NEWSLETTER

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Editorial Note

Bilthoven, 7 April 2017

Dear colleague,

After a mild winter, spring really started here in the Netherlands, bringing many beautiful flowers. A very nice time of the year, but also a busy period.
In the first quarter of this year, we have (again) been busy with several activities in relation to the EURL-*Salmonella* interlaboratory studies.

In January/February 2017, the analysis of the serotyping results of the **21st EURL-*Salmonella* interlaboratory comparison study on typing of *Salmonella*** was performed. By mid-February the laboratories 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: http://www.eurlsalmonella.eu/dsresource?type=pdf&disposition=inline&objectid=rivmp:327826&versionid=&subobjectname=. Thirty-two of the 34 participants scored a good performance with the serotyping of the different *Salmonella* serovars. For two NRLs a follow-up study will soon be organised. The results of the PFGE typing part of the interlaboratory study are still under analysis and will soon be reported to the participants.

In March 2017, the **20th interlaboratory comparison study on the detection of *Salmonella* in samples from the primary production stage** was organised.
For a while we were not sure whether we could transport poultry faeces because of the outbreak of Avian Influenza in poultry in several EU Member States this winter. Luckily it turned out to be no problem to mail the interlaboratory samples, also because the faeces was obtained from a SPF (Avian Influenza free) flock. The samples were sent to the participants by mid-March and the deadline for reporting the results of the interlaboratory study is mid-April.

In fall last year, and still in the first quarter of this year, several Member States were involved in the **multi-country outbreak of *Salmonella* Enteritidis** linked to eggs. As EURL we helped several NRLs by performing MLVA and/or WGS typing of suspect non-human *Salmonella* Enteritidis isolates. We also performed cluster analysis of the WGS data of non-human isolates to find a possible link with the outbreak. More details on the outbreak can be found in the joint rapid outbreak assessment of EFSA and ECDC: http://www.efsa.europa.eu/sites/default/files/scientific_output/documents/1188e.pdf.

By the end of last year and earlier this year, we have sent information about the **EURL-*Salmonella* workshop of 2017**. This years' workshop will be organised in the Netherlands, more precisely in Zaandam (the same location as the workshop of 2014). For the registration we introduced, for the first time, a web-based form and by now we have received all completed registration forms. Our secretary, Jeanette van Essen, will soon start booking the flights and the participants will be informed about the details. Currently we are preparing the draft program and as soon as this is worked out in more detail, the participants will be informed as well.

Earlier this year we noticed that the link to the **White Kauffmann Le Minor scheme** was no longer working. Please find below the correct (new) link: https://www.pasteur.fr/sites/default/files/veng_0.pdf.

In January 2017, we sent a **questionnaire** to all NRLs-*Salmonella* to gain an update on the **use of MLVA** by the NRLs. The outcome of this questionnaire is summarised in this newsletter.

Last month (March 2017), the final version of **EN ISO 6579-1** ('Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* – Part 1: Detection of *Salmonella* spp.') has been published. This first edition of ISO 6579-1 cancels and replaces ISO 6579:2002 and ISO 6785:2001, which have been technically revised. It also incorporates ISO 6579:2002/Amd 1:2007 and ISO 6579:2002/Cor 1:2004. The systematic review of ISO 6579 (2002) took place in 2007, meaning that it took us approximately 10 years to get the updated document published!

It is good to know that EN ISO 6579-1 is not really a 'new' EN ISO document, but an update of EN ISO 6579:2002. Additionally, EN ISO 6579-1 is now applicable for all samples of the food chain, including all categories of food, even milk and milk products (originally described in ISO 6785:2001), animal feed, and samples from the primary production stage. The procedure originally described in EN ISO 6579:2002/Amd 1:2007 for detection of *Salmonella* in samples from the primary production stage has been incorporated in the new EN ISO 6579-1. In clause 9 of EN ISO 6579-1 a distinction is made in the procedure for detection of *Salmonella* in food and animal feed (selective enrichment in MKTTn and RVS or MSRV) and detection of *Salmonella* in samples from the primary production stage (selective enrichment on MSRV only).

In the Introduction of ISO 6579-1 it is indicated that 'the main changes in the document, compared to ISO 6579:2002 are considered as minor', meaning that the changes have little to no effect on the performance characteristics of the method. For the introduction of the new EN ISO 6579-1 in your laboratory there is generally a transition time of one year. If and to what extent a re-verification of the performance characteristics in a laboratory is necessary may need to be discussed with the national accreditation board.

The main changes introduced in EN ISO 6579-1:2007 compared to EN ISO 6579:2002 were presented at the International Symposium for *Salmonella* and Salmonellosis (I3S) in Saint Malo, France in 2016. This information has been summarised in a manuscript for publication in a special issue of Journal Food Microbiology (likely to be published in 2017) and is currently in press and available online: http://dx.doi.org/10.1016/j.fm.2017.03.001.

By the end of March we have sent the **annual technical report of the activities of EURL-*Salmonella* performed in 2016** to EC DG-Sante. For your information this report is also included in this newsletter.

Best wishes,
Kirsten Mooijman
Coordinator EURL-*Salmonella*

Contribution of the EURL-*Salmonella*

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

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

Question	**Number of replies (countries)**
For which work field are you NRL-*Salmonella*?	
Animal feed	20
Food	17
Primary production – cattle	20
Primary production – pigs	21
Primary production – poultry	21
Typing	23
Does your laboratory (either internally or by outsourcing) perform MLVA typing of *Salmonella* isolates from food, feed, and/or animals?	
No	15 (CY, CZ, EE, FI, GR, LV, LT, NO, PL, PT, RO, SK, SI, ES, CH)
Yes	10 (AT, BE, BG, DK, FR, DE, IT, NL, SE. UK)
For which *Salmonella* serovar(s) do you perform MLVA?	
Salmonella Typhimurium	10
Salmonella Enteritidis	7
Other	2 (monophasic *S.* Typhimurium, *S.* Dublin, *S.* Derby)
Do you perform MLVA typing on a routine basis or occasionally?	
On a routine basis	6
Occasionally	4
Where do the isolates come from?	
Samples related to official controls, national control programmes or surveys carried out by competent authorities	8
Samples related to outbreak investigations	8
Samples related to research	8

Question	**Number of replies (countries)**
Which MLVA protocol do you use for *Salmonella* Typhimurium?	
EFSA SOP (2014), incl. set of standardisation strains http://dx.doi.org/10.2903/sp.efsa.2014.EN-703	4
EFSA SOP (2014), **not** incl. set of standardisation strains	-
ECDC SOP (2011), incl. set of standardisation strains http://dx.doi.org/10.29000/56328	6
ECDC SOP (2011), **not** incl. set of standardisation strains	-
Which MLVA protocol do you use for *Salmonella* Enteritidis?	
ECDC SOP (2016), incl. set of standardisation strains http://dx.doi.org/10.29000/973540	7
ECDC SOP (2016), **not** incl. set of standardisation strains	-
Which MLVA protocol do you use for other *Salmonella* serovars?	
Not applicable	9
Other	Internal method for *S.* Dublin and *S.* Derby
How many *Salmonella* isolates did you MLVA type in 2016?	
0	1
10-100	3
100-500	2
500-1000	3
>1000	1 (including human isolates)

Remarks

- Estonia (EE): In case of outbreaks the public health laboratory will decide which kind of molecular typing is used. Usually they use subcontracting from Finnish Public Health Laboratory.
- Finland (FI): Certain *Salmonella* Typhimurium and *Salmonella* Enteritidis strains (beforehand defined in method instructions) have been sent to the National Institute for Health and Welfare (THL) for phage typing. During the years THL has typed by MLVA part of the strains.
- Norway (NO): When we need a MLVA-typing on our non-human isolates, we send them to Norwegian Institute of Public Health.
- Romania (RO): We intend to implement the MLVA technique in our laboratory in this year.
- Switzerland (CH): We planned the implementation of MLVA for *S*. Typhimurium and *S*. Enteritidis about two years ago. Unfortunately we do not get resources for that task from our government until now, but we are still engaged in that field.
- Belgium (BE): CODA-CERVA is no longer NRL for *Salmonella* from January 2017. This competency has been transferred to WIV-ISP.
- Bulgaria (BG): We do not carry out research on MLVA for technical reasons related to the sequencer machine. I rule MLVA profiles of our strains in Denmark. I was supported by a grant of the Bulgarian Ministry of Education, Youth and Science, 2012.

- Denmark (DK): From January 1, 2017 DTU Food, Denmark has stopped using MLVA for molecular typing of *Salmonella* from food, feed and animal samples. Instead isolates are analysed by WGS.
- France (FR): we submitted in journal of Frontiers in Microbiology a MLVA subtyping method for *Salmonella* Dublin suitable for inter-laboratory surveillance. *Salmonella* Dublin is one of the most frequently encountered *Salmonella* in cattle in the EU. We defined a MLVA method with 6 VNTRs and a list of reference strains. To normalize the MLVA results we published in 2016 a pipeline, MLVA_normalizer (Bachelerie, et al., 2016. MLVA_Normalizer: Workflow for Normalization of MLVA Profiles and Data Exchange between Laboratories. Journal of Proteomics & Bioinformatics 09(02), 25-27. doi: 10.4172/jpb.1000385).
- Germany (DE): We are going to shut down MLVA within the next two years with establishing of WGS for outbreak and other epidemiological studies.
- United Kingdom (UK) – APHA: We would only do MLVA if specifically asked by government or a customer. WGS is now the routine method for outbreak investigation and it is likely that the use of MLVA will be completely discontinued soon as it is not economic to maintain the capability.
- United Kingdom (UK) – PHE: The information in this survey is based on the activity of the Scottish *Salmonella* Reference Laboratory (SSRL) (Scottish Microbiology Reference Laboratories, Glasgow, Scotland), who also receive mostly clinical strains. *Salmonella* isolates from England, Wales, and Northern Ireland are served by the Gastrointestinal Bacteria Reference Laboratory in Public Health England, where whole genome sequencing is now performed, and MLVA has been discontinued. The SSRL also intend to implement WGS for *Salmonella* in the current year.

Technical report on activities of the European Union Reference Laboratory for *Salmonella* in 2016

K.A. Mooijman
23 March 2017

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

Letter-report 028/2017 Z&O Mo/km
RIVM project-number: E/114506/16

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Introduction

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

General

In 2016, the following activities were carried out:

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

Deliverables

Reports

In 2016, the following reports were published:
Mooijman, K.A. The 20th EURL-*Salmonella* workshop – 28 and 29 May 2015, Berlin, Germany. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM Report no.: 2015-0083. The presentations were published on the EURL-*Salmonella* website on 2 June 2015:
http://www.eurlsalmonella.eu/Workshops/Workshop_2015
The draft report was sent to DG-Sanco in October 2015. The final report was published in January 2016 and is available through the EURL-*Salmonella* website:
http://www.rivm.nl/bibliotheek/rapporten/2015-0083.pdf

Kuijpers A.F.A., van de Kassteele, J. and Mooijman, K.A. EU Interlaboratory comparison study animal feed III (2014) - Detection of *Salmonella* in chicken feed. National Institute for Public Health and the Environment, Bilthoven, The Netherlands. RIVM Report no.: 2015-0080. The interim summary of this interlaboratory comparison study was published

in November 2014 and is available through the EURL-*Salmonella* website: http://www.eurlsalmonella.eu/dsresource?type=pdf&disposition=inline&objectid=rivmp:264448&versionid=&subobjectname=. The final report was published in March 2016 and is available at the following link: http://www.rivm.nl/bibliotheek/rapporten/2015-0080.pdf

Jacobs-Reitsma, W.F., Maas, H.M.E., de Pinna, E., Mensink, M.E. and Mooijman, K.A. Nineteenth EURL-*Salmonella* interlaboratory comparison study (2014) on typing of *Salmonella* spp. RIVM report no.: 2015-0081. The interim summary of this study was published in February 2015 and is available through the EURL-*Salmonella* website: http://www.eurlsalmonella.eu/dsresource?type=pdf&disposition=inline&objectid=rivmp:271634&versionid=&subobjectname=. The final report was published in March 2016 and is available at the following link: http://www.rivm.nl/bibliotheek/rapporten/2015-0081.pdf

Pol-Hofstad, I.E. and Mooijman, K.A. Interlaboratory comparison study Primary Production XVIII (2015) - Detection of *Salmonella* in pig faeces. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM Report no.: 2015-0082. The interim summary of this interlaboratory comparison study was published in May 2015 and is available through the EURL-*Salmonella* website: http://www.eurlsalmonella.eu/dsresource?type=pdf&disposition=inline&objectid=rivmp:280206&versionid=&subobjectname=. The final report was published in August 2016 and is available at the following link: http://www.rivm.nl/bibliotheek/rapporten/2015-0082.pdf

Mooijman, K.A. The 21st EURL-*Salmonella* workshop – 9 June 2016, Saint Malo, France. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM Report no.: 2016-0045. The presentations were published on the EURL-*Salmonella* website on 13 June 2016: http://www.eurlsalmonella.eu/Workshops/Workshop_2016. The draft report was sent to DG-Sante in September 2016. The final report was published in December 2016 and is available through the EURL-*Salmonella* website: http://www.rivm.nl/bibliotheek/rapporten/2016-0045.pdf.

The following reports are in the pipeline for publication early 2017:
Pol-Hofstad, I.E. and Mooijman, K.A. The 19th EU Interlaboratory comparison study in Primary Production (2016) - Detection of *Salmonella* in chicken faeces adhering to boot socks. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM Report no.: 2016-0044. The interim summary of this interlaboratory comparison study was published in April 2016 and is available through the EURL-*Salmonella* website: http://www.eurlsalmonella.eu/dsresource?type=pdf&disposition=inline&objectid=rivmp:315274&versionid=&subobjectname=.

Kuijpers A.F.A. and Mooijman, K.A. EU Interlaboratory comparison study food VII (2015) - Detection of *Salmonella* in whole liquid chicken egg. National Institute for Public Health and the Environment, Bilthoven, The Netherlands. RIVM Report no.: 2016-0042. The interim summary of this interlaboratory comparison study was published in November 2015 and is available through the EURL-*Salmonella* website: http://www.eurlsalmonella.eu/dsresource?type=pdf&disposition=inline&objectid=rivmp:293851&versionid=&subobjectname=.

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

Kuijpers A.F.A. and Mooijman, K.A. EU Interlaboratory comparison study food VIII (2016) - Detection of *Salmonella* in minced chicken meat. The interim summary of this interlaboratory comparison study was published in November 2016 and is available through the EURL-*Salmonella* website:

http://www.eurlsalmonella.eu/dsresource?type=pdf&disposition=inline&objectid=rivmp:325328&versionid=&subobjectname=. The final report is under preparation.

ISO and CEN
A consolidated report of 8 EURLs (coordinated by the EURL-*Salmonella*) of the meetings of ISO/TC34/SC9 and CEN/TC275/WG6 held in Paris, France on 9-13 May 2016, was sent to DG-Sante on 24 June 2016.

ISO 6579-1: Detection of *Salmonella*
The voting on ISO/FDIS 6579-1 was launched on 12 November 2015 and finished on 12 January 2016. Due to important technical comments which had to be incorporated into ISO/FDIS 6579-1, a second FDIS voting was launched on 31 October 2016 and finished on 26 December 2016. The final version of EN ISO 6579-1 was published in February 2017.

ISO 16140-6: Validation of confirmation and typing methods
The voting on ISO/CD 16140-6 was launched on 11 February 2016 and finished on 12 April 2016.

In 2016 several updates of the draft document 'Template and guidance for drafting ISO/CEN Standard methods for microbiology of the food chain' were prepared.
A third Working Draft (WD3) was sent for comments to the project leaders of ISO/TC34/SC9 and CEN/TC275/WG6 on 14 December 2015 (deadline 24 January 2016). The comments were incorporated in WD4 of the document and sent to the members of ISO/TC34/SC9 and CEN/TC275/WG6 for further comments (deadline 25 April 2016). WD5 was prepared in August 2016 and further updated in January 2017.

1. Interlaboratory comparison studies

General

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

For the reporting of the results of the interlaboratory comparison studies by the NRLs-*Salmonella,* web based test reports are used. In the course of 2015 these test reports were further improved. The number of questions on general laboratory information was reduced as this information can be requested at the NRL in case of deviating results. For the reporting of the results, details per medium combination were no longer requested as this is not done for routine analysis. Reporting if a sample was found positive or negative for *Salmonella,* independent on the medium combination used, was considered sufficient.

Details on the performance of third countries in the EURL-*Salmonella* interlaboratory comparison studies are reported annually to DG-Sante. In 2016 this report was prepared and sent to DG-Sante in October.

Follow-up studies from interlaboratory comparison studies organised in 2015

In September 2015, the seventh EURL-*Salmonella* interlaboratory comparison study on the detection of *Salmonella* in a food matrix was organised.

In this study, 36 NRLs for *Salmonella* participated: 30 NRLs from the (28) EU Member States and 6 NRLs from third countries (EU candidate MS or potential EU candidate MS, members of the European Free Trade Association (EFTA) and a non-European country). Each NRL analysed in total 20 samples: 18 samples of each 25 g whole liquid egg artificially contaminated with three different levels (blank, low and high level) of *Salmonella* Enteritidis (SE) and 2 control samples. The used contamination levels of the diluted cultures were: 20 cfu/sample and 100 cfu/sample.
The prescribed method for analyses was ISO 6579:2002 (with selective enrichment in Muller Kauffmann Tetrathionate novobiocin (MKTTn) broth and Rappaport Vassiliadis broth with soya (RVS). For this study it was anticipated that the Final Draft International Standard (FDIS) version of ISO 6579-1 was published in fall 2015. This latter document describes the (final) updated technical steps for the detection of *Salmonella* in food, animal feed and samples from the primary production stage. An important change in this document, compared to the 2002 version of ISO 6579, is the possibility to choose between RVS and MSRV for the selective enrichment of *Salmonella* from food and animal feed samples. For that reason, this choice was already introduced in the current study, meaning that additional to MKTTn, either RVS or MSRV could be used for selective enrichment. It was also allowed to use all three selective enrichment media. For the reporting of the results, the participants were asked to report what would have been reported in case these samples would have been routine samples, meaning that the indication 'positive' (1) or 'negative' (0) per sample (after confirmation) was sufficient (irrespective of the combination of selective enrichment medium and isolation medium).
All NRLs reported the results by mid-October 2015. In November 2015, the participants received information on their performance as well as an interim summary report including the results of all participants. One NRL (EU-MS) scored below the level of good performance as it tested two blank samples false positive for *Salmonella*. This laboratory participated in a follow-up study in January 2016 and scored eventually a good performance.
The results of all laboratories were presented at the EURL-*Salmonella* workshop in June 2016 and the full report of this study is likely to be published in the first half of 2017 (see 'Introduction').

