NEWSLETTER

European Union Reference Laboratory for *Salmonella*

Vol. 27 No. 1
March 2021

ISSN 2211-6877

Continuation of Newsletter Community Reference Laboratory for *Salmonella*
ISSN 1572-3836

Produced by

European Union Reference Laboratory for *Salmonella*

National Institute of Public Health and the Environment
P.O. Box 1, 3720 BA Bilthoven, The Netherlands

phone: +31 30 274 3537 (Kirsten Mooijman)
+31 30 274 4290 (Wilma Jacobs)
e-mail: kirsten.mooijman@rivm.nl
wilma.jacobs@rivm.nl

Contents

Editorial Note

Bilthoven, 6 April 2021

Dear colleague,

I hope that you are all doing well and have not been hit too hard by the **SARS-CoV-2 virus**? The vaccinations against the virus have started, but of course it takes a lot of time before the majority of the population is vaccinated and we can go back to the 'old normal'. Unfortunately, the number of infected persons are again increasing in many countries, resulting in a third (fourth, fifth,…) wave of infections. Hence, we still have to hold on to all measures against the virus and we do hope that the situation improves in the course of this year.

In the previous Newsletter we informed you that we had sent the **EURL-*Salmonella* work program 2021** to DG SANTE shortly before the Christmas break in December 2020. Due to late adoption of the Multiannual Financial Framework (MFF) 2021-2027 and of the new Single Market Program Regulation (SMP) by the Council and the European Parliament, the grant applications for EURLs work programs can be submitted only in the course of 2021. In order to ensure the continuity of the activities, the EURLs had to informally submit to the relevant DG SANTE technical desk officer the annual work program for the year 2021. Early January 2021, the technical desk officer at DG SANTE informally agreed with the EURL-*Salmonella* work program for 2021. This, informal, work program is included in this Newsletter. The formal agreement will follow later this year.

The COVID-19 pandemic has learned us to be flexible and that it is quite possible to work from home. Although this latter is of course impossible when you have to perform analysis in the laboratory. We are also getting used to the online meetings and learn that many ICT options exist to organise these meetings. However, we also experience a variety of technical problems that we may face when having these online meetings. Organising our first online EURL-*Salmonella* workshop was quite a challenge last year, but we also learned that it was possible to still meet all NRLs-*Salmonella* even in this challenging time. Of course it is much nicer to meet each other in person, but when this is not possible, an online meeting is a relatively good alternative. Many of you also indicated this in the evaluation of the workshop of September 2020. We had hoped that the workshop of 2021 could be a physical meeting again, but for the moment this is not a realistic option. For that reason, the **EURL-*Salmonella* workshop of 2021** will be an online workshop again. This workshop will be organised on 28 May 2021 and will be a one-day meeting as this years' workshop is organised relatively shortly after the workshop of 2020. Early March 2021, all NRLs-*Salmonella*, and other contacts at EC and EFSA, were informed about the planning of the workshop and were requested to register before mid-April 2021. Only registered persons will receive log-in information for the meeting.

In January/February 2021, the evaluation of the serotyping results of the **PT on typing of *Salmonella* 2020** was performed. Early March 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-serotyping-2020. I am glad to notice that the performance of the participants in the PT Serotyping 2020 was very good, all participants met the level of good performance at the first stage of this PT. In addition to the

obligatory serotyping part, this study included the second pilot on cluster analysis. The results on the (optional) cluster analysis part, using PFGE and/or MLVA and/or WGS data are under analysis and will be reported separately in April/May 2021.

In March 2021 we organised the **PT on detection of *Salmonella* in a food sample**. The matrix of choice was liquid whole egg. The deadline for reporting the results was by the end of March 2021 and we recently started analysing the data.

Before the end of March 2021 we submitted the **financial and technical reports of the activities of the EURL-*Salmonella* performed in 2019 and 2020** to DG SANTE. After review by DG SANTE, we will publish the technical report in a next version of the Newsletter.

A new activity in 2020 was the monitoring of the incidence of ***Salmonella* Mikawasima** in food (products), animals, animal feed or the environment. From mid-March 2020 until January 2021 it was possible to report findings of *Salmonella* Mikawasima through a link at the EURL-*Salmonella* website. In total 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. The WGS data of these isolates were reported to EFSA, and compared with the human isolates collected by ECDC by cluster analysis. I would like to thank all NRLs for their cooperation in this monitoring activity.

Since 2017, a **joint EURLs working group exists on Next Generation Sequencing (NGS)**. This working group exists of 8 (biological) EURLs and has prepared/ is preparing several (harmonised) documents which 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 websites. Currently the following guidance documents have been published:

- Overview of conducted and planned PTs
  - curated by EURL-Antimicrobial Resistance.
- 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*.
- Guidance document for NGS-Benchmarking
  - curated by EURL-*Listeria monocytogenes.*
- 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 section on the EURL-*Salmonella* website: https://www.eurlsalmonella.eu/publications/analytical-methods

You may remember that last year the ISO New Work Item Proposal (NWIP) of **ISO/TS 6579-4 on identification of monophasic *Salmonella* Typhimurium** was launched. The outcome of the NWIP voting was 100% approval in ISO and CEN. After this, the first working draft (WD1) of ISO/TS 6579-4 was prepared and discussed in the first meeting of ISO-WG10 in November 2020. After this meeting, WD2 of ISO/TS 6579-4 was prepared and sent to the members of ISO-WG10 for further comments and discussed at the second meeting of ISO-WG10 in March 2021. The next step is to prepare the draft Committee Draft (CD) for voting and comments by the members of ISO/TC34/SC9.

In February 2021, **EN ISO 16140-3** 'Microbiology of the food chain - Method validation - Part 3: Protocol for the verification of reference methods and validated alternative methods in a single laboratory' was published. To facilitate the implementation of this document, a webinar was organised by the Dutch standardisation organisation, NEN, in March 2021. Presentations on the use of EN ISO 16140-3, an Excel calculation tool, as well as the recording of the webinar can be found at the website of ISO/TC34/SC9:
https://committee.iso.org/sites/tc34sc9/home/essential-information/content-left-area/validation-of-methods/method-validation-and-method-ver.html
At this website also information is given on the other parts of EN ISO 16140.

For your information, EFSA and ECDC have published their EU One Health 2019 Zoonoses Report, earlier this year:
https://www.efsa.europa.eu/fr/efsajournal/pub/6406: 'The report presents the results of zoonoses monitoring activities carried out in 2019 in 36 European countries (28 Member States (MS) and eight non-MS). The first and second most reported zoonoses in humans were campylobacteriosis and salmonellosis, respectively. The EU trend for confirmed human cases of these two diseases was stable (flat) during 2015–2019. The proportion of human salmonellosis cases due to *Salmonella* Enteritidis acquired in the EU was similar to that in 2017–2018. Of the 26 MS reporting on *Salmonella* control programmes in poultry, 18 met the reduction targets, whereas eight failed to meet at least one. The EU prevalence of *Salmonella* target serovar-positive flocks has been stable since 2015 for breeding hens, laying hens, broilers and fattening turkeys, with fluctuations for breeding turkey flocks. *Salmonella* results from competent authorities for pig carcases and for poultry tested through national control programmes were more frequently positive than those from food business operators.'

Best wishes,
Kirsten Mooijman
Coordinator EURL-*Salmonella*

Contribution of the EURL-*Salmonella*

EURL-*Salmonella* work-program 2021

INTRODUCTION

In this document the activities of the EURL-*Salmonella* are described for the year 2021. These activities are based on the responsibilities and tasks described in Article 94 of Regulation (EU) 2017/625 for European Union reference laboratories.

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

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

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

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

Sub-activity 1.1 *Analytical methods*

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

Description:

Standardisation of methods

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

- ISO/TC34/SC9: International Standardisation Organisation, Technical Committee 34 on Food products, Subcommittee 9 – Microbiology of the food chain.
- CEN/TC463: European Committee for Standardisation, Technical Committee 463 Microbiology of the food chain.

For the following activities in working groups of ISO/TC34/SC9 and CEN/TC463, staff members of EURL-*Salmonella* have the leadership, and these activities will be continued in 2021:

ISO - Ad hoc group 'Checklist to avoid ambiguity in drafting standards in food microbiology'. The project leader is Kirsten Mooijman (EURL-*Salmonella*) and the co-project leader is Laura Mout (Dutch Standardisation organisation NEN).

In 2018 the first edition, and in 2020 the second edition of guidance document for writing standards for ISO/TC34/SC9 and CEN/TC463 were published as an internal ISO/CEN document for convenors and project leaders. This guidance document is a 'dynamic' document and may need regular updating to keep the information up to date. It is expected that in 2021 the third edition will be drafted and published.

ISO-WG3 'Method validation'.

For the revision of EN ISO 17468 'Microbiology of the food chain - Technical requirements and guidance on establishment or revision of a standardized reference method', Wilma Jacobs-Reitsma (EURL-*Salmonella*) is project leader, together with Bertrand Lombard from ANSES, France. The revision started in 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.

The revision of EN ISO 17468 will be discussed in one or more meetings of WG3 in 2021 (physical and/or teleconferences).

ISO-WG10 'ISO/TS 6579-4 PCR identification of monophasic *Salmonella* Typhimurium'. Project leader for this activity is Kirsten Mooijman (EURL-*Salmonella*). A draft Technical Specification (TS) has been prepared by Task Advisory Group (TAG) 3 of CEN/TC275/WG6 (since fall 2019: CEN/TC463) in 2017. In this document, three PCR procedures are described. For determination of the performance characteristics of the PCR procedures, a 'standard set of test strains' is needed. After a call for strains, the EURL-*Salmonella* received approximately 400 strains in 2017. A subset of 172 strains (target and non-target) was used to verify the performance of the PCR procedures by the NRL-*Salmonella* in Germany and by the EURL-*Salmonella* in 2018. The first analysis of the results of this verification was performed in fall 2018 and some discrepancies were seen, which were further investigated in 2019. Early 2020, the activity was moved from CEN to ISO-WG10 and the New Work Item proposal (NWIP), including the draft document prepared by CEN-TAG3 as well as the report of testing 172 strains, was launched in May 2020. The comments to the NWIP were discussed in a meeting of WG10 in November 2020, after which a second Working Draft of the document will be circulated among the members of ISO-WG10 early 2021. Next a draft Committee Draft (CD) version of the document will be prepared for comments among the members of ISO/TC34/SC9. An interlaboratory study to determine the performance characteristics of the three PCR protocols can only be organised when the final draft CD version of ISO/TS 6579-4 is available. Depending on the number of comments and the outcome of the voting steps it may be the case that this final draft CD version will become available by the end of 2021 or early 2022. As soon as this version of ISO/TS 6579-4 is available, the preparatory work for organisation of the interlaboratory

study (ILS) will start, to be able to organise this ILS as soon as possible after publication of final draft CD ISO/TS 6579-4 (e.g. early 2022).
ISO-WG10 is expected to meet (physically and/or by teleconference) once or twice in 2021.

In the following groups in ISO and CEN, a staff member of EURL-*Salmonella* participates and contributes to the projects. Activities for these groups will be continued in 2021:
CEN-TAG9 'Improvement of the pre-enrichment step to enhance the recovery of Gram negative bacteria'. TAG9 has prepared a draft protocol to evaluate pre-enrichment media. This protocol was tested by the members of ISOTC34/SC9 and CEN/TC463 and a draft report was prepared. However, due to the fact that the project leader changed jobs, no further progress was made with the activities in this group in 2020. By the end of 2020 a call for a new project leader was made among the members of CEN/TC463 and it is likely that the activities will be continued in 2021 (and will become a working group in CEN/TC463 instead of a task advisory group).
ISO-AHG 'Validation status of ISO standards'. Wilma Jacobs-Reitsma 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-Reitsma will do this for the validation data of EN ISO 6579-1 on detection of *Salmonella*.
ISO-WG3 'Method validation'. Wilma Jacobs-Reitsma of the EURL-*Salmonella* is member of ISO-WG3 and will participate in the activities of this working group, including:
- Development of Amendment 1 of EN ISO 16140-2 'Microbiology of the food chain – Method validation - Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method - Amendment 1: Revision of the qualitative method study data evaluation'.
- Revision of EN ISO 16140-2 'Microbiology of the food chain – Method validation - Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method'.
- Development of ISO 16140-7 'Microbiology of the food chain – Method validation - Part 7: Protocol for validation of methods for the identification and characterization of microorganisms'.
- Development of a protocol for validation of larger samples sizes in detection methods.
- Subgroup to review questions and documents concerning validation of methods from working groups of ISO/TC34/SC9 and CEN/TC463.
ISO-WG3 will organise one or more meetings in 2021 (physical and/or teleconferences) in which EURL-*Salmonella* will participate.
ISO-WG25 Development of EN ISO 23418 'Microbiology of the Food Chain - Whole genome sequencing for typing and genomic characterization of foodborne bacteria - General requirements and guidance'. Angela van Hoek and Kirsten Mooijman (EURL-*Salmonella*) are members of this working group and commented on the Committee Draft (CD) version on behalf of the joint EURLs WG NGS (1.2) in 2019, and participated in teleconferences organised since the start of the activities in 2018. In fall 2020 the voting on the Draft International Standard (DIS) version of this document took place. It is expected that the comments to the DIS version will be discussed in a meeting of WG25 in spring 2021 so that the next version, the Final Draft International Standard (FDIS) version, can be launched for voting.

The plenary meetings of both ISO/TC34/SC9 and CEN/TC463 are planned to be organised in Sydney Australia in June 2021. However, it will depend on the situation with the pandemic of

COVID-19 if a physical meeting is possible or whether this meeting will (again) become a teleconference (or a combination).
One representative of the EURL-*Salmonella* will participate in these meetings to present the progress with the activities for which EURL-*Salmonella* has the leadership.

Development of (alternative) methods
Several (proprietary) alternative methods have been developed for the detection of *Salmonella*. The application of these methods depends on its validation. Certificates of validated methods (following EN ISO 16140-2) are published by the relevant validation organisations (Afnor validation, MicroVal). The EURL-*Salmonella* will keep track of developments in alternative methods by regularly checking the literature and the information from validation organisations.
In 2019, EN ISO 16140-6 was published so that by then also an internationally accepted protocol for validation of alternative confirmation and typing methods became available. A draft version of this ISO protocol (prEN ISO/DIS 16140-6) was already used for (pilot) validation studies of MicroVal for an alternative confirmation method to confirm four different pathogens, including *Salmonella*. With these studies, the applicability of (draft) EN ISO 16140-6 was shown. It may be expected that in the near future validation studies will follow for other alternative confirmation and typing methods, such as the use of WGS for serotyping of *Salmonella*. The EURL-*Salmonella* will keep track of these alternative methods and when relevant, will also test these methods. Additionally, the EURL can advise NRLs on the protocol for internal validation.

Expected Output:
- First draft revised version of EN ISO 17468.
- Publication of (draft) third edition of the Guidance document for drafting standards for microbiology of the food chain.
- Second working draft (WD2) of CEN ISO/TS 6579-4, followed by draft Committee Draft (CD).
- Summary of performance characteristics for detection of *Salmonella,* extracted from validation studies of proprietary methods.
- Report annual meetings ISO/TC34/SC9 and CEN/TC275/WG6 2021.
- Overview literature on new/alternative methods published in the EURL-*Salmonella* Newsletters.

Duration:
Continuous activities in 2021. Several ISO and CEN activities will be continued after 2021.

Meetings (expected) in 2021 (physically and/or by teleconference):
ISO-WG3: 2 meetings of 1-2 days each (1x EU-MS, 1x outside EU).
ISO-WG10: 2 meetings of 1 day each (EU-MS).
ISO/TC34/SC9 and CEN/TC463 plenary meetings: 1 meeting of 5 days (Sydney, Australia).

