 10.4% (38/365) (hanging area: 5.0%, scalding tank: 5.8%, plucking machine: 17.0%); in the evisceration room, 1.5% (2/138); and in the cutting area, 2.0% (3/149). No Salmonella (0/28) was found in samples from the chilling line. On neck skin samples taken from the various lines, Salmonella prevalence was 16.1% (48/299) after plucking, 16.0% (48/300) after evisceration, 23.3% (70/300) after chilling; on thighs, prevalence was 10.0% (24/240). Nine Salmonella serotypes were identified of which Salmonella Infantis was the most common serovar (53.8%), especially in slaughterhouse A. Two contamination causes were identified; first, although all flocks had an official Salmonella negative status, this was in one case incorrect and led to an enormous contamination of the neck skins of the flock and the slaughterline (i.e., cooling water). Second, molecular typing revealed cross-contamination from flocks slaughtered 1 d before sampling. Salmonella was apparently not always eliminated by the cleaning and disinfection process and able to contaminate the carcasses of the first slaughtered flock. In conclusion, the results of this study provided practical insights for poultry production to further improve their Salmonella control, for example, Salmonella status determination closer to the slaughter date, to adapt cleaning and disinfection protocols especially for critical machinery and better hygienic designed equipment. ISSN: 00325791

Kreitlow, A., Becker, A., Schotte, U., Malorny, B., Plötz, M., Abdulmawjood, A.
Evaluation of different target genes for the detection of Salmonella sp. by loop-mediated isothermal amplification
(2021) Letters in Applied Microbiology, 72 (4), pp. 420-426.
ABSTRACT: The loop-mediated isothermal amplification (LAMP) technique was used to investigate six salmonella-specific sequences for their suitability to serve as targets for the pathogen identification. Sequences selected for designing LAMP primers were genes invA, bcfD, phoP, siiA, gene62181533 and a region within the ttrRSBCA locus. Primers including single nucleotide polymorphisms were configured as degenerate primers. Specificity of the designed primer sets was determined by means of 46 salmonella and 32 other food- and waterborne bacterial reference species and strains. Primers targeting the ttrRSBCA locus showed 100 % inclusivity of target and exclusivity of other test species and strains. Other primer sets revealed deficiencies, especially regarding Salmonella enterica subsp. II–IV and Salmonella bongori. Additionally, primers targeting the siiA gene failed to detect S. enterica subsp. enterica serotypes Newport and Stanley, whereas bcfD primers did not amplify DNA of S. enterica subsp. enterica serotype Schleissheim. TtrRSBCA primers, providing short detection times and constant melting temperatures of amplification products, achieved best overall performance. ISSN: 02668254

Sarjit, A., Ravensdale, J.T., Coorey, R., Fegan, N., Dykes, G.A.
Salmonella survival after exposure to heat in a model meat juice system
(2021) Food Microbiology, 94, art. no. 103628, .

ABSTRACT: The effect of heat against eleven Salmonella strains in model meat juices was examined. Juices from beef, lamb and goat were made from either the fatty layer (FL), muscle (M) or a mixture of both (FLM). The pH of each FLM sample was altered to match the pH of PBS and vice versa to determine the pH effect on the survival of Salmonella against the effect of heat. Salmonella were exposed to either gradual heating to 70 °C in FLM, M and FL or heat shock at 70 °C for 5 min in FLM. Fat, fatty acid profile and iron

reduced Salmonella as compared to the untreated controls (~1.92–

controls (~5.80–
than other juices. The fat content in lamb FL (3.25%) was s

The omega 6 and linoleic acid content in goat FLM (~36.0% and ~34.4%) was significantly

differentially protect Salmonella against the effect of heat in these juices. ISSN: 07400020

Levent, G., Schlochtermeier, A., Ives, S.E., Norman, K.N., Lawhon, S.D., Loneragan, G.H., Anderson, R.C., Vinasco, J., den Bakker, H.C., Scott, H.M.
High-Resolution Genomic Comparisons within Salmonella enterica Serotypes Derived from Beef Feedlot Cattle: Parsing the Roles of Cattle Source, Pen, Animal, Sample Type, and Production Period
(2021) Applied and environmental microbiology, 87 (12), p. e0048521.
ABSTRACT: Salmonella enterica is a major foodborne pathogen, and contaminated beef products have been identified as one of the primary sources of Salmonella-related outbreaks. Pathogenicity and antibiotic resistance of Salmonella are highly serotype and subpopulation specific, which makes it essential to understand high-resolution Salmonella population dynamics in cattle. Time of year, source of cattle, pen, and sample type (i.e., feces, hide, or lymph nodes) have previously been identified as important factors influencing the serotype distribution of Salmonella (e.g., Anatum, Lubbock, Cerro, Montevideo, Kentucky, Newport, and Norwich) that were isolated from a longitudinal sampling design in a research feedlot. In this study, we performed high-resolution genomic comparisons of Salmonella isolates within each serotype using both single-nucleotide polymorphism-based maximum-likelihood phylogeny and hierarchical clustering of core-genome multilocus sequence typing. The importance of the aforementioned features in clonal Salmonella expansion was further explored using a supervised machine learning algorithm. In addition, we identified and compared the resistance genes, plasmids, and pathogenicity island profiles of the isolates within each subpopulation. Our findings indicate that clonal expansion of Salmonella strains in cattle was mainly influenced by the randomization of block and pen, as well as the origin/source of the cattle, i.e., regardless of sampling time and sample type (i.e., feces, lymph node, or hide). Further research is needed concerning the role of the feedlot pen environment prior to cattle placement to better understand carryover contributions of existing strains of Salmonella and their bacteriophages. IMPORTANCESalmonella serotypes isolated from outbreaks in humans can also be found in beef cattle and feedlots. Virulence factors and antibiotic resistance are among the primary defense mechanisms of Salmonella, and are often associated with clonal expansion. This makes understanding the subpopulation dynamics of Salmonella in cattle critical for effective mitigation. There remains a gap in the literature concerning subpopulation dynamics within Salmonella serotypes in feedlot cattle from the beginning of feeding up until slaughter. Here, we explore Salmonella population dynamics within each serotype using core-genome phylogeny and hierarchical classifications. We used machine learning to quantitatively parse the relative importance of both hierarchical and longitudinal clustering among cattle host samples. Our results reveal that Salmonella populations in cattle are highly clonal over a 6-month study period and that clonal dissemination of Salmonella in cattle is mainly influenced spatially by experimental block and pen, as well by the geographical origin of the cattle. ISSN: 10985336