In October/November 2015 the 20th interlaboratory comparison study on typing of *Salmonella* spp. was organised. This study contained a compulsory part on serotyping and an optional part on PFGE (Pulsed Field Gel Electrophoresis) typing.
In total 34 NRLs for *Salmonella* participated: 29 NRLs from the (28) EU Member States and 5 NRLs from third countries (EU candidate MS or potential EU candidate MS and member countries of the EFTA).
All (34) NRLs participated in the serotyping part of the study. For this, 20 different serovars of *Salmonella enterica* subsp. *enterica* had to be analysed. Additionally a 21st strain of an uncommon *Salmonella* serovar was added for serotyping. The serotyping of this latter serovar was optional, and the results were not taken into account for the evaluation of the performance of the NRLs.
Sixteen NRLs participated in the PFGE part of the study. For this, 10 different *Salmonella* strains had to be analysed. The *Salmonella* strains for this part of the study were selected in close cooperation with the Statens Serum Institute (SSI; Copenhagen, Denmark), who organises EQA schemes for PFGE typing for the laboratories analysing human samples of the ECDC-FWD network.
The NRLs reported the results of the serotyping before early December 2015. The PFGE results were reported separately by December 2015/January 2016.
The analysis of the serotyping results was performed in January 2016 after which the NRLs received their own results and an interim summary report, including the results of all laboratories in February 2016. One laboratory (EU-MS) did not fulfil the criteria of good performance for the serotyping. This laboratory participated in a follow-up study in April 2016 and scored eventually a good performance. The results of the study on PFGE typing were analysed in February/March 2016 and reported to the participants in April 2016.
The results of all laboratories and for both methods (serotyping and PFGE) were presented at the EURL-*Salmonella* workshop in June 2016 and the full report of this study is likely to be published early 2017 (see 'Introduction').

Interlaboratory comparison studies on detection of *Salmonella* organised in 2016

In February 2016, the 19th interlaboratory comparison study on the detection of *Salmonella* in samples from the primary production stage was organised.
In this study, 36 NRLs for *Salmonella* participated: 29 NRLs from the (28) EU Member States and 7 NRLs from third countries (member countries of the EFTA, (potential) EU candidate Member States and one NRL from a non-European country).
Each NRL had to analyse a total of 20 samples: 18 samples of boot socks with 10 g chicken faeces artificially contaminated with three different levels (blank, low and high level) of *Salmonella* Typhimurium and 2 control samples. The used contamination levels of the diluted cultures were: 11 cfu/sample and 95 cfu/sample.
The prescribed method for analyses was Annex D of ISO 6579 (2007), with selective enrichment on Modified Semi-solid Rappaport Vassiliadis (MSRV) agar. All NRLs reported the results before mid-March 2016, after which the analysis of the results was performed.
In April 2016, the participants received information on their performance as well as an interim summary report including the results of all participants. One laboratory scored a 'moderate performance' because of a transcription error in copying raw data onto the electronic reporting form. One laboratory (non EU-MS) scored a 'poor performance' for detecting *Salmonella* in three of the six blank samples, for which no explanation could be found. This laboratory could not accept the invitation for a follow-up study for financial reasons. The results of the study were presented at the EURL-*Salmonella* workshop in June 2016. The final report is likely to be published early 2017 (see 'Introduction').

In October 2016, the eighth EURL-*Salmonella* interlaboratory comparison study on the detection of *Salmonella* in a food matrix was organised.
In this study, 34 NRLs for *Salmonella* participated: 30 NRLs from the (28) EU Member States and 4 NRLs from third countries (EU candidate MS or potential EU candidate MS, members of the European Free Trade Association (EFTA) and a non-European country).
Each NRL analysed in total 20 samples: 18 samples of each 25 g minced chicken meat artificially contaminated with three different levels (blank, low and high level) of *Salmonella* Stanley and 2 control samples. It was initially announced that minced turkey meat would be used as matrix for this study, but as it turned out that this batch was naturally contaminated with *Salmonella,* the choice of the matrix was changed (at the very last minute) to minced chicken meat.
The used contamination levels of the diluted cultures were: 16 cfu/sample and 73 cfu/sample.
The prescribed method for analyses was ISO 6579:2002 (with selective enrichment in RVS and MKTTn). Like for the interlaboratory comparison study on detection of *Salmonella* in a food matrix of 2015, it was also allowed to follow ISO/FDIS 6579-1 (Anonymous 2015), meaning that in addition to MKTTn, either RVS or MSRV could be used for selective enrichment. It was also allowed to use all three selective enrichment media.
All NRLs reported the results by mid-October 2016. In November 2016, the participants received information on their performance as well as an interim summary report including the results of all participants. All laboratories scored a good performance.

In November 2016 the 21st interlaboratory comparison study on typing of *Salmonella* spp. was organised. This study contained a compulsory part on serotyping and an optional part on PFGE (Pulsed Field Gel Electrophoresis) typing.
In total 34 NRLs for *Salmonella* participated: 29 NRLs from the (28) EU Member States and 5 NRLs from third countries (EU candidate MS or potential EU candidate MS and member countries of the European Free Trade Association (EFTA)).
All (34) NRLs participated in the serotyping part of the study. For this, 20 different serovars of *Salmonella enterica* subsp. *enterica* had to be analysed. Additionally a 21st strain of an uncommon *Salmonella* serovar was added for serotyping. The serotyping of this latter serovar was optional, and the results were not taken into account for the evaluation of the performance of the NRLs.
Fifteen NRLs participated in the PFGE part of the study. For this, 10 different *Salmonella* strains had to be analysed. Like for the typing study organised in 2015, the *Salmonella* strains for this part of the study were also selected in close cooperation with SSI.

The NRLs reported the results of the serotyping before mid-December 2016. The PFGE results were reported separately by December 2016/January 2017.
The analyses of the serotyping results were performed in January 2017 after which the NRLs received their own results and an interim summary report, including the results of all laboratories in February 2017. Two laboratories (non-EU MS) did not fulfil the criteria of good performance for the serotyping. Both laboratories were contacted for a possible explanation for the deviating results and a follow-up study is offered for, probably, April 2017. The results of the study on PFGE typing are under analysis.

2. Workshop

On 9 June 2016, the annual EURL-*Salmonella* workshop was organised in Saint Malo, France.
A total of 45 participants were present at the workshop:
34 participants from the 28 EU-MS
2 participants from the EFTA countries
2 participants from EU candidate MSs or potential EU candidate MSs
3 participants from EURL-*Salmonella*
1 guest speaker
1 participant from EFSA
1 participant from DG-Sante
1 participant from ECDC
Five participants of NRLs from two EU Member States, one EFTA country and two (potential) candidate countries, were unable to come to the workshop due to lack of staff or due to problems with public transport.

Presentations were given on the following subjects:

- Results of the interlaboratory comparison studies as organised by the EURL-*Salmonella* since the previous workshop (May 2015);
- Proposals for new interlaboratory comparison studies;
- *Salmonella* monitoring data and food-borne outbreaks for 2014 in the EU;
- Update on the joint EFSA/ECDC molecular typing database;
- Information on joint cluster management, introduction to EPIS-FWD;
- Information on activities in ISO and CEN related to *Salmonella*, including standardisation of PCR method(s) for identification of monophasic *Salmonella* Typhimurium;
- The use of whole genome sequencing (WGS) for typing of *Salmonella* at Public Health England;
- Nordic cooperation for Proficiency Testing;
- Investigations on *Salmonella* Enteritidis in poultry production in France;
- Work-programme of the EURL-*Salmonella* for the coming year.

During the workshop an evaluation form about the workshop was distributed and the participants were requested to complete it (anonymously). The evaluation form was handed over to 43 participants of the workshop (excluding the organisers) and 37 completed forms were returned, being a response of 86%. From the answers of the respondents, it could be concluded that the participants considered the scientific programme as interesting. However, the accessibility to the meeting venue and the meeting room itself got some low scores. For the majority of participants, the travel time to Saint Malo was long. Additionally, at the time of the workshop public transport strikes took place in France. This certainly had an effect on the scores for the accessibility of the meeting venue.

More details on the presentations, discussion and evaluation of the workshop is summarised in the report of the workshop. The draft version of this report was sent to EC DG-Sante in September 2016 and the final report was published in December 2016 (see 'Introduction'). All presentations were placed on the EURL-*Salmonella* website (http://www.eurlsalmonella.eu/Workshops/Workshop_2016) on 13 June 2016.

3. Supporting activities

Activities in ISO and CEN

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

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

Both groups organised their annual meeting in Paris, France from 9 to 13 May 2016.

Kirsten Mooijman and Wilma Jacobs of the EURL-*Salmonella* are (co-)project leaders of groups in CEN and ISO dealing with methods for *Salmonella*, validation of typing methods and drafting of a guidance document for drafting ISO/CEN standard methods. Kirsten presented the progress of these groups at the plenary meeting of SC9 and of WG6. A consolidated report of 8 EURLs (coordinated by the EURL-*Salmonella*) on the meetings was sent to DG-Sante on 24 June 2016 (see 'Introduction').

EN ISO 6579-1: Detection of* Salmonella *(activity in CEN/TC275/WG6)

At the meeting in Paris, Kirsten Mooijman (project leader of CEN Task Group TAG8) informed the members of CEN-WG6 on the progress with drafting part 1 of EN ISO 6579 on 'Detection of *Salmonella*'.
From November 2015 to January 2016, the first FDIS voting of EN ISO 6579-1 took place, with the following results:
ISO: 24 approvals (96%) and 1 disapproval (Canada)
CEN: 20 approvals (100%)
Additionally, some editorial comments were given, but also a few technical comments which had to be taken into account. As the Final Draft International Standard (FDIS) stage of an ISO standard concerns the last voting step, only editorial comments are allowed at this stage. Therefore a written consultation at the members of ISO-SC9 & CEN-WG6 was organised from 9 March to 20 April 2016 to ask for acceptance of the technical changes. The outcome was positive, but at CEN central level it was decided that a consultation was not sufficient and that a second FDIS voting was needed. Before doing so, the members of CEN-WG6 and ISO-SC9 were consulted for their approval to launch the amended draft document for second FDIS voting (13 July – 11 August 2016). The outcome of this consultation was positive with a few comments. After introducing the last remarks into the document, the second FDIS voting (ISO FDIS 6579-1.2) was launched on 31 October 2016 and finished at 26 December 2016. The outcome was positive (26 approvals (96%) and 1 disapproval (Canada)) and again a few editorial comments were added. In January and February 2017 the last amendments were introduced into the document and by the end of February 2017 the final version of EN ISO 6579-1 was published.

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

At the meeting in Paris, Kirsten Mooijman (convenor of ISO/TC34/SC9 WG10) presented the cooperating activities with CEN/TC275/WG6 TAG3 (project leader in TAG3: Burkhard Malorny, NRL-*Salmonella* Germany).
Summary of the work in progress:

- It was agreed that CEN/TC275/WG6 TAG3 will continue the technical work to develop a PCR for identification on monophasic *Salmonella* Typhimurium. Once CEN-TAG3 agrees on the (preliminary) draft document, the work will be transferred to ISO/TC34/SC9 WG10.
- Concerning the content of the document the following was agreed:
  - Priority should be given to a protocol for identification of monophasic *S*. Typhimurium lacking the second phase (1,4,[5],12:i:-). For the time being,

the protocol does not yet have to be able to also identify the monophasic variant lacking the first phase (1,4,[5],12:-:1,2).
  - A gel-based and real-time PCR method covering monophasic variant *S.* 1,4,[5],12:i:- should be standardised including data of their performance characteristics. Additionally, gel-based singleplex PCR using the reagents of the real time assay will be included.
- It is preferred to prepare a new part 4 of ISO 6579 instead of making the method an annex to ISO/TR 6579-3, as it concerns a different technique. This part 4 will become a Technical Specification: ISO/TS 6579-4, entitled: 'Identification of monophasic *Salmonella* Typhimurium (1,4,[5],12:i:-) by Polymerase Chain Reaction'.
- In February 2016 a request was sent to CEN-TAG8 ('Detection of *Salmonella'*), ISO-WG10 ('Serotyping of *Salmonella'*) and NRLs-*Salmonella* to indicate their interest to review draft PCR protocols and to participate in a future verification study to determine performance characteristics and to indicate possible interesting strains for use in a verification study. In total 23 replies (including one from USA) were received.
- Between March and October 2016 three draft versions of ISO/TS 6579-4 were prepared by Burkhard Malorny and sent for comments to the EURL-*Salmonella*. The third Working Draft was presented at the meeting of CEN-TAG3 in November 2016 and distributed to the members of TAG3 for comments until March 2017.
- It was agreed that performance characteristics of the PCR protocols will be determined in a 'validation study' as soon as the final draft version of ISO/TS 6579-4 is available. This study will be organised by EURL-*Salmonella* with a 'standard set of strains'. For this, a call for interesting strains was made among the NRLs-*Salmonella* in November 2016. By March 2017, approximately 400 different isolates (target and non-target strains) were received at the EURL-*Salmonella*. The (sero)typing results of all isolates are confirmed at the EURL. In case of discrepancies between the typing results of the EURL and the sender, the typing will be repeated by the EURL, and the results will be discussed with the relevant NRL, if necessary. Next, a selection of the strains will be used to test the PCR protocols of draft ISO/TS 6579-4 at the laboratories of the NRL-*Salmonella* in Germany (Burkhard Malorny) and of the EURL-*Salmonella*. From these results, a final selection of the set of test strains will be made for the 'validation study'.

ISO/TC34/SC9 WG3 on validation of microbiological methods

The Committee Draft (CD) version of ISO 16140-6 ('Microbiology of the food chain — Method validation — Part 6: Protocol for the validation of alternative (proprietary) methods for microbiological confirmation and typing procedures) was launched in February 2016. The voting closed on 12 April 2016, and the outcome was positive with comments. The drafting group leader, Wilma Jacobs, incorporated the comments into the amended document and the draft DIS (Draft International Standard) version of the document was sent to the members of ISO-WG3 in October 2016 for further comments and discussion at the meeting of ISO-WG3 in December 2016. After this meeting a second draft DIS version has been prepared and sent to the members of ISO-WG3, asking for agreement to launch the DIS voting in 2017. The commenting round to the second DIS version started on 25 February 2017 and will last until 31 March 2017.

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

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

A third draft version was sent for comments to the project leaders of ISO-SC9 and CEN-WG6 in December 2015. The comments were incorporated in the fourth draft version of the document and sent for comments to all members of ISO-SC9 and CEN-WG6 in March 2016. The comments to the draft document were discussed at the annual meeting of ISO-

SC9 and CEN-WG6 in May 2016. In fall 2016 a fifth draft document was prepared and forwarded to the secretary of the Dutch Standardisation Organisation (NEN) for further finalisation in spring 2017.

ISO/TC34/SC9 ad hoc group on Harmonization of incubation temperatures
In 2014, an ad hoc group was raised to investigate the possibilities to broaden the temperature range for incubation of selective media to 34-38 °C instead of 37 °C ± 1 °C. In 2013 it was already agreed that this would be possible for non-selective media, but for selective media additional investigations were considered necessary. In 2015, the French project leader of the ad hoc group, in cooperation with the EURL-*Salmonella,* drafted a protocol to compare the growth of *Salmonella* and of background flora in MKTTn broth and on subsequent plating out media (XLD and second plate) after incubation at 37 °C and at 35 °C. Some first experiments following this protocol were performed at the laboratory of the EURL-*Salmonella* in January 2016. As a result of these first experiments the protocol and reporting form were further amended and sent to the members of ISO-SC9 and CEN-WG6 for comments in April 2016. At that time, the project leader from France changed jobs and the EURL-*Salmonella* was asked to take over the activities. In September 2016 the final version of the amended protocol and reporting form were sent to the members of ISO-SC9 and CEN-WG6, asking them to perform some experiments and report the results to the EURL-*Salmonella* by February 2017. By the end of February 2017, the EURL-*Salmonella* has received datasets from six different laboratories for further analysis.

ISO/34/SC9 WG4 Revision of ISO/TS 22117 Proficiency testing (PT)
ISO/TS 22117 was published in 2010 and it was decided in 2014 to revise the document for several reasons (e.g. to make it a full standard, to include PT schemes for viruses, parasites, primary production, yeasts and moulds, and molecular methods). The involvement of the EURLs in this working group is considered important as they have much experience with the organisation of PT schemes. In March 2016 a meeting was organised to discuss the amendment of the different parts of the document.

ISO/TC34/SC9 WG8 Revision of ISO 6887 Sample preparation series
Parts 1 to 4 of ISO 6887 are, for several years, under revision. Kirsten Mooijman is partly involved in drafting of the documents, especially in relation to pooling and compositing of samples (part 1) and for sample preparation of specific products like cocoa and acid and acidifying products. The preparation of the latter products was moved from ISO 6579:2002 (for detection of *Salmonella*) to the new part 4 of ISO 6887. The voting for Final Draft International Standard (FDIS) of the four parts was launched in October 2016 and finished in December 2016.

CEN/TC275/WG6 TAG 9 on pre-enrichment
In 2012 a Technical Advisory Group (TAG) was raised in CEN on the improvement of the pre-enrichment step to enhance the recovery of Gram-negative bacteria. For the detection of several Gram-negative bacteria like *Salmonella, Cronobacter*, STEC and *Enterobacteriaceae* a pre-enrichment step is included in the procedure. As convenor of the two groups for *Salmonella*, Kirsten Mooijman has become member of this TAG 9. Up to mid-2015 no activities were performed due to lack of a project leader. In June 2015 a new project leader was appointed and a first teleconference was organised in December 2015 to restart the activities. In February 2016 a physical meeting was organised in Germany and it was decided to prioritize the development of a dedicated protocol to determine performance characteristics for the pre-enrichment step (e.g. in BPW). Such a protocol should become included in a future revision of ISO 11133. Furthermore, the impact of modifications on sample preparation protocols should be considered for the pre-enrichment step (e.g. soaking for dry samples, pooling test portions, different dilution factors, pre-warming of Buffered Peptone Water or new BPW formula for acidifying food items). In November 2016 this was further discussed at a teleconference.