Sub-activity 1.2 *EURLs working group on NGS*

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

Description:
In 2017 a working group of 8 EURLs was raised on Next Generation Sequencing (NGS), including the following EURLs: Antimicrobial resistance, *Campylobacter*, Coagulase positive

staphylococci, *E. coli* (including VTEC), Food borne Viruses, *Listeria monocytogenes,* Parasites, *Salmonella*. EURL-*E. coli* has been appointed as coordinator of the working group.
The working group works or will work on the following activities in relation to NGS:
1) Proficiency Testing
2) NGS laboratory procedures (SOPs)
3) Bioinformatics tools
4) NGS cluster analysis
5) Bench marking
6) Trainings on NGS
7) Reference and confirmatory testing using NGS
8) Follow-up of ISO activities on WGS
9) Quality parameters for sequencing

The lead for each activity is distributed over the 8 EURLs. EURL-*Salmonella* has the lead of activity 7and participates in the other activities. For each activity information is gathered and (draft) guidance documents have been set up and discussed in the working group. The first versions of these guidance documents have become available at the website of the EURL leading the activity by the end of 2020. The other EURLs will provide a link at their own websites to the documents on the other websites. The guidance documents will need regular updating which will be discussed at the meetings of the working group.
For NGS trainings, a physical meeting with participants is preferred, but also the possibility of joint EURL e-trainings will be considered. For this it will be investigated if presentations used in on-site trainings can be used for e-trainings as well. A possible physical training is foreseen in the fall of 2021 (also see activity 2.2).
In 2020, the joint EURLs working group organised an online conference with the support of the Med-Vet-Net association on 'Modern technologies to enable response to crises: Next Generation Sequencing to tackle food-borne diseases in the EU'. The conference was very well received (over 500 participants) and the working group plans to organise a second conference, to give a follow-up to the questions from the first conference, in fall 2021. The Med-Vet-Net association will be requested to support this second conference as well. Depending on the situation with COVID-19, this conference will be a physical meeting in Rome or an online meeting, or a combination of both.

The working group will meet twice a year.

Expected Output:
This concerns output of the whole working group:
- Guidance documents for the listed activities, made available for all NRL networks through the websites of the EURLs.
- Investigation to set up joint EURL e-trainings or plan a physical training in the fall of 2021

Duration:
Continuous activities in 2021.

Meetings (expected) in 2021 (physically and/or by teleconference):
2x 1 day meeting of the working group (EU-MS).
Conference on NGS (1 day, Rome, Italy)

Sub-activity 1.3 *Proficiency Tests*

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

Description:
Organisation of 3 Proficiency Tests (PTs) per year:
1. One PT on detection of *Salmonella* in samples from the primary production stage (PPS).
2. One PT on detection of *Salmonella* in food or animal feed samples, including a PT on detection of *Salmonella* in Live Bivalve Molluscs (LBM) every 2-3 years (last PT on LBM was organised in 2020).
3. One PT on typing of *Salmonella* (serotyping, molecular typing).

For the set-up of the PTs on detection of *Salmonella*, EN ISO 22117 ('Microbiology of the food chain – Specific requirements and guidance for proficiency testing by interlaboratory comparison') is followed as much as possible.
The choice of the *Salmonella* serovars, the contamination levels of the samples, the type of matrix, the number of samples, as well as the protocol for artificially contaminating the samples will be established for each PT. Whenever possible, the samples will be artificially contaminated individually at the laboratory of the EURL-*Salmonella.* Homogeneity and stability of the samples will be tested in advance of each PT.

The PTs for typing of *Salmonella* consist of an obligatory part on serotyping of *Salmonella* and on an optional part for molecular typing of *Salmonella*. For the serotyping part, the EURL-*Salmonella* will select different serovars from *Salmonella enterica* subsp. *enterica*, including serovars with public health significance, serovars with antigens similar to those of public health significant strains and serovars that caused typing problems in previous studies. Since the study of 2011, one additional *Salmonella* serovar from another subspecies than *Salmonella enterica* subsp. *enterica* is included in the study. Analysis of this additional strain is optional.
For the part on molecular typing, the new set-up, initially introduced in the PT on Typing of 2019, will be continued. The optional part of this PT concerns a cluster identification of a selected set of strains, and gives the NRLs the possibility to choose their 'routinely' used molecular methods, like Pulsed Field Gel Electrophoresis (PFGE) and/or Multiple-Locus Variable-number tandem repeat Analysis (MLVA) and/or Whole Genome Sequencing (WGS). The analytical method will be free of choice, but detailed information on the methodology will be requested, as well as uploading of data like raw NGS reads (fastq files).

All samples of each PT are blindly coded and send to the NRLs-*Salmonella* one week before the performance of the PT. For the transport of the samples to the NRLs, the materials will be packed and shipped in accordance with the IATA rules for shipping UN 3373 materials (biological substance category B), using a classified courier service.
For the reporting of the results by the NRLs-*Salmonella* to the EURL, electronic (web-based) test reports will be used. These test reports are amended for each study.

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

Additional to the judgement 'good performance', or 'unsatisfactory performance', the results of an NRL can also be judged as 'moderate performance'. The criteria to define a performance as 'moderate' are described per study. The actions after moderate performance are less

stringent than after unsatisfactory performance. In case of moderate performance, the performance of the NRL over several consecutive PTs is judged. If moderate performance is seen in three consecutive PTs, the NRL will be contacted by the EURL to discuss a proper follow-up. The type of follow-up will be considered on a case by case basis depending on the nature of the moderate performance. A visit of a staff member(s) of the EURL-*Salmonella* to the NRL can be considered as a possible follow-up. In case of repeated moderate performance (like for unsatisfactory performance), DG SANTE will be informed.

Additional to the NRLs of the 27 EU Member States, also NRLs-*Salmonella* of other countries regularly participate in one or more Proficiency Tests (when capacity of the EURL-*Salmonella* allows), either for budget of the EURL-*Salmonella* or for own costs.

Participation in the PTs for budget of EURL-*Salmonella*:
- NRLs-*Salmonella* of candidate countries Albania, Republic of North Macedonia and Serbia.
- NRLs-*Salmonella* of potential candidate countries Bosnia and Herzegovina, and Kosovo*.
*: only when mailing of parcels classified as UN3373 in according with the IATA Dangerous Goods Regulations is possible.

Participation in the PTs for own costs:
- NRL-*Salmonella* of United Kingdom.
- NRLs-*Salmonella* of EFTA countries Iceland, Norway and Switzerland.
- NRL-*Salmonella* of candidate country Turkey.
- NRL-*Salmonella* of Israel (upon request of DG SANTE and only in the PT for PPS).

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

Duration:
Preparation and testing of samples, organisation and reporting of the three PTs will be divided over each year. Some activities may continue in a following year (like follow-up study, reporting).

2 TO PROVIDE SCIENTIFIC AND TECHNICAL ASSISTANCE TO NRLs

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

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

Sub-activity 2.1 *Workshop*

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

Description:
Every year, the EURL-*Salmonella* organises a workshop for the NRLs- *Salmonella*. The workshops are generally organised in May and will last approx. 1,5 days. Due to the COVID-19 pandemic it is foreseen that the workshop of 2021 will, like in 2020, again become an online workshop.

The programme of the workshop may contain the following items:
- Introductory presentations (e.g., by EU representative and EURL-*Salmonella*);
- Zoonoses in Europe (EFSA, DG SANTE);
- Results of (research) activities of EURL-*Salmonella*;
- Results of Proficiency Tests organised by EURL-*Salmonella*;
- Experiences, problems, results in relation to monitoring surveys for *Salmonella*;
- *Salmonella* outbreaks;
- Plans and results of (research) activities of the NRLs-*Salmonella*;
- Discussion on methods (e.g. typing, molecular, serological);
- Activities in ISO and CEN;
- Future working plan of EURL-*Salmonella*;
- Information on research in relation to *Salmonella* by one or more guest speakers.

Additional to the NRLs of the 27 EU Member States, also NRLs-*Salmonella* of other countries regularly participate in the workshops, either for budget of the EURL-*Salmonella* or for own costs. However, in case of an online workshop no costs will be made for travel or stay. In addition it is possible to invite more than one participant per NRL to an online workshop.

Participation in the workshops for budget of EURL-*Salmonella*:
- NRLs-*Salmonella* of candidate countries Albania, Republic of North Macedonia and Serbia.
- NRLs-*Salmonella* of potential candidate countries Bosnia and Herzegovina, and Kosovo.
- 2-3 guest speakers from different European countries.

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

The NRLs-*Salmonella* responsible for analysing bivalve molluscs will be advised to participate in the extension of the annual workshop of the EURL-marine biotoxins (when organised). It is planned that this extended workshop will focus on exchange of information related to microbiological aspects of production of bivalve molluscs.

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

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

Sub-activity 2.2 *Training courses*

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

Description:
Due to the COVID-19 pandemic, no physical training courses could be organised in 2020. Depending on the situation with the pandemic, the following training courses may be organised in 2021:
1. Upon request of an NRL, the EURL can give a training course for a specific need of an NRL, which can be on detection and typing of *Salmonella* (including serotyping and molecular typing).
2. Upon advise of the EURL, an NRL will follow a training at the EURL or staff members of the EURL give a training at the laboratory of the NRL, especially in case of (repeated) unsatisfactory performance of the NRL in Proficiency Tests.
3. Joint EURLs training on Next Generation Sequencing (NGS), organised in cooperation with the joint EURLs working group on NGS (sub-activity 1.2). If no physical meeting can be organised for this training, it will be discussed with the other EURLs of the joint EURLs WG on NGS if an online training can be organised instead.

Additional to the NRLs of the 27 EU Member States, training is also offered to NRLs-*Salmonella* of other countries (when capacity of the EURL-*Salmonella* allows), either for budget of the EURL-*Salmonella* or for own costs. However, in case of an online training no costs will be made for travel or stay. In addition it may be possible to invite more participants to an online training than to a physical training. The disadvantage of an online training is the (physical) distance between trainer and trainee so that it is more difficult to solve problems which a trainee is facing.

Participation in training courses for budget of EURL-*Salmonella*:
- NRLs-*Salmonella* of candidate countries Albania, Republic of North Macedonia and Serbia.
- NRLs-*Salmonella* of potential candidate countries Bosnia and Herzegovina, and Kosovo.

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

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

Expected Output:
The training courses intend to result in improved performance of the NRLs in the relevant work field and to build their capacity for new work fields like NGS. Details on each training course as well as the results of the evaluation will be summarised in the annual technical report of the EURL-*Salmonella*.

Duration:
The duration of training courses 1 and 2 will depend on the set up of the course and the needs of the NRLs, but in general will vary between 2 and 5 days.
Training course 3 will last 1.5-2 days, but the organisation and reporting will last several weeks (before and after the training course).

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

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

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

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

The EURL-*Salmonella* will perform confirmation and/or (sub)typing of samples/isolates from NRLs-*Salmonella* for e.g. second opinion analysis or outbreak investigations, whenever needed.

Expected Output:
- Scientific and technical support of NRLs and other parties in the relevant work field.
- An up to date website.
- Publication of 4 newsletters (per year) through the website.
- Confirmation and (sub)typing (WGS) of samples/isolates when applicable.

Duration:
Continuous activities in 2021.

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

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

- *Art. 94.2.f* ***Providing scientific and technical assistance to the Commission within the scope of their mission.***
- *Art. 94.2.h* ***Collaborating within the scope of their mission with laboratories in third countries and with the European Food Safety Authority (EFSA), the European Medicines Agency (EMA) and the European Centre for Disease Prevention and Control (ECDC).***

- *Art. 94.2.i* ***Assisting actively in the diagnosis of outbreaks in Member States of foodborne, zoonotic or animal diseases, or of pests of plants, by carrying out confirmatory diagnosis, characterisation and taxonomic or epizootic studies on pathogen isolates or pest specimens.***

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

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

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

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

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

The EURL-*Salmonella* is member of the joint EFSA-ECDC Steering Committee for management of the (joint) EFSA-ECDC molecular typing database. In this Steering Committee members of EFSA, ECDC and three EURLs (*Salmonella*, VTEC, *Listeria monocytogenes*) participate. The EFSA molecular database is intended for the collection of molecular typing data from *Salmonella*, VTEC and *Listeria monocytogenes* isolated from food, animal feed and animals and its environment. The ECDC molecular database is intended for the collection of molecular typing data of the same pathogens isolated from humans. Up to 2020, both molecular databases were intended for collection of PFGE data of *Salmonella*, VTEC and *Listeria monocytogenes* and MLVA data of *Salmonella* Typhimurium and *Salmonella* Enteritidis. However, for outbreak investigations WGS has become the sub-typing method of choice nowadays. Therefore, ECDC and EFSA received a mandate from the EC to 'set up in ECDC and EFSA two interoperable systems for the collection and sharing of WGS data provided by Member States, allowing the joint analysis of WGS data for at least *Salmonella, L. monocytogenes,* and *VTEC* for the purpose of multi-country outbreak detection and assessment'. The development of these WGS database at EFSA and ECDC started early 2020 and the EURLs are regularly informed about the progress with building this database and are also requested for their opinions on details (e.g. visibility of some metadata or not, access to the database) and asked to test prototypes of the database.

The Steering Committee will meet twice a year, generally in the country of the chair of that year (the chairmanship alternates between EFSA and ECDC). However, due to the COVID-19 pandemic the meeting in fall 2020 was changed into an online meeting and it is expected that the spring 2021 meeting will become an online meeting as well. If in the course of 2021 the pandemic is fought successfully, the fall meeting might be a physical meeting again.

Expected Output:
- Scientific and technical advices when needed.
- Summary of (substantial) advices in the annual technical report.
- Assistance in case of outbreaks, including calls to the NRLs-*Salmonella,* collecting isolates and/or WGS data, (sub)typing of isolates (generally by WGS), (cluster) analysis of WGS data and reporting results to EFSA.
- Testing EFSA WGS database under development by submitting WGS data and metadata (from NRLs), and testing the EFSA selected core genome MLST scheme for *Salmonella*.
- Minutes of meetings EFSA-ECDC steering committee.

Duration:
Continuous activities in 2021.

4 REAGENTS AND REFERENCE COLLECTIONS

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

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

Sub-activity 4.1 *Reference strains and reference materials*

Objectives:
Supply information on available culture collections and suppliers of microbiological reference materials. Investigation to the possibility for setting up a reference collection of WGS data.

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

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

Microbiological reference materials for use in, for example, first line quality control are produced by different organisations. Examples of reference material producers are given at the EURL-*Salmonella* website.

The working group EURLs on NGS (sub-activity 1.2) agreed to try to build a reference collection of genomes (and potentially informative genes) of the different pathogens to be able to validate tools and pipelines. For building this reference collection, the results of Proficiency Tests for WGS will be used, with the permission of the NRLs. In fall 2020 a first start was made for building a reference collection of genomes (and genes) of *Salmonella* Typhimurium (including the monophasic variant). The information will be published at the EURL-*Salmonella* website and extended to other *Salmonella* serovars when information comes available (e.g. from PTs on Typing of *Salmonella*).

Expected Output:
- Up-to-date information on reference strains and reference materials at the EURL-*Salmonella* website. This work is considered to be part of the sub-activity for keeping all information at the EURL-*Salmonella* website up to date. Therefore the planning and output of this part of sub-activity 4.1 is merged with sub-activity 2.3.
- Publication at the EURL-*Salmonella* website of a reference collection of genomes (and genes) of *Salmonella* Typhimurium (including the monophasic variant) and extension to other *Salmonella* serovars when information comes available. This work is also part of sub-activity 1.2 (EURLs working group on NGS) so that for the planning and output of this part of sub-activity 4.1, reference is made to sub-activity 1.2.

Duration:
Continuous activities in 2021.