González-Santamarina, B., García-Soto, S., Hotzel, H., Meemken, D., Fries, R., Tomaso, H.
Salmonella Derby: A Comparative Genomic Analysis of Strains From Germany
(2021) Frontiers in Microbiology, 12, art. no. 591929, .
ABSTRACT: Salmonella enterica subspecies enterica serovar Derby (S. Derby) is one of the most frequent causes for salmonellosis in humans and animals. Understanding the genetic diversity of S. Derby, as well as the nature and origin of its resistance to antimicrobial treatment are thus the key to epidemiological control and surveillance. Here, we report an analysis of 15 S. Derby strains isolated from pig and cattle in slaughterhouses across

Germany (2000–2015), which belonged to multilocus sequence types (ST) ST39, ST40 and ST682. Strains were compared to publicly available S. Derby sequence data of these three STs from Germany, comprising 65 isolates collected between 2004 and 2018 from different sources (i.e., pigs, humans, cattle, wild boar, and poultry). A total of 80 sequences (ST39 = 34, ST40 = 21, and ST682 = 25) were analyzed to assess genetic diversity, to identify virulence-associated and antimicrobial resistance genes (ARGs), and to characterize plasmid content. Strains belonging to all three STs were identified in each source examined. Strains with the same ST were closely related regardless of origin. Altogether, 72.5% of the isolates carried at least one resistance gene, furthermore ST40 carried most of the ARGs and the plasmid replicons. The IncI1 replicon was detected in eleven isolates, four of them carried IncI1 plasmid ST26 with clonal complex 2. The comparison of these four isolates with an IncI1 ST26 plasmid reported in 2010 from a German pig (JX566770), showed only variations in a region carrying different ARGs and mobile genetic elements. The strains of our collection had similar genetic diversity as the strains taken from the public database. Moreover, we found that strains harboring multidrug resistant IncI plasmid were found in different animal species, indicating that S. Derby may be implicated in the spread of antimicrobial resistance among animal species. Results may contribute to the knowledge about the diversity in S. Derby in Germany, which may be useful for the future surveillance and antimicrobial resistance of this serovar. ISSN: 1664302X

Cadel-Six, S., Cherchame, E., Douarre, P.-E., Tang, Y., Felten, A., Barbet, P., Litrup, E., Banerji, S., Simon, S., Pasquali, F., Gourmelon, M., Mensah, N., Borowiak, M., Mistou, M.-Y., Petrovska, L.

The Spatiotemporal Dynamics and Microevolution Events That Favored the Success of the Highly Clonal Multidrug-Resistant Monophasic Salmonella Typhimurium Circulating in Europe

(2021) Frontiers in Microbiology, 12, art. no. 651124, .

ABSTRACT: The European epidemic monophasic variant of Salmonella enterica serovar Typhimurium (S. 1,4,[5],12:i:-) characterized by the multi locus sequence type ST34 and the antimicrobial resistance ASSuT profile has become one of the most common serovars in Europe (EU) and the United States (US). In this study, we reconstructed the time-scaled phylogeny and evolution of this Salmonella in Europe. The epidemic S. 1,4,[5],12:i:- ST34 emerged in the 1980s by an acquisition of the Salmonella Genomic Island (SGI)-end of the phenylalanine phe tRNA locus conferring resistance to copper and arsenic toxicity. Subsequent integration of the Tn21 transposon into the fljAB locus gave resistance to mercury toxicity and several classes of antibiotics used in food-producing animals (ASSuT profile). The second step of the evolution occurred in the 1990s, with the integration of mTmV and mTmV-like prophages carrying the perC and/or sopE genes involved in the ability to reduce nitrates in intestinal contents and facilitate the disruption of the junctions of the host intestinal epithelial cells. Heavy metals are largely used as food supplements or pesticide for cultivation of seeds intended for animal feed so the expansion of the epidemic S. 1,4,[5],12:i:- ST34 was strongly related to the multiple-heavy metal resistance acquired by transposons, integrative and conjugative elements and facilitated by the escape until 2011 from the regulatory actions applied in the control of S. Typhimurium in Europe. The genomic plasticity of the epidemic S. 1,4,[5],12:i:- was demonstrated in our study by the analysis of the plasmidome. We were able to identify plasmids harboring genes mediating resistance to phenicols, colistin, and fluoroquinolone and also describe for the first time in six of the analyzed genomes the presence of two plasmids (pERR1744967-1 and pERR2174855-2) previously described only in strains of enterotoxigenic Escherichia coli and E. fergusonii. ISSN: 1664302X

Rodrigues, L.A., Wellington, M.O., González-Vega, J.C., Htoo, J.K., Van Kessel, A.G., Columbus, D.A.

A longer adaptation period to a functional amino acid-supplemented diet improves growth performance and immune status of Salmonella Typhimurium-challenged pigs

(2021) Journal of animal science, 99 (5), .