Other standardisation activities
Within AOAC, an ISPAM (International Stakeholder Panel on Alternative Methods) working group on Harmonization of *Salmonella* methods was raised in January 2015. This group

was formed to determine how and if the US and ISO reference methods for *Salmonella* can be harmonised. Members of this group are, amongst others, representatives from AOAC, FDA, USDA and Health Canada. Kirsten Mooijman of the EURL-*Salmonella* also participates in this working group as representative for the ISO working groups on *Salmonella*. The meetings of the group concern mainly teleconferences and most contacts are through e-mail. The group started very active with four teleconferences in 2015, but in 2016 no further activities were performed.

Samples for interlaboratory comparison studies

Samples for interlaboratory comparison study primary production, February 2016
For this study it was decided to use boot socks to which chicken faeces is adhered (10 g of chicken faeces added to one pair of boot socks which were pre-moistened with 15 ml peptone saline solution). This type of samples has also been used in the interlaboratory comparison study on detection of *Salmonella* in samples from the primary production stage (pps) in 2013. In this latter study the samples were artificially contaminated with *Salmonella* Typhimurium (ATCC 14028), resulting in sufficient stable materials for the interlaboratory comparison study. As the results of the pps study of 2015 could not be used to analyse the performance of the laboratories it was considered important for the 2016 study to choose samples which have proven to be stable in the past. The pre-studies performed in fall 2015 showed good stability for the high contaminated as well as for the low contaminated samples. The high contaminated samples (approximately 65 cfu/25 g) were all tested positive (5/5) after 2 weeks of storage at +5 °C, as well as at +10 °C. Of the low contaminated samples (approximately 10 cfu/25 g) still 4/5 and 3/5 samples were tested positive for *Salmonella* after storage at respectively +5 °C and +10 °C for 2 weeks. Information on the contamination levels of *Salmonella* Typhimurium in the boot sock samples used for the interlaboratory study is given in Table 1. In this Table information is given on the inoculum levels of *Salmonella* Typhimurium at the day of preparation of the boot sock samples as well as on the number of *Salmonella* in the samples at the day of the study. For this latter an MPN format (Most Probable Number) of Annex D of ISO 6579 was used.
The amount of background flora in the chicken faeces was determined shortly after arrival at the laboratory of the EURL and after one week of storage at 5 °C. For this the total aerobic count (following ISO 4833-1) as well as the number of *Enterobacteriaceae* (following ISO 21528-2) was tested. The results are summarised in Table 2.

Table 1 Salmonella *Typhimurium (STM) concentrations in inoculum culture and in boot sock samples with contaminated chicken faeces used in the EURL-*Salmonella *PPS interlaboratory study, organised in February 2016*

Date of testing	Low level *S.* Typhimurium (cfu/25 g)	High level *S.* Typhimurium (cfu/25 g)
9 February 2016 Inoculum level diluted culture	11	95
22 February 2016 (date of the study; after storage at 5 °C). MPN of boot socks with artificially contaminated chicken faeces (95 % confidence limit)	5 (1.5 -16.3)	>> (65 - >>)

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

Date of testing	Number of aerobic bacteria (cfu/g)	Number of *Enterobacteriaceae* (cfu/g)
2 February 2016	7.1×10^7	3.5×10^6
9 February 2016, after storage at 5 °C	1.5×10^7	2.8×10^5

Samples for interlaboratory comparison study Food, October 2016

For the study on detection of *Salmonella* in a food matrix in 2016 it was initially announced that minced turkey meat would be used as matrix, but as it turned out that this batch was naturally contaminated with *Salmonella,* the choice of the matrix was changed, at the very last minute, to minced chicken meat. Still the pre-studies were performed with minced turkey meat. Initially, two *Salmonella* serovars (*S.* Typhimurium and *S.* Stanley) were tested for their stability when added to minced turkey meat at a low concentration (respectively 12 cfu/25 g and 6 cfu/25 g) and stored at +5 °C, +10 °C and -20 °C. Both strains showed to be stable at all temperatures for at least 2 weeks. It was decided to choose *Salmonella* Stanley for artificially contaminating the samples in the interlaboratory study. This serovar has not been used before in EURL-*Salmonella* interlaboratory comparison studies and was reported to be related with turkey meat according to the EFSA/ECDC zoonoses report of 2014.

Information on the contamination levels of *Salmonella* Stanley in the minced chicken meat samples used for the interlaboratory study is given in Table 3. In this Table information is given on the inoculum levels of *Salmonella* Stanley at the day of preparation of the minced chicken meat samples as well as on the number of *Salmonella* in the samples at the day of the study. For this latter an MPN format (Most Probable Number) of Annex D of ISO 6579 was used.

The amount of background flora in the minced chicken meat was determined only at the (starting) day of the interlaboratory study, as the time was too short between receipt of the meat at the laboratory and the mailing of the samples to the NRLs (only 3 working days) to be able to perform an additional control in advance. Immediate after receipt of the meat at the laboratory of the EURL-*Salmonella* it was stored at +5 °C for 3 days, followed by storage at -20 °C. For determination of the amount of background flora, the total aerobic count (following ISO 4833-1) as well as the number of *Enterobacteriaceae* (following ISO 21528-2) was tested. The results are summarised in Table 4.

Table 3 Salmonella *Stanley concentrations in inoculum culture and in minced chicken meat samples used in the EURL-*Salmonella *Food interlaboratory study, organised in October 2016.*

Date of testing	Low level *S.* Stanley (cfu/25 g)	High level *S.* Stanley (cfu/25 g)
21 September 2016 Inoculum level diluted culture	16	73
3 October 2016 MPN of meat, inoculated with *S.* Stanley after storage for 10 days at -20 °C (95 % confidence limit)	35 (11-110)	55 (16-188)

Table 4 Amount of background flora in the minced chicken meat samples, tested at the (starting) day of the interlaboratory comparison study in October 2016 (after storage at +5 °C and -20 °C)

Date of testing	Number of aerobic bacteria (cfu/g)	Number of *Enterobacteriaceae* (cfu/g)
3 October 2016 After storage for 3 days at +5 °C followed by 10 days at -20 °C	2×10^6	4×10^4

Samples for interlaboratory comparison study primary production, March 2017

For the study on detection of *Salmonella* in samples from the primary production stage (pps) to be organised in spring 2017, it was decided to use chicken faeces. For the artificial contamination of the chicken faeces three different strains of two *Salmonella* serovars were tested for their stability in the samples when stored at 5 °C and at 10 °C. Depending on the results, one of the strains will be chosen to artificially contaminate the samples for the interlaboratory comparison study.

Additional to the stability studies of *Salmonella* in chicken faeces, a stability study was done for the same *Salmonella* strain in hygiene swabs in the presence of a mixture of a high level of other *Enterobacteriaceae* (approx. 10^7 cfu/sample). These samples were tested as an alternative in case chicken faeces could not be used due to the Avian influenza outbreak in several EU Member States during winter 2016/2017.

4. Giving assistance to the Commission and ad hoc activities

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

- information on content and publication of the revised ISO 6579 (ISO 6579-1);
- information on difference in sensitivity between ISO 6579-2 (enumeration of *Salmonella*) and ISO 6579-1 (detection of *Salmonella*);
- information on a method for detection of *Salmonella* Gallinarum;
- validation of (alternative) methods;
- possible use of alternative methods for confirmation (Maldi-tof) and serotyping (Whole Genome Sequencing) of *Salmonella*. For this latter a document was drafted and reported to DG-Sante in October 2016;
- help with (WGS) typing of *Salmonella*;
- advice on reporting of (typing) results;
- information on sampling of seeds;
- information on the possible effect of pooling of samples on detection of *Salmonella*;
- ISO and CEN activities by the EURL;
- artificial contamination of samples, especially cocoa powder;
- confirmation of strains (serotyping/genotyping) of NRLs-*Salmonella* (4 EU MS);
- information on activities EURL;
- information on different *Salmonella* serovars;
- anaerobic digestion of manure and possible effect on *Salmonella*;
- information on the required consumables and time for the different steps of the methods for detection, serotyping and antimicrobial resistance testing of *Salmonella*. Information was reported to DG-Sante in October and December 2016.

On average the EURL-*Salmonella* received 5 questions every week, varying from simple to complex. All questions were answered as quickly as possible. Depending on the complexity of the questions, answers could be given immediately by the experts of the EURL-*Salmonella*, or further information was gained from other experts (inside or outside the RIVM) or from literature.

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

In 2016, the EURL-*Salmonella* was requested to give assistance in a multi-country outbreak of *Salmonella* Enteritidis by performing MLVA and WGS typing of non-human isolates (also see 7. 'Molecular typing of *Salmonella* spp.').

5. Communication

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

- In April 2016, volume 22 no 1 of the newsletter was published, which included the new link to the White Kauffmann Le Minor scheme and the technical report on the activities of the EURL performed in 2015.
- In June 2016, volume 22 no 2 of the newsletter was published including the timetables of the interlaboratory comparison studies on the detection of *Salmonella* spp. in minced (turkey) meat (October 2016) and on typing of *Salmonella* (November 2016). Additionally, a summary was given of the '*Salmonella*-items' as discussed at

the annual meetings of ISO/TC34/SC9 and CEN/TC275/WG6 in Paris, France in May 2016.

- In October 2016, volume 22 no 3 of the newsletter was published, which included again the time table of the 21st interlaboratory comparison study on serotyping and PFGE typing of *Salmonella* spp.
- In January 2017, volume 22 no 4 of the newsletter was published, which included the time table of the 19th interlaboratory comparison studies on the detection of *Salmonella* spp. in samples from the primary production stage (March 2017).

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

In June 2016, two staff members of the EURL-*Salmonella* participated in the International Symposium for *Salmonella* and Salmonellosis (I3S) in Saint Malo, France. Angelina Kuijpers gave a poster presentation on the use of artificially contaminated matrices in interlaboratory comparison studies. Kirsten Mooijman gave an oral presentation on the new (draft) ISO 6579-1 (changes compared to ISO 6579:2002). Additionally, this latter information has been summarised in a manuscript for publication in a special issue of Journal Food Microbiology (likely to be published in 2017) and is already available online: http://dx.doi.org/10.1016/j.fm.2017.03.001.

6. Training activities

In May 2016, one staff member (Kirsten Mooijman) of the EURL-*Salmonella* gave two lectures at the workshop of the Greek NRL-*Salmonella* in Chalkida, to inform the official Greek laboratories on the activities of the EURL-*Salmonella*.

On 5 and 6 July 2016, a joint training course of three EURLs (*Listeria monocytogenes*, STEC and *Salmonella*) was organised on the use of BioNumerics software to analyse PFGE data. The training was organised at the premises of the EURL-*Listeria monocytogenes*, Maisons-Alfort, France. Of each EURL network, 4 NRLs participated, resulting in a total of 12 participants. From EURL-*Salmonella*, two staff members (Wilma Jacobs and El Bouw) were part of the group of trainers at this course. The evaluation of this training course is summarised in Annex 1.

In 2016 a request for training on serotyping and molecular typing of *Salmonella* for three staff members of the NRL-*Salmonella* form the Former Yugoslav Republic of Macedonia (FYROM) was received through TAIEX. The actual training was organised from 12 to 16 September 2016. The evaluation of this training is summarised in Annex 2.

7. Molecular typing of *Salmonella*

In relation to molecular typing of *Salmonella*, the following activities were performed in 2016:

- Throughout 2016, monthly meetings were organised with members of two centres of the RIVM: the Centre for Zoonoses and Environmental Microbiology (Z&O; the centre where the coordination of the EURL-*Salmonella* is hosted) and the Centre for Infectious diseases, Diagnostics and Screening (IDS; the centre performing the typing of strains). The group was raised in 2013 to regularly exchange information in relation to (molecular) typing of *Salmonella*.
- Throughout 2016, one member of the EURL-*Salmonella* participated in meetings of the joint EFSA-ECDC steering committee on 'the collection and management of molecular typing data from animal, food, feed and the related environment, and human isolates.' In 2016, the steering committee organised two physical meetings in Brussels, Belgium (April) and Stockholm, Sweden (October), in which Kirsten Mooijman participated.
- The EFSA pilot database for the collection of molecular data was activated in December 2014, but for *Salmonella* little activities were employed so far. To a large extent this was caused by the fact that agreement on and signature of the collaboration agreement by all parties lasted until April 2016. Additionally, each Member State needs to agree for its own country which molecular typing data are suitable for uploading in the database and who in the MS is allowed and able to do so. To help the NRLs-*Salmonella* with the discussions in their MS, they have regularly

been updated on the EFSA-ECDC database, e.g. at the EURL-*Salmonella* workshop and by sending an informative document drafted by EFSA in June 2016.

- In July 2016, Wilma Jacobs and El Bouw of the EURL-*Salmonella* were part of the group of trainers of the joint training course for NRLs on the use of BioNumerics software (see 6. 'Training activities'). Next to this training, a meeting was organised for the curators of the three EURLs (*Listeria monocytogenes*, STEC and *Salmonella*) to discuss and harmonise curation of molecular (PFGE) data.
- In November 2015, PFGE was included for the third time as optional typing method in the EURL-*Salmonella* interlaboratory comparison study on typing of *Salmonella.* Sixteen NRLs for *Salmonella* participated in the PFGE part of the study. The *Salmonella* strains for this part of the study were selected in close cooperation with Statens Serum Institute (SSI; Copenhagen, Denmark), who organises EQA schemes for PFGE typing for the laboratories analysing human samples of the ECDC-FWD network (FWD: Food- and Waterborne Diseases). The NRLs reported their results by December 2015/January 2016. The NRLs were asked to send their PFGE images, and additionally it was possible to send results after analysis of the gel in BioNumerics. The evaluation of the PFGE results was done by 3 staff members of the EURL to make sure that the evaluation was performed in a harmonised way. The quality grading of the PFGE images was done according to the PulseNet International/ECDC guidelines. The results of the individual laboratories were sent to the participants in April 2016. The overall results were presented at the EURL-*Salmonella* workshop in June 2016 and will be summarised in the report of this study (see 'Introduction'). All PFGE images were scored for 7 parameters. Each parameter was given a score from 1 (poor) to 4 (excellent) and also for each participant the total score for the 7 parameters was calculated. Like for the studies of 2013 and 2014, there was some variation in results between the participants. Over time some improvements in the quality of the PFGE images were seen: the number of poor scores decreased and the number of good to excellent scores increased.
- In November 2016, PFGE was again included as optional typing method in the EURL-*Salmonella* interlaboratory comparison study on typing of *Salmonella.* Fifteen NRLs for *Salmonella* participated in the PFGE part of the study. The *Salmonella* strains for this part of the study were also selected in close cooperation with SSI. The NRLs reported their results by December 2016/January 2017 and the results are currently being evaluated.
- In August 2016, DG-Sante requested the EURL-*Salmonella* to draft a document on the possible use of WGS for serotyping of *Salmonella*, containing information on i) development and availability of WGS-based serotyping methods; ii) validation information of WGS serotyping methods against the conventional serotyping; iii) pros and cons on the use of WGS serotyping vs conventional serotyping; iv) possible changes needed in the legislation in order to eliminate legislative barriers in the use of WGS-based serotyping for Salmonella controls. For this, information from literature and experts was summarised and sent to DG-Sante in October 2016.
- The activities for standardisation of PCR methods for identification of monophasic *Salmonella* Typhimurium is described in 3. 'Supporting activities'.
- In 2016, DG-Sante asked the EURL-*Salmonella* to help in a multi-country outbreak of *Salmonella* Enteritidis (mainly MLVA 2-9-7-3-2) related to polish eggs. The EURL was asked to perform MLVA and WGS typing and to perform cluster analysis of the WGS results of non-human *Salmonella* Enteritidis (SE) strains which were likely to be part of the outbreak. Some first analyses were done in spring 2016 and more from October 2016 up to March 2017. Thirty-five non-human isolates from six different countries were received for MLVA typing and, in case of a match with the MLVA type(s) of the outbreak definition, also WGS typing. Additionally, four more countries uploaded their WGS data of SE strains likely to be related to the outbreak to the RIVM server for cluster analysis (28 isolates). The results have been included in the joint rapid outbreak assessment of ECDC and EFSA of March 2017.

8. Missions

*The following missions in relation with the EURL-*Salmonella *activities and budget were performed in 2016.*

Related to activity 3. 'Supporting activities'

- Meeting CEN/TC275/WG6 – TAG9 on improvement of pre-enrichment
  23 February 2016: Darmstadt, Germany
  Participant: Kirsten Mooijman (member TAG9)
- Meetings ISO/TC34/SC9 – WG3 on validation of microbiological methods (including draft ISO 16140-6 on validation of confirmation/typing methods)
  6-8 April 2016: Campden, United Kingdom
  Participant: Wilma Jacobs (project leader drafting ISO 16140-6 and member WG3)
  5-7 December 2016: Minneapolis, USA
  Participant: Wilma Jacobs
- Annual meetings of CEN/TC275/WG6 and ISO/TC34/SC9 (Microbiology of the food chain)
  9-13 May 2016: Paris, France
  Participant: Kirsten Mooijman
- Workshop NRL-*Salmonella* Greece
  19 May 2016: Chalkida, Greece
  Participant: Kirsten Mooijman

Related to activity 6. 'Training activities' and 7. 'Molecular typing of Salmonella'

- Joint training course on the use of BioNumerics, followed by curators meeting
  5-7 July 2016: Maisons-Alfort, France
  Participants (trainers): Wilma Jacobs, El Bouw

Related to activity 7. 'Molecular typing of Salmonella'

- Meetings of the EFSA-ECDC steering committee on the collection and management of molecular typing data
  7-8 April 2016: Brussels, Belgium
  27-28 October 2016: Stockholm, Sweden
  Participant: Kirsten Mooijman (all meetings)

Mrs. Drs. K.A. Mooijman,
Head EURL-*Salmonella*
Bilthoven, 23 March 2017

Abbreviations

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

References

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

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

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

ISO/FDIS 6579-1: 2015. Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* – Part 1: Detection of *Salmonella* spp.