From the Literature

Salmonella-related Literature from Scopus: January – March 2021

Groves, P.J., Williamson, S.L., Ahaduzzaman, M., Diamond, M., Ngo, M., Han, A., Sharpe, S.M.
Can a combination of vaccination, probiotic and organic acid treatment in layer hens protect against early life exposure to Salmonella Typhimurium and challenge at sexual maturity?
(2021) Vaccine, 39 (5), pp. 815-824.
ABSTRACT: Day old layer chicks were challenged with Salmonella Typhimurium using a seeder bird technique. Treatment groups were untreated control, administration of a probiotic in drinking water weekly, vaccination by intramuscular injection of a live aro-A deletion mutant vaccine at 10 weeks of age (woa) followed by an oral dose at 16 woa, probiotic administration plus vaccination, vaccination plus the administration of an organic acid preparation in feed from 16 woa and a combination of probiotic, vaccine and organic acid. Faecal shedding was monitored by culture at 1, 2, 3, 4, 8, 12, 15, 17, 20, 21, 23 and 25 woa and in dust from settle plates by PCR at intervals from 8 woa. Birds from each group were separated at 17 and 18 woa and challenged orally with 106 CFU of S. Typhimurium. Both untreated and probiotic groups shed Salmonella until 56 days. Salmonella was also detected in dust from 8 until 12 woa but little after this. After vaccination, from sexual maturity (18 woa) all groups except those that were vaccinated with and without probiotic re-excreted Salmonella. The probiotic alone was ineffective against this re-excretion and all groups receiving organic acids shed Salmonella. At 17 woa, unchallenged controls were fully susceptible to caecal colonization, however all other groups showed reduced susceptibility, including the untreated challenged group. However, at 18 woa (sexual maturity) only the groups that were vaccinated with or without probiotic showed reduced susceptibility to colonization. The organic acid treated groups (including the vaccinated group) did not show a difference to the untreated controls. S. Typhimurium demonstrated an ability to re-emerge at sexual maturity, similar to other serovars. The vaccine assisted in limiting the re-excretion at sexual maturity and decreased susceptibility to subsequent challenge. Use of a probiotic augmented the vaccine's protective capacity. ISSN: 0264410X

Arnold, M., Smith, R.P., Tang, Y., Guzinski, J., Petrovska, L.
Bayesian Source Attribution of Salmonella Typhimurium Isolates From Human Patients and Farm Animals in England and Wales
(2021) Frontiers in Microbiology, 12, art. no. 579888, .
ABSTRACT: The purpose of the study was to apply a Bayesian source attribution model to England and Wales based data on Salmonella Typhimurium (ST) and monophasic variants (MST), using different subtyping approaches based on sequence data. The data consisted of laboratory confirmed human cases and mainly livestock samples collected from surveillance or monitoring schemes. Three different subtyping methods were used, 7-loci Multi-Locus Sequence Typing (MLST), Core-genome MLST, and Single Nucleotide Polymorphism distance, with the impact of varying the genetic distance over which isolates would be grouped together being varied for the latter two approaches. A Bayesian frequency matching method, known as the modified Hald method, was applied to the data from each of the subtyping approaches. Pigs were found to be the main contributor to human infection for ST/MST, with approximately 60% of human cases attributed to them, followed by other mammals (mostly horses) and cattle. It was found that the use of different clustering methods based on sequence data had minimal impact on the estimates of source attribution. However, there was an impact of genetic distance over which isolates were grouped: grouping isolates which were relatively closely related increased uncertainty but tended to have a better model fit. ISSN: 1664302X

Sekhon, A.S., Singh, A., Unger, P., Babb, M., Yang, Y., Michael, M.
Survival and thermal resistance of Salmonella in dry and hydrated nonfat dry milk and whole milk powder during extended storage
(2021) International Journal of Food Microbiology, 337, art. no. 108950, .
ABSTRACT: Foodborne pathogens such as Salmonella can endure dry environments of milk powders for extended periods due to the increased adaptability at a low water activity (aw) and proliferate when powders are hydrated. This study compared the survivability and the thermal resistance of a 5-serovar Salmonella cocktail in dry and hydrated nonfat dry milk

(NFDM) and whole milk powder (WMP) stored for 180 days at ambient temperature (~20 °C). This study was designed as two factorial (storage days and milk powder type) randomized complete block design with three replications as blocks. The milk powders were spray inoculated with 5-serovar Salmonella cocktail and dried back to the original pre-inoculation aw. The D-values of Salmonella in inoculated NFDM and WMP were determined periodically (every 30 days, starting from day one). The milk powders were also individually hydrated on each analysis day to determine D- and z-values of Salmonella in hydrated powders. The D-values were determined using thermal-death-time disks and hot-water baths at 80, 85 and 90 °C for milk powders, and 59, 62 and 65 °C for hydrated powders. The D- and z-values of Salmonella at specific temperatures within dry or hydrated powders during the storage period were compared at $P \leq 0.05$ using two-way ANOVA and Tukey's Test. The D-values of Salmonella in WMP on day 1 were 18.9, 9.9 and 4.4 min at 80, 85 and 90 °C, respectively, which increased to 29.4, 13.6 and 6.5 min at 80, 85 and 90 °C, respectively, on day 180. Whereas, D-values of Salmonella in NFDM on day 1 were 17.9, 9.2 and 4.4 min at 80, 85 and 90 °C, respectively, and stayed similar during the storage. The D-values of Salmonella in milk powder remained similar throughout the storage once hydrated. The overall z-value of Salmonella in NFDM and WMP was 16.3 °C, whereas in hydrated NFDM and WMP, the overall z-value was 6.4 °C. ISSN: 01681605

Kürekci, C., Sahin, S., Iwan, E., Kwit, R., Bomba, A., Wasyl, D.
Whole-genome sequence analysis of Salmonella Infantis isolated from raw chicken meat samples and insights into pESI-like megaplasmid
(2021) International Journal of Food Microbiology, 337, art. no. 108956, .
ABSTRACT: There has been an increase in the number of reports on Salmonella enterica subsp. enterica serovar Infantis (S. Infantis) isolated from animals and humans. Recent studies using whole genome sequencing (WGS) have provided evidence on the likely contribution of a unique conjugative megaplasmid (pESI; ~280 kb) to the dissemination of this serovar worldwide. In the present study, twenty-two unrelated Salmonella strains [S. Infantis (n = 20) and Salmonella 6,7:r:- (n = 2)] and their plasmids were investigated using next generation sequencing technologies (MiSeq and MinION) to unravel the significant expansion of this bacteria in Turkey. Multi-locus sequence typing, plasmid replicons, resistance gene contents as well as phylogenetic relations between strains were determined. According to the WGS data, all S. Infantis possessed the relevant megaplasmid backbone genes and belonged to sequence type 32 (ST32) with the exception of a single novel ST7091. Tetracycline and trimethoprim/sulfamethoxazole resistance were found to be widespread in S. Infantis strains and the resistant strains exclusively carried the tetA, sul1, sul2 and dfrA14 genes. One S. Infantis isolate was also a carrier of the plasmid-mediated ampC via blaCMY-2, gene. Moreover, full genomes of four S. Infantis isolates were reconstructed based on hybrid assembly. All four strains contained large plasmids (240–290 kb) similar to previously published megaplasmid (pESI) and accompanied by several small plasmids. The megaplasmid backbone contained a toxin-antitoxin system, two virulence cassettes and segments associated with heavy metals resistance, while variable regions possessed several antibiotic resistance genes flanked by mobile elements. This study indicated that pESI-like megaplasmid is widely disseminated within the tested S. Infantis strains of chicken meat, warranting further genomic studies on clinical strains from humans and animals to uncover the overall emergence and spread of this serovar. ISSN: 01681605

Møretrø, T., Moen, B., Almli, V.L., Teixeira, P., Ferreira, V.B., Åsli, A.W., Nilsen, C., Langsrud, S.
Dishwashing sponges and brushes: Consumer practices and bacterial growth and survival
(2021) International Journal of Food Microbiology, 337, art. no. 108928, .
ABSTRACT: Sponges are frequently used in kitchens and have been shown to harbor large numbers of bacteria, occasionally also pathogens. Less is known about kitchen brushes regarding usage and presence of bacteria. In the present study, the use of sponges and brushes was studied in a survey among 9966 European consumers in ten countries, and growth and survival of bacteria in sponges and brushes were examined in laboratory experiments. Sponges were the preferred hand-cleaning utensils for washing-up in the majority of countries, while brushes were most frequently used in Denmark and Norway. Consumers mostly change their sponges at regular times, but also sensory cues (looks dirty, smelly, slimy) and usage occurrences such as wiping up meat juices may trigger replacement. Besides cleaning the dishes, over a quarter of the dish brush users also use it to clean a chopping board after soilage from chicken meat juices. The water uptake and drying rate varied considerably, both between different sponges and between brushes and sponges, where brushes dried fastest. Campylobacter survived one day in all sponges and

Salmonella more than seven days in two of three types of sponges. In the type of sponge that dried slowest, Salmonella grew on the first day and was always found in higher levels than in the other sponges. Non-pathogenic bacteria grew in the sponges and reached levels around 9 log CFU/sponge. In brushes all types of bacteria died over time. Campylobacter and Salmonella were reduced by more than 2.5 log to below the detection limit after one and three days, respectively. Bacteriota studies revealed a tendency for a dominance by Gram-negative bacteria and a shift to high relative prevalence of Pseudomonas over time in sponges. Both enumeration by agar plating and bacteriota analysis confirmed that the pathogens were in a minority compared to the other bacteria. Treatments of sponges and brushes with chlorine, boiling or in the dishwasher were effective to reduce Salmonella. We conclude that brushes are more hygienic than sponges and that their use should be encouraged. Contaminated sponges or brushes should be replaced or cleaned when they may have been in contact with pathogenic microorganisms, e.g. used on raw food spills. Cleaning of sponges and brushes with chlorine, boiling or dishwasher may be a safe alternative to replacing them with new ones. ISSN: 01681605

Marin, C., Lorenzo-Rebenaque, L., Laso, O., Villora-Gonzalez, J., Vega, S.
Pet Reptiles: A Potential Source of Transmission of Multidrug-Resistant Salmonella
(2021) Frontiers in Veterinary Science, 7, art. no. 613718, .
ABSTRACT: Salmonella spp. is widely considered one of the most important zoonotic pathogens worldwide. The close contact between reptiles and their owners provides favourable conditions for the transmission of zoonotic pathogen infections, and ~6% of human salmonellosis cases are acquired after direct or indirect contact with reptiles. Moreover, antimicrobial resistance is one of the most important health threats of the twenty-first century and has been reported in Salmonella strains isolated from pet reptiles, which could entail therapeutic consequences for their owners and breeders. The aim of this study was to assess Salmonella carriage by pet reptiles in pet shops and households, and their role in the transmission of antimicrobial resistance, to inform the owners about the possible risks factors. During the period between January 2019 and December 2019, 54 reptiles from pet shops and 69 reptiles from households were sampled in the Valencian Region (Eastern Spain). Three different sample types were collected from each reptile: oral cavity, skin, and cloacal swabs. Salmonella identification was based on ISO 6579-1:2017 (Annex D), serotyped in accordance with Kauffman-White-Le-Minor technique, and antibiotic susceptibility was assessed according to Decision 2013/652. The results of this study showed that 48% of the pet reptiles examined from households and pet shops carry Salmonella spp. All the strains isolated presented resistance to at least one antibiotic, and 72% were multidrug-resistant strains, the most frequently observed resistance patterns being gentamicin-colistin and gentamicin-colistin-ampicillin. The present study demonstrates that pet reptiles could be a source of human multidrug-resistant Salmonella infection. In this context, the most optimal prevention of multidrug-resistant Salmonella infections necessarily involves strict control of the sanitary status of reptile pet shops and hygienic handling by the individual owners at home. ISSN: 22971769

Sevilla, E., Vico, J.P., Delgado-Blas, J.F., González-Zorn, B., Marín, C.M., Uruén, C., Martín-Burriel, I., Bolea, R., Mainar-Jaime, R.C.
Resistance to colistin and production of extended-spectrum β-lactamases and/or AmpC enzymes in Salmonella isolates collected from healthy pigs in Northwest Spain in two periods: 2008–2009 and 2018
(2021) International Journal of Food Microbiology, 338, art. no. 108967, .
ABSTRACT: Salmonellosis is a common subclinical infection in pigs and therefore apparently healthy animals may represent a reservoir of antibiotic-resistant Salmonella for humans. This study estimates and characterizes resistance to two classes of antimicrobials considered of the highest priority within the critically important antimicrobials for humans, i.e. colistin (CR) and 3rd generation cephalosporins (3GC), on a collection of Salmonella isolates from pigs from two periods: between 2008 and 09, when colistin was massively used; and in 2018, after three years under a National Plan against Antibiotic Resistance. Prevalence of CR was low (6 out of 625; 0.96%; 95%CI: 0.44–2.1) in 2008–09 and associated mostly to the mcr-1 gene, which was detected in four S. 4,5,12:i:- isolates. Polymorphisms in the pmrAB genes were detected in a S. 9,12:-:- isolate. No CR was detected in 2018 out of 59 isolates tested. Among 270 Salmonella isolates considered for the assessment of resistance to 3GC in the 2008–2009 sampling, only one Salmonella Bredeney (0.37%; 95%CI: 0.07–2.1) showed resistance to 3GC, which was associated with the blaCMY-2 gene (AmpC producer). In 2018, six isolates out of 59 (10.2%; 95%CI: 4.7–20.5) showed resistance to 3GC, but only two different strains were identified (S. 4,12:i:- and S. Rissen), both confirmed as extended-spectrum β-lactamases (ESBL) producers. The blaCTX-M-3 and blaTEM-1b genes in S. 4,12:i:- and the blaTEM-1b gene in

S. Rissen seemed to be associated with this resistance. Overall, the prevalence of CR in Salmonella appeared to be very low in 2008–2009 despite the considerable use of colistin in pigs at that time, and seemed to remain so in 2018. Resistance to 3GC was even lower in 2008–2009 but somewhat higher in 2018. Resistance was mostly coded by genes associated with mobile genetic elements. Most serotypes involved in these antimicrobial resistances displayed a multidrug resistance pattern and were considered zoonotic. ISSN: 01681605

Peruzy, M.F., Houf, K., Joossens, M., Yu, Z., Proroga, Y.T.R., Murru, N.
Evaluation of microbial contamination of different pork carcass areas through culture-dependent and independent methods in small-scale slaughterhouses
(2021) International Journal of Food Microbiology, 336, art. no. 108902, .
ABSTRACT: Routine evaluation of the slaughter process is performed by the enumeration of the aerobic colony count, Enterobacteriaceae and Salmonella spp. on the carcass through destructive or non-destructive methods. With non-destructive methods, bacteria are counted from a minimum area of 100 cm2 in different sampling sites on the pork carcasses, and the results of these investigated areas are pooled to one value for the complete carcass evaluation (a total of 400 cm2). However, the composition of the bacterial community present on the different sampling areas remains unknown. The aim of the study was to characterize the microbial population present on four areas (ham, back, jowl and belly) of eight pork carcasses belonging to two different slaughterhouses through culture-dependent (Matrix-assisted laser desorption/ionization time-of-flight Mass Spectrometry MALDI-TOF MS, combined with 16S rRNA gene sequencing) and complementary culture-independent (16S rRNA amplicon sequencing) methods. The presence of Salmonella spp. and Y. enterocolitica was additionally assessed. Using MALDI-TOF MS, Staphylococcus, Pseudomonas, and Escherichia coli were found to dominate the bacterial cultures isolated from the 8 carcasses. Based on the 16S rRNA amplicon sequencing analyses however, no specific genus clearly dominated the bacterial community composition. By using this culture-independent method, the most abundant genera in microbial populations of the ham, back, jowl and belly were found to be similar, but important differences between the two slaughterhouses were observed. Thus, present data suggests that the indigenous bacterial population of individual animals is overruled by the microbial population of the slaughterhouse in which the carcass is handled. Also, our data suggests that sampling of only one carcass area by official authorities may be appropriate for the evaluation of the hygienic status of the carcasses and therefore of the slaughter process. ISSN: 01681605