ABSTRACT: We recently showed that dietary supplementation with key functional amino acids (FAA) improves growth performance and immune status of Salmonella Typhimurium (ST)-challenged pigs. It is not known if ST-challenged pigs will benefit from a longer adaptation period to FAA. The objective of this study was to evaluate the effects of different adaptation periods to diets containing FAA above requirements for growth on performance and immune response of weaned pigs subsequently challenged with ST. A total of 32 mixed-sex weanling pigs (11.6 ± 0.3 kg) were randomly assigned to 1 of 4 dietary treatments, being a basal amino acid (AA) profile fed throughout the experimental period (FAA-) or a functional AA profile (FAA+; Thr, Met, and Trp at 120% of requirements) fed only in the postinoculation (FAA+0), for 1 wk pre- and postinoculation

(FAA+1), or throughout the experimental period (FAA+2). After a 14-d adaptation period, pigs were inoculated with ST (2.15 × 109 CFU/mL). Growth performance, body temperature, fecal score, acute-phase proteins, oxidant/antioxidant balance, score for ST shedding in feces and intestinal colonization, and fecal and digesta myeloperoxidase (MPO) were measured pre- and postinoculation. Postinoculation body temperature and fecal score, serum haptoglobin, plasma superoxide dismutase (SOD), malondialdehyde (MDA), and fecal MPO were increased while serum albumin and plasma reduced glutathione (GSH):oxidized glutathione (GSSG) were reduced compared to pre-inoculation ($P < 0.05$). Average daily gain and G:F were greater in FAA+2 pigs compared to FAA- pigs ($P < 0.05$). Serum albumin was higher in FAA+2 and FAA+1 compared to FAA+0 and FAA- pigs ($P < 0.05$) while FAA+2 pigs had lower haptoglobin compared to FAA- ($P < 0.05$). Plasma SOD was increased and GSH:GSSG was decreased in FAA- pigs compared to the other treatments ($P < 0.05$). Score for ST shedding in feces was progressively lower from d 1 to 6 regardless of treatment ($P < 0.05$) and was lower in FAA+2 pigs compared to FAA- and FAA+0 ($P < 0.05$). Counts of ST in colon digesta were higher in FAA- and FAA+0 pigs compared to FAA+2 ($P < 0.05$). Fecal and colonic digesta MPO were lower in FAA+2 and FAA+1 pigs compared to FAA- ($P < 0.05$). These results demonstrate a positive effect of a longer adaptation period to FAA-supplemented diets on performance and immune status of weaned pigs challenged with Salmonella. ISSN: 15253163

Razzuoli, E., Listorti, V., Martini, I., Migone, L., Decastelli, L., Mignone, W., Berio, E., Battistini, R., Ercolini, C., Serracca, L., Andreoli, T., Dellepiane, M., Adriano, D., Pitti, M., Meloni, D., Modesto, P.
Prevalence and antimicrobial resistances of salmonella spp. Isolated from wild boars in Liguria region, Italy
(2021) Pathogens, 10 (5), art. no. 568, .
ABSTRACT: Salmonella spp. is an important zoonotic agent. Wild boars might host this pathogen in the intestinal tract and might represent a risk for Salmonella spp. transmission to humans. Wild boars are widely spread in Liguria, due to the environmental characteristics of the region. The aim of the study was the isolation, typing, and investigation of antimicrobial susceptibility of the isolated strains of Salmonella spp. During the 2013–2017 hunting seasons, 4335 livers of wild boars were collected and analyzed for the presence of Salmonella spp. A total of 260 strains of Salmonella spp. were isolated and characterized, with a prevalence of 6%. The isolated strains belonged to all six Salmonella enterica subspecies. Most of them were identified as Salmonella enterica subs. Enteric of which 31 different serotypes were identified. The dominating serotype identified was S. Enteritidis. The antimicrobial resistance profiles of the isolated strains were analyzed against sixteen molecules. Of the isolated strains, 94.6% were resistant to at least one of the tested antimicrobials. This study showed the circulation of resistant Salmonella spp. strains in the wild boar population living in this area of Italy, underling the potential risk for these animals to disseminate this pathogen and its antimicrobial resistances.
ISSN: 20760817

Chen, S.-H., Parker, C.H., Croley, T.R., McFarland, M.A.
Genus, species, and subspecies classification of salmonella isolates by proteomics
(2021) Applied Sciences (Switzerland), 11 (9), art. no. 4264, .
ABSTRACT: Identification of bacteria by mass spectrometry offers the potential of a high-throughput non-targeted method to determine the presence of Salmonella. While MALDI-TOF mass spectrometry can identify Salmonella at the genus and species level, few studies have reported subtyping beyond the species level due to the diversity and complexity of Salmonella that includes more than 2600 serovars. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) approaches enable profiling of a greater number of proteins over a larger dynamic range and offer the potential to detect small differences between closely related isolates. We evaluate the discriminatory power of bottom-up LC-MS/MS with a collection of nineteen isolates that differ at the genus, species, subspecies, or strain level. Isolates were classified by matching the sequence of identified peptides to reference proteomes translated from genomes with known taxonomic ranks. The degree of proteomic similarity between the tested isolates and reference strains correlated with how closely they were related. All tested Salmonella isolates were easily distinguished from their close relatives, E. coli and Shigella, and readily grouped by species and subspecies. Additionally, each Salmonella isolate most closely matched to its correct serovar. This approach presents a simple and effective proteomic approach to identification of Salmonella genus, species, and subspecies. ISSN: 20763417

Ehuwa, O., Jaiswal, A.K., Jaiswal, S.
Salmonella, food safety and food handling practices