CEN ISO/TS 6579-2:2012. Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* – Part 2: Enumeration by a miniaturized most probable number technique.

CEN ISO/TR 6579-3:2014. Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* – Part 3: Guidelines for serotyping of *Salmonella* spp.

ISO 6785:2007, Milk and milk products — Detection of *Salmonella* spp.

ISO/DIS 6887-1:2013. Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions

ISO/DIS 6887-2:2013. Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 2: Specific rules for the preparation of meat and meat products

ISO/DIS 6887-3: 2013. Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 3: Specific rules for the preparation of fish and fishery products

ISO/DIS 6887-4:2013. Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 4: Specific rules for the preparation of miscellaneous products

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

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

EC, 2004. European Regulation EC No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. Official Journal of the European Union L 165: 30 April 2004. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32004R0882&qid=1458664362396&from=EN (access date 16/03/2017)

ECDC and EFSA, 2017. Multicountry outbreak of *Salmonella* Enteritidis phage type 8, MLVA type 2-9-7-3-2 and 2-9-6-3-2 infections. ECDC and EFSA: Stockholm and Parma; 7 March 2017. http://dx.doi.org/10.2903/sp.efsa.2017.EN-1188

EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), 2015. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2014. EFSA Journal 2015;13(12):4329, 190 pp. doi:10.2903/j.efsa.2015.4329. http://www.efsa.europa.eu/en/efsajournal/pub/4329 (access date 07/03/2017).

Annex 1 Evaluation joint training course organized by 3 EURLs on the use of BioNumerics (5-6 July 2016)

Evaluation Joint Training Course of 3 EURLs on the use of BioNumerics Software to analyse PFGE data of STEC, *Salmonella* and *Listeria monocytogenes*
5-6 July 2016. Location: EURL-*Listeria monocytogenes,* ANSES; Laboratory for Food Safety, Maisons-Alfort, France

Number of participants training course	12
Number of participants completing evaluation	11
Participating countries	Finland, Ireland, Italy, Latvia, Poland (2x), Romania, Slovenia, Slovakia, Spain, Switzerland, United Kingdom
How would you rate your general satisfaction with the training session?	
Satisfied	4
Very satisfied	7
Comments	Nice, short overview about the topic. Was nice to see the possible problems that can occur with PFGE
Are you satisfied with the practical aspects of the training session (venue, date, times, etc.)?	
Yes	9
No	2
Comments	More time for hands on sessions would have been nice. Idea of practical working was good, but there was sometimes a mess between the different steps and the different groups. Would be better to separate the groups, and do it step by step. Maybe more time for practical exercise is needed.
Did the content of the training session match what was on the programme?	
Yes	11
No	0
Did the training session meet your goals?	
Yes	9
No	2
Comments	I hoped to have more time on cluster analysis and even on how to send data to EURL/Joint database.
How would you rate the facilitator's performance?	
Satisfied	3
Very satisfied	8
Comments	I suppose that the issues related to the interpretation of results have been comprehensively considered, the theoretical aspects were discussed in detail. As regards the practical part of the courses, I find it difficult to estimate its efficiency as we have no experience in this point yet. I would have like the training to be more in depth. I think we spent too long on the basics.

Annex 2 Evaluation laboratory training at EURL-*Salmonella* (12-16 September 2016)

Evaluation of the laboratory training of three participants of NRL-*Salmonella* of the Former Yugoslav Republic of Macedonia (FYROM), on serotyping and genotyping of *Salmonella* (funded by TAIEX).

1. What were the dates of your training at the EURL-*Salmonella*?

12 – 16 September 2016

2. Please describe what you expected to learn from this training (on forehand):

Salmonella serotyping, PFGE, MLVA and PCR

3. Who were your main trainees at the EURL-*Salmonella*?

Mrs. Anjo Verbruggen and Mr. Sjoerd Kuiling

4. Were the trainees able to fulfill your expectations?

X Yes
☐ No

The trainers were very communicative and helpful.

5. Was the time sufficient for your training?

X Yes
☐ No, too short; would have needed … days more
☐ No, too long; training could have been done in … days

6. Can you please describe (in short) what you have learned during the training?

We learned what we expected (see 2.) and additional we learned about the use of Luminex for serotyping and received information on other bacteria.

7. Is what you have learned during the training applicable in your laboratory?

X Yes
☐ No

One participant indicated that MLVA and Luminex are not applicable in its laboratory.

8. Overall, did the training fulfill your expectations?

X Yes
☐ No

From the Literature

Salmonella-related Literature from Scopus: January – March 2017

Kloska, F., Beyerbach, M., Klein, G.
Infection dynamics and antimicrobial resistance profile of salmonella paratyphi B d-tartrate positive (Java) in a persistently infected broiler barn
(2017) International Journal of Environmental Research and Public Health, 14 (1), art. no. 101, .
ABSTRACT: The infection dynamics of S. Java were examined in three consecutive rearing periods on a broiler farm in Northwestern Germany which had been persistently infected with S. Java for more than five years. The barn was investigated for Salmonella occurrence after cleaning and disinfection to verify the persistent contamination of the broiler house with S. Java before the start of the first rearing cycle. Confirmation of Salmonella absence in day-old chicks (time-point 1) as well as early establishment of infection between days 5–7 (time-point 2) were confirmed by caecal swabs prepared for qPCR and classical microbiological methods. At three time-periods (between days 11–15 (time-point 3), days 25–28 (time-point 4), and days 38–40 (time-point 5)) caecal content was examined for colony forming units (CFU) Salmonella/g. In general, there was an increase in Salmonella Java load at time-point 4 compared to time-points 3 and 5. Therefore, we observed a bell-shaped course of infection resulting in higher rates of Salmonella CFU/g prior to prethinning than at final slaughter. The antimicrobial susceptibility testing revealed resistance to tetracycline, fluorquinolones, trimethoprim, and cefoxitin. ISSN: 16617827

Yuan, M.-Y., Xu, L.-Y., Liu, E.-L., Cao, J.-J., Chen, B.-L.
Development of a duplex real-time PCR method with Taqman-based probes for detecting Salmonella enterica and Salmonella enteritidis
(2017) Modern Food Science and Technology, 33 (1), pp. 248-252.
ABSTRACT: In order to detect Salmonella enterica (SP) and Salmonella enteritidis (SE) using a Taqman-based duplex real-time PCR method, primers and Taqman probes were designed based on the aceA (GenBank: U43344.1) sequence of SP and the SEP sequence (GenBank: AF370707.1) of SE. Probes were separately labeled with FAM and VIC. The results showed that all 58 Salmonella strains, with 29 different serotypes, could be amplified with the aceA sequence. Only 15 SE strains could be amplified with the SEP primers and probe, while the other 28 strains, with 29 different serotypes of Salmonella, the 17 strains of Proteus, as well as the negative control strains showed negative results. The amplification efficiency of aceA and SEP were 100% and 104%, respectively. R2 values were estimated to be 0.999 and 0.998, respectively. The detection limits of aceA and SEP for this method were 280 cfu/mL and 260 cfu/mL, respectively. The duplex real-time PCR assay developed in this study showed high sensitivity and specificity, and could be used as a rapid and effective method for detecting SP and SE in foods.
ISSN: 16739078

Forkus, B., Ritter, S., Vlysidis, M., Geldart, K., Kaznessis, Y.N.
Antimicrobial Probiotics Reduce Salmonella enterica in Turkey Gastrointestinal Tracts
(2017) Scientific Reports, 7, art. no. 40695, .
ABSTRACT: Despite the arsenal of technologies employed to control foodborne nontyphoidal Salmonella (NTS), infections have not declined in decades. Poultry is the primary source of NTS outbreaks, as well as the fastest growing meat sector worldwide. With recent FDA rules for phasing-out antibiotics in animal production, pressure is mounting to develop new pathogen reduction strategies. We report on a technology to reduce Salmonella enteritidis in poultry. We engineered probiotic E. coli Nissle 1917, to express and secrete the antimicrobial peptide, Microcin J25. Using in vitro experiments and an animal model of 300 turkeys, we establish the efficacy of this technology. Salmonella more rapidly clear the ceca of birds administered the modified probiotic than other treatment groups. Approximately 97% lower Salmonella carriage is measured in a treated group, 14 days post-Salmonella challenge. Probiotic bacteria are generally regarded as safe to consume, are bile-resistant and can plausibly be modified to produce a panoply of antimicrobial peptides now known. The reported systems may provide a foundation for

platforms to launch antimicrobials against gastrointestinal tract pathogens, including ones that are multi-drug resistant. ISSN: 20452322

Sanchez-Maldonado, A.F., Aslam, M., Service, C., Narváez-Bravo, C., Avery, B.P., Johnson, R., Jones, T.H.
Prevalence and antimicrobial resistance of Salmonella isolated from two pork processing plants in Alberta, Canada
(2017) International Journal of Food Microbiology, 241, pp. 49-59.
ABSTRACT: This study investigated the frequency of Salmonella serovars on pig carcasses at various processing steps in two commercial pork processing plants in Alberta, Canada and characterized phenotypic and genotypic antimicrobial resistance (AMR) and PFGE patterns of the Salmonella isolates. Over a one year period, 1000 swab samples were collected from randomly selected pigs at two slaughter plants. Sampling points were: carcass swabs after bleeding (CSAB), carcass swabs after de-hairing (CSAD, plant A) or skinning (CSASk, plant B), carcass swabs after evisceration (CSAE), carcass swabs after pasteurization (CSAP, plant A) or washing (CSAW, plants B) and retail pork (RP). For plant A, 87% of CSAB and 8% of CSAE were positive for Salmonella while at plant B, Salmonella was recovered from 94% of CSAB and 10% of CSAE. Salmonella was not recovered from the RP samples at either plant, indicating that the plants used effective control measures. Salmonella enterica serovar Derby was the most common serotype (23%, 29/127) recovered in plant A and plant B (61%, 76/124). For plant A, 35% (45/127) of isolates were resistant to at least one antimicrobial. Five isolates (3.9%), 4 serovar Ohio strains and one serovar I:Rough-O:I,v:-, strain were simultaneously resistant to antimicrobials of very high (Category I), high (Category II), and medium (Category III) importance to human medicine. The 4 S. Ohio isolates were recovered from 3 different steps of pork processing on the same sampling day and displayed resistance to 5–7 antimicrobials, with all of them displaying resistance to ceftiofur and ceftriaxone (Category I). An I:Rough-O:I,v:- isolate, recovered on a different sampling day, was resistant to 7 antimicrobials that included resistance to ampicillin/clavulanic acid, ceftiofur and ceftriaxone (Category I). Salmonella strains isolated from plant A harbored 12 different AMR genes. The most prevalent genes were sul1, sul2, tet(A), tet(B), aadA, strA/strB, aac(3)IV and aphA1. For Salmonella isolates from plant B, 7 resistance genes were identified alone or in combination where tet(B) was found in 77 (62.3%) of the isolates. For plant A, 19 different PFGE subtypes of Salmonella isolates that displayed phenotypic and/or genotypic resistance were observed while 13 different PFGE subtypes were observed for plant B. The lack of detection of Salmonella on the surfaces of RP suggests that current pork processing practices can dramatically reduce Salmonella. Salmonella isolates from pig carcasses at various steps displayed multidrug resistance, including to those of very high importance in human medicine, which represent a public health concern. ISSN: 01681605
CODEN: IJFMD

Acar, S., Bulut, E., Durul, B., Uner, I., Kur, M., Avsaroglu, M.D., Kirmaci, H.A., Tel, Y.O., Zeyrek, F.Y., Soyer, Y.
Phenotyping and genetic characterization of Salmonella enterica isolates from Turkey revealing arise of different features specific to geography
(2017) International Journal of Food Microbiology, 241, pp. 98-107.
ABSTRACT: 192 Food samples (commonly consumed 8 food types), 355 animal samples (animal feces of bovine, ovine, goat and chicken) and 50 samples from clinical human cases in Sanliurfa city, Turkey in a year were collected to determine the Salmonella enterica subsp. enterica mosaic in Turkey. 161 Salmonella isolates represented 17 serotypes, 20 sequence types (STs) and 44 PFGE patterns (PTs). 3 serotypes, S. Enteritidis, S. Typhimurium and S. Kentucky, were recovered from three different hosts. The highest discriminatory power was obtained by PFGE (SID = 0.945), followed by MLST (SID = 0.902) and serotyping (SID = 0.885) for all isolates. The prevalence of antimicrobial resistance genes (aadA1, aadA2, strA, strB, aphA1-Iab, blaTEM-1, blaPSE-1, tetA) was highly correlated with phenotypic profiles of aminoglycoside, ß-lactam and tetracycline groups (kappa > 0.85). From our knowledge, this is the first study reporting spatial and temporal distribution of Salmonella species through phenotypic and genetic approaches over farm to fork chain in Turkey. Thus, our data provided further information for evolution, ecology and transmission of Salmonella in Turkey. ISSN: 01681605

Lee, H.K., Abdul Halim, H., Thong, K.L., Chai, L.C.
Assessment of food safety knowledge, attitude, self-reported practices, and microbiological hand hygiene of food handlers
(2017) International Journal of Environmental Research and Public Health, 14 (1), art. no. 55, .

ABSTRACT: Institutional foodborne illness outbreaks continue to hit the headlines in the country, indicating the failure of food handlers to adhere to safe practices during food preparation. Thus, this study aimed to compare the knowledge, attitude, and self-reported practices (KAP) of food safety assessment and microbiological assessment of food handlers' hands as an indicator of hygiene practices in food premises. This study involved 85 food handlers working in a university located in Kuala Lumpur, Malaysia. The food safety KAP among food handlers (n = 67) was assessed using a questionnaire; while the hand swabs (n = 85) were tested for the total aerobic count, coliforms, and Escherichia coli, Staphylococcus aureus, Salmonella, Vibrio cholerae and Vibrio parahaemolyticus. The food handlers had moderate levels of food safety knowledge (61.7%) with good attitude (51.9/60) and self-reported practices (53.2/60). It is noteworthy that the good self-reported practices were not reflected in the microbiological assessment of food handlers' hands, in which 65% of the food handlers examined had a total aerobic count ≥20 CFU/cm2 and Salmonella was detected on 48% of the food handlers' hands. In conclusion, the suggestion of this study was that the food handlers had adequate food safety knowledge, but perceived knowledge failed to be translated into practices at work.
ISSN: 16617827

Gast, R.K., Jones, D.R.
Salmonella and Impact on Egg Production
(2017) Egg Innovations and Strategies for Improvements, pp. 515-521.
ABSTRACT: There is a strong association between the incidence of human illness and the prevalence of Salmonella in commercial egg-producing poultry. Although most egg-associated disease is attributed to Salmonella Enteritidis, other serovars are sometimes implicated. Deposition of S. Enteritidis within the edible contents of eggs results from colonization of reproductive tissues in infected hens. Environmental conditions influence opportunities for Salmonella introduction, transmission, and persistence in laying flocks. Environmental influences are shaped by the various housing systems used in egg production. Substantial public and private resources have been invested in comprehensive S. Enteritidis testing and risk reduction programs for egg-laying chickens. A strategy involving multiple interventions throughout the egg production cycle is recommended as the most effective approach for controlling S. Enteritidis. Controlling temperature is critical for restricting Salmonella growth inside eggs. ISBN: 9780128011515; 9780128008799

Bollerslev, A.M., Nauta, M., Hansen, T.B., Aabo, S.
A risk modelling approach for setting microbiological limits using enterococci as indicator for growth potential of Salmonella in pork
(2017) International Journal of Food Microbiology, 240, pp. 102-107.
ABSTRACT: Microbiological limits are widely used in food processing as an aid to reduce the exposure to hazardous microorganisms for the consumers. However, in pork, the prevalence and concentrations of Salmonella are generally low and microbiological limits are not considered an efficient tool to support hygiene interventions. The objective of the present study was to develop an approach which could make it possible to define potential risk-based microbiological limits for an indicator, enterococci, in order to evaluate the risk from potential growth of Salmonella. A positive correlation between the concentration of enterococci and the prevalence and concentration of Salmonella was shown for 6640 pork samples taken at Danish cutting plants and retail butchers. The samples were collected in five different studies in 2001, 2002, 2010, 2011 and 2013. The observations that both Salmonella and enterococci are carried in the intestinal tract, contaminate pork by the same mechanisms and share similar growth characteristics (lag phase and maximum specific growth rate) at temperatures around 5–10 °C, suggest a potential of enterococci to be used as an indicator of potential growth of Salmonella in pork. Elevated temperatures during processing will lead to growth of both enterococci and, if present, also Salmonella. By combining the correlation between enterococci and Salmonella with risk modelling, it is possible to predict the risk of salmonellosis based on the level of enterococci. The risk model used for this purpose includes the dose–response relationship for Salmonella and a reduction factor to account for preparation of the fresh pork. By use of the risk model, it was estimated that the majority of salmonellosis cases, caused by the consumption of pork in Denmark, is caused by the small fraction of pork products that has enterococci concentrations above 5 log CFU/g. This illustrates that our approach can be used to evaluate the potential effect of different microbiological limits and therefore, the perspective of this novel approach is that it can be used for definition of a risk-based microbiological limit for enterococci. The limit for enterococci can then be used for development of a process hygiene criterion in cutting plants and retail butcher shops, with the purpose of reducing the risk of Salmonella for the consumer. ISSN: 01681605