Thomson, J.L., Cernicchiaro, N., Zurek, L., Nayduch, D.
Cantaloupe Facilitates Salmonella Typhimurium Survival within and Transmission among Adult House Flies (Musca domestica L.)
(2021) Foodborne Pathogens and Disease, 18 (1), pp. 49-55.
ABSTRACT: Salmonella enterica serovar Typhimurium is a pathogen harbored by livestock and shed in their feces, which serves as an acquisition source for adult house flies. This study used a green fluorescent protein (GFP) expressing strain of Salmonella Typhimurium to assess its acquisition by and survival within house flies, and transmission from and between flies in the presence or absence of cantaloupe. Female house flies were exposed to manure inoculated with either sterile phosphate-buffered saline or GFP-Salmonella Typhimurium for 12 h, then used in four experiments each performed over 24 h. Experiment 1 assessed the survival of GFP-Salmonella Typhimurium within inoculated flies. Experiment 2 determined transmission of GFP-Salmonella Typhimurium from inoculated flies to cantaloupe. Experiment 3 assessed fly acquisition of GFP-Salmonella Typhimurium from inoculated cantaloupe. Experiment 4 evaluated transmission of GFP-Salmonella Typhimurium between inoculated flies and uninoculated flies in the presence and absence of cantaloupe. GFP-Salmonella Typhimurium survived in inoculated flies but bacterial abundance decreased between 0 and 6 h without cantaloupe present and between 0 and 6 h and 6 and 24 h with cantaloupe present. Uninoculated flies acquired GFP-Salmonella Typhimurium from inoculated cantaloupe and bacterial abundance increased in cantaloupe and flies from 6 to 24 h. More uninoculated flies exposed to inoculated flies acquired GFP-Salmonella Typhimurium when cantaloupe was present than when absent. We infer that the presence of a shared food source facilitated the transfer of GFP-Salmonella Typhimurium from inoculated to uninoculated flies. Our study demonstrated that house flies acquired, harbored, and excreted viable GFP-Salmonella Typhimurium and transferred bacteria to food and each other. Understanding the dynamics of bacterial acquisition and transmission of bacteria between flies and food helps in assessing the risk flies pose to food safety and human health. ISSN: 15353141

Oscar, T.
Salmonella Prevalence Alone Is Not a Good Indicator of Poultry Food Safety
(2021) Risk Analysis, 41 (1), pp. 110-130.
ABSTRACT: Salmonella is a leading cause of foodborne illness (i.e., salmonellosis) outbreaks, which on occasion are attributed to ground turkey. The poultry industry uses Salmonella prevalence as an indicator of food safety. However, Salmonella prevalence is only one of several factors that determine risk of salmonellosis. Consequently, a model for predicting risk of salmonellosis from individual lots of ground turkey as a function of Salmonella prevalence and other risk factors was developed. Data for Salmonella contamination (prevalence, number, and serotype) of ground turkey were collected at meal preparation. Scenario analysis was used to evaluate effects of model variables on risk of salmonellosis. Epidemiological data were used to simulate Salmonella serotype virulence in a dose-response model that was based on human outbreak and feeding trial data. Salmonella prevalence was 26% (n = 100) per 25 g of ground turkey, whereas Salmonella number ranged from 0 to 1.603 with a median of 0.185 log per 25 g. Risk of salmonellosis (total arbitrary units (AU) per lot) was affected ($p \leq 0.05$) by Salmonella prevalence, number, and virulence, by incidence and extent of undercooking, and by food consumption behavior and host resistance but was not ($p > 0.05$) affected by serving size, serving size distribution, or total bacterial load of ground turkey when all other risk factors were held constant. When other risk factors were not held constant, Salmonella prevalence was not correlated ($r = -0.39$; $p = 0.21$) with risk of salmonellosis. Thus, Salmonella prevalence alone was not a good indicator of poultry food safety because other factors were found to alter risk of salmonellosis. In conclusion, a more holistic approach to poultry food safety, such as the process risk model developed in the present study, is needed to better protect public health from foodborne pathogens like Salmonella. Published 2020. This article is a U.S. Government work and is in the public domain in the USA. ISSN: 02724332

Zeng, H., Rasschaert, G., De Zutter, L., Mattheus, W., De Reu, K.
Identification of the source for salmonella contamination of carcasses in a large pig slaughterhouse
(2021) Pathogens, 10 (1), art. no. 77, pp. 1-12.
ABSTRACT: To identify the major source of Salmonella contamination in a pig slaughterhouse, samples were collected from the clean and unclean area and Salmonella isolates were further typed. Carcasses entering the clean area showed a Salmonella contamination rate of 96.7% in the oral cavity and 55.0% in the rectum content samples. Evisceration seemed not to be critical as the contamination rate of the carcasses was similar before (16.7%) and after (18.3%) this slaughter step. In the unclean area, a limited number of oral cavity samples were positive after bleeding, while a dramatic increase of positives was observed after dehairing. Salmonella was detected in up to 0.01 mL of the recycled water collected from the dehairing machine. Genotyping of Salmonella isolates showed that similar pulsotypes were present in the oral cavity and recycled water. Based on these observations it can be concluded that the recycled water used in the dehairing machine was the major source for the carcass contamination in this slaughterhouse. ISSN: 20760817

Yamasaki, E., Matsuzawa, S., Takeuchi, K., Morimoto, Y., Ikeda, T., Okumura, K., Kurazono, H.
Rapid Serotyping of Salmonella Isolates Based on Single Nucleotide Polymorphism-Like Sequence Profiles of a Salmonella-Specific Gene
(2021) Foodborne Pathogens and Disease, 18 (1), pp. 31-40.
ABSTRACT: Although serotyping is the most important method of identification of taxonomy in Salmonella, conventional serotype determination with a complete set of antisera is time consuming and laborious. Recently, rapid serotyping procedures with polymerase chain reaction (PCR) have been developed. In this study, we established a novel PCR-based rapid serotyping method that employs a unique target gene. Alignment study of Salmonella-specific gene (Salmonella enterotoxin [stn]) revealed a correlation between the stn gene sequence and the serotype of the organism. In 750 bp of stn gene, 55 nucleotides indicated single nucleotide polymorphism (SNP)-like polymorphism, and the correlation between the SNP-like polymorphism and the serotype of the organism suggests that SNP-like sequences in stn gene can serve as an index for serotyping. To develop a rapid serotyping method based on the SNP-like polymorphism, we selected serotype-associated 12 SNP-like sites in the stn gene and established a method based on high-resolution melting (HRM) and PCR, which identifies nucleotides at SNP-like sites within 1.5 h. This newly established rapid serotyping procedure (stn-HRM) could identify nine serotypes, including the frequently isolated serovar Enteritidis. These nine serotypes cover 64.3% of cases of Salmonella, as reported by the World Health Organization/Global

Foodborne Infection Network (WHO/GFN) Country Databank from 2001 to 2010. In this study, we employed a unique target gene, stn, which is completely independent of the genes that were targeted in previously reported rapid serotyping procedures. Therefore, the results obtained by our newly developed stn-HRM procedure are independent of the results obtained by other procedures. Besides, stn-HRM can ensure accurate identification of the bacterial species as stn is a Salmonella-specific gene. It is expected that the combination of newly constructed stn-HRM and previously reported procedures could further improve the credibility of Salmonella isolate serotyping. ISSN: 15353141

Shen, Y., Xu, L., Li, Y.
Biosensors for rapid detection of Salmonella in food: A review
(2021) Comprehensive Reviews in Food Science and Food Safety, 20 (1), pp. 149-197.
ABSTRACT: Salmonella is one of the main causes of foodborne infectious diseases, posing a serious threat to public health. It can enter the food supply chain at various stages of production, processing, distribution, and marketing. High prevalence of Salmonella necessitates efficient and effective approaches for its identification, detection, and monitoring at an early stage. Because conventional methods based on plate counting and real-time polymerase chain reaction are time-consuming and laborious, novel rapid detection methods are urgently needed for in-field and on-line applications. Biosensors provide many advantages over conventional laboratory assays in terms of sensitivity, specificity, and accuracy, and show superiority in rapid response and potential portability. They are now recognized as promising alternative tools and one of the most on-site applicable and end user–accessible methods for rapid detection. In recent years, we have witnessed a flourishing of studies in the development of robust and elaborate biosensors for detection of Salmonella in food. This review aims to provide a comprehensive overview on Salmonella biosensors by highlighting different signal-transducing mechanisms (optical, electrochemical, piezoelectric, etc.) and critically analyzing its recent trends, particularly in combination with nanomaterials, microfluidics, portable instruments, and smartphones. Furthermore, current challenges are emphasized and future perspectives are discussed. ISSN: 15414337

Prabhakar, P., Lekshmi, M., Joseph, T.C., Balange, A.K., Nayak, B.B., Kumar, S.H.
Performance evaluation of a selective-enrichment-isolation protocol for Salmonella enterica from seafood
(2021) Journal of Microbiological Methods, 180, art. no. 106120, .
ABSTRACT: In this study using 57 finfish samples of marine origin, selective enrichment in Rappaport-Vassiliadis (RV) broth followed by isolation on the Hektoen enteric agar (HEA) yielded 50 (53.2%) of 94 isolates. The results suggest RV-HEA as the most suitable media combination for the recovery of Salmonella from tropical seafood. ISSN: 01677012

Silveira, L., Nunes, A., Pista, A., Isidro, J., Belo Correia, C., Saraiva, M., Batista, R., Castanheira, I., MacHado, J., Gomes, J.P.
Characterization of Multidrug-Resistant Isolates of Salmonella enterica Serovars Heidelberg and Minnesota from Fresh Poultry Meat Imported to Portugal
(2021) Microbial Drug Resistance, 27 (1), pp. 87-98.
ABSTRACT: Salmonella enterica serovars Heidelberg and Minnesota frequently display several genetic mobile elements making them potential spreaders of resistance genes. Here, we phenotypically determined the antibiotic resistance profile and subsequently performed whole-genome sequencing on 36 isolates recovered from samples of fresh poultry meat, within the Portuguese Official Inspection Plan for Imported Foodstuffs. Several isolates of both serovars showed high genetic relatedness either with isolates from raw poultry meat imported to the Netherlands from Brazil or with isolates from samples from the broiler production chain in Brazil. The multidrug-resistant (MDR) character was common to the vast majority (94.4%) of isolates from both serovars, and several isolates carried the plasmid IncA/C2 containing the β-lactamase gene blaCMY-2 and IncX1 containing a type IV secretion system. These results somehow mirror the scenario observed in the Netherlands, showing the introduction, through fresh imported poultry meat in compliance with European legislation, of MDR Salmonella enterica serovars Heidelberg and Minnesota in Europe, with the potential spread of resistance markers. These data suggest the need to revise the hygiene criteria for foodstuffs monitoring before its placement on the market, with the determination of the resistome being an invaluable contribute to limit the dissemination of resistance markers. ISSN: 10766294

Zhao, B.C., Hanson, E.J., Ingham, B.H.
Holding fresh-cut produce under refrigeration may not prevent pathogen growth: Implications for time-temperature control to reduce risk

(2021) Food Protection Trends, 41 (1), pp. 46-55.
ABSTRACT: The U.S. Food and Drug Administration Food Code suggests that holding fresh-cut produce at < 5°C will limit growth of pathogenic microorganisms. Here, we determined whether cucumber, onion, pepper, mango, and tomato supported growth of Listeria monocytogenes (LM), Shiga toxin-producing Escherichia coli (STEC), and Salmonella enterica (SALM) at 5, 10, and 22°C. Produce was surface-pasteurized, diced, inoculated with single-pathogen cocktails, and incubated. Survivors were then enumerated with change in population (Δ-log CFU per gram) determined over time. Mango did not support pathogen growth at 5 or 10°C, but SALM and STEC exhibited significant ($P < 0.05$) growth on mango at 22°C (2.85 and 1.41 Δ-log CFU/g, respectively). At 5°C, significant ($P < 0.05$) growth was seen on cucumber inoculated with SALM and LM; onion and pepper inoculated with LM; and tomato inoculated with STEC. At 10°C, fresh-cut cucumber, onion, and pepper supported significant ($P < 0.05$) increases in SALM, STEC, and LM, along with SALM on tomato; Δ-log ranged from 3.37 (onion, LM) to 5.40 CFU/g (pepper, SALM). Growth of pathogens was not significantly different ($P < 0.05$) at 10 and 22°C for SALM or STEC inoculated onto onion, pepper, cucumber, or tomato. Results suggest that holding fresh-cut produce at or near refrigeration temperatures (5 or 10°C) may not control risk of pathogen growth. ISSN: 15419576

Zhao, L., Wang, J., Sun, X.X., Wang, J., Chen, Z., Xu, X., Dong, M., Guo, Y.-N., Wang, Y., Chen, P., Gao, W., Geng, Y.
Development and Evaluation of the Rapid and Sensitive RPA Assays for Specific Detection of Salmonella spp. in Food Samples
(2021) Frontiers in Cellular and Infection Microbiology, 11, art. no. 631921, .
ABSTRACT: Salmonella spp. is among the main foodborne pathogens which cause serious foodborne diseases. An isothermal real-time recombinase polymerase amplification (RPA) and lateral flow strip detection (LFS RPA) were used to detect Salmonella spp. targeting the conserved sequence of invasion protein A (invA). The Real-time RPA was performed in a portable florescence scanner at 39°C for 20 min. The LFS RPA was performed in an incubator block at 39°C for 15 min, under the same condition that the amplifications could be inspected by the naked eyes on the LFS within 5 min. The detection limit of Salmonella spp. DNA using real-time RPA was 1.1 × 101 fg, which was the same with real-time PCR but 10 times higher than that of LFS RPA assay. Moreover, the practicality of discovering Salmonella spp. was validated with artificially contaminated lamb, chicken, and broccoli samples. The analyzing time dropped from 60 min to proximately 5–12 min on the basis of the real-time and LFS RPA assays compared with the real-time PCR assay. Real-time and LFS RPA assays' results were equally reliable. There was no cross-reactivity with other pathogens in both assays. In addition, the assays had good stability. All of these helped to show that the developed RPA assays were simple, rapid, sensitive, credible, and could be a potential point-of-need (PON) test required mere resources. ISSN: 22352988

Abdul-Rahiman, U.A., Nordin, N., Abdul-Mutalib, N.A., Sanny, M.
Holistic approaches to reducing salmonella contamination in poultry industry
(2021) Pertanika Journal of Tropical Agricultural Science, 44 (1), pp. 1-23.
https://www.scopus.com/inward/record.uri?eid=2-s2.0-
ABSTRACT: Salmonella are widely found in the poultry industry, which subsequently may pose a risk to animal and human health. The aim of this review is to highlight strategies for the prevention and control of Salmonella at each stage in the poultry production chain by monitoring risks from the farm to the retailer. Among the primary approaches for control of Salmonella at the farm level includes the administration of synthetic and natural compounds to live chickens (vaccination and antibiotic), litter management as well as fortification of feed and acidification of drinking water. In the poultry processing plant, multiple hurdle technology and different chilling conditions to reduce Salmonella were discussed. In the retail level, an effective monitoring program to control Salmonella contamination by good manufacturing practices and hazard analysis and critical control points has been reviewed. Overall, we conclude that these approaches play a role in reducing the dissemination of Salmonella in the poultry industry. However, there is no published data related to logistic scheduling of poultry processing. ISSN: 15113701

Cochet, M.-F., Baron, F., Bonnassie, S., Jan, S., Leconte, N., Jardin, J., Briard-Bion, V., Gautier, M., Andrews, S.C., Guérin-Dubiard, C., Nau, F.
Identification of new antimicrobial peptides that contribute to the bactericidal activity of egg white against salmonella enterica serovar enteritidis at 45 °c
(2021) Journal of Agricultural and Food Chemistry, 69 (7), pp. 2118-2128.
ABSTRACT: A recent work revealed that egg white (EW) at 45 °C exhibits powerful bactericidal activity against S. enterica serovar Enteritidis, which is surprisingly little

affected by removal of the >10 kDa EW proteins. Here, we sought to identify the major EW factors responsible for this bactericidal activity by fractionating EW using ultrafiltration and nanofiltration and by characterizing the physicochemical and antimicrobial properties of the resulting fractions. In particular, 22 peptides were identified by nano-LC/MSMS and the bactericidal activities of representative peptides (with predicted antimicrobial activity) were further assessed. Two peptides (FVPPVQR and GDPSAWSWGAEAHS) were found to be bactericidal against S. enterica serovar Enteritidis at 45 °C when provided in an EW environment. Nevertheless, these peptides contribute only part of this bactericidal activity, suggesting other, yet to be determined, antimicrobial factors. ISSN: 00218561