(2021) Foods, 10 (5), art. no. 907, .
ABSTRACT: Salmonellosis is the second most reported gastrointestinal disorder in the EU resulting from the consumption of Salmonella-contaminated foods. Symptoms include gastroenteritis, abdominal cramps, bloody diarrhoea, fever, myalgia, headache, nausea and vomiting. In 2018, Salmonella accounted for more than half of the numbers of foodborne outbreak illnesses reported in the EU. Salmonella contamination is mostly associated with produce such as poultry, cattle and their feeds but other products such as dried foods, infant formula, fruit and vegetable products and pets have become important. Efforts aimed at controlling Salmonella are being made. For example, legislation and measures put in place reduced the number of hospitalizations between 2014 and 2015. However, the number of hospitalizations started to increase in 2016. This calls for more stringent controls at the level of government and the private sector. Food handlers of "meat processing" and "Ready to Eat" foods play a crucial role in the spread of Salmonella. This review presents an updated overview of the global epidemiology, the relevance of official control, the disease associated with food handlers and the importance of food safety concerning salmonellosis. ISSN: 23048158

Cardoso, M.J., Nicolau, A.I., Borda, D., Nielsen, L., Maia, R.L., Møretrø, T., Ferreira, V., Knøchel, S., Langsrud, S., Teixeira, P.
Salmonella in eggs: From shopping to consumption—A review providing an evidence-based analysis of risk factors
(2021) Comprehensive Reviews in Food Science and Food Safety, 20 (3), pp. 2716-2741.
ABSTRACT: Nontyphoidal salmonellae are among the most prevalent foodborne pathogens causing gastrointestinal infections worldwide. A high number of cases and outbreaks of salmonellosis are associated with the consumption of eggs and egg products, and several of these occur at the household level. The aim of the current study is to critically evaluate the current status of knowledge on Salmonella in eggs from a consumer's perspective, analyzing the hazard occurrence and the good practices that should be applied to reduce salmonellosis risk. Following a HACCP (Hazard Analysis and Critical Control Point) based approach, some steps along the food journey were identified as Critical Consumer Handling (CCH)—steps in which consumers, through their behavior or choice, can significantly reduce the level of Salmonella in eggs and egg products. From shopping/collecting to consumption, each of these steps is discussed in this review to provide an evidence-based overview of risk factors of human salmonellosis related to egg consumption. The main message to consumers is to choose Salmonella-free eggs (those that some official entity or producer guarantees that does not contain Salmonella), when available, especially for dishes that are not fully heat treated. Second, as guaranteed Salmonella-free eggs are only available in a few countries, refrigerated storage from the point of collection and proper cooking will significantly reduce the risk of salmonellosis. This will require a revision of the actual recommendations/regulations, as not all ensure that eggs are maintained at temperatures that prevent growth of Salmonella from collection until the time of purchasing. ISSN: 15414337

Reid, A.N., Conklin, C., Beaton, K., Donahue, N., Jackson, E., Locascio, B., Marsocci, C., Szemreylo, E., Szemreylo, K.
Inoculum preparation conditions influence adherence of salmonella enterica serovars to red leaf lettuce (lactuca sativa)
(2021) Journal of Food Protection, 84 (5), pp. 857-868.
ABSTRACT: Salmonella enterica has been increasingly linked to outbreaks involving consumption of fresh produce. Although researchers have identified genes whose products are involved in mediating S. enterica-plant interactions, the use of various experimental approaches, serovars, and plant types has generated variable and conflicting data. The purpose of this study was to determine whether conditions under which inocula are prepared for in vitro plant interaction studies influence the outcome of these studies. Seven S. enterica serovars were grown in media that differed in salinity and physical state with incubation at 25 or 378C. These cultures were then used to inoculate red leaf lettuce, and adherent microbes were subsequently recovered. Although all Salmonella serovars were influenced by inoculum preparation conditions, the amount of variation differed. Analysis of pooled serovar data revealed that inocula prepared from either agar plates or biphasic cultures had higher levels of interaction with red leaf lettuce than those prepared from broth cultures. Incubation at 378C enhanced adherence after 30 s or 5 days of contact time, and adherence after 1 h of contact time was increased in low-salt medium. Broth inoculum cultures were highly influenced by medium salinity and incubation temperature, whereas plate and biphasic inoculum cultures were only minimally affected. Therefore, inocula prepared from bacteria grown on plates or in biphasic culture would be most suitable for evaluation of strategies used to interfere with plant-Salmonella

interactions. However, pooled data mask serovar-specific responses, and care should be taken when extrapolating these findings to individual serovars. The previous association of a serovar with outbreaks involving leafy greens was not correlated with levels of interaction with red leaf lettuce, suggesting that the occurrence of these serovars in or on these commodities does not reflect their fitness in the plant environment. ISSN: 0362028X

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P., Szulowski, K., Wasyl, D.
Salmonella in captive reptiles and their environment—can we tame the dragon?
(2021) Microorganisms, 9 (5), art. no. 1012, .
ABSTRACT: Reptiles are considered a reservoir of a variety of Salmonella (S.) serovars. Nevertheless, due to a lack of large-scale research, the importance of Reptilia as a Salmonella vector still remains not completely recognized. A total of 731 samples collected from reptiles and their environment were tested. The aim of the study was to assess the prevalence of Salmonella in exotic reptiles kept in Poland and to confirm Salmonella contamination of the environment after reptile exhibitions. The study included Salmonella isolation and identification, followed by epidemiological analysis of the antimicrobial resistance of the isolates. Implementation of a pathway additional to the standard Salmonella isolation protocol led to a 21% increase in the Salmonella serovars detection rate. The study showed a high occurrence of Salmonella, being the highest at 92.2% in snakes, followed by lizards (83.7%) and turtles (60.0%). The pathogen was also found in 81.2% of swabs taken from table and floor surfaces after reptile exhibitions and in two out of three egg samples. A total of 918 Salmonella strains belonging to 207 serovars and serological variants were obtained. We have noted the serovars considered important with respect to public health, i.e., S. Enteritidis, S. Typhimurium, and S. Kentucky. The study proves that exotic reptiles in Poland are a relevant reservoir of Salmonella.
ISSN: 20762607