Possas, A., Posada-Izquierdo, G.D., Pérez-Rodríguez, F., Valero, A., García-Gimeno, R.M., Duarte, M.C.T.
Application of predictive models to assess the influence of thyme essential oil on Salmonella Enteritidis behaviour during shelf life of ready-to-eat turkey products
(2017) International Journal of Food Microbiology, 240, pp. 40-46.
ABSTRACT: Consumers' demand for ready-to-eat (RTE) turkey meat is attributed to its convenience and healthy properties. However, as cooked meat product it is subjected to post-process contamination, thus allowing presence and growth of microbial pathogens, such as Salmonella spp. The aim of this study was to include a natural antimicrobial, thyme essential oil (TEO), on RTE turkey products in order to evaluate its effectiveness throughout the shelf life. To do so, the effect of four different formulations of cooked RTE turkey products on Salmonella Enteritidis behaviour was investigated. Products' slices were surface inoculated with S. Enteritidis (ca. 4 to 5 log cfu/g), subsequently stored at 10 and 25 °C and microbiologically analysed during 18 and 12 days, respectively. Predictive microbiology models fitted to count data were used to evaluate microbial behaviour. Results showed that S. Enteritidis behaviour on RTE turkey products slices during storage was strongly dependent on temperature. The pathogen was able to grow on slices at all tested conditions during storage at 25 °C and no statistical differences were detected ($p > 0.05$) between growth parameters. At 10 °C, different behaviour patterns were observed. The application of TEO led to higher Salmonella inactivation rates on a product exempt of chemical preservatives. The addition of this novel antimicrobial on meat products or its incorporation on meat active packaging systems as a part of hurdle technology could increase RTE turkey products safety while satisfying the demand of more natural foods. ISSN: 01681605

Prado-Rebolledo, O.F., Delgado-Machuca, J.D.J., Macedo-Barragan, R.J., Garcia-Márquez, L.J., Morales-Barrera, J.E., Latorre, J.D., Hernandez-Velasco, X., Tellez, G.
Evaluation of a selected lactic acid bacteria-based probiotic on Salmonella enterica serovar Enteritidis colonization and intestinal permeability in broiler chickens
(2017) Avian Pathology, 46 (1), pp. 90-94.
ABSTRACT: Two experiments were conducted to evaluate the effect of a lactic acid bacteria-based probiotic (FloraMax-B11®) against Salmonella enterica serovar Enteritidis intestinal colonization and intestinal permeability in broiler chickens. Experiment 1 consisted of two independent trials. In each trial, day-old broiler chicks were assigned to one of two groups: control + S. Enteritidis or probiotic + S. Enteritidis. At 72 h post-S. Enteritidis challenge, haematology and caecal content were evaluated for S. Enteritidis colonization. In Experiment 2, day-old broiler chicks were assigned to one of four groups: negative control; probiotic; control + S. Enteritidis; or probiotic + S. Enteritidis. At 72 h post-S. Enteritidis challenge, chickens in all groups were given an oral gavage dose of fluorescein isothiocyanate dextran (FITC-d). In both trials of Experiment 1, a significant reduction (P < 0.05) in colony-forming units/gram of S. Enteritidis in caecal content and a reduction in the incidence of S. Enteritidis enriched caecal samples were observed in probiotic + S. Enteritidis chickens. In addition, significant heterophilia and lymphopaenia were observed in control + S. Enteritidis chickens. In Experiment 2, a decrease in numbers of S. Enteritidis in caeca were observed in probiotic + S. Enteritidis chickens when compared to control + S. Enteritidis. Also, an increase in serum FITC-d concentration was detected in control + S. Enteritidis. These results suggest that early infection with S. Enteritidis can increase intestinal permeability, but the adverse effects can be prevented by the administration of the probiotic tested. ISSN: 03079457

Métris, A., George, S.M., Ropers, D.
Piecewise linear approximations to model the dynamics of adaptation to osmotic stress by food-borne pathogens
(2017) International Journal of Food Microbiology, 240, pp. 63-74.
ABSTRACT: Addition of salt to food is one of the most ancient and most common methods of food preservation. However, little is known of how bacterial cells adapt to such conditions. We propose to use piecewise linear approximations to model the regulatory adaptation of Escherichia coli to osmotic stress. We apply the method to eight selected genes representing the functions known to be at play during osmotic adaptation. The network is centred on the general stress response factor, sigma S, and also includes a module representing the catabolic repressor CRP-cAMP. Glutamate, potassium and supercoiling are combined to represent the intracellular regulatory signal during osmotic stress induced by salt. The output is a module where growth is represented by the concentration of stable RNAs and the transcription of the osmotic gene osmY. The time course of gene expression of transport of osmoprotectant represented by the symporter

proP and of the osmY is successfully reproduced by the network. The behaviour of the rpoS mutant predicted by the model is in agreement with experimental data. We discuss the application of the model to food-borne pathogens such as Salmonella; although the genes considered have orthologs, it seems that supercoiling is not regulated in the same way. The model is limited to a few selected genes, but the regulatory interactions are numerous and span different time scales. In addition, they seem to be condition specific: the links that are important during the transition from exponential to stationary phase are not all needed during osmotic stress. This model is one of the first steps towards modelling adaptation to stress in food safety and has scope to be extended to other genes and pathways, other stresses relevant to the food industry, and food-borne pathogens. The method offers a good compromise between systems of ordinary differential equations, which would be unmanageable because of the size of the system and for which insufficient data are available, and the more abstract Boolean methods. ISSN: 01681605

Lynch, H., Arguëllo, H., Walia, K., Lawlor, P.G., Duffy, G., Gardiner, G.E., Leonard, F.C.
Evaluation of an Alternative Experimental Infection Method, Which Closely Mimics the Natural Route of Transmission of Monophasic Salmonella Typhimurium in Pigs
(2017) Foodborne Pathogens and Disease, 14 (1), pp. 23-28.
ABSTRACT: Salmonella carriage in pigs is a significant food safety issue. This study describes a new protocol of Salmonella infection based on exposure to an artificially contaminated environment that closely mimics natural exposure to the organism. The aim of the study was to develop and evaluate the effectiveness of this protocol, which could then be used as a tool in the investigation of control measures. In addition, Salmonella shedding pattern and growth performance of the pigs were examined. Trial pigs (n = 10) were placed in a pen that had been previously contaminated by housing two pigs experimentally challenged with a monophasic Salmonella Typhimurium (mST). A further 10 pigs were placed in a Salmonella-free pen. Pigs were weighed on days 0 and 28. Feces was collected on days 0, 2, 3, 5, 7, 14, 21, and 28 and examined for the presence and quantity of Salmonella. The trial was replicated once. All pigs in the contaminated pens shed Salmonella within the first 2 days of exposure with values ranging from 100 to 104 CFU/g. The noninfected pigs had significantly higher final body weights on day 28 than those exposed to the Salmonella contaminated environment in both replicates. The pigs in the Salmonella-free pen had significantly higher average daily weight gain over the 28-day period compared to the infected animals (p < 0.001). Although not significant, numerical improvements in average daily feed intake and feed conversion efficiency were observed in the Salmonella-free pigs when compared to the contaminated pigs. The approach used was successful in infecting pigs with Salmonella without the need for direct inoculation or exposure to seeder pigs. This "natural" method of infection in which pigs are exposed to low levels of environmental contamination with Salmonella may be an effective tool that could be utilized when investigating control measures. ISSN: 15353141

Jeong, S., Marks, B.P., James, M.K.
Comparing thermal process validation methods for salmonella inactivation on almond Kernels
(2017) Journal of Food Protection, 80 (1), pp. 169-176.
ABSTRACT: Ongoing regulatory changes are increasing the need for reliable process validation methods for pathogen reduction processes involving low-moisture products; however, the reliability of various validation methods has not been evaluated. Therefore, the objective was to quantify accuracy and repeatability of four validation methods (two biologically based and two based on timetemperature models) for thermal pasteurization of almonds. Almond kernels were inoculated with Salmonella Enteritidis phage type 30 or Enterococcus faecium (NRRL B-2354) at ~108 CFU/g, equilibrated to 0.24, 0.45, 0.58, or 0.78 water activity (aw), and then heated in a pilot-scale, moist-Air impingement oven (dry bulb 121, 149, or 1778C; dew point ,33.0, 69.4, 81.6, or 90.68C; vair = 2.7 m/s) to a target lethality of ~4 log. Almond surface temperatures were measured in two ways, and those temperatures were used to calculate Salmonella inactivation using a traditional (D, z) model and a modified model accounting for process humidity. Among the process validation methods, both methods based on time-Temperature models had better repeatability, with replication errors approximately half those of the surrogate (E. faecium). Additionally, the modified model yielded the lowest root mean squared error in predicting Salmonella inactivation (1.1 to 1.5 log CFU/g); in contrast, E. faecium yielded a root mean squared error of 1.2 to 1.6 log CFU/g, and the traditional model yielded an unacceptably high error (3.4 to 4.4 log CFU/g). Importantly, the surrogate and modified model both yielded lethality predictions that were statistically equivalent (a = 0.05) to actual Salmonella lethality. The results demonstrate the importance of methodology, aw,

and process humidity when validating thermal pasteurization processes for low-moisture foods, which should help processors select and interpret validation methods to ensure product safety. ISSN: 0362028X

Silveira, J.B., Hessel, C.T., Tondo, E.C.
Inactivation of Salmonella enteritidis on lettuces used by minimally processed vegetable industries
(2017) Journal of Infection in Developing Countries, 11 (1), pp. 34-41.
ABSTRACT: Introduction: Washing and disinfection methods used by minimally processed vegetable industries of Southern Brazil were reproduced in laboratory in order to verify their effectiveness to reduce Salmonella Enteritidis SE86 (SE86) on lettuce. Methodology: Among the five industries investigated, four carried out washing with potable water followed by disinfection with 200 ppm sodium hypochlorite during different immersion times. Results: The washing procedure alone decreased approximately 1 log CFU/g of SE86 population and immersion times of 1, 2, 5, and 15 minutes in disinfectant solution demonstrated reduction rates ranging from 2.06±0.10 log CFU/g to 3.01±0.21 log CFU/g. Rinsing alone was able to reduce counts from 0.12±0.63 log CFU/g to 1.90±1.07 log CFU/g. The most effective method was washing followed by disinfection with 200 ppm sodium hypochlorite for 15 minutes and final rinse with potable water, reaching 5.83 log CFU/g of reduction. However, no statistical differences were observed on the reduction rates after different immersion times. Conclusion: A time interval of 1 to 2 minutes may be an advantage to the minimally vegetable processed industries in order to optimize the process without putting at risk food safety. ISSN: 20366590

Mishra, A., Guo, M., Buchanan, R.L., Schaffner, D.W., Pradhan, A.K.
Prediction of Escherichia coli O157:H7, salmonella, and listeria monocytogenes growth in leafy greens without temperature control
(2017) Journal of Food Protection, 80 (1), pp. 68-73.
ABSTRACT: A recent study by the Centers for Disease Control and Prevention reported that between 1998 and 2008, leafy greens outbreaks accounted for 22.3% of foodborne outbreaks in the United States. Several studies on the growth of bacteria at different temperatures have been conducted; however, there is a need for the prediction of bacterial growth when leafy greens are transported without temperature control. Food products, when taken out of refrigeration, undergo a temperature change, with the rate of temperature change being proportional to the difference in the temperature of food and its surroundings. The objective of this study was to estimate the growth of Escherichia coli O157:H7, Salmonella enterica, and L. monocytogenes in leafy greens during transportation from retail to home at ambient temperatures ranging from 10 to 40°C for up to 10 h. Experiments were conducted to monitor the temperature increase in fresh spinach taken from refrigeration temperature to ambient temperature. The growth of pathogens was predicted using these changing temperature profiles with the three-phase linear model as a primary model and the square root model as the secondary model. The levels of E. coli O157:H7, S. enterica, and L. monocytogenes increased by 3.12, 2.43, and 3.42 log CFU at 40°C for the 10-h period, respectively, when no lag phase was assumed. If leafy greens are not kept out of refrigeration for more than 3 h, when the air temperature is 40°C or more, pathogen growth should be less than 1 log CFU. These results would assist in developing recommendations for food transportation without refrigeration.
ISSN: 0362028X

Songe, M.M., Hang'ombe, B.M., Knight-Jones, T.J.D., Grace, D.
Antimicrobial resistant enteropathogenic escherichia coli and salmonella spp. In houseflies infesting fish in food markets in Zambia
(2017) International Journal of Environmental Research and Public Health, 14 (1), art. no. 21, .
ABSTRACT: Diarrhea is one of the most common diseases and is a leading cause of death in developing countries. This is often caused by contaminated food. Poor food hygiene standards are exacerbated by the presence of flies which can transmit a variety of infectious microorganisms, particularly through animal source foods. This fact becomes especially important in developing countries like Zambia, where fish is a highly valued source of protein. Our interest in this study was to identify if the flies that beset food markets in Zambia carry important pathogenic bacteria on their bodies, and subsequently if these bacteria carry resistance genes to commonly used antibiotics, which would indicate problems in eradicating these pathogens. The present study took into account fish vendors' and consumers' perception of flies and interest in interventions to reduce their numbers. We conducted semi-structured interviews with (1) traders (comprised of randomly selected males and females) and (2) consumers (including randomly selected males and females).

Thereafter, we collected flies found on fish in markets in Mongu and Lusaka districts of Zambia. For the entire study, a total of 418 fly samples were analyzed in the laboratory and Salmonella spp. and enteropathogenic Escherichia coli were isolated from the flies. Further laboratory screening revealed that overall, 17.2% (72/418) (95% CI; 43.2%–65.5%) of total samples analyzed contained Extended-Spectrum Beta-Lactamase (ESBL)-producing E. coli. These significant findings call for a strengthening of the antibiotic administering policy in Zambia and the development of sustainable interventions to reduce fly numbers in food markets and improve food safety and hygiene. ISSN: 16617827

Edlind, T., Brewster, J.D., Paoli, G.C.
Enrichment, amplification, and sequence-based typing of salmonella enterica and other foodborne pathogens
(2017) Journal of Food Protection, 80 (1), pp. 15-24.
ABSTRACT: Detection of Salmonella enterica in foods typically involves microbiological enrichment, molecular-based assay, and subsequent isolation and identification of a pure culture. This is ideally followed by strain typing, which provides information critical to the investigation of outbreaks and the attribution of their sources. Pulsed-field gel electrophoresis is the ''gold standard'' for S. enterica strain typing, but its limitations have encouraged the search for alternative methods, including whole genome sequencing. Both methods typically require a pure culture, which adds to the cost and turnaround time. A more rapid and costeffective method with sufficient discriminatory power would benefit food industries, regulatory agencies, and public health laboratories. To address this need, a novel enrichment, amplification, and sequence-based typing (EAST) approach was developed involving (i) overnight enrichment and total DNA preparation, (ii) amplification of polymorphic tandem repeat- containing loci with electrophoretic detection, and (iii) DNA sequencing and bioinformatic analysis to identify related strains. EAST requires 3 days or less and provides a strain resolution that exceeds serotyping and is comparable to pulsed-field gel electrophoresis. Evaluation with spiked ground Turkey demonstrated its sensitivity (with a starting inoculum of ≤1 CFU/g) and specificity (with unique or nearly unique alleles relative to databases of >1,000 strains). In tests with unspiked retail chicken parts, 3 of 11 samples yielded S. enterica-specific PCR products. Sequence analysis of three distinct typing targets (SeMT1, SeCRISPR1, and SeCRISPR2) revealed consistent similarities to specific serotype Schwarzengrund, Montevideo, and Typhimurium strains. EAST provides a time-saving and cost-effective approach for detecting and typing foodborne S. enterica, and postenrichment steps can be commercially outsourced to facilitate its implementation. Initial studies with Listeria monocytogenes and Shiga toxigenic Escherichia coli suggest that EAST can be extended to these foodborne pathogens. ISSN: 0362028X

Zadernowska, A., Chajęcka-Wierzchowska, W.
Prevalence, biofilm formation and virulence markers of Salmonella sp. and Yersinia enterocolitica in food of animal origin in Poland
(2017) LWT - Food Science and Technology, 75, pp. 552-556.
ABSTRACT: Examination of 330 food samples (meat, white raw sausage, smoked meat and cheeses) was conducted and the Salmonella spp. and Y. enterocolitica prevalence were determined. For isolated strains of Salmonella spp., it was determined whether they were of S. Typhi, S. Typhimurium or S. Enteritidis serotypes. Isolated Y. enterocolitica strains were biotyped. Moreover, the strains' ability to form a biofilm and presence of virulence factors was determined. Salmonella spp. were found in 16 (5.5%) samples, whereas Yersinia enterocolitica in 7 (2.1%) samples. Serotyping resulted in classifying 4 strains as S. Typhimurium, 2 as S. Enteritidis and none as S. Typhi. Ten strains were not classified as any of the determined serotypes. The gene invA was found in all Salmonella strains and spvC was found in 3 (18.7%) strains. All of the Y. enterocolitica were classified as biotype 1A, none of the strains had the genes ail or ystA, and the ystB gene was found in five strains. None of the 16 strains of Salmonella spp. and the 7 strains of Y. enterocolitica showed any strong ability to form a biofilm, while 7 strains of Salmonella spp. and 3 strains of Y. enterocolitica showed a moderate ability to form a biofilm. ISSN: 00236438

Weaver, T., Valcanis, M., Mercoulia, K., Sait, M., Tuke, J., Kiermeier, A., Hogg, G., Pointon, A., Hamilton, D., Billman-Jacobe, H.
Longitudinal study of Salmonella 1,4,[5],12:i:- shedding in five Australian pig herds
(2017) Preventive Veterinary Medicine, 136, pp. 19-28.
ABSTRACT: The shedding patterns of Salmonella spp. and MLVA profiles of Salmonella enterica subspecies enterica (I) serotype 1,4,[5],12:i:- were monitored in a 12-month longitudinal observational study of five pig herds to inform management; provide indications of potential hazard load at slaughter; and assist evaluation of MLVA for use by animal and public health practitioners. Twenty pooled faecal samples, stratified by age

group, were collected quarterly. When Salmonella was cultured, multiple colonies were characterized by serotyping and where S. Typhimurium-like serovars were confirmed, isolates were further characterized by phage typing and multiple locus variable number tandem repeat analysis (MLVA). Salmonella was detected in 43% of samples. Salmonella 1,4,[5],12:i- was one of several serovars that persisted within the herds and was found among colonies from each production stage. Virtually all Salmonella 1,4,[5],12:i:- isolates were phage type 193, but exhibited 12 different, closely-related MLVA profiles. Salmonella 1,4,[5],12:i:- diversity within herds was low and MLVA profiles were stable indicating colonization throughout the herds and suggesting each farm had an endemic strain. High prevalence of S. 1,4,[5],12:i:- specific shedding among terminal animals indicated high hazard load at slaughter, suggesting that primary production may be an important pathway of S. 1,4,[5],12:i:- into the human food chain, this has implications for on-farm management and the application and targeting control measures and further evidence of the need for effective process control procedures to be in place during slaughter and in pork boning rooms. These findings have implications for animal health and food safety risk mitigation and risk management. ISSN: 01675877