Uelze, L., Becker, N., Borowiak, M., Busch, U., Dangel, A., Deneke, C., Fischer, J., Flieger, A., Hepner, S., Huber, I., Methner, U., Linde, J., Pietsch, M., Simon, S., Sing, A., Tausch, S.H., Szabo, I., Malorny, B.
Toward an Integrated Genome-Based Surveillance of Salmonella enterica in Germany
(2021) Frontiers in Microbiology, 12, art. no. 626941, .
ABSTRACT: Despite extensive monitoring programs and preventative measures, Salmonella spp. continue to cause tens of thousands human infections per year, as well as many regional and international food-borne outbreaks, that are of great importance for public health and cause significant socio-economic costs. In Germany, salmonellosis is the second most common cause of bacterial diarrhea in humans and is associated with high hospitalization rates. Whole-genome sequencing (WGS) combined with data analysis is a high throughput technology with an unprecedented discriminatory power, which is particularly well suited for targeted pathogen monitoring, rapid cluster detection and assignment of possible infection sources. However, an effective implementation of WGS methods for large-scale microbial pathogen detection and surveillance has been hampered by the lack of standardized methods, uniform quality criteria and strategies for data sharing, all of which are essential for a successful interpretation of sequencing data from different sources. To overcome these challenges, the national GenoSalmSurv project aims to establish a working model for an integrated genome-based surveillance system of Salmonella spp. in Germany, based on a decentralized data analysis. Backbone of the model is the harmonization of laboratory procedures and sequencing protocols, the implementation of open-source bioinformatics tools for data analysis at each institution and the establishment of routine practices for cross-sectoral data sharing for a uniform result interpretation. With this model, we present a working solution for cross-sector interpretation of sequencing data from different sources (such as human, veterinarian, food, feed and environmental) and outline how a decentralized data analysis can contribute to a uniform cluster detection and facilitate outbreak investigations. ISSN: 1664302X

Ban, G.-H., Dai, Y., Huan, T., Ke, A., Delaquis, P., Wang, S.
Endogenous metabolites released by sanitized sprouting alfalfa seed inhibit the growth of salmonella enterica
(2021) mSystems, 6 (1), art. no. e00898-20, .
ABSTRACT: Sprouts are the leading cause of foodborne disease outbreaks globally, mainly because the specialized conditions required to germinate seed sprouts for human consumption contribute to an environment that allows pathogenic bacteria to flourish. To reduce risk of illness, current food safety guidelines in the United States and Canada recommend hypochlorite treatment for seed sanitation. However, many growers and consumers have become wary of the impact of hypochlorite on human health and the environment and are actively seeking less caustic approaches. Here, we evaluated the effects of both the traditional hypochlorite treatment and a milder alternative on nontyphoidal Salmonella enterica colonization of germinating alfalfa seed. Moreover, we explored three biological factors as potential contributors for inhibition of S. enterica growth: colonization by indigenous bacteria, seed composition changes, and seed metabolite release. In this experimental setting, we found that a combinatorial treatment of heat, peroxide, and acetic acid was as effective as hypochlorite for inhibiting S. enterica growth. Notably, we pinpointed N-acetyl-spermidine as an endogenous metabolite exuded by treated seeds that strongly inhibits S. enterica growth. In doing so, we both elucidated one of the mechanisms of chemical sanitation and highlighted a potential seed-derived mode of antimicrobial treatment that may apply to modernized food safety protocols. IMPORTANCE Warm, humid, and nutrient-rich conditions that are used to produce sprouts encourage Salmonella enterica to proliferate. However, many disparate sanitation methods exist, and there is currently no single treatment that can guarantee pathogen-free seeds. Here, we compared the ability of traditional hypochlorite treatment against a combinatorial treatment of heat, peroxide, and vinegar (HPA) commonly used in organic farming practices to inhibit S. enterica colonization and growth during alfalfa germination and found HPA to be at least as effective. Furthermore, we explored seed-based changes

following sanitization treatments using metabolomics and identified polyamines as strong inhibitors of Salmonella growth on germinating alfalfa. Our findings enable a better understanding of host-pathogen interactions in sprout microbial communities and promote in-depth, evidence-based research in seed sprout safety. ISSN: 23795077

Dhakal, J., Aldrich, C.G.
A comparison of salmonella survival and detection using an enrichment technique in dry-And wet-inoculated rendered chicken fat treated with sodium bisulfate
(2021) Journal of Food Protection, 84 (2), pp. 249-254.
ABSTRACT: The differences in the recovery of Salmonella from rendered chicken fat treated with sodium bisulfate (SBS) when inoculated with a dry versus wet inoculum were evaluated. Food-grade rendered chicken fat was inoculated with a dry inoculum and a wet inoculum containing a cocktail of Salmonella serovars (Enteritidis, Heidelberg, and Typhimurium). In addition, the effect of an antimicrobial treatment (SBS) against Salmonella in both the aqueous phase and fat phase of the chicken fat was evaluated. The untreated control samples in the aqueous phase had a consistent level of Salmonella (~7 log) when both the dry and wet inocula were used. In the SBS-treated aqueous phase, Salmonella pathogens were not detectable after 6 h when the wet inoculum was used; when the dry inoculum was used, Salmonella pathogens were not detectable at 24 h. Salmonella pathogens were detected for up to 6 h in the SBS-treated fat phase when the dry inoculum was used compared with 2 h with the wet inoculum. The 24-h fat samples that failed to show growth on Trypticase soy agar were enriched for Salmonella isolation, followed by confirmation by PCR using primers for the invA gene. SBS-treated and control samples from the dry-inoculated rendered chicken fat and the inoculated control from the wet-inoculated rendered chicken fat tested positive for Salmonella. However, the SBS-treated sample from the wet-inoculated fat was negative for Salmonella. The use of dry SBS powder against dry Salmonella inoculum in the fat matrix caused only ~2.8-log reduction after 24 h compared with ~2.2-log reduction in the positive control. However, the recovery of Salmonella from untreated control fat was lower and was not different (P. 0.05) from that recovered from the SBS-treated fat. The results suggest that viable but nonculturable states of Salmonella may develop in rendered chicken fat or that injured cells may be present, which indicates that testing should include an enrichment and appropriate molecular confirmation instead of agar plating alone. ISSN: 0362028X

Tzani, M., Mandilara, G., Dias, J.G., Sideroglou, T., Chrysostomou, A., Mellou, K.
Impact of salmonella control programmes in poultry on human salmonellosis burden in Greece
(2021) Antibiotics, 10 (2), art. no. 121, pp. 1-10.
ABSTRACT: Since 2008, veterinary authorities in Greece have implemented national control programmes (NSCPs) targeting S. Enteritidis (SE) and S. Typhimurium (ST) in poultry. We assessed the effect of the programs on the reported number of human isolates. Using monthly data for 2006–2017, we defined two groups (SE, ST) and one control group with serotypes unrelated to poultry or eggs. For SE we also analysed data for 2006–2015 due to a multi-county SE outbreak in 2016. We performed an interrupted time series analysis and used a negative binominal regression model. For both SE and ST, there was no significant trend of the isolation rate before or after NSCPs' introduction. After the NSCPs' introduction there was an increasing rate (IRR: 1.005, 95% CI: 1.001–1.008) for control serotypes and a decreasing one for SE (IRR: 0.990, 95% CI: 0.986–0.995) (for 2009 to 2015 analysis). From 2006 to 2017, NSCPs had a statistically significant impact on the number of SE isolates that decreased by 49% (IRR:0.511, 95% CI: 0.353–0.739). No impact was shown on the number of ST (p-value = 0.741) and control isolates (p = 0.069). As a conclusion, NSCP's implementation was associated with decreased SE isolates and overall burden of salmonellosis; however further measures aiming at human salmonellosis due to ST, should be considered. ISSN: 20796382

Daigle, F.
Special issue "salmonella: Pathogenesis and host restriction"
(2021) Microorganisms, 9 (2), art. no. 325, pp. 1-2.
ISSN: 20762607

Grasso-Kelley, E.M., Liu, X., Halik, L.A., Douglas, B.
Evaluation of hot-air drying to inactivate salmonella and enterococcus faecium on apple pieces
(2021) Journal of Food Protection, 84 (2), pp. 240-248.
ABSTRACT: Hot-air drying processes are used to provide specific quality attributes to products, such as dehydrated apple pieces. To comply with the U.S. Food and Drug

Administration Food Safety Modernization Act, there is a need to understand microbial lethality during these processes. The objective of this study was to determine the level of inactivation provided by hot-air drying on a Salmonella cocktail inoculated onto apple cubes and to evaluate the performance of Enterococcus faecium as a surrogate. A cocktail of Salmonella serovars (Agona, Tennessee, Montevideo, Mbandaka, and Reading) and E. faecium were individually inoculated onto cored, peeled Gala apple cubes at 9.2 6 0.3 and 8.8 6 0.1 log CFU per sample, respectively. Apple cubes were dried at 104 or 1358C in ~1.5-kg batches using a hot-air dryer with a vertically directed heat source and without mixing. Three subsamples, consisting of four inoculated cubes, were enumerated at each time point (n ≥ 5) from multiple product bed depths. Water activity decreased throughout the duration of the study, with samples drying faster at 135 than 1048C. Samples at the bottom bed depth, closer to the heat source, dried faster than those at the higher bed depth, regardless of temperature. Significant microbial inactivation was not seen immediately. It took.10 min at the bottom bed depth or.40 min of drying at the top bed depth, regardless of temperature (P, 0.05). By the end of drying, average Salmonella inactivation of greater than 5 log CFU per sample was achieved. At temperature conditions evaluated, E. faecium inactivation was slower than Salmonella, indicating that it would likely serve as a good surrogate for in-plant validation studies. Case hardening did not inhibit microbial inactivation in the conditions tested. Hot-air drying under the conditions evaluated may provide a preventive control in the production of dehydrated products, such as apples. ISSN: 0362028X

Bernad-Roche, M., Casanova-Higes, A., Marín-Alcalá, C.M., Cebollada-Solanas, A., Mainar-Jaime, R.C.
Salmonella infection in nursery piglets and its role in the spread of salmonellosis to further production periods
(2021) Pathogens, 10 (2), art. no. 123, pp. 1-14.
ABSTRACT: Few studies have focused on assessing Salmonella infection in the nursery and its role in further pig production periods. Mesenteric lymph nodes, intestinal content, and meat juice from 389 6-week-old male piglets intended for human consumption from five breeding farms and 191 pooled floor fecal samples from gilt development units (GDU) from the same farms were analyzed to estimate and characterize (by pulsed-field gel electrophoresis and antimicrobial resistance analyses) Salmonella infection. The prevalence of infection and shedding among piglets was 36.5% and 37.3%, respectively, shedding being significantly associated with infection (Odds Ratio = 12.7; CI 7.3–22.0). Salmonella Rissen; S. 4,[5],12:i:-; and S. Derby were the most common serotypes. A low level of Salmonella-specific maternal antibodies at the beginning of the nursery period suggested it was a period of high risk of infection. Resistance to 3rd-and 4th-generation cephalosporins was detected in piglet isolates although the piglets never received antibiotics, indicating they could be vectors of antimicrobial resistance. The same Salmonella clones were detected in piglet and GDU isolates, suggesting that infected piglets play a significant role in the infection of gilts and consequently of finishing pigs in the case of production farms. The control of Salmonella infection in nursery piglets may decrease the risk of abattoir and carcass contamination. ISSN: 20760817

Chatzopoulos, D.C., Vasileiou, N.G.C., Ioannidi, K.S., Katsafadou, A.I., Mavrogianni, V.S., Michael, C.K., Katsarou, E.I., Karavanis, E., Papadopoulos, N., Sbiraki, A., Athanasiou, L.V., Billinis, C., Fthenakis, G.C.
Experimental study of the potential role of salmonella enterica subsp. Diarizonae in the diarrhoeic syndrome of lambs
(2021) Pathogens, 10 (2), art. no. 113, pp. 1-16.
ABSTRACT: The objectives of this experimental work were the evaluation of the potential role of Salmonella enterica subsp. diarizonae in diarrhoeic syndrome in lambs and the investigation of facets of the pathogenesis of the infection. In total, 12 lambs were challenged orally on the first day of life, with a S. enterica subsp. diarizonae isolate from a clinical case of diarrhoeic syndrome. Sequential blood, faecal and buccal samples were collected from lambs and faecal and milk samples were taken from their dams. Lambs were euthanised 1, 2, 4, 7, 10, 14 and 21 days after challenge. Samples were processed for recovery of the challenge organism; they were also subjected to examination by PCR for detection of the invA gene. Tissue samples from lambs were also examined as above and histopathologically. S. enterica subsp. diarizonae was recovered from faecal samples of all lambs, in total, from 45/77 samples (median duration: 2.4 days post-inoculation). It was also recovered from buccal samples (10/77) from seven lambs (median duration: 0.8 days), and from tissue samples (small intestine, abomasum, liver, gallbladder) of nine lambs. It was recovered from two consecutive milk samples from the same ewe, but not from any faecal sample from ewes. The invA gene was detected in samples from all lambs

(median duration: 5.5 days in faecal and 1.3 days in buccal samples), as well as in milk samples from three ewes. Histopathological findings included abomasitis with subepithelial presence of eosinophils, lymphocytes and plasma cells, consistently observed in all lambs. In the small intestine, salient lesions initially included distension and oedema of intestinal villi, leucocytic infiltration and hyperplasia of lymphoid nodules with apparent germinal centres; this was followed at later stages by atrophy and/or degeneration of the lymphoid tissue of the intestine with marked subepithelial infiltration of lymphocytes, plasma cells and eosinophils. ISSN: 20760817

Zheng, J., Reed, E., Ramachandran, P., Ottesen, A., Brown, E.W., Wang, Y.
Taxonomic and Functional Shifts in the Sprout Spent Irrigation Water Microbiome in Response to Salmonella Contamination of Alfalfa Seeds
(2021) Applied and Environmental Microbiology, 87 (3), pp. 1-18.
ABSTRACT: Despite recent advances in Salmonella-sprout research, little is known about the relationship between Salmonella and the sprout microbiome during sprouting. Sprout spent irrigation water (SSIW) provides an informative representation of the total microbiome of this primarily aquaponic crop. This study was designed to characterize the function and taxonomy of the most actively transcribed genes in SSIW from Salmonella enterica serovar Cubana-contaminated alfalfa seeds throughout the sprouting process. Genomic DNA and total RNA from SSIW was collected at regular intervals and sequenced using Illumina MiSeq and NextSeq platforms. Nucleic acid data were annotated using four different pipelines. Both metage-nomic and metatranscriptomic analyses revealed a diverse and highly dynamic SSIW microbiome. A "core" SSIW microbiome comprised Klebsiella, Enterobacter, Pantoea, and Cronobacter. The impact, however, of Salmonella contamination on alfalfa seeds influenced SSIW microbial community dynamics not only structurally but also functionally. Changes in genes associated with metabolism, genetic information processing, environmental information processing, and cellular processes were abundant and time dependent. At time points of 24 h, 48 h, and 96 h, totals of 541, 723, and 424 S. Cubana genes, respectively, were transcribed at either higher or lower levels than at 0 h in SSIW during sprouting. An array of S. Cubana genes (107) were induced at all three time points, including genes involved in biofilm formation and modulation, stress responses, and virulence and tolerance to antimicrobials. Taken together, these findings expand our understanding of the effect of Salmonella seed contamination on the sprout crop microbiome and metabolome. IMPORTANCE Interactions of human enteric pathogens like Salmonella with plants and plant microbiomes remain to be elucidated. The rapid development of next-generation sequencing technologies provides powerful tools enabling investigation of such interactions from broader and deeper perspectives. Using metagenomic and metatranscriptomic approaches, this study identified not only changes in microbiome structure of SSIW associated with sprouting but also changes in the gene expression patterns related to the sprouting process in response to Salmonella contamination of alfalfa seeds. This study advances our knowledge on Salmonella-plant (i.e., sprout) interaction. ISSN: 00992240