Ceyssens, P.-J., Van den Bossche, A., Phan, L.K., Van Hoorde, K., Mattheus, W.
A molecular assay for rapidly distinguishing the AviPro SALMONELLA VAC T vaccine strain from wild-type field isolates
(2021) Journal of Microbiological Methods, 184, art. no. 106190, .
ABSTRACT: Rapid differentiation of the AviPro Salmonella VAC T strain from wild-type Salmonella ser. Typhimurium isolates is essential for the monitoring of veterinary isolates and targeted control actions. The distinction between the two strain types is routinely made by phenotypic antimicrobial resistance testing, but this sometime leads to ambiguous results with major economic implications. In this study, we used whole-genome sequencing to identify conserved and specific mutations in resistance and virulence genes which enable to distinguish field and vaccine strains. Based on this information, we developed and validated (n = 199) a Luminex-based assay targeting seven specific single-nucleotide polymorphisms. This molecular test is able to distinguish both Salmonella ser. Typhimurium types with 100% sensitivity and specificity within one working day.
ISSN: 01677012

Sarjit, A., Ravensdale, J.T., Coorey, R., Fegan, N., Dykes, G.A.
Survival of Salmonella Under Heat Stress is Associated with the Presence/Absence of CRISPR Cas Genes and Iron Levels
(2021) Current Microbiology, 78 (5), pp. 1741-1751.
ABSTRACT: Clustered regularly interspaced short palindromic repeats (CRISPR) cas genes have been linked to stress response in Salmonella. Our aim was to identify the presence of CRISPR cas in Salmonella and its response to heat in the presence of iron. Whole genomes of Salmonella (n = 50) of seven serovars were compared to identify the presence of CRISPR cas genes, direct-repeats and spacers. All Salmonella genomes had all cas genes present except S. Newport 2393 which lacked these genes. Gene-specific primers were used to confirm the absence of these genes in S. Newport 2393. The presence/absence of CRISPR cas genes was further investigated among 469 S. Newport genomes from PATRIC with 283 genomes selected for pan-genome analysis. The response of eleven Salmonella strains of various serovars to gradual heat in ferrous and ferric forms of iron was investigated. A total of 32/283 S. Newport genomes that lacked all CRISPR cas genes clustered together. S. Newport 2393 was the most heat-sensitive strain at higher iron levels (200 and 220 pm) in ferrous and ferric forms of iron. The absence of CRISPR cas genes in S. Newport 2393 may contribute to its increase in heat sensitivity and iron may play a role in this. The high reduction in numbers of most Salmonella strains exposed to heat makes it unfeasible to extract RNA and conduct transcription studies. Further studies should be conducted to validate the survival of Salmonella when exposed to heat in the presence/absence of CRISPR cas genes and different iron levels. ISSN: 03438651

Shang, Y., Ye, Q., Cai, S., Wu, Q., Pang, R., Yang, S., Xiang, X., Wang, C., Zha, F., Ding, Y., Zhang, Y., Wang, J., Sun, X., Zhang, J.
Loop-mediated isothermal amplification (LAMP) for rapid detection of Salmonella in foods based on new molecular targets
(2021) LWT, 142, art. no. 110999, .
ABSTRACT: Various molecular techniques have been introduced for Salmonella detection. Loop-mediated isothermal amplification (LAMP) is a simple and easy-to-operate detection method compared with conventional PCR. However, the specificity and efficiency of DNA amplification relies on the selected target sequence. In this study, we aimed to identify new molecular targets and develop a LAMP-based method for the rapid detection of Salmonella in food samples. Using bioinformatics analysis and PCR verification, 3 and 10 molecular targets respectively unique to Salmonella genus and S. enterica were identified. The limit of detection (LOD) for these targets ranged from 2.1 × 102 to 2.1 × 103 CFU/mL. Of these, the ssaQ gene yielded 100% positive results upon testing with 23 most commonly reported Salmonella serovars and showed no cross-reaction with 22 non-Salmonella strains. Furthermore, its LOD was 2.1 × 101 CFU/mL, making it 10-fold more sensitive than the optimal PCR-based method. Therefore, the ssaQ gene was selected as the target gene to detect Salmonella using LAMP. The ability of the ssaQ-based LAMP method of detecting Salmonella in food samples was consistent with that of the standard culture method. The developed method has potential applications in identifying Salmonella contamination in food as well as for rapid and precise tracing of contamination sources in clinical diagnostics. ISSN: 00236438

Tirloni, E., Stella, S., Bernardi, C., Rosshaug, P.S.
A new predictive model for the description of the growth of Salmonella spp. in Italian fresh ricotta cheese
(2021) LWT, 143, art. no. 111163, .
ABSTRACT: In this study, cardinal parameter models were developed for the growth of Salmonella spp. in different brands of Italian fresh ricotta cheese. Two models were proposed, including the effect of temperature or the combined effect of temperature, pH, and concentration of lactic, citric and, acetic acid. Validation of the models included an assessment of the ability to predict maximum specific growth rate max using two indices: bias-factor (Bf) and accuracy factor (Af), and the acceptable simulation zone (ASZ). The new models for Salmonella spp. showed good performances with Bf of 1.11–1.10 (model with 1 or 5 variables), and an average of 91% and 89% of observations within the ASZ (model with 1 or 5 variables, respectively). Comparing the performances of other existing models when applied to ricotta cheese, a general underprediction of the growth rate was evidenced. The proposed models can be applied by a high number of users with the aim to assess levels of this pathogen in ricotta cheese under both static and dynamic environmental conditions, being useful for the dairy business as the tested conditions cover a wide range of the available brands on the market. ISSN: 00236438