Lebel, P., Letellier, A., Longpré, J., Laplante, B., Yergeau, E., Fravalo, P.
Feed presentation options in Swine early fattening mitigates Salmonella shedding and specifically modulates the faecal microbiota
(2017) Journal of Applied Microbiology, 122 (1), pp. 30-39.
ABSTRACT: Aims: The object of this study was to determine the impact of only modifying the processing and/or particle size of pig feed on Salmonella shedding and faecal microbiota. Methods and Results: Pigs were fed a diet that varied only by their processing (pellet or mash) and their particle size (500, 750 or 1250 μm) for 21 days. Salmonella detection in faeces and seroconversion were determined. Faecal microbiota was assessed by Ion Torrent amplicon sequencing and real-time PCR. Significantly fewer pigs ($P < 0{\cdot}05$) shed Salmonella in the groups fed mash 500 (1) and mash or pellet 1250 (5 each) compared to the commercial reference group (15) fed pellet 500. Both mash processing and large particle size raised the proportion and number of bacteria from the Bifidobacterium genus in the faecal microbiota of the pigs. Thirteen other taxa significantly varied ($P < 0{\cdot}0005$) with feed presentation. Conclusion: Mash processing and/or large particle size in pig feed reduces Salmonella shedding prevalence and promotes beneficial populations of digestive microbiota. Significance and Impact of the Study: This study is the first to demonstrate a difference in Salmonella shedding through only modifying pig feed presentation and is the first to extensively describe modifications of faecal microbiota. ISSN: 13645072

Inns, T., Ashton, P.M., Herrera-Leon, S., Lighthill, J., Foulkes, S., Jombart, T., Rehman, Y., Fox, A., Dallman, T., De Pinna, E., Browning, L., Coia, J.E., Edeghere, O., Vivancos, R.
Prospective use of whole genome sequencing (WGS) detected a multi-country outbreak of Salmonella Enteritidis
(2017) Epidemiology and Infection, 145 (2), pp. 289-298.
ABSTRACT: Since April 2015, whole genome sequencing (WGS) has been the routine test for Salmonella identification, surveillance and outbreak investigation at the national reference laboratory in England and Wales. In May 2015, an outbreak of Salmonella Enteritidis cases was detected using WGS data and investigated. UK cases were interviewed to obtain a food history and links between suppliers were mapped to produce a food chain network for chicken eggs. The association between the food chain network and the phylogeny was explored using a network comparison approach. Food and environmental samples were taken from premises linked to cases and tested for Salmonella. Within the outbreak single nucleotide polymorphism defined cluster, 136 cases were identified in the UK and 18 in Spain. One isolate from a food containing chicken eggs was within the outbreak cluster. There was a significant association between the chicken egg food chain of UK cases and phylogeny of outbreak isolates. This is the first published Salmonella outbreak to be prospectively detected using WGS. This outbreak in the UK was linked with contemporaneous cases in Spain by WGS. We conclude that UK and Spanish cases were exposed to a common source of Salmonella-contaminated chicken eggs. ISSN: 09502688

Salvat, G., Guyot, M., Protino, J.
Monitoring Salmonella, Campylobacter, Escherichia coli and Staphylococcus aureus in traditional free-range 'Label Rouge' broiler production: a 23-year survey programme
(2017) Journal of Applied Microbiology, 122 (1), pp. 248-256.

ABSTRACT: Aim: 'Label Rouge' broiler free-range carcasses have been monitored since 1991, and broiler flocks since 2010, for contamination by the main foodborne zoonotic bacteria. Methods and Results: Initially, the monitoring plan mainly focused on the surveillance of Salmonella, and on indicators of the overall microbiological quality of free-range broiler carcasses such as Staphylococcus aureus and coliforms, but was extended in 2007 to include Campylobacter enumeration on carcasses and in 2010, to Salmonella in the environment of live birds. Salmonella contamination of free-range broiler carcasses rose to a peak of 16% in 1994 but less than 1% of carcasses are now regularly found to be positive. Indicators of the overall microbiological quality of carcasses are also improving. These results correlate with the low prevalence of Salmonella in free-range broiler breeding and production flocks, and with the continuous improvement of hazard analysis and critical control points in slaughterhouses, the implementation of a good manufacturing practice guide since 1997 and the application of EU regulations on Salmonella since 1998 in France. Regarding Campylobacter counts on carcasses, the situation has been improving continuously over the last few years, even if 2·5% of the carcasses are still contaminated by more than 1000 Campylobacter per g of skin. Conclusions: Although the current control system focusing on Salmonella is based on firm epidemiologic data and offers effective means of control (e.g. slaughtering of positive breeder flocks), existing information on Campylobacter makes it more difficult to formulate an effective control plan for free-range broilers, due to their particular exposure to environmental contamination. Significance and Impact of the Study: This long-term surveillance programme provided an extended view of the evolution of the contamination of free-range broilers and a direct measurement of the impact of mandatory and profession-driven interventions on the microbiological quality of carcasses. ISSN: 13645072

Gabriel, A.A., Tongco, A.M.P., Barnes, A.A., Jr.
Utility of UV-C radiation as anti-Salmonella decontamination treatment for desiccated coconut flakes
(2017) Food Control, 71, pp. 117-123.
ABSTRACT: This study established the ultraviolet-C (UV-C)-mediated reduction of a cocktail of Salmonella enterica serovars, artificially inoculated onto desiccated coconut flakes. Inoculated cells exhibited biphasic inactivation behavior, characterized by an initial, log-linear population reduction, followed by a slower log-linear population decline where sublethal injury accumulated. Decimal reduction times in the faster inactivation phase (Dfast) ranged from 0.65 to 0.82 min, equivalent to UV-C energy dose of 86.58–109.22 mJ/cm2. The Dslow values ranged from 21.19 to 24.21 min, equivalent to energy dose of 2822.51–3224.78 mJ/cm2. A total of 3-log cycles reduction in inoculated Salmonella were observed after 40 min exposure of desiccated coconut to UV-C. Further, this 40-min process resulted in changes in the Hunter L*, a* and b* color parameter values, but were not detected by a test consumer panel as evident in the non-significant difference in the color acceptability of UV-C treated and untreated coconut flakes. The UV-C process also did not affect the general acceptability of baked coconut macaroons made from UV-C treated coconut flakes. The results obtained in this work may serve as baseline information in the development of an in- or post-process integration of a UV-C radiation step against Salmonella spp. in the desiccated coconut production process flow. ISSN: 09567135

Damaso, A.F., Rushton, J.
Economic impact of a Salmonella outbreak on a Welsh dairy farm and an estimation of the breakeven point for vaccination
(2017) Veterinary Record Case Reports, 4 (2), art. no. e000359, .
ABSTRACT: Salmonellosis is a disease present in the cattle herds of England with clinical presentations such as abortion storms that can have devastating farm-level economic consequences. Using farm-specific costs and revenues, a partial budget analysis estimated the economic impact of a Salmonella outbreak. A breakeven analysis was used to estimate the point at which vaccination as a preventative measure would be economically more profitable than managing acute salmonellosis as it occurs. The analysis showed that the costs of disease were greater than the costs of controlling the disease on farm, and that vaccination would be beneficial versus a scenario of managing an outbreak every 16 or less years. Vaccination against salmonellosis is a preventative measure that incorporates high costs, but can reveal itself as an investment to prevent higher losses. Practitioners should use economic analysis tools to discuss with their clients the best options for disease management. ISSN: 20526121

van Asselt, E.D., van der Fels-Klerx, H.J., Marvin, H.J.P., van Bokhorst-van de Veen, H., Groot, M.N.

Overview of Food Safety Hazards in the European Dairy Supply Chain
(2017) Comprehensive Reviews in Food Science and Food Safety, 16 (1), pp. 59-75.
ABSTRACT: Monitoring of dairy products should preferably focus on the most relevant food safety hazards in the dairy supply chain. For this purpose, the possible presence of microbiological, chemical, and physical hazards as well as trends in the dairy supply chain that may affect their presence were assessed. A literature review was combined with available data from EFSA, RASFF, and the Dutch monitoring program on chemical hazards as well as expert information. This study revealed that microbiological hazards are encountered more frequently in dairy products than chemical and physical hazards. Listeria monocytogenes, Staphylococcus aureus, Salmonella, and human pathogenic Escherichia coli were identified as the most important microbiological hazards in dairy products. Soft and semisoft cheeses are most frequently associated with L. monocytogenes and S. aureus enterotoxins, whereas raw milk is most frequently associated with human pathogenic E. coli and Campylobacter spp., Cronobacter spp., and Salmonella spp. are the microbiological hazards of most concern in powdered infant formula. Based on literature, monitoring, and RASFF data, the most relevant chemical hazards in dairy products are aflatoxin M1, dioxins, and dioxin-like compounds and residues of veterinary drugs. Chemical hazards primarily occur at the dairy farm and may accumulate during further processing. The most relevant physical hazards are metal, glass, and plastic particles introduced during processing. Analysis of trends in the near future revealed that increased milk production is seen as most relevant in relation to food safety. Other trends affecting food safety are climate change and changes at the farm level, which aim to improve animal welfare and environmental sustainability. ISSN: 15414337

Bover-Cid, S., Belletti, N., Aymerich, T., Garriga, M.
Modelling the impact of water activity and fat content of dry-cured ham on the reduction of Salmonella enterica by high pressure processing
(2017) Meat Science, 123, pp. 120-125.
ABSTRACT: This work aimed to quantify the impact of aw and fat content of dry-cured ham on the Log reduction of Salmonella enterica by high pressure (HP). Dry-cured ham with adjusted aw (0.86–0.96) and fat content (10–50%) was inoculated with S. enterica and pressurised (347–852 MPa, 5 min/15 °C), following a Central Composite Design. Polynomial regression indicated a significant impact of pressure and aw on S. enterica HP-lethality. By lowering aw a clear piezoprotection was observed. At low aw (0.88) the S. enterica reduction was little affected by increasing pressure (e.g. 2.3 to 3.2 Logs at 450 to 750 MPa, respectively). At the highest aw the estimated inactivation ranged from 3.3 to 8.9 Logs at 450 to 750 MPa, respectively. No significant piezoprotective effect on S. enterica was recorded by the fat content. The relevance of food characteristics on the HP-lethality of S. enterica indicate the need to validate the HP effectiveness on the specific product. ISSN: 03091740

Chai, S.J., Cole, D., Nisler, A., Mahon, B.E.
Poultry: The most common food in outbreaks with known pathogens, United States, 1998-2012
(2017) Epidemiology and Infection, 145 (2), pp. 316-325.
ABSTRACT: As poultry consumption continues to increase worldwide, and as the United States accounts for about one-third of all poultry exports globally, understanding factors leading to poultry-associated foodborne outbreaks in the United States has important implications for food safety. We analysed outbreaks reported to the United States' Foodborne Disease Outbreak Surveillance System from 1998 to 2012 in which the implicated food or ingredient could be assigned to one food category. Of 1114 outbreaks, poultry was associated with 279 (25%), accounting for the highest number of outbreaks, illnesses, and hospitalizations, and the second highest number of deaths. Of the 149 poultry-associated outbreaks caused by a confirmed pathogen, Salmonella enterica (43%) and Clostridium perfringens (26%) were the most common pathogens. Restaurants were the most commonly reported location of food preparation (37% of poultry-associated outbreaks), followed by private homes (25%), and catering facilities (13%). The most commonly reported factors contributing to poultry-associated outbreaks were food-handling errors (64%) and inadequate cooking (53%). Effective measures to reduce poultry contamination, promote safe food-handling practices, and ensure food handlers do not work while ill could reduce poultry-associated outbreaks and illnesses. ISSN: 09502688

Oyinlola, L.A., Obadina, A.O., Omemu, A.M., Oyewole, O.B.
Prevention of microbial hazard on fresh-cut lettuce through adoption of food safety and hygienic practices by lettuce farmers
(2017) Food Science and Nutrition, 5 (1), pp. 67-75.

ABSTRACT: Lettuce is consumed raw in salads and is susceptible to microbial contamination through environment, agricultural practices, and its morphology, thus, a potential vehicle for food-borne illness. This study investigated the effect of adoption of food safety and hygienic practices by lettuce farmers on the microbial safety of field sourced lettuce in Lagos State, Nigeria. Ten structured questionnaires were administered randomly to 10 lettuce farmers to assess food safety and hygienic practices (FSH). Two farmers who practice FSH and two farmers who do not practice NFSH were finally used for this study. Samples of ready-to-harvest lettuce, manure applied, and irrigation water were obtained for a period of five months (August – December 2013) and analyzed for total plate count (TPC), total coliform count (TCC), Escherichia coli, Listeria spp., Salmonella spp., and Shigella spp. counts. Result of microbial analyses of lettuce samples was compared with international microbiological specification for ready-to-eat foods. Results showed that the range of TPC on lettuce was 6.00 to 8.11 LogCFU/g from FSH farms and TPC of lettuce samples from NFSH farms ranged from 6.66 to 13.64 LogCFU/g. 1.49 to 4.85LogCFU/g were TCC ranges from lettuce samples obtained from FSH farms while NFSH farms had TCC ranging between 3.95 and 10.86 LogCFU/g, respectively. The range of isolated pathogen count on lettuce from FSH and NFSH farms exceeded the international safety standard; there was a significant difference in the microbial count of lettuce from FSH farms and NFSH farms. This study concludes that the lettuce samples obtained did not pass the international microbial safety standards. FSH compliance is a major determinant of the microbial safety of lettuce. Hence, the institution of FSH on farm to improve microbial safety of lettuce produced for public consumption is emphasized.
ISSN: 20487177

Vignaud, M.-L., Cherchame, E., Marault, M., Chaing, E., Le Hello, S., Michel, V., Jourdan-Da Silva, N., Lailler, R., Brisabois, A., Cadel-Six, S.
MLVA for Salmonella enterica subsp. enterica serovar Dublin: Development of a method suitable for inter-laboratory surveillance and application in the context of a raw milk cheese outbreak in France in 2012
(2017) Frontiers in Microbiology, 8 (FEB), art. no. 295, .
ABSTRACT: Salmonella enterica subspecies enterica serovar Dublin (S. Dublin) figures among the most frequently isolated Salmonella strains in humans in France. This serovar may affect production and animal health mainly in cattle herds with corresponding high economic losses. Given that the current gold standard method, pulsed-field gel electrophoresis (PFGE), provides insufficient discrimination for epidemiological investigations, we propose a standard operating procedure in this study for multiple-locus variable number tandem repeat analysis (MLVA) of S. Dublin, suitable for inter-laboratory surveillance. An in silico analysis on the genome of S. Dublin strains CT_02021853 was performed to identify appropriate microsatellite regions. Of 21 VNTR loci screened, six were selected and 401 epidemiologically unrelated and related strains, isolated from humans, food and animals were analyzed to assess performance criteria such as typeability, discriminatory power and epidemiological concordance. The MLVA scheme developed was applied to an outbreak involving Saint-Nectaire cheese for which investigations were conducted in France in 2012, making it possible to discriminate between epidemiologically related strains and sporadic case strains, while PFGE assigned only a single profile. The six loci selected were sequenced on a large set of strains to determine the sequence of the repeated units and flanking regions, and their stability was evaluated in vivo through the analysis of the strains investigated from humans, food and the farm environment during the outbreak. The six VNTR selected were found to be stable and the discriminatory power of the MLVA method developed was calculated to be 0.954 compared with that for PFGE, which was only 0.625. Twenty-four reference strains were selected from the 401 examined strains in order to represent most of the allele diversity observed for each locus. This reference set can be used to harmonize MLVA results and allow data exchange between laboratories. This original MLVA protocol could be used easily and routinely for monitoring of serovar Dublin isolates and for conducting outbreak investigations. ISSN: 1664302X

Wierup, M., Wahlström, H., Lahti, E., Eriksson, H., Jansson, D.S., Odelros, A., Ernholm, L.
Occurrence of Salmonella spp.: A comparison between indoor and outdoor housing of broilers and laying hens
(2017) Acta Veterinaria Scandinavica, 59 (1), art. no. 13, .
ABSTRACT: Background: Outdoor production of poultry is rapidly increasing, which could be associated with increased risks for exposure to different environmental sources of Salmonella. We report a comparison on the occurrence of Salmonella during 2007-2015 in broilers and laying hens in outdoor and indoor production subjected to the same

requirements for the prevention and control of Salmonella as applied in Sweden. Results: Our results give no indication that, during the period studied, the exposure to Salmonella in outdoor poultry production was higher than in the indoor production. The annual incidence of Salmonella infected flocks in outdoor production remained at a very low and at a similar level as for indoor production. For laying hens the annual proportion of birds in test positive flocks ranged from 0 to 1.3% for indoor production from 0 to 2.0% for outdoor production. For broilers the proportion of Salmonella infected flocks (2013-2015) was 0.16% for indoor, and 0% in outdoor production. The difference was not statistically significant and was further reduced when flocks infected due to vertical transmission or from a hatchery source were excluded. It should, however, be considered that the number of outdoor flocks included in this evaluation is very small and continuous evaluation is needed. Conclusions: New animal production systems, including those driven by consumer and welfare demands, may be associated with a higher risk for the exposure of potential pathogens to food animals and possibly also subsequent outbreaks of food borne infections. In this study no increase in the risk for exposure of flocks to Salmonella in outdoor poultry production was found. The situation may well change and the possibility of Salmonella contamination in outdoor poultry production requires continuous attention. ISSN: 0044605X