Vallejo, C.V., Tere, C.P., Calderon, M.N., Arias, M.M., Leguizamon, J.E.
Development of a genomic DNA reference material for Salmonella enteritidis detection using polymerase chain reaction
(2021) Molecular and Cellular Probes, 55, art. no. 101690, .
ABSTRACT: Several rapid methods based on nucleic acids can detect foodborne pathogens, such as Salmonella spp. However, a common reference that enables metrological traceability among measurement results is not available. Reference materials (RM) are thus key to guarantee methodological comparability. This study developed a candidate genomic DNA reference material for Salmonella enteritidis quantification to establish performance conditions and reference values for normalized RM production. The growth of Salmonella enteritidis ATCC® 13076 in Rappaport Vassiliadis selective medium was characterized, and we optimized a method of DNA extraction using cetrimonium bromide (CTAB) and LiCl. In a first stage six concentrations of DNA were prepared with and without yeast RNA (40 ng/μL) to evaluate its effect as a stabilizer in terms of homogeneity and short-term stability. Based on the findings, in a second stage two DNA concentrations were prepared and a reference value with its uncertainty was assigned based on the results of characterization, homogeneity, and stability studies using digital polymerase chain reaction and the gene targets, invA, ttr, and hilA. The material was stable for 9 months at 4 °C, with a expanded uncertainty contribution range of 11%–14%. The novel candidate RM is the first to be developed nationwide and will improve the quality of measurements in the area of food safety. ISSN: 08908508

Brasão, S.C., Melo, R.T.D., Prado, R.R., Monteiro, G.P., Santos, F.A.L.D., Braz, R.F., Rossi, D.A.
Characterization and control of biofilms of Salmonella Minnesota of poultry origin
(2021) Food Bioscience, 39, art. no. 100811, .
ABSTRACT: Biofilms characterize sessile form that allows bacterial maintenance under hostile conditions. Salmonella represents an important foodborne zoonotic agent, capable of forming biofilms on diverse surfaces. The emergence of Salmonella Minnesota in the Brazilian poultry production expresses the need for more specific knowledge related to its maintenance in the environment and consequent food contamination, as well as control measures. Our approach combines the analysis of genetic determinants linked to biofilm formation and the phenotypic study of biomass on different surfaces, together with the determination of the effect of chemical agents on the control of sessile structure. It also evaluates the genetic similarity profile of 29 S. Minnesota strains isolated from food and environmental samples in two full-cycle poultry industries from 2009 to 2014. Genetic analysis showed low heterogeneity with the identification of six clonal groups, three clusters with homology greater than 80% and seven distinct genotypes. It was compatible with the presence of important genes in biofilm formation, with 28/29 (97.0%) presenting adrA and csgD and 27 (93.0%) presenting the luxS gene. Allied to this, 19/29 of the strains (66.0%) presented biomass that varied the intensity in weak (9/19–47.0%), moderate (6/19–32.0%) and strong (4/19–21.0%), with characteristic ultrastructure of a mature biofilm. Sodium hypochlorite showed greater efficiency in the control of sessile cells, even after incubation process. The study shows the presence of biofilm-producing S. Minnesota characterizing the problem of persistence in broiler slaughterhouses and indicates as a control strategy the use of sodium hypochlorite in an appropriate manner. ISSN: 22124292

Cox, L.A., Jr.
Higher line speed in young chicken slaughter establishments does not predict increased Salmonella contamination risks
(2021) Poultry Science, 100 (2), pp. 635-642.
ABSTRACT: Do faster slaughter line speeds for young chickens increase risk of Salmonella contamination? We analyze data collected in 2018–2019 from 97 slaughter establishments processing young chickens to examine the extent to which differences in slaughter line speeds across establishments operating under the same inspection system explain observed differences in their microbial quality, specifically frequencies of positive Salmonella samples. A variety of off-the-shelf statistical and machine learning techniques applied to the data to identify and visualize correlations and potential causal relationships among variables showed that the presence of Salmonella or other indicators of process control, such as noncompliance records for regulations associated with process control and food safety, are not significantly increased in establishments with higher line speeds (e.g., above 140 birds per min) compared with establishments with lower line speeds when establishments are operating under the conditions present in this study. This included some establishments operating under specific criteria to obtain a waiver for line speed. A null hypothesis advanced over 30 yr ago by the National Research Council that increased line speeds result in a product that is not contaminated more often than before line speeds were increased, appears to be fully consistent with these recent data. ISSN: 00325791

Mencía-Ares, O., Argüello, H., Puente, H., Gómez-García, M., Álvarez-Ordóñez, A., Manzanilla, E.G., Carvajal, A., Rubio, P.
Effect of antimicrobial use and production system on Campylobacter spp., Staphylococcus spp. and Salmonella spp. resistance in Spanish swine: A cross-sectional study
(2021) Zoonoses and Public Health, 68 (1), pp. 54-66.
ABSTRACT: Antimicrobial resistance is a worldwide public health threat; hence, current trends tend to reduce antimicrobial use in food-producing animals and to monitor resistance in primary production. This study aimed at evaluating the impact of antimicrobial use and production system on swine farms in the antimicrobial resistance of Campylobacter, Salmonella and Staphylococcus, the main zoonotic pathogens in pig herds, in order to assess their potential value as sentinel microorganisms in antimicrobial resistance surveillance schemes. A total of 37 Spanish swine farms, 18 intensive and 19 organic/extensive farms, were included in the study. The antimicrobial resistance of 104 Campylobacter, 84 Staphylococcus and 17 Salmonella isolates was evaluated using Sensititre plates following the EUCAST guidelines. Mixed-effects logistic regression was used to evaluate the influence of production system and antimicrobial use in resistant and multidrug-resistant (MDR) phenotypes to the antimicrobials tested. The results showed that antimicrobial use was higher ($p < .001$) on intensive farms than on organic/extensive farms. MDR in Campylobacter and Staphylococcus was lower on organic/extensive farms

(OR <.01p <.001). Antimicrobial resistance in Campylobacter and Staphylococcus isolates was, also for most of the antimicrobials studied, significantly higher in intensive than organic/extensive pig herds. Tetracycline resistance was associated with total antimicrobial consumption in both microbial species (p <.05), and some cross-associations between distinct antimicrobial substances were established, for instance resistance to erythromycin was associated with macrolide and phenicol consumption. No significant associations could be established for Salmonella isolates. The results demonstrate the link between antimicrobial consumption and resistance in zoonotic bacteria and evidence the potential value of using Campylobacter and Staphylococcus species in monitoring activities aimed at determining the impact of antimicrobials use/reduction on the occurrence and spread of antimicrobial resistance. ISSN: 18631959

Larivière-Gauthier, G., Thibodeau, A., Yergeau, É., Fravalo, P.
Sows affect their piglets' faecal microbiota until fattening but not their Salmonella enterica shedding status
(2021) Letters in Applied Microbiology, 72 (2), pp. 113-120.
ABSTRACT: Recent studies have shown that Salmonella shedding status affects sows' microbiota during gestation and that these modifications are reflected in the faecal microbiota of their piglets at weaning. The aims of this study were: (a) to evaluate the persistence, up to the fattening period, of the previously measured link between the microbiota of piglets and their mothers' Salmonella shedding status; and (b) measure the impact of the measured microbiota variations on their Salmonella excretion at this stage. To achieve this, 76 piglets born from 19 sows for which the faecal microbiota was previously documented, were selected in a multisite production system. The faecal matter of these swine was sampled after 4 weeks, at the fattening stage. The Salmonella shedding status and faecal microbiota of these animals were described using bacteriological and 16S rRNA gene amplicon sequencing respectively. The piglet digestive microbiota association with the Salmonella shedding status of their sows did not persist after weaning and did not affect the risk of Salmonella excretion during fattening, while the birth mother still affected the microbiota of the swine at fattening. This supports the interest in sows as a target for potentially transferrable microbiota modifications. ISSN: 02668254

Országh, E., Pitter, J.G., Kaló, Z., Vokó, Z., Józwiak, Á.
Retrospective cost-utility analysis of the Non-typhoidal Salmonella control programme in Hungary
(2021) Food Control, 120, art. no. 107529, .
ABSTRACT: Salmonellosis is one of the most important foodborne infections in the European Union (EU), causing more than 90,000 human salmonellosis cases with an overall economic burden of 3 billion Euro annually (EFSA, 2014). Salmonella enterica serovar Enteritidis (S. Enteritidis or SE) and Salmonella enterica serovar Typhimurium (S. Typhimurium or ST) are the most pathogenic serotypes, also the most frequently reported serovars in humans in the EU. To fight against zoonotic diseases, including Salmonella, the EU established an extended control programme (Regulation (EC) No 2160/2003) that was launched in 2007. The cost-effectiveness of the control programme has not been examined at EU-level and there are only a limited number of national assessments available in the subject. The authors of the present paper conducted a retrospective cost-utility analysis of the Hungarian Salmonella Control Programme (HSCP) for the years 2007–2017. Costs and outcomes were considered from the state's perspective. Country-specific cost of illness estimates and a quality-adjusted life year (QALY) -based burden estimate have been developed for human salmonellosis cases. The programme's results were compared to a reference arm where incidence, hospitalization and mortality rates were extrapolated after 2007 by adjusting for the slight annual changes in population demographics, as if no control measures had been introduced in Hungary. The incremental cost-effectiveness ratio (ICER) was calculated and compared to the national health technology assessment (HTA) threshold, defined as 3xGDP per capita (EMMI, 2017). Since the calculated ICER value (27,150 EUR/QALY gain) was below the threshold (35,790 EUR/QALY gain), the HSCP was found to be cost-effective for the investigated time period. The analysis can serve as a model to carry out further analyses in relation to other pathogens or interventions and help the priority setting and decision-making processes of food safety in Hungary. Further discussion is needed on methodological questions, such as the perspective of the analysis or the inclusion or exclusion of various cost types. ISSN: 09567135

Harhay, D.M., Weinroth, M.D., Bono, J.L., Harhay, G.P., Bosilevac, J.M.
Rapid estimation of Salmonella enterica contamination level in ground beef – Application of the time-to-positivity method using a combination of molecular detection and direct plating

(2021) Food Microbiology, 93, art. no. 103615, .
ABSTRACT: Little progress has been made in decreasing the incidence rate of salmonellosis in the US over the past decade. Mitigating the contribution of contaminated raw meat to the salmonellosis incidence rate requires rapid methods for quantifying Salmonella, so that highly contaminated products can be removed before entering the food chain. Here we evaluated the use of Time-to-Positivity (TTP) as a rapid, semi-quantitative approach for estimating Salmonella contamination levels in ground beef. Growth rates of 14 Salmonella strains (inoculated at log 1 to −2 CFU/g) were characterized in lean ground beef mTSB enrichments and time-to-detection was determined using culture and molecular detection methods. Enrichments were sampled at five timepoints and results were used to construct a prediction model of estimated contamination level by TTP (superscript indicates time in hours) defined as TTP4: ≥5 CFU/g; TTP6: ≤5, ≥1 CFU/g; TTP8: ≤1, ≥0.01 CFU/g; with samples negative at 8 h estimated ≤0.01 CFU/g. Model performance measures showed high sensitivity (100%) and specificity (83% and 93% for two detection methods) for samples with a TTP4, with false negative rates of 0%. ISSN: 07400020

Monte, D.F.M., Nethery, M.A., Barrangou, R., Landgraf, M., Fedorka-Cray, P.J.
Whole-genome sequencing analysis and CRISPR genotyping of rare antibiotic-resistant Salmonella enterica serovars isolated from food and related sources
(2021) Food Microbiology, 93, art. no. 103601, .
ABSTRACT: For decades, Salmonella Typhimurium and Salmonella Enteritidis have prevailed in several countries as agents of salmonellosis outbreaks. In Brazil, the largest exporter of poultry meat, relatively little attention has been paid to infrequent serovars. Here, we report the emergence and characterization of rare serovars isolated from food and related sources collected between 2014 and 2016 in Brazil. Twenty-two Salmonella enterica isolates were analyzed through the use of whole-genome sequencing (WGS) and clustered regularly interspaced short palindromic repeats (CRISPR) genotyping. These isolates were classified into 10 infrequent serovars, including S. Abony, S. Isangi, S. Rochdale, S. Saphra, S. Orion, S. Ouakam, S. Grumpensis, S. Carrau, S. Abaetetuba, and S. Idikan. The presence of six antimicrobial resistance (AMR) genes, qnrB19, blaCMY-2, tetA, aac(6')-Iaa, sul2 and fosA7, which encode resistance to quinolones, third-generation cephalosporin, tetracycline, aminoglycoside, sulfonamide and fosfomycin, respectively, were confirmed by WGS. All S. Isangi harbored qnrB19 with conserved genomic context across strains, while S. Abony harbored blaCMY-2. Twelve (54.5%) strains displayed chromosomal mutations in parC (Thr57→Ser). Most serovars were classified as independent lineages, except S. Abony and S. Abaetetuba, which phylogenetically nested with Salmonella strains from different countries. CRISPR analysis revealed that the spacer content was strongly correlated with serovar and multi-locus sequence type for all strains, independently confirming the observed phylogenetic patterns, and highlighting the value of CRISPR-based genotyping for Salmonella. These findings add valuable information to the epidemiology of S. enterica in Brazil, where the emergency of antibiotic-resistant Salmonella continues to evolve. ISSN: 07400020

Wu, X., Luo, H., Xu, F., Ge, C., Li, S., Deng, X., Wiedmann, M., Baker, R.C., Stevenson, A., Zhang, G., Tang, S.
Evaluation of Salmonella Serotype Prediction With Multiplex Nanopore Sequencing
(2021) Frontiers in Microbiology, 12, art. no. 637771, .
ABSTRACT: The use of whole genome sequencing (WGS) data generated by the long-read sequencing platform Oxford Nanopore Technologies (ONT) has been shown to provide reliable results for Salmonella serotype prediction in a previous study. To further meet the needs of industry for accurate, rapid, and cost-efficient Salmonella confirmation and serotype classification, we evaluated the serotype prediction accuracy of using WGS data from multiplex ONT sequencing with three, four, five, seven, or ten Salmonella isolates (each isolate represented one Salmonella serotype) pooled in one R9.4.1 flow cell. Each multiplexing strategy was repeated with five flow cells, and the loaded samples were sequenced simultaneously in a GridION sequencer for 48 h. In silico serotype prediction was performed using both SeqSero2 (for raw reads and genome assemblies) and SISTR (for genome assemblies) software suites. An average of 10.63 Gbp of clean sequencing data was obtained per flow cell. We found that the unevenness of data yield among each multiplexed isolate was a major barrier for shortening sequencing time. Using genome assemblies, both SeqSero2 and SISTR accurately predicted all the multiplexed isolates under each multiplexing strategy when depth of genome coverage ≥50× for each isolate. We identified that cross-sample barcode assignment was a major cause of prediction errors when raw sequencing data were used for prediction. This study also demonstrated that, (i) sequence data generated by ONT multiplex sequencing can be used to simultaneously predict serotype for three to ten Salmonella isolates, (ii) with three to ten Salmonella

isolates multiplexed, genome coverage at ≥50× per isolate was obtained within an average of 6 h of ONT multiplex sequencing, and (iii) with five isolates multiplexed, the cost per isolate might be reduced to 23% of that incurred with single ONT sequencing. This study is a starting point for future validation of multiplex ONT WGS as a cost-efficient and rapid Salmonella confirmation and serotype classification tool for the food industry.
ISSN: 1664302X

Yan, R., Pinto, G., Taylor-Roseman, R., Cogan, K., D'Alesandre, G., Kovac, J.
Evaluation of the Thermal Inactivation of a Salmonella Serotype Oranienburg Strain During Cocoa Roasting at Conditions Relevant to the Fine Chocolate Industry
(2021) Frontiers in Microbiology, 12, art. no. 576337, .
ABSTRACT: Cocoa roasting produces and enhances distinct flavor of chocolate and acts as a critical control point for inactivation of foodborne pathogens in chocolate production. In this study, the inactivation kinetics of Salmonella enterica subsp. enterica serotype Oranienburg strain was assessed on whole cocoa beans using roasting protocols relevant to the fine chocolate industry. Beans were inoculated with 107–108 log10 CFU/bean of Salmonella Oranienburg and roasted at 100–150°C for 2–100 min. A greater than 5 log10 reduction of S. Oranienburg was experimentally achieved after 10-min roasting at 150°C. Data were fitted using log-linear and Weibull models. The log-linear models indicated that the roasting times (D) needed to achieve a decimal reduction of Salmonella at 100, 110, 115, 120, 130, and 140°C were 33.34, 18.57, 12.92, 10.50, 4.20, and 1.90 min, respectively. A Weibull model indicated a decrease in the Salmonella inactivation rate over time (β < 1). Statistical analysis indicated that the Weibull model fitted the data better compared to a log-linear model. These data demonstrate the efficacy of cocoa roasting in inactivation of Salmonella and may be used to guide food safety decision-making.
ISSN: 1664302X