Somorin, Y.M., Odeyemi, O.A., Ateba, C.N.
Salmonella is the most common foodborne pathogen in African food exports to the European Union: Analysis of the Rapid Alert System for Food and Feed (1999–2019)
(2021) Food Control, 123, art. no. 107849, .
ABSTRACT: Global food imports, including those from Africa, constitute an integral part of the food chain in the European Union (EU) and a potential source of food hazards. Foodborne pathogens are among the food hazards that do not only impact on public health but also have economic implications for exporters. The Rapid Alert System for Food and Feed (RASFF) is an important tool for reporting and communicating food safety risks among EU Member States and EEA countries. This study aimed to identify the common foodborne pathogens in foods originating from African countries to the EU between 1999 and 2019 by analysing RASFF notifications. A total of 596 notifications were reported by 19 countries due to the presence of pathogenic microorganisms (PM) in food originating from 27 African countries. The highest number of notifications related to Greece (n = 228) and most of the PM notifications were border rejections (60.6%). PM notifications increased from 17 (2016) to 46 (2017) and 173 (2019). Salmonella was the most predominant pathogen notified, accounting for 523 (87.8%) of PM notifications. Over half (52%) of the Salmonella notifications were from foods originating from Eastern Africa, followed by Western Africa (n = 145; 28%), and the country with the highest Salmonella contamination was Sudan (n = 182). The most important product category contaminated with Salmonella was “nuts, nut products and seeds” (n = 343), with majority (n = 335) being sesame seeds. Evaluation of the RASFF risk decision listed for each notification showed that 97% of Salmonella-contaminated sesame seeds posed serious risks to consumers. African countries exporting food products to the EU must strengthen their food

safety systems to prevent the huge economic losses resulting from non-compliance with EU food safety standards. ISSN: 09567135

He,Y., Chen, R., Qi,Y., Salazar,J.K., Zhang,S., Tortorello,M.L., Deng,X., Zhang,W.
Survival and transcriptomic response of Salmonella enterica on fresh-cut fruits
(2021) International Journal of Food Microbiology, 348, art. no. 109201, .
ABSTRACT: Salmonella enterica is frequently implicated in foodborne disease outbreaks associated with fresh-cut fruits. In the U.S., more than one third of fruit-related outbreaks have been linked to two S. enterica serotypes Newport and Typhimurium. Approximately 80% of fruit-related human salmonellosis cases were associated with tomatoes, cantaloupes and cucumbers. In this study, we investigated the population dynamics of S. Newport and S. Typhimurium on fresh-cut tomato, cantaloupe, cucumber and apple under short-term storage conditions. We further compared the transcriptomic profiles of a S. Newport strain on fresh-cut tomato and cantaloupe using high-throughput RNA-seq. We demonstrated that both S. enterica Newport and Typhimurium survived well on various fresh-cut fruit items under refrigeration storage conditions, independent of inoculation levels. However, S. enterica displayed variable survival behaviors on different types of fruits. For example, at 7 d storage, the population of S. enterica reduced less than 0.2 log ($p > 0.05$) on fresh-cut tomato and cantaloupe, in contrast to ~0.5 log ($p < 0.05$) on cucumber and apple. RNA-seq analysis suggested that S. enterica mediates its survival on fresh-cut fruits through differentially regulating genes involved in specific carbon utilization and metabolic pathways. Several known bacterial virulence factors (e.g., pag gene) were found to be differentially regulated on fresh-cut tomato and cantaloupe, suggesting a link between the events of food contamination and subsequent human infection. Findings from this study contribute to a better understanding of S. enterica survival mechanisms on fresh-cut produce. ISSN: 01681605

Møretrø, T., Nguyen-The, C., Didier, P., Maître, I., Izsó, T., Kasza, G., Skuland, S.E., Cardoso, M.J., Ferreira, V.B., Teixeira, P., Borda, D., Dumitrascu, L., Neagu, C., Nicolau, A.I., Anfruns-Estrada, E., Foden, M., Voysey, P., Langsrud, S.
Consumer practices and prevalence of Campylobacter, Salmonella and norovirus in kitchens from six European countries
(2021) International Journal of Food Microbiology, 347, art. no. 109172, .
ABSTRACT: About 40% of foodborne infections are acquired in the home. The aim of the present study was to track contamination of pathogens during domestic food preparation and link the contamination to preparation practices. Research participants from 87 households in six European countries were observed and interviewed during shopping and preparation of a chicken and vegetable meal. The presence of Salmonella spp., Campylobacter spp. and norovirus on raw chicken, kitchen surfaces, cloths and sponges was determined. The prevalence of Campylobacter on raw chicken varied from 8.3% in Norway (NO) to 80% in France (FR) and Portugal (PT), with a mean prevalence of 57%. Campylobacter was found on half of the products that had been frozen and appeared to be less prevalent on chicken from supermarkets than other sources. Salmonella was found in 8.6% of raw chicken samples, exclusively from Hungary (HU). A relationship between observed practices and spread of pathogens to kitchen surfaces was found only for the use of cutting boards for chicken and/or vegetables. After food preparation, Campylobacter and Salmonella were isolated from 23% (samples derived from HU, RO, UK) and 8.7% (HU), respectively of cutting boards. Research participants in France and Portugal were more likely to buy products that fitted their recipe, with less need for using cutting boards. Using the same board and knife for vegetables after using it for chicken and without washing with detergent was common in Portugal and Romania, but not in the other countries. Contamination with Campylobacter to other kitchen surfaces or washing utensils were found in five households (UK, RO, PT). Rinsing chicken in sinks was common in three countries (PT, HU, RO), and washing vegetables in the same sink was also usual. Prevalence of Norovirus was low, with detection in one out of 451 samples. The participants' awareness of the risk posed by pathogens from raw chicken differed among the six countries, with higher awareness in Norway and the UK than the other countries studied. In conclusion, practices intended to avoid cross-contamination from chicken to kitchen surfaces and washing utensils are not established among consumers in all European countries. Nevertheless, cross-contamination events that disseminate infectious doses of pathogens seems to be rare, probably due to the relatively low levels of pathogens in food combined with food preferences. Food safety interventions must consider the national food culture, preferences, practices and the prevalence and levels of pathogens in food. Emphasis should be on providing and promoting chicken products with lower risk (prevalence of pathogens, ready-to-cook) and safe use of cutting boards. ISSN: 01681605