Kumar, G.D., Williams, R.C., Al Qublan, H.M., Sriranganathan, N., Boyer, R.R., Eifert, J.D.
Airborne soil particulates as vehicles for Salmonella contamination of tomatoes
(2017) International Journal of Food Microbiology, 243, pp. 90-95.
ABSTRACT: The presence of dust is ubiquitous in the produce growing environment and its deposition on edible crops could occur. The potential of wind-distributed soil particulate to serve as a vehicle for S. Newport transfer to tomato blossoms and consequently, to fruits, was explored. Blossoms were challenged with previously autoclaved soil containing S. Newport (9.39 log CFU/g) by brushing and airborne transfer. One hundred percent of blossoms brushed with S. Newport-contaminated soil tested positive for presence of the pathogen one week after contact ($P < 0.0001$). Compressed air was used to simulate wind currents and direct soil particulates towards blossoms. Airborne soil particulates resulted in contamination of 29% of the blossoms with S. Newport one week after contact. Biophotonic imaging of blossoms post-contact with bioluminescent S. Newport-contaminated airborne soil particulates revealed transfer of the pathogen on petal, stamen and pedicel structures. Both fruits and calyxes that developed from blossoms contaminated with airborne soil particulates were positive for presence of S. Newport in both fruit (66.6%) and calyx (77.7%). Presence of S. Newport in surface-sterilized fruit and calyx tissue tested indicated internalization of the pathogen. These results show that airborne soil particulates could serve as a vehicle for Salmonella. Hence, Salmonella contaminated dust and soil particulate dispersion could contribute to pathogen contamination of fruit, indicating an omnipresent yet relatively unexplored contamination route. ISSN: 01681605

Wang, X., Devlieghere, F., Geeraerd, A., Uyttendaele, M.
Thermal inactivation and sublethal injury kinetics of Salmonella enterica and Listeria monocytogenes in broth versus agar surface
(2017) International Journal of Food Microbiology, 243, pp. 70-77.
ABSTRACT: The objective of the present study was to compare the thermal inactivation and sublethal injury kinetics of Salmonella enterica and Listeria monocytogenes in broth (suspended cells) and on solid surface (agar-seeded cells). A 3-strain cocktail of S. enterica or L. monocytogenes inoculated in broth or on agar was subjected to heating in a water bath at various set temperatures (55.0, 57.5 and 60.0 °C for S. enterica and 60.0, 62.5 and 65 °C for L. monocytogenes). The occurrence of sublethally injured cells was determined by comparing enumerations on nonselective (TSAYE) and selective (XLD or ALOA) media. Results showed that the inactivation curves obtained from selective media were log-linear, and significant shoulders ($p < 0.05$) were observed on some of the inactivation curves from TSAYE media. The D-values derived from the total population were higher than those from the uninjured cells. Generally, cells on agar surface exhibited higher heat resistance than those in broth. For S. enterica, cell injury increased with the exposure time, no difference was observed when treated at temperatures from 55.0 to 60.0 °C, while for L. monocytogenes, cell injury increased significantly with heating time and treatment temperature (from 60.0 to 65 °C). Moreover, the degree of sublethal injury affected by thermal treatment in broth or on agar surface depended upon the target microorganism. Higher proportions of injured S. enterica cells were observed for treatment in broth than on agar surface, while the opposite was found for L. monocytogenes. The provided information may be used to assess the efficacy of thermal treatment processes on surfaces for inactivation of S. enterica and L. monocytogenes, and it provides insight

into the sublethally injured survival state of S. enterica and L. monocytogenes treated in liquid or on solid food. ISSN: 01681605

Wang, F., Horikawa, S., Hu, J., Wikle, H.C., III, Chen, I.-H., Du, S., Liu, Y., Chin, B.A.
Detection of Salmonella typhimurium on spinach using phage-based magnetoelastic biosensors
(2017) Sensors (Switzerland), 17 (2), art. no. 386, .
ABSTRACT: Phage-based magnetoelastic (ME) biosensors have been studied as an in-situ, real-time, wireless, direct detection method of foodborne pathogens in recent years. This paper investigates an ME biosensor method for the detection of Salmonella Typhimurium on fresh spinach leaves. A procedure to obtain a concentrated suspension of Salmonella from contaminated spinach leaves is described that is based on methods outlined in the U.S. FDA Bacteriological Analytical Manual for the detection of Salmonella on leafy green vegetables. The effects of an alternative pre-enrichment broth (LB broth vs. lactose broth), incubation time on the detection performance and negative control were investigated. In addition, different blocking agents (BSA, Casein, and Superblock) were evaluated to minimize the effect of nonspecific binding. None of the blocking agents was found to be superior to the others, or even better than none. Unblocked ME biosensors were placed directly in a concentrated suspension and allowed to bind with Salmonella cells for 30 min before measuring the resonant frequency using a surface-scanning coil detector. It was found that 7 h incubation at 37◦C in LB broth was necessary to detect an initial spike of 100 cfu/25 g S. Typhimurium on spinach leaves with a confidence level of difference greater than 95% (p < 0.05). Thus, the ME biosensor method, on both partly and fully detection, was demonstrated to be a robust and competitive method for foodborne pathogens on fresh products. ISSN: 14248220

Helke, K.L., McCrackin, M.A., Galloway, A.M., Poole, A.Z., Salgado, C.D., Marriott, B.P.
Effects of antimicrobial use in agricultural animals on drug-resistant foodborne salmonellosis in humans: A systematic literature review
(2017) Critical Reviews in Food Science and Nutrition, 57 (3), pp. 472-488.
ABSTRACT: Controversy continues concerning antimicrobial use in food animals and its relationship to drug-resistant infections in humans. We systematically reviewed published literature for evidence of a relationship between antimicrobial use in agricultural animals and drug-resistant meat or dairy-borne non-typhoidal salmonellosis in humans. Based on publications from the United States (U.S.), Canada, and Denmark from January 2010 to July 2014, 858 articles received title and abstract review, 104 met study criteria for full article review with 68 retained for which data are presented. Antibiotic exposure in both cattle and humans found an increased likelihood of Salmonella colonization, whereas in chickens, animals not exposed to antibiotics (organic) were more likely to be Salmonella positive and those that had antibiotic exposure were more likely to harbor antimicrobial resistant Salmonella organisms. In swine literature, only tylosin exposure was examined and no correlation was found among exposure, Salmonella colonization, or antimicrobial resistance. No studies that identified farm antimicrobial use also traced antimicrobial-resistant Salmonella from farm to fork. ISSN: 10408398

Kim, H.B., Isaacson, R.E.
Salmonella in Swine: Microbiota Interactions
(2017) Annual Review of Animal Biosciences, 5, pp. 43-63.
ABSTRACT: For the important foodborne pathogen Salmonella enterica to cause disease or persist in pigs, it has evolved an intricate set of interactions between itself, the host, and the indigenous microflora of the host. S. enterica must evade the host's immune system and must also overcome colonization resistance mediated by the pig's indigenous microflora. The inflammatory response against S. enterica provides the bacteria with unique metabolites and is thus exploited by S. enterica for competitive advantage. During infection, changes in the composition of the indigenous microflora occur that have been associated with a breakdown in colonization resistance. Healthy pigs that are low-level shedders of S. enterica also exhibit alterations in their indigenous microflora similar to those in ill animals. Here we review the literature on the interactions that occur between swine, S. enterica, and the indigenous microflora and discuss methods to reduce or prevent colonization of pigs with S. enterica. ISSN: 21658102

Sannat, C., Patyal, A., Rawat, N., Ghosh, R.C., Jolhe, D.K., Shende, R.K., Hirpurkar, S.D., Shakya, S.
Characterization of Salmonella Gallinarum from an outbreak in Raigarh, Chhattisgarh

(2017) Veterinary World, 10 (2), pp. 144-148.
ABSTRACT: Aim: The present investigation was conducted to isolate and characterize Salmonella Gallinarum from an outbreak of fowl typhoid in layer birds. Materials and Methods: Clinically ill and dead layer birds from an outbreak were investigated. History, clinical signs, and postmortem lesions were suggestive of fowl typhoid. Postmortem samples including heart blood, intestinal contents, pieces of ovary, and liver were collected and processed immediately for bacterial culture, serotyping and antibiotic sensitivity tests. Isolates were further screened for the presence of extended spectrum beta lactamase (ESBL) (blaTEM) gene by polymerase chain reaction. Results: On the basis of cultural, staining and biochemical characteristics; three bacterial isolates were confirmed as S. Gallinarum. On serotyping, somatic antigen O: 9 and 12 with nonflagellated antigen were detected in all three isolates. Isolates were intermediate sensitive to amoxycillin, amoxyclav, gentamicin and ciprofloxacin and resistant to most of the antibiotics including chloramphenicol, ampicillin, ceftazidime, cefexime, cefepime, azithromycin, nalidixin, tetracycline, oxytetracycline, and streptomycin. Two isolates were found to harbor ESBL (blaTEM) gene. Conclusion: Beta lactamase producer S. Gallinarum was confirmed as cause of increased mortality in layer birds during present investigation. Existence of multi drug resistant Salmonella poses serious threat to poultry industry in Chhattisgarh.
ISSN: 09728988

Pesciaroli, M., Cucco, L., De Luca, S., Massacci, F.R., Maresca, C., Medici, L., Paniccià, M., Scoccia, E., Staffolani, M., Pezzotti, G., Magistrali, C.F.
Association between pigs with high caecal Salmonella loads and carcass contamination (2017) International Journal of Food Microbiology, 242, pp. 82-86.
ABSTRACT: Contaminated pork is a significant source of foodborne Salmonella infections. Pork is contaminated at the slaughterhouse; however, the mechanisms driving Salmonella contamination of carcasses are still poorly understood. The aim of this study was to investigate whether the amount of Salmonella carried by slaughtered pigs in their guts has an influence on carcass contamination. On that account, we tested whether the number of carcasses contaminated during a slaughter day was associated with the prevalence of highly contaminated pigs (HCP: Salmonella caecal loads ≥ 3log/g), or with the prevalence of pigs that simply carry Salmonella spp. in their guts. Three hundred and six pigs were sampled in a slaughterhouse from Central Italy. Salmonella loads in the caecum and on the carcass of each pig were estimated by the most probable number (MPN) technique. The overall prevalence of Salmonella was 34.64% and 7.19% for the caeca and carcasses, respectively. S. Derby and S. 4, (Bonardi et al., 2003),12:i:- were the most frequently isolated serovars. The prevalence of HCP was 11.44%. We found a higher number of contaminated carcasses on days of high prevalence of HCP than on days of low prevalence of HCP ($p = 0.0011$). Conversely, carcass contamination did not vary with the prevalence of pigs that simply carried Salmonella spp. in their guts ($p = 0.7970$). Therefore, the prevalence of HCP, but not the prevalence of pigs carrying Salmonella spp., was related to carcass contamination. Taken together, these findings suggest that reduction of Salmonella loads in the guts of slaughtered pigs would result in fewer contaminated carcasses, and consequently, help to minimise the risk of human infection due to the consumption of contaminated pork. ISSN: 01681605

Gong, C., Jiang, X.
Characterizing Salmonella contamination in two rendering processing plants (2017) Journal of Food Protection, 80 (2), pp. 265-270.
ABSTRACT: A microbiological investigation on Salmonella contamination was conducted in two U.S. rendering plants to investigate the potential cross-contamination of Salmonella in the rendering processing environment. Sampling locations were predetermined at the areas where Salmonella contamination may potentially occur, including raw materials receiving, crax (rendered materials before grinding process) grinding, and finished meal loading-out areas. Salmonella was either enumerated directly on xylose lysine Tergitol 4 agar plates or enriched in Rappaport-Vassiliadis and tetrathionate broths. The presumptive Salmonella isolates were confirmed using CHROMagar plating and latex agglutination testing and then characterized using pulsed-field gel electrophoresis, serotyping, and biofilm-forming determination. Among 108 samples analyzed, 79 (73%) samples were Salmonella positive after enrichment. Selected Salmonella isolates ($n = 65$) were assigned to 31 unique pulsed-field gel electrophoresis patterns, with 16 Salmonella serotypes, including Typhimurium and Mbandaka, identified as predominant serotypes and 10 Salmonella strains determined as strong biofilm formers. Our results indicated that the raw materials receiving area was the primary source of Salmonella and that the surfaces surrounding crax grinding and finished meal loading-out areas harbor Salmonella in biofilms that may recontaminate the finished meals. The same Salmonella serotypes found

in both raw materials receiving and the finished meal loading-out areas suggested a potential of cross-contamination between different areas in the rendering processing environment. ISSN: 0362028X

Ogunremi, D., Nadin-Davis, S., Dupras, A.A., Márquez, I.G., Omidi, K., Pope, L., Devenish, J., Burke, T., Allain, R., Leclair, D.
Evaluation of a multiplex pcr assay for the identification of Salmonella serovars enteritidis and typhimurium using retail and abattoir samples
(2017) Journal of Food Protection, 80 (2), pp. 295-301.
ABSTRACT: A multiplex PCR was developed to identify the two most common serovars of Salmonella causing foodborne illness in Canada, namely, serovars Enteritidis and Typhimurium. The PCR was designed to amplify DNA fragments from four Salmonella genes, namely, invA gene (211-bp fragment), iroB gene (309-bp fragment), Typhimurium STM 4497 (523-bp fragment), and Enteritidis SE147228 (612-bp fragment). In addition, a 1,026-bp ribosomal DNA (rDNA) fragment universally present in bacterial species was included in the assay as an internal control fragment. The detection rate of the PCR was 100% among Salmonella Enteritidis (n = 92) and Salmonella Typhimurium (n = 33) isolates. All tested Salmonella isolates (n = 194) were successfully identified based on the amplification of at least one Salmonella-specific DNA fragment. None of the four Salmonella DNA amplicons were detected in any of the non-Salmonella isolates (n = 126), indicating an exclusivity rate of 100%. When applied to crude extracts of 2,001 field isolates of Salmonella obtained during the course of a national microbiological baseline study in broiler chickens and chicken products sampled from abattoir and retail outlets, 163 isolates, or 8.1%, tested positive for Salmonella Enteritidis and another 80 isolates, or 4.0%, tested as Salmonella Typhimurium. All isolates identified by serological testing as Salmonella Enteritidis in the microbiological study were also identified by using the multiplex PCR. The new test can be used to identify or confirm pure isolates of the two serovars and is also amenable for integration into existing culture procedures for accurate detection of Salmonella colonies. ISSN: 0362028X

Voss-Rech, D., Potter, L., Vaz, C.S.L., Pereira, D.I.B., Sangioni, L.A., Vargas, Á.C., De Avila Botton, S.
Antimicrobial resistance in nontyphoidal salmonella isolated from human and poultry-related samples in Brazil: 20-year meta-analysis
(2017) Foodborne Pathogens and Disease, 14 (2), pp. 116-124.
ABSTRACT: Nontyphoidal Salmonella are one of the leading causes of foodborne diseases in the world. As poultry products are recognized as main sources of human salmonellosis, nontyphoidal Salmonella control has become a global issue for the poultry industry. The increasing antimicrobial resistance in poultry-related nontyphoidal Salmonella serovars is a global matter of concern. By monitoring the evolution of antimicrobial resistance, alternative treatments can be identified and possible restrictions in the treatment of systemic human salmonellosis foreseen. A meta-analysis was conducted to assess the profile and temporal evolution of the antimicrobial resistance of nontyphoidal Salmonella of poultry and human origin in Brazil, isolated in the period from 1995 to 2014. Four databases were researched; twenty-nine articles met the eligibility criteria and were included in the meta-analysis. In the nontyphoidal isolates of poultry origin, the highest levels of antimicrobial resistance were verified for sulfonamides (44.3%), nalidixic acid (42.5%), and tetracycline (35.5%). In the human-origin isolates, the resistance occurred mainly for sulfonamides (46.4%), tetracycline (36.9%), and ampicillin (23.6%). Twenty-two articles described results of antimicrobial resistance specifically for Salmonella Enteritidis, also enabling the individual meta-analysis of this serovar. For most antimicrobials, the resistance levels of Salmonella Enteritidis were lower than those found when considering all the nontyphoidal serovars. In the poultry-origin isolates, a quadratic temporal distribution was observed, with reduced resistance to streptomycin in Salmonella Enteritidis and in all nontyphoidal serovars, and a linear increase of resistance to nalidixic acid in Salmonella Enteritidis. In the human-origin isolates, a linear increase was identified in the resistance to nalidixic acid in Salmonella Enteritidis and in all the nontyphoidal isolates, and to gentamicin in Salmonella Enteritidis. Continuous monitoring of the development and spread of antimicrobial resistance could support the measurement of the consequences on poultry and human health. ISSN: 15353141

Iwamoto, M., Reynolds, J., Karp, B.E., Tate, H., Fedorka-Cray, P.J., Plumblee, J.R., Hoekstra, R.M., Whichard, J.M., Mahon, B.E.
Ceftriaxone-resistant nontyphoidal salmonella from humans, retail meats, and food animals in the United States, 1996-2013
(2017) Foodborne Pathogens and Disease, 14 (2), pp. 74-83.