Lee, H., Park, J.H., Park, Y.K., Kim, H.J.
Mathematical modeling for the growth of salmonella spp. and staphylococcus aureus in cake at fluctuating temperatures
(2021) Applied Sciences (Switzerland), 11 (6), art. no. 2475, .
ABSTRACT: This study aimed to develop dynamic mathematical models to predict the growth of Salmonella spp. and Staphylococcus aureus in a cake under fluctuating temperatures. Among the nine different types of cakes frequently served during school meals, one type of cake was selected based on bacterial growth and water activity. Cocktails of Salmonella spp. and S. aureus were inoculated in the samples and stored at 4–35◦C for up to 336 h. The growth of Salmonella spp. and S. aureus was observed above 20 and 15◦C, respectively. The bacterial cell counts were fitted in the Baranyi model, and the maximum specific growth rate (μmax; log CFU/g/h) and lag phase duration (LPD; h) were analyzed using a polynomial model as a function of temperature (R2 = 0.968–0.988), and the performance of the developed models was appropriate. Furthermore, dynamic models were developed, and the predictions were acceptable in changing the temperature, indicating that the developed dynamic models can successfully predict the outcomes of Salmonella spp. and S. aureus in cake. These results provide useful information for assessing and managing microbial risk in foods by predicting the behavior of Salmonella spp. and S. aureus in cake, especially in changing temperature. ISSN: 20763417

Ford, L., Glass, K., Williamson, D.A., Sintchenko, V., Robson, J.M.B., Lancsar, E., Stafford, R., Kirk, M.D.
Cost of whole genome sequencing for nontyphoidal Salmonella enteric
(2021) PLoS ONE, 16 (3 March), art. no. e0248561, .
ABSTRACT: Background While whole genome sequencing (WGS) may be more expensive than traditional testing and polymerase chain reaction (PCR), simple cost comparisons ignore the potential for WGS to reduce the societal costs of non-typhoidal Salmonella enterica through public health action to prevent illness. Methods We determined how many cases the use of WGS data would need to prevent to be costequal to serotyping and MLVA, or culture independent testing based on PCR in Australia. We then examined the costs and cost-savings of current typing methods compared with WGS in outbreak scenarios. Results A median of 275 (90% CrI-55-775) or 1.9% (90% CrI-0.4%-5.4%) of notified serotyped Salmonella cases would need to be prevented for WGS to be cost-equal to current typing methods and 1,550 (90% CrI 820-2,725) or 9.6% of all notified Salmonella cases would need to be prevented to be cost-equal to PCR. WGS is likely to result in cost savings in prolonged outbreaks, where data can support earlier public health action. Conclusions Despite currently having a higher cost per isolate, routine WGS of Salmonella was no more expensive than existing typing methods or PCR where >2% of illness was averted.
ISSN: 19326203

Mthembu, T.P., Zishiri, O.T., El Zowalaty, M.E.
Genomic characterization of antimicrobial resistance in food chain and livestock-associated salmonella species
(2021) Animals, 11 (3), art. no. 872, pp. 1-16.
ABSTRACT: The rising trend of antimicrobial resistance (AMR) by foodborne bacteria is a public health concern as these pathogens are easily transmitted to humans through the food chain. Non-typhoid Salmonella spp. is one of the leading foodborne pathogens which infect humans worldwide and is associated with food and livestock. Due to the lack of discovery of new antibiotics and the pressure exerted by antimicrobial resistance in the pharmaceutical industry, this review aimed to address the issue of antibiotic use in livestock which leads to AMR of Salmonella. Much attention was given to resistance to carbapenems and colistin which are the last-line antibiotics used in cases of multi drug resistant bacterial infections. In the present review, we highlighted data published on antimicrobial resistant Salmonella species and serovars associated with livestock and food chain animals. The importance of genomic characterization of carbapenem and colistin resistant Salmonella in determining the relationship between human clinical isolates and food animal isolates was also discussed in this review. Plasmids, transposons, and insertion sequence elements mediate dissemination of not only AMR genes but also genes for resistance to heavy metals and disinfectants, thus limiting the thera-peutic options for treatment and control of Salmonella. Genes for resistance to colistin (mcr-1 to mcr- 9) and carbapenem (blaVIM-1, blaDNM-1, and blaNDM-5) have been detected from poultry, pig, and human Salmonella isolates, indicating food animal-associated AMR which is a threat to human public health. Genotyping, plasmid characterization, and phylogenetic analysis is important in understanding the epidemiology of livestock-related Salmonella so that measures of preventing foodborne threats to humans can be improved. ISSN: 20762615

Deaven, A.M., Ferreira, C.M., Reed, E.A., See, J.R.C., Lee, N.A., Almaraz, E., Rios, P.C., Marogi, J.G., Lamendella, R., Zheng, J., Bell, R.L., Shariat, N.W.
Salmonella Genomics and Population Analyses Reveal High Inter- and Intraserovar Diversity in Freshwater
(2021) Applied and Environmental Microbiology, 87 (6), art. no. e02594-20, pp. 1-14.
ABSTRACT: Freshwater can support the survival of the enteric pathogen Salmonella, though temporal Salmonella diversity in a large watershed has not been assessed. At 28 locations within the Susquehanna River basin, 10-liter samples were assessed in spring and summer over 2 years. Salmonella prevalence was 49%, and increased river discharge was the main driver of Salmonella presence. The amplicon-based sequencing tool, CRISPR-SeroSeq, was used to determine serovar population diversity and detected 25 different Salmonella serovars, including up to 10 serovars from a single water sample. On average, there were three serovars per sample, and 80% of Salmonella-positive samples contained more than one serovar. Serovars Give, Typhimurium, Thompson, and Infantis were identified throughout the watershed and over multiple collections. Seasonal differences were evident: serovar Give was abundant in the spring, whereas serovar Infantis was more frequently identified in the summer. Eight of the ten serovars most commonly associated with human illness were detected in this study. Crucially, six of these serovars often existed in the background, where they were masked by a more abundant serovar(s) in a sample. Serovars Enteritidis and Typhimurium, especially, were masked in 71 and 78% of samples where they were detected, respectively. Whole-genome sequencing-based phylogeny demonstrated that strains within the same serovar collected throughout the watershed were also very diverse. The Susquehanna River basin is the largest system where Salmonella prevalence and serovar diversity have been temporally and spatially investigated, and this study reveals an extraordinary level of inter- and intraserovar diversity. ISSN: 00992240

Martinez-Sanguiné, A.Y., D'Alessandro, B., Langleib, M., Traglia, G.M., Mónaco, A., Durán, R., Chabalgoity, J.A., Betancor, L., Yim, L.
Salmonella enterica serovars Dublin and enteritidis comparative proteomics reveals differential expression of proteins involved in stress resistance, virulence, and anaerobic metabolism
(2021) Infection and Immunity, 89 (3), art. no. e00606-20, .
ABSTRACT: The Enteritidis and Dublin serovars of Salmonella enterica are phylogenetically closely related yet differ significantly in host range and virulence. S. Enteritidis is a broad-host-range serovar that commonly causes self-limited gastroenteritis in humans, whereas S. Dublin is a cattle-adapted serovar that can infect humans, often resulting in invasive extraintestinal disease. The mechanism underlying the higher invasiveness of S. Dublin

remains undetermined. In this work, we quantitatively compared the proteomes of clinical isolates of each serovar grown under gut-mimicking conditions. Compared to S. Enteritidis, the S. Dublin proteome was enriched in proteins linked to response to several stress conditions, such as those encountered during host infection, as well as to virulence. The S. Enteritidis proteome contained several proteins related to central anaerobic metabolism pathways that were undetected in S. Dublin. In contrast to what has been observed in other extraintestinal serovars, most of the coding genes for these pathways are not degraded in S. Dublin. Thus, we provide evidence that S. Dublin metabolic functions may be much more affected than previously reported based on genomic studies. Single and double null mutants in stress response proteins Dps, YciF, and YgaU demonstrate their relevance to S. Dublin invasiveness in a murine model of invasive salmonellosis. All in all, this work provides a basis for understanding interserovar differences in invasiveness and niche adaptation, underscoring the relevance of using proteomic approaches to complement genomic studies. Copyright ISSN: 00199567

Domesle, K.J., Young, S.R., Ge, B.
Rapid screening for salmonella in raw pet food by loop-mediated isothermal amplification (2021) Journal of Food Protection, 84 (3), pp. 399-407.
ABSTRACT: Raw pet food, composed of raw meat and vegetables, has increased in popularity in recent years. Multiple surveys and frequent recalls indicate that this commodity has a high risk of contamination with Salmonella and other foodborne pathogens. Improved screening methods are needed to meet the growing demand for testing. This matrix verification study aimed to apply a Salmonella loop-mediated isothermal amplification (LAMP) method, recently completed multilaboratory validation in dry dog food, in several raw pet food matrices, following the U.S. Food and Drug Administration (FDA)'s method validation guidelines. Five types of raw pet food, consisting of freeze-dried beef and chicken treats and frozen beef, pork, and turkey complete foods, were evaluated. For each matrix, two sets of ten 25-g test portions (seven inoculated with ≤30 cells of Salmonella Typhimurium and three uninoculated controls) were examined. One set was preenriched in buffered peptone water and the other one was preenriched in lactose broth, which was followed by LAMP screening using two isothermal master mixes (ISO-001 and ISO-004). All results were confirmed by culture as specified in the FDA Bacteriological Analytical Manual (BAM). The LAMP method accurately detected Salmonella in all inoculated test portions of the five raw pet food samples, regardless of the preenrichment broth used. Positive results could be obtained within 4 min of the LAMP run using the ISO-004 master mix. All uninoculated controls tested negative using LAMP or BAM. In addition, one turkey-based complete pet food sample was found to be already contaminated with three Salmonella serovars harboring multiple antimicrobial resistance genes. The Salmonella LAMP method offers a rapid, reliable, and robust tool for routine screening of Salmonella in raw pet food, which will help better ensure product safety and protect public health. ISSN: 0362028X

Jung, J., Schaffner, D.W.
Quantification of survival and transfer of salmonella on fresh cucumbers during waxing (2021) Journal of Food Protection, 84 (3), pp. 456-462.
ABSTRACT: Cucumbers found in retail markets are often waxed to improve visual appeal and retard moisture loss. This waxing may affect bacterial survival, and the waxing process may facilitate cross-contamination between cucumbers. This study assessed the survival of Salmonella on waxed and unwaxed cucumbers and the potential for Salmonella cross-contamination during the waxing process. Fresh waxed or unwaxed cucumbers were spot inoculated with a cocktail of Salmonella enterica strains. Three different wax coatings (mineral oil, vegetable oil, or petroleum wax) were manually applied to unwaxed cucumbers using polyethylene brushes. Salmonella transfer from inoculated cucumbers to the brush or to uninoculated cucumbers was quantified. Higher Salmonella concentrations were observed on waxed cucumbers during the first 3 days of storage, but the final concentration on unwaxed cucumbers was higher than on waxed cucumbers at the end of storage, regardless of storage temperature. The wax formulation did affect the survival of Salmonella inoculated directly into waxes, with a significant decline in Salmonella populations observed in vegetable-based wax coating but with populations unchanged over 7 days at 7 or 218C in mineral oil–based and petroleum-based waxes. Salmonella cells could transfer from inoculated unwaxed cucumbers to brushes used for waxing and then to uninoculated cucumbers during waxing. A significantly higher log percentage of transfer to brushes was observed when cucumbers were waxed with vegetable oil (0.71 log percent, P ¼ 0.00441) than with mineral oil (0.06 log percent) or petroleum (0.05 log percent). Transfer to uninoculated cucumbers via brushes was also quantified (0.18 to 0.35 log percent transfer). Salmonella remaining on contaminated cucumbers after waxing could be

detected for up to 7 days, and Salmonella survived better on cucumbers treated with a petroleum-based wax. These findings should be useful in managing the risk of Salmonella contamination in cucumbers during postharvest handling. ISSN: 0362028X

Unger, P., Channaiah, L.H., Singh, A., Singh Sekhon, A., Babb, M., Yang, Y., Michael, M.
Validation of brownie baking step for controlling Salmonella and Listeria monocytogenes
(2021) Food Science and Nutrition, 9 (3), pp. 1574-1583.
ABSTRACT: Pathogens, such as Salmonella and Listeria monocytogenes, can survive under the dry environment of flour for extended periods of time and could multiply when flour is hydrated to prepare batter or dough. Therefore, inactivation of these pathogens during the cooking/baking step is vital to ensure the microbiological safety of bakery products such as brownies. The aim of this research was to validate a simulated commercial baking process as a kill-step for controlling Salmonella and L. monocytogenes in brownies and to determine thermal inactivation parameters of these pathogens in brownie batter. Independent studies were conducted in a completely randomized design for each pathogen. All-purpose flour was inoculated with a 5-serovar Salmonella and 3-strain L. monocytogenes cocktails. For baking validation, brownie batters were prepared from inoculated flour, and cooked in the oven set at 350°F (176.7°C) for 40 min followed by 15 min of ambient air cooling. For calculating D-values, brownie batter was transferred into thermal-death-time disks, sealed, and placed in hot-water baths. The samples were held for pre-determined time intervals in hot-water baths and immediately transferred to cold-water baths. Microbial populations were enumerated using injury-recovery media. At the end of baking, Salmonella and L. monocytogenes populations decreased by 6.3 and 5.9 log CFU/g, respectively. D-values of Salmonella and L. monocytogenes cocktails were 53.4 and 37.5 min at 64°C; 27.2 and 16.9 min at 68°C; 10.7 and 9.1 min at 72°C; and 4.6 and 7.3 min at 76°C; respectively. The z-values of Salmonella and L. monocytogenes cocktails were 11.1 and 16.4°C, respectively. This study can be used as a supporting document for the validation of similar brownie baking processes to control Salmonella and L. monocytogenes. The data from this study can also be employed for developing basic prediction models for the survival and thermal resistance of these pathogens during brownie baking step. ISSN: 20487177

Khan, M.N.K., Das, M.R., Sabur, M.A., Rahman, M.M., Uddin, M.B., Cho, H.S., Hossain, M.M.
Isolation, identification, molecular detection and sensitivity to antibiotics of salmonella from cattle faeces
(2021) Bulgarian Journal of Veterinary Medicine, 24 (1), pp. 57-66.
ABSTRACT: The present study was designed with the aim of isolation and identification of Salmonella by conventional culture method and their confirmation by polymerase chain reaction (PCR). Antibacterial sensitivity study of isolated Salmonella from cattle faeces was also performed. During the study period of July 2017 to June 2018, a total of 200 faecal samples were collected from different government and private farms in Sylhet district of Bangladesh. Out of 200 samples, 24 (12%) were found to be positive for Salmonella by conventional culture methods. Among the twenty four suspected colonies of Salmonella, seventeen were confirmed by biochemical test and same number was detected by PCR estimating a prevalence of 8.5% (17/200). The prevalence was higher in calves under 1 year of age (16%) compared with older animals (11.25% of 1–2 years; 10% of above 2 years of age) but without statistically significant differences (χ2=4.835, P=0.089). Moreover, in diarrhoeic animals the prevalence was significantly higher (32.14%, χ2=49.414, P<0.01) than in apparently healthy animals (8.72%). The antibiotic sensitivity test showed that highest number of Salmonella isolates were sensitive to ciprofloxacin (100%), gentamicin (100%) and neomycin (100%). On the other hand, significantly high resistance of Salmonella isolates was detected to erythromycin (100%), amoxicillin (100%), cotrimoxazole (81.48%), streptomycin (62.96%) followed by tetracycline (55.56%). ISSN: 13111477