López-Gálvez, F., Rasines, L., Conesa, E., Gómez, P.A., Artés-Hernández, F., Aguayo, E.
Reusable plastic crates (RPCs) for fresh produce (case study on cauliflowers): Sustainable packaging but potential salmonella survival and risk of cross-contamination
(2021) Foods, 10 (6), art. no. 1254, .
ABSTRACT: The handling of fresh fruits and vegetables in reusable plastic crates (RPCs) has the potential to increase the sustainability of packaging in the fresh produce supply chain. However, the utilization of multiple-use containers can have consequences related to the microbial safety of this type of food. The present study assessed the potential cross-contamination of fresh cauliflowers with Salmonella enterica via different contact materials (polypropylene from RPCs, corrugated cardboard, and medium-density fiberboard (MDF) from wooden boxes). Additionally, the survival of the pathogenic microorganism was studied in cauliflowers and the contact materials during storage. The life cycle assessment (LCA) approach was used to evaluate the environmental impact of produce handling containers made from the different food-contact materials tested. The results show a higher risk of cross-contamination via polypropylene compared with cardboard and MDF. Another outcome of the study is the potential of Salmonella for surviving both in cross-contaminated produce and in contact materials under supply chain conditions. Regarding environmental sustainability, RPCs have a lower environmental impact than single-use containers (cardboard and wooden boxes). To exploit the potential environmental benefits of RPCs while ensuring food safety, it is necessary to guarantee the hygiene of this type of container. ISSN: 23048158

Priyanka, Meena, P.R., Meghwanshi, K.K., Rana, A., Singh, A.P.
Leafy greens as a potential source of multidrug-resistant diarrhoeagenic Escherichia coli and Salmonella
(2021) Microbiology (Reading, England), 167 (6), .
ABSTRACT: A continued rise in leafy green-linked outbreaks of disease caused by pathogenic Escherichia coli or Salmonella, particularly strains exhibiting multidrug resistance (MDR), has emerged as a major threat to human health and food safety worldwide. Thus, the present study was conducted to examine antimicrobial resistance, including MDR, in diarrhoeagenic E. coli (DEC) and Salmonella isolates obtained from leafy greens from rural and urban areas of India. Of the collected samples (830), 14.1 and 6.5% yielded 117 E. coli (40 DEC and 77 non-DEC) and 54 Salmonella isolates, respectively. Among the DEC pathotypes, enteroaggregative E. coli was the most prevalent (10.2%), followed by enteropathogenic E. coli (9.4%), enteroinvasive E. coli (7.6%) and enterohemorrhagic E. coli (6.8%). Antimicrobial susceptibility testing of all bacterial isolates with respect to drugs categorized as critically or highly important in both human and veterinary medicine revealed moderate to high (30-90%) resistance for amoxicillin/clavulanic acid, ampicillin, gentamycin and colistin, but relatively low resistance (>30%) for ciprofloxacin, trimethoprim/sulfamethoxazole and fosfomycin. Notably, all DEC and more than 90% non-DEC or Salmonella isolates were found to be multidrug-resistant to drugs of both human and animal importance. Overall, the results of the present study suggest that leafy greens are potential reservoirs or sources of multidrug-resistant DEC and Salmonella strains in the rural or urban areas of India. ISSN: 14652080

Hamilton, A.M., Paulsen, D.J., Trout Fryxell, R.T., Orta, V.E., Gorman, S.J., Smith, D.M., Buchanan, J.R., Wszelaki, A.L., Critzer, F.J.
Prevalence of salmonella enterica in flies on a diversified cattle and fresh produce farm across two growing seasons
(2021) Journal of Food Protection, 84 (6), pp. 1009-1015.
ABSTRACT: Flies are a vector for spreading foodborne pathogens pertinent to fresh produce, such as Shiga toxigenic Escherichia coli and Salmonella; however, most studies focus on concentrated animal feeding operations, which do not reflect low-density animal farming practices that often adjoin fruit and vegetable acreage. In this study, we determined the prevalence of Salmonella in flies collected biweekly on an integrated animal and produce operation over two growing seasons. Eleven of 889 pooled samples tested positive for Salmonella. Flies from the Calliphoridae, Muscidae, Sarcophagidae, and Tachinidae families were associated with Salmonella carriage, but fly family was not a significant factor for isolation of Salmonella (P ¼ 0.303). Fly species were a significant factor (P ¼ 0.026), with five Pentacricia aldrichii pools testing positive for Salmonella. With the exception of single specimen isolation, prevalence ranged from 2.2 to 15.2%. With the exception of the Tachinidae family, these results reflect a strong association of flies that are commonly associated with feces or are pests of animals. Trap location was not significantly associated with isolation of Salmonella-positive flies (P ¼ 0.236). Overall, the population of flies was not as abundant as studies conducted with produce grown close to

concentrated animal feeding operations, indicating a reduced risk of transmission; however, similar to these studies, fly families that are commonly isolated from fecal and decaying matter were most frequently associated with Salmonella isolation. Further work is warranted to elucidate the foodborne pathogen transmission rates to produce and subsequent survival over time. ISSN: 0362028X