Gast, R.K., Jones, D.R., Guraya, R., Anderson, K.E., Karcher, D.M.
Applied Research Note: Internal organ colonization and horizontal transmission of experimental Salmonella Enteritidis and Salmonella Kentucky infection in vaccinated laying hens in indoor cage-free housing
(2021) Journal of Applied Poultry Research, 30 (1), art. no. 100132, .
ABSTRACT: Cage-free housing of laying hens may provide opportunities for widespread environmental distribution of Salmonella contamination and horizontal transmission of infection within flocks. Salmonella Enteritidis in commercial laying flocks presents an ongoing public health concern because reproductive organ colonization in hens leads to

deposition inside eggs. Many S. Enteritidis control programs include vaccination to induce protective immunity against infection. Salmonella Kentucky is common in egg production environments but has not been associated with egg contamination. This study compared the invasion of internal organs and horizontal spread of infection during the first 2 wk after experimental S. Enteritidis and S. Kentucky infection of previously vaccinated laying hens in indoor cage-free housing. Two groups of 72 hens each were housed in isolation rooms simulating commercial cage-free barns and 1/3 of the hens were orally inoculated with either S. Enteritidis (1 room) or S. Kentucky (1 room). At 6 and 12 d after inoculation, half of the hens in each room were euthanized and samples of the liver, spleen, ovary, oviduct, and intestinal tract were removed for bacteriologic culturing. Among hens inoculated with S. Enteritidis, 66.7% of the intestinal, liver, and spleen samples were positive for the pathogen at 6 d after infection, as well as 41.7% of intestines and 16.7% of livers from contact-exposed hens. Significantly ($P < 0.05$) fewer hens were colonized by S. Kentucky. These results demonstrate that vaccines may not always provide complete exclusion of Salmonella. In cage-free housing systems, vaccination should be supplemented with a comprehensive risk reduction effort to prevent extensive horizontal dissemination of Salmonella. ISSN: 10566171

Steghöfer, S., Limburn, R., Margas, E.
Microbiological assessment of heat treatment of broiler mash at laboratory scale to evaluate Salmonella reduction during feed conditioning
(2021) Journal of Applied Poultry Research, 30 (1), art. no. 100122, .
ABSTRACT: Salmonella is the pathogen mostly associated with feed and dry food safety incidents and is a cause of many foodborne outbreaks. In most feed mills conditioning and retentioning systems are installed to improve the pelleting process but also as a kill-step to reduce the microbial load of the feed material. As knowledge about the efficiency of this kill-step at different process conditions in specific feed matrices is lacking, a study was carried out to characterize the inactivation kinetics of Salmonella spp. in broiler feed treated with superheated steam in laboratory scale. First, Salmonella strains commonly associated with feed materials were identified through a literature review. Five Salmonella strains were screened for heat resistance allowing selection of Salmonella Agona as one of the most heat-resistant serotype. Screening of triplicate strains of this serotype allowed selection of S. Agona RA1052 (isolated from animal feed) as the most suitable strain to be used in determination of D- and z-values at selected moisture levels and temperatures in broiler feed. D-values for S. Agona strain RA1052 in broiler feed mash adjusted to 12% moisture, determined within a customized autoclave at 65°C and 85°C, were 178.2 s and 3.1 s, respectively. At 19% moisture, D-values were 81.1 s at 65°C and 0.7 s at 85°C. To perform on-site challenge tests, the surrogate Enterococcus faecium ATCC 8459 (NRRL B-2354) with an equivalent heat resistance to S. Agona RA1052 was selected. The collected data will serve as a basis for the validation of conditioning and retentioning as a Salmonella spp. kill step in pilot and industrial scale. ISSN: 10566171

Tan, Z., Lu, P., Adewole, D., Diarra, M.S., Gong, J., Yang, C.
Iron requirement in the infection of Salmonella and its relevance to poultry health
(2021) Journal of Applied Poultry Research, 30 (1), art. no. 100101, .
ABSTRACT: Iron is essential for DNA synthesis, respiration, energy metabolism, and key metabolic reactions intrinsic to life because of its capability to accept and release electrons easily and its indispensability for most of the creatures on the earth including bacteria and poultry. Salmonella species, the gram-negative foodborne facultative anaerobes, are notorious enteric pathogens that have a wild range of animal hosts, causing the deterioration of animal health, compromised animal welfare, enormous agricultural loss and medical burden around the world. Nontyphoidal Salmonella enterica (NTS) infections in poultry are the important cause of human salmonellosis. Poultry can be infected by NTS and have no obvious symptoms and are sold, transported and slaughtered as healthy animals. However, birds may carry in their intestine this organism undetected into the abattoir at the time of slaughter, thereby representing a food-safety risk for consumers. To reduce the risk of salmonellosis, the prevention of S. enterica serovars from colonization and invasion of the gut through limiting their iron acquisition can be an effective approach. This review summarizes the biological roles of iron and iron homeostasis in both Salmonella and poultry, competition for iron between Salmonella and hosts, and potential strategies targeting iron acquisitions to control Salmonella infection in poultry.
ISSN: 10566171

Ksibi, B., Ktari, S., Othman, H., Ghedira, K., Maalej, S., Mnif, B., Abbassi, M., Fabre, L., Rhimi, F., Le Hello, S., Hammami, A.
Comparison of conventional molecular and whole-genome sequencing methods for subtyping Salmonella enterica serovar Enteritidis strains from Tunisia
(2021) European Journal of Clinical Microbiology and Infectious Diseases, 40 (3), pp. 597-606.
ABSTRACT: We sought to determine the relative value of conventional molecular methods and whole-genome sequencing (WGS) for subtyping Salmonella enterica serovar Enteritidis recovered from 2000 to 2015 in Tunisia and to investigate the genetic diversity of this serotype. A total of 175 Salmonella Enteritidis isolates were recovered from human, animal, and foodborne outbreak samples. Pulsed-field gel electrophoresis (PFGE), multiple locus variable-number tandem repeat analysis (MLVA), and whole-genome sequencing were performed. Eight pulsotypes were detected for all isolates with PFGE (DI = 0.518). Forty-five Salmonella Enteritidis isolates were selected for the MLVA and WGS techniques. Eighteen MLVA profiles were identified and classified into two major clusters (DI = 0.889). Core genome multilocus typing (cgMLST) analysis revealed 16 profiles (DI = 0.785). Whole-genome analysis indicated 660 single-nucleotide polymorphism (SNP) divergences dividing these isolates into 43 haplotypes (DI = 0.997). The phylogenetic tree supported the classification of Salmonella Enteritidis isolates into two distinct lineages subdivided into five clades and seven subclades. Pairwise SNP differences between the isolates ranged between 302 and 350. We observed about 311 SNP differences between the two foodborne outbreaks, while only less or equal to 4 SNP differences within each outbreak. SNP-based WGS typing showed an excellent discriminatory power comparing with the conventional methods such as PFGE and MLVA. Besides, we demonstrate the added value of WGS as a complementary subtyping method to discriminate outbreak from non-outbreak isolates belonging to common subtypes. It is important to continue the survey of Salmonella Enteritidis lineages in Tunisia using WGS. ISSN: 09349723

Dehghani, Z., Nguyen, T., Golabi, M., Hosseini, M., Rezayan, A.H., *Mohammadnejad, J., Wolff, A., Vinayaka, A.C.*
Magnetic beads modified with Pt/Pd nanoparticle and aptamer as a catalytic nano-bioprobe in combination with loop mediated isothermal amplification for the on-site detection of Salmonella Typhimurium in food and fecal samples
(2021) Food Control, 121, art. no. 107664, .
ABSTRACT: Concentration of pathogens directly from food samples by using magnetic beads is a potential strategy in the on-site food sample analysis. In this study, magnetic beads, double modified with platinum/palladium nanoparticle (Pt/Pd NP) and DNA aptamer, is presented as catalytic nano-bioprobes for the on-site detection of Salmonella enterica serovar Typhimurium in food and fecal samples. Combination of the developed catalytic nano-bioprobes with loop mediated isothermal amplification (LAMP) enabled rapid detection of low levels of S. Typhimurium in food and chicken fecal samples without culture enrichment. S. Typhimurium-specific DNA aptamer immobilized on the magnetic bead could efficiently concentrate S. Typhimurium with a capturing efficiency higher than 76% in phosphate-buffered saline (PBS). Further, DNA-mediated inhibition of peroxidase-mimic activity of Pt/Pd NP in combination with LAMP was used as a unique approach to detect S. Typhimurium. With this unique approach, it was possible to capture and detect S. Typhimurium as low as 10–15 CFU/mL in chicken meat sample and 3–10 CFU/mL in both whole egg and chicken fecal samples within less than 3 h. Analysis of S. Typhimurium-spiked chicken meat, whole egg and chicken fecal materials have confirmed the precision of the method. A relative accuracy of 90% with an intra and inter assay precision of 8.36% and 9.92% respectively was achieved in the S. Typhimurium-spiked food samples. This unique approach has the potential for the integration in to Lab-on-a-chip based biosensors for on-site monitoring of foodborne pathogens in future. ISSN: 09567135

Kim, W.-J., Kim, S.-H., Kang, D.-H.
Combination effect of 915 MHz microwave heating and carvacrol for inactivation of Escherichia coli O157:H7, Salmonella Typhimurium and Listeria monocytogenes in hot chili sauce
(2021) Food Control, 121, art. no. 107578, .
ABSTRACT: Hot chili sauce is a type of condiment whose popularity is steadily increasing with the incline of a risk for foodborne outbreaks. The combination effect of 915 MHz microwave and carvacrol was studied to inactivate the foodborne pathogens inoculated in hot chili sauce. The addition of 3.25 mM carvacrol did not change the dielectric properties including dielectric constant and dielectric loss factor, which remained at 54.1 and 80.5, respectively. Also, the heating rate was not influenced by the addition of carvacrol ($p > 0.05$). Time to reach 100 °C was 65 s for both samples with or without carvacrol. However,

adding 3.23 mM carvacrol generated a synergistic effect against E. coli O157:H7, Salmonella Typhimurium, and Listeria monocytogenes when combined with 915 MHz microwave. An additional population reduction of 1.0–2.6 log CFU/ml for the different pathogens was observed under the combined treatment of 40 s. No significant amounts of injured cells were observed. Furthermore, combination treatment did not result in a significant change in the color or water activity of hot chili sauce. No significant difference in volatile compound contents was found. This study demonstrates the potential of combining carvacrol and 915 MHz microwave for sauce processing without significant quality changes. ISSN: 09567135

Giacometti, F., Pezzi, A., Galletti, G., Tamba, M., Merialdi, G., Piva, S., Serraino, A., Rubini, S.
Antimicrobial resistance patterns in Salmonella enterica subsp. enterica and Escherichia coli isolated from bivalve molluscs and marine environment
(2021) Food Control, 121, art. no. 107590, .
ABSTRACT: The current study presents data on the antimicrobial resistance (AMR) patterns of 102 S. enterica subsp. enterica (72 Salmonella ser. Typhimurium and 30 monophasic S. Typhimurium serovar) and 79 Escherichia coli (and their phylogenetic group determination) isolates from different species of bivalve molluscs and from the water samples collected from the sub-areas of a mollusc production area near Ferrara (Italy). These areas were classified as Long-line, Lupini, B-Out, B-in, and Sacca. A retrospective evaluation was performed to assess the spatial trends of the resistance patterns of Salmonella and E. coli and the temporal trend for Salmonella; the role of molluscs as AMR indicators and the potential use of E. coli as a microorganism indicator of AMR occurrence in a seawater environment were also investigated. Overall, 81% of Salmonella spp. and 75% of E. coli isolates were resistant to, at least, one antimicrobial agent (AA) and 44% and 38% of the isolates were multidrug resistant (MDR), respectively. The resistance levels of Salmonella were influenced by the investigated serovars. Monophasic S. Typhimurium serovar showed the highest resistance value with 70% of MDR isolates, in contrast with only 33% in S. Typhimurium. In monophasic S. Typhimurium versus S. Typhimurium, twofold resistance levels were observed to streptomycin (97 versus 43%), ampicillin (80 versus 40%) and tetracyclines (67 versus 36%). Regarding the temporal trend for Salmonella, strains were resistant to, at least, one AA, but this resistance was significantly lower during the first years of this 17-year sampling; however, in parallel MDR isolates, the resistance increased from 23% to a maximum level of 57% during the 2008–2012 period. On assessing the spatial trends, the Sacca area was found to show the lowest number of Salmonella spp. and E. coli strains resistant to, at least, one AA and MDR. For E. coli, the most commonly observed resistance was towards ampicillin (56%), streptomycin (52%), sulphonamides (30%) and ceftiofur (24%). The great majority (65%) of E. coli isolates belonged to the commensal phylogroups A and B1, with B1 as the dominant one, whereas most MDR isolates belonged to phylogroup C. Molluscs may be an efficient tool for antimicrobial resistance monitoring, and E. coli could be used as a microorganism indicator of the occurrence of antimicrobial resistance in seawater environment. ISSN: 09567135

Huth, P., Wirth, S.E., Baker, D., Nicholas, D.C., Douris, A., Freiman, J., Kline, K.E., Devinney, K., Gläsker, S., Schwensohn, C.
Ability of whole-genome sequencing to refine a salmonella i 4,[5],12:I:-cluster in new york state and detect a multistate outbreak linked to raw poultry
(2021) Food Protection Trends, 41 (2), pp. 239-245.
ABSTRACT: Whole-genome sequencing (WGS) has proven to be a more powerful tool than pulsed-field gel electrophoresis for foodborne illness cluster definition because of improved resolution. Between November 2017 and May 2018, the New York State (NYS) Dept. of Health investigated 10 cases of Salmonella I 4,[5],12:i:-with pulsed-field gel electrophoresis pattern JPXX01.0621; comparison of case exposures did not identify a common source of infection. In June 2018, the NYS Dept. of Health's Wadsworth Center analyzed the isolates using WGS and defined a subcluster of five isolates related within zero to six single-nucleotide polymorphisms. The National Center for Biotechnology Information Pathogen Detection browser advanced this investigation by identifying additional clinical and food (chicken) isolates related within zero to eight single-nucleotide polymorphisms to the original subcluster. Comparison of WGS-related isolates would support the hypothesis that illness was associated with exposure to a kosher poultry product. This outbreak ultimately consisted of 25 cases from six states. Of 20 cases interviewed, all reported chicken consumption, and of those able to recall brand information, 83% cited a brand produced at a facility linked to the WGS-related chicken isolates. This paper demonstrates how WGS was able to refine a Salmonella I 4,[5],12:i:-cluster in NYS to uncover a multistate outbreak linked to raw poultry. ISSN: 15419576

Lau, S.K., Wei, X., Kirezi, N., Panth, R., See, A., Subbiah, J.
A comparison of three methods for determining thermal inactivation kinetics: A case study on Salmonella enterica in whole milk powder
(2021) Journal of Food Protection, 84 (3), pp. 521-530.
ABSTRACT: Different methods for determining the thermal inactivation kinetics of microorganisms can result in discrepancies in thermal resistance values. In this study, thermal resistance of Salmonella in whole milk powder was determined with three methods: thermal death time (TDT) disk in water bath, pouches in water bath, and the TDT Sandwich system. Samples from three production lots of whole milk powder were inoculated with a five-strain Salmonella cocktail and equilibrated to a water activity of 0.20. The samples were then subjected to three isothermal treatments at 75, 80, or 858C. Samples were removed at six time points and cultures were enumerated for survivors. The inactivation data were fitted to two consolidated models: two primary models (log linear and Weibull) and one secondary model (Bigelow). Normality testing indicated that all the model parameters were normally distributed. None of the model parameters for both consolidated models were significantly different (α ¼ 0.05). The amount of inactivation during the come-up time was also not significantly different among the methods (α ¼ 0.05). However, the TDT Sandwich resulted in less inactivation during the come-up time and overall less variation in model parameters. The survivor data from all three methods were combined and fitted to both consolidated models. The Weibull had a lower root mean square error and a better fit, according to the corrected Akaike's information criterion. The three thermal treatment methods produced results that were not significantly different; thus, the methods are interchangeable, at least for Salmonella in whole milk powder. Comparisons with more methods, other microorganisms, and larger varieties of food products using the same framework presented in this study could provide guidance for standardizing thermal inactivation kinetics studies for microorganisms in foods.
ISSN: 0362028X