Siceloff, A.T., Ohta, N., Norman, K.N., Loneragan, G.H., Norby, B., Morgan Scott, H., Shariat, N.W.
Antimicrobial resistance hidden within multiserovar salmonella populations
(2021) Antimicrobial Agents and Chemotherapy, 65 (6), art. no. e00048, .
ABSTRACT: Salmonella enterica can exist in food animals as multiserovar populations, and different serovars can harbor diverse antimicrobial resistance (AMR) profiles. Conventional Salmonella isolation assesses AMR only in the most abundant members of a multiserovar population, which typically reflects their relative abundance in the initial sample. Therefore, AMR in underlying serovars is an undetected reservoir that can readily be expanded upon antimicrobial use. CRISPR-SeroSeq profiling demonstrated that 60% of cattle fecal samples harbored multiple serovars, including low levels of Salmonella serovar Reading in 11% of samples, which were not found by culture-based Salmonella isolation. An in vitro challenge revealed that Salmonella serovar Reading was tetracycline resistant, while more abundant serovars were susceptible. This study highlights the importance of AMR surveillance in multiserovar populations. ISSN: 00664804

Casalino, G., Bellati, A., Pugliese, N., Camarda, A., Faleo, S., Lombardi, R., Occhiochiuso, G., Circella, E., D'onghia, F.
Salmonella infection in turtles: A risk for staff involved in wildlife management?
(2021) Animals, 11 (6), art. no. 1529, .
ABSTRACT: Monitoring of infections that may be transmitted to humans by animals in wildlife rescue centres is very important in order to protect the staff engaged in rehabilitation practices. Salmonella may be a natural inhabitant of the intestinal tract of turtles, rarely causing disease. This may represent a potential risk for humans, increasing the sanitary risk for operators in wildlife rescue centres. In this paper, the occurrence of non typhoidal Salmonella among terrestrial turtles housed in a wildlife rescue centre in Southern Italy was investigated, in order to assess the serovars more frequently carried by turtles and identify those that may represent a risk for operators involved in wildlife management. Sixty-nine adult turtles (Testudo hermanni hermanni, T. h. boettgeri, T. graeca, and T. marginata) were tested. Detection and serotyping of Salmonella strains were performed according to ISO 6579 1 and ISO/TR 6579 3:2013, respectively. The distribution of Salmonella spp. was significantly higher in T. hermanni hermanni than in other species, independent of the age and gender of the animals. Two different Salmonella species, S. enterica and S. bongori, three S. enterica subspecies (enterica, diarizonae, salamae), and five different serovars (Hermannswerder, Abony, Ferruch, Richmond, Vancouver) within the group S. enterica subspecies enterica were identified. Different combinations of Salmonella types were simultaneously found in specimens of T. h. hermanni. Most of detected Salmonella types may represent a potential risk for public health. Adopting correct animal husbandry procedures and informing on potential sanitary risks may be useful for minimising the risk of transmission of Salmonella to workers involved in wildlife management. ISSN: 20762615

Rosniawati,T., Rahayu,W.P., Kusumaningrum,H.D., Indrotristanto,N., Nikastri,E.
Prevalence and level of salmonella spp. Contamination on selected pathways of preparation and cooking of fried chicken at the household level
(2021) Food Science and Technology (Brazil), 41, pp. 41-46.
ABSTRACT: Homemade foods have been reported as an important contributor to some foodborne outbreaks. This study determined the prevalence and number of Salmonella spp. on selected pathways of fried chicken preparation and cooking at the household level and investigated their antimicrobial resistance. Salmonella serovar was confirmed by polymerase chain reaction (PCR) and partial sequencing using primer 785F and 907R. Samples consisted of chicken meat (raw, pre-cooked and fried), seasoning, water, mortar, and the hands of the food handler. The results showed that Salmonella spp. was found in 22.1% of 104 samples. The level of Salmonella found was in the range of 0.3 MPN/g (in fried chicken and water) to 920 MPN/g (in marinated raw chicken). Six Salmonella serovars were identified, namely S. Typhimurium, S. Bergen, S. Enteritidis strain FORC_052, S. Enteritidis strain GD1011, S. Typhi strain 541, and S. Typhi strain 3N4. Three were resistant to nalidixic acid, while one was resistant to streptomycin. ISSN: 1678457X

Kaavya, R., Pandiselvam, R., Abdullah, S., Sruthi, N.U., Jayanath, Y., Ashokkumar, C., Chandra Khanashyam, A., Kothakota, A., Ramesh, S.V.
Emerging non-thermal technologies for decontamination of Salmonella in food (2021) Trends in Food Science and Technology, 112, pp. 400-418.
ABSTRACT: Background: Salmonella infection has become a foremost health issue as it is the causative agent of several foodborne outbreaks. Currently, there is a huge demand for safe, healthy, and nutritious, fresh-like food products. It strongly suggests the food manufacturers to develop appropriate practices like expeditious testing, detection, and inactivation of foodborne pathogens as well as to prevent the pathogen entry into the supply chain. Scope and approach: In this decade, a lot of innovative ideas and technologies have been investigated as a substitute for conventional thermal technologies employed to inactivate foodborne pathogens. This review presents the potential of such technologies for instance, cold plasma, light-emitting diode, ozone, ultrasound, and pulsed electric field in decontaminating the Salmonella in food production and supply chain. These emerging innovative decontamination practices not only ensure the freshness of food but also enhance the microbial safety and quality of a food product. The synergistic effect of the cold plasma technique arrests the pathogenic cells' viability and multiplication. Oxidative response and the free radical generation capability of ozone treatment destroy the bacterial cells and accord antimicrobial activity. Applications of acoustic cavitation mechanism of ultrasound and non-ionizing electromagnetic radiations of UV light progressively inactivate the pathogenic microorganisms. The high-intensity usage of electric field strength by utilizing the electroporation method resulting in microbial cell death. Key findings: The effect of emerging non-thermal technologies and the processing parameters involved in the decontamination have been reviewed comprehensively along with the summary of different food products. A thorough understanding and deep insights into the mechanisms underlying the optimization of the process conditions will pave the way for upscaling these technologies for improved quality and sustaining the nutritional components of the food product. ISSN: 09242244