ABSTRACT: Background: Ceftriaxone resistance in Salmonella is a serious public health threat. Ceftriaxone is commonly used to treat severe Salmonella infections, especially in children. Identifying the sources and drivers of ceftriaxone resistance among nontyphoidal Salmonella is crucial. Materials and Methods: The National Antimicrobial Resistance Monitoring System (NARMS) tracks antimicrobial resistance in foodborne and other enteric bacteria from humans, retail meats, and food animals. We examined NARMS data reported during 1996-2013 to characterize ceftriaxone-resistant Salmonella infections in humans. We used Spearman rank correlation to examine the relationships between the annual percentage of ceftriaxone resistance among Salmonella isolates from humans with isolates from retail meats and food animals. Results: A total of 978 (2.9%) of 34,100 nontyphoidal Salmonella isolates from humans were resistant to ceftriaxone. Many (40%) ceftriaxone-resistant isolates were from children younger than 18 years. Most ceftriaxone-resistant isolates were one of three serotypes: Newport (40%), Typhimurium (26%), or Heidelberg (12%). All were resistant to other antimicrobials, and resistance varied by serotype. We found statistically significant correlations in ceftriaxone resistance between human and ground beef Newport isolates (r = 0.83), between human and cattle Typhimurium isolates (r = 0.57), between human and chicken Heidelberg isolates (r = 0.65), and between human and turkey Heidelberg isolates (r = 0.67). Conclusions: Ceftriaxone resistance among Salmonella Newport, Typhimurium, and Heidelberg isolates from humans strongly correlates with ceftriaxone resistance in isolates from ground beef, cattle, and poultry, respectively. These findings support other lines of evidence that food animals are important reservoirs of ceftriaxone-resistant Salmonella that cause human illness in the United States. ISSN: 15353141

Morishige, Y., Koike, A., Tamura-Ueyama, A., Amano, F.
Induction of viable but nonculturable Salmonella in exponentially grown cells by exposure to a low-humidity environment and their resuscitation by catalase
(2017) Journal of Food Protection, 80 (2), pp. 288-294.
ABSTRACT: Salmonella is a major cause of foodborne disease that sometimes occurs in massive outbreaks around the world. This pathogen is tolerant of low-humidity conditions. We previously described a method for induction of viable but nonculturable (VBNC) Salmonella enterica serovar Enteritidis by treatment with hydrogen peroxide (H2O2) and subsequent resuscitation with 0.3 mM sodium pyruvate. Here, we report a new method for the induction of the VBNC state in Salmonella Enteritidis cells, one involving dehydration. Exposure of Salmonella Enteritidis cells to dehydration stress under poor nutritional conditions (0.9% [wt/vol] NaCl) and 10 to 20% relative humidity at room temperature decreased the presence of culturable population to 0.0067%, but respiratory and glucose uptake active populations were maintained at 0.46 and 1.12%, respectively, meaning that approximately 1% may have entered the VBNC state. Furthermore, these VBNC cells could be resuscitated to acquire culturability by incubation with catalase in M9 minimal medium without glucose in a manner dependent on the dose of catalase but not sodium pyruvate. These results suggest that a low-humidity environment could cause Salmonella Enteritidis cells to enter the VBNC state and the cells could then be resuscitated for growth by treatment with catalase, suggesting a potential risk of Salmonella Enteritidis to survive in low water activity foods in the VBNC state and to start regrowth for foodborne illness. ISSN: 0362028X

Kim, H.-S., Choi, D., Kang, I.-B., Kim, D.-H., Yim, J.-H., Kim, Y.-J., Chon, J.-W., Oh, D.-H., Seo, K.-H.
A single-step enrichment medium for nonchromogenic isolation of healthy and cold-injured salmonella spp. from fresh vegetables
(2017) Foodborne Pathogens and Disease, 14 (2), pp. 84-88.
ABSTRACT: Culture-based detection of nontyphoidal Salmonella spp. in foods requires at least four working days; therefore, new detection methods that shorten the test time are needed. In this study, we developed a novel single-step Salmonella enrichment broth, SSE-1, and compared its detection capability with that of commercial single-step ONE broth-Salmonella (OBS) medium and a conventional two-step enrichment method using buffered peptone water and Rappaport-Vassiliadis soy broth (BPW-RVS). Minimally processed lettuce samples were artificially inoculated with low levels of healthy and cold-injured Salmonella Enteritidis (10 0 or 10 1 colony-forming unit/25 g), incubated in OBS, BPW-RVS, and SSE-1 broths, and streaked on xylose lysine deoxycholate (XLD) agar. Salmonella recoverability was significantly higher in BPW-RVS (79.2%) and SSE-1 (83.3%) compared to OBS (39.3%) ($p < 0.05$). Our data suggest that the SSE-1 single-step enrichment broth could completely replace two-step enrichment with reduced enrichment time from 48 to 24 h, performing better than commercial single-step enrichment medium

in the conventional nonchromogenic Salmonella detection, thus saving time, labor, and cost. ISSN: 15353141

Jensen, D.A., Danyluk, M.D., Harris, L.J., Schaffner, D.W.
Quantifying bacterial cross-contamination rates between fresh-cut produce and hands (2017) Journal of Food Protection, 80 (2), pp. 213-219.
ABSTRACT: This study quantifies the cross-contamination rates between fresh-cut produce and hands using a nalidixic acid-resistant nonpathogenic Enterobacter aerogenes and cocktails of rifampin-resistant Salmonella or Escherichia coli O157:H7 strains. Volunteers performed the E. aerogenes experiments (n = 20), and one of the authors performed the Salmonella and E. coli O157:H7 experiments multiple times (n=15 and n=10, respectively). Each participant handled 25 g of fresh-cut carrots, celery, or cantaloupe in two different scenarios. In the first scenario, gloved hands were inoculated with 6 log CFU per hand of the bacteria, and in the second scenario, five 25-g pieces of fresh produce were inoculated to a concentration of 6 log CFU/25 g. The glove juice method was used to quantify the bacterial concentration on the gloved hands. About 30% of E. aerogenes on gloved hands was transferred to the carrots and celery, and 18% of E. aerogenes on gloved hands was transferred to the cantaloupe. When carrots or cantaloupe was inoculated with E. aerogenes, 1% was transferred to gloved hands; from inoculated celery, about 0.3% of E. aerogenes was transferred to gloved hands. There was not a significant difference between E. aerogenes and Salmonella cross-contamination rates (P > 0.05). When gloved hands were contaminated with E. coli O157:H7, about 30% was transferred to carrots, about 10% to celery, and about 3% to cantaloupe. When carrots and celery were inoculated with E. coli O157:H7, about 1% was transferred to gloved hands, but from inoculated cantaloupe only about 0.3% was transferred. Direction of transfer (to versus from produce), difference in type of produce, and differences among the bacterial species all had significant effects on the transfer rate. Understanding transfer rates to and from fresh-cut produce will allow for better risk assessment and management of microbial food safety risk related to fresh-cut produce. ISSN: 0362028X

Zappelini, L., Martone-Rocha, S., Dropa, M., Matté, M.H., Tiba, M.R., Breternitz, B.S., Razzolini, M.T.P.
Effective characterization of Salmonella Enteritidis by most probable number (MPN) followed by multiplex polymerase chain reaction (PCR) methods
(2017) Environmental Science and Pollution Research, 24 (5), pp. 4828-4834.
ABSTRACT: Nontyphoidal Salmonella (NTS) is a relevant pathogen involved in gastroenteritis outbreaks worldwide. In this study, we determined the capacity to combine the most probable number (MPN) and multiplex polymerase chain reaction (PCR) methods to characterize the most important Salmonella serotypes in raw sewage. A total of 499 isolates were recovered from 27 raw sewage samples and screened using two previously described multiplex PCR methods. From those, 123 isolates were selected based on PCR banding pattern—identical or similar to Salmonella Enteritidis and Salmonella Typhimurium—and submitted to conventional serotyping. Results showed that both PCR assays correctly serotyped Salmonella Enteritidis, however, they presented ambiguous results for Salmonella Typhimurium identification. These data highlight that MPN and multiplex PCR can be useful methods to describe microbial quality in raw sewage and suggest two new PCR patterns for Salmonella Enteritidis identification. ISSN: 09441344

Kheiri, R., Ranjbar, R., Memariani, M., Akhtari, L.
Multiplex PCR for detection of water-borne bacteria
(2017) Water Science and Technology: Water Supply, 17 (1), pp. 169-175.
ABSTRACT: Microbial water-borne diseases still affect developing countries and are major water quality concerns throughout the world. Routine culture-based methods of identifying bacterial pathogens in water sources are laborious and time-consuming. Recently, the use of molecular techniques such as the polymerase chain reaction (PCR) has provided rapid and highly promising detection methods. In this study, we developed two multiplex PCR assays for simultaneous detection of six water-borne bacteria. Two triplex PCR protocols were developed to detect six target genes. The first protocol targets uidA (Escherichia coli), int (Shigella spp.), and gyrB (Pseudomonas aeruginosa) genes, while invA (Salmonella spp.), ompW (Vibrio cholera), and lacZ (coliforms) were amplified by the second protocol. Specificity testing was carried out for 12 reference strains. Furthermore, the applicability of the multiplex PCR assays for detection of these bacteria was investigated for 52 surface water samples. The results indicated that all primer pairs showed specificities only for their corresponding target organisms. The detection sensitivity of both multiplex PCR assays was 3 × 102 3 × 103 colony forming units. The developed assays represent simple and efficient diagnostic procedures for codetection of water-borne

bacteria and have the potential to provide earlier warnings of possible public health threats and more accurate surveillance of these organisms. ISSN: 16069749

Carter, A., Adams, M., La Ragione, R.M., Woodward, M.J.
Colonisation of poultry by Salmonella Enteritidis S1400 is reduced by combined administration of Lactobacillus salivarius 59 and Enterococcus faecium PXN-33
(2017) Veterinary Microbiology, 199, pp. 100-107.
ABSTRACT: Salmonella Enteritidis remains a significant issue within the poultry industry and one potential solution is to use probiotic bacteria to prevent Salmonella colonisation through competitive exclusion (CE). We demonstrate that combined administration of Lactobacillus salivarius 59 and Enterococcus faecium PXN33 were effective competitive excluders of Salmonella Enteritidis S1400 in poultry. Two models were developed to evaluate the efficacy of probiotic where birds received Salmonella Enteritidis S1400 by a) oral gavage and b) sentinel bird to bird transmission. A statistically significant ($p < 0.001$) 2 log reduction of Salmonella Enteritidis S1400 colonisation was observed in the ileum, caecum and colon at day 43 using combined administration of the two probiotic bacteria. However, no Salmonella Enteritidis S1400 colonisation reduction was observed when either probiotic was administered individually. In the sentinel bird model the combined probiotic administered at days 12 and 20 was more effective than one-off or double administrations at age 1 and 12 days. In vitro cell free culture supernatant studies suggest the mechanism of Salmonella Enteritidis S1400 inhibition was due to a reduction in pH by the probiotic bacteria. Our current study provides further evidence that probiotics can significantly reduce pathogenic bacterial colonisation in poultry and that mixed preparation of probiotics provide superior performance when compared to individual bacterial preparations. ISSN: 03781135

Mathole, M.A., Muchadeyi, F.C., Mdladla, K., Malatji, D.P., Dzomba, E.F., Madoroba, E.
Presence, distribution, serotypes and antimicrobial resistance profiles of Salmonella among pigs, chickens and goats in South Africa
(2017) Food Control, 72, pp. 219-224.
ABSTRACT: Salmonellosis is an infectious zoonotic disease of socio-economic importance worldwide. Food animals with subclinical infection as well as farm effluents are usually the sources of contaminated meat, eggs and milk, which cause diarrhoea and systemic infections in humans. The indiscriminate use of antibiotics to curb salmonellosis in both animals and humans has contributed to the emergence and spread of drug-resistant bacteria among both pathogenic and commensal organisms. The aim of the study was therefore to determine the presence, serovar distribution and antimicrobial resistance profiles of Salmonella isolated from domestic livestock species in South Africa. For this purpose,1069 rectal and cloacal swabs were collected from pigs (n = 322), chickens (n = 286) and goats (n = 461) from smallholder farms in Limpopo, Eastern Cape, Northern Cape, North West and KwaZulu Natal provinces of South Africa. The frequency of occurrence of Salmonella per animal species was highest in pigs (5.90%; n = 19), followed by chickens (3.15%; n = 9) and goats had the lowest proportion of 0.43% (n = 2). Nine Salmonella serovars were obtained including S. Techimani, a serovar that was not previously observed in South African animals. Six isolates were assigned to Salmonella II. Some of the Salmonella were untypable (n = 6). All Salmonella isolates were sensitive to cefotaxime, enrofloxacin, florphenicol and polymyxin B. Most of the Salmonella isolates were resistant to at least one antimicrobial (n = 20; 66.7%) and resistance was predominant towards trimethoprim (n = 11; 36.7%), followed by ampicillin (n = 5; 16.7%), oxytetracycline (n = 3; 10%), and kanamycin (n = 1; 3.3%). The results illustrate the presence of diverse and rare Salmonella serovars that were not previously isolated from animals in South Africa. The pattern of development of antibiotic resistance should be monitored and followed-up. The occurrence of elevated trimethoprim resistant Salmonella in South African food animals could lead to the emergence and distribution of drug resistant salmonellosis in human beings. ISSN: 09567135

Topalcengiz, Z., Danyluk, M.D.
Thermal inactivation responses of acid adapted and non-adapted stationary phase Shiga toxin-producing Escherichia coli (STEC), Salmonella spp. and Listeria monocytogenes in orange juice
(2017) Food Control, 72, pp. 73-82.
ABSTRACT: All published D-values for Shiga toxin-producing Escherichia coli (STEC), Salmonella, and Listeria monocytogenes in orange juice were obtained using strain cocktails. The objective of this study was to evaluate the heat resistance of individual strain of stationary phase non-adapted and acid adapted STEC, Salmonella spp., and

L. monocytogenes in orange juice. Three STEC and Salmonella isolates were grown in TSB, and three L. monocytogenes strains grown in BHI, supplemented with 1% glucose for acid adaption. Sealed microcapillary tubes with inoculated single-strength pasteurized orange juice without pulp were treated at 56, 58, and 60 °C for STEC and L. monocytogenes and at 55, 58, and 60 °C for Salmonella. Thermal tolerance was increased significantly ($P < 0.05$) for acid adapted STEC strains, however, no improvement was observed for Salmonella spp., and L. monocytogenes strains at most temperatures tested. Salmonella serotypes are less heat resistant, at all temperatures tested, than L. monocytogenes and STEC. STEC, especially strain O111, are the most heat resistant at 56 and 58 °C; L. monocytogenes strains are the most thermal tolerance at 60 °C. Combining individual results of all pathogens tested, the formula of $\log D = 8.2167 - 0.1356\ T(°C)$ was used to calculate a general process for orange juice at 71.1 °C. Using this equation, a 5-log reduction of all three pathogens in single strength orange juice requires 11 s at 71.1 °C, with a z-value of 7.1 °C. ISSN: 09567135

Wales, A.D., Davies, R.H.
Salmonella Vaccination in Pigs: A Review
(2017) Zoonoses and Public Health, 64 (1), pp. 1-13.
ABSTRACT: The control of Salmonella enterica in pig production is necessary for both public and animal health. The persistent and frequently asymptomatic nature of porcine Salmonella infection and the organism's abilities to colonize other animal species and to survive in the environment mean that effective control generally requires multiple measures. Vaccination is one such measure, and the present review considers its role and its future, drawing on studies in pigs from the 1950s to the present day. Once established in the body as an intracellular infectious agent, Salmonella can evade humoral immunity, which goes some way to explaining the often disappointing performance of inactivated Salmonella vaccines. More recent approaches, using mucosal presentation of antigens, live vaccines and adjuvants to enhance cell-mediated immunity, have met with more success. Vaccination strategies that involve stimulating both passive immunity from the dam plus active immunity in offspring appear to be most efficacious, although either approach alone can yield significant control of Salmonella. Problems that remain include relatively poor control of Salmonella serovars that are dissimilar to the vaccine antigen mix, and difficulties in measuring and predicting the performance of candidate vaccines in ways that are highly relevant to their likely use in commercial production. ISSN: 18631959

Parada, J., Carranza, A., Alvarez, J., Pichel, M., Tamiozzo, P., Busso, J., Ambrogi, A.
Spatial distribution and risk factors associated with Salmonella enterica in pigs
(2017) Epidemiology and Infection, 145 (3), pp. 568-574.
ABSTRACT: SUMMARY The importance of pork in the transmission of Salmonella spp. to humans has led to the development of control programmes worldwide. For this, knowledge on the epidemiology of the infection in the production system is fundamental to the efficacy of the regulations. Our objective was to determine the prevalence and spatial distribution of Salmonella-infected farms in the central region of Argentina, and to identify the predominant serotypes and epidemiological factors associated with an increased risk of infection. Salmonella was isolated from 22 of 52 sampled farms, for a farm prevalence of 42 3% (95% confidence interval 28 4-56 1). The most frequent serotypes isolated were S. Typhimurium and S. Derby, which have often been considered of public health concern in the region. Limited evidences of global and local clustering in the region under study were found, and the type of feed and presence of diarrhoeic pigs were significantly associated with having Salmonella shedders in the farm. This highlights the need to evaluate microbiological controls at the farm level, and demonstrates the usefulness of the spatial tools to identify areas of greatest risk when processing pork at slaughterhouse, which could contribute to increasing the food safety of pork products. ISSN: 09502688

Speranza, B., Monacis, N., Sinigaglia, M., Corbo, M.R.
Approaches to Removal and Killing of Salmonella Spp. Biofilms
(2017) Journal of Food Processing and Preservation, 41 (1), art. no. e12758, .
ABSTRACT: This study aims to assess the effectiveness of three sanitizers (an alkaline solution, an acid solution and a quaternary ammonium salt) to remove biofilm formed by Salmonella spp. on stainless steel surfaces. To evaluate the individual or combined effects of sanitizer concentration, time (5.0–25 min) and temperature (25–65C) on survival of Salmonella spp. biofilms three different Central Composite Designs (CCD) were developed. Results highlighted that treatments containing NaOH were able to eliminate more than 82–83% of sessile population, whereas the total removal of biofilm was obtained using a solution at least 800 ppm for 15 min at 45C. Peracetic acid seemed to be more effective

than NaOH assuring the total removal of biofilm at low temperature. Even the quaternary ammonium salt was able to remove completely Salmonella spp. biofilms at 60C using a solution at 12 ppm, or at higher temperature (65C), using a less concentrated solution (6 ppm). Practical Applications: Due to the wide range of contributing factors (surface type, availability of nutrients and oxygen, microbial species, etc.) biofilms are quite diverse; in essence it can be said that each biofilm is different. Even if numerous attempts have been made to find standardized systems to prevent, remove and kill biofilm cells, until now there is no unique system that is able to remove all biofilms. Thus, a study on the factors affecting the resistance of Salmonella spp. biofilms against sanitizers may be useful into the development of new sanitation strategies in food industries. ISSN: 01458892

Kilroy, S., Raspoet, R., Martel, A., Bosseler, L., Appia-Ayme, C., Thompson, A., Haesebrouck, F., Ducatelle, R., Van Immerseel, F.
Salmonella Enteritidis flagellar mutants have a colonization benefit in the chicken oviduct (2017) Comparative Immunology, Microbiology and Infectious Diseases, 50, pp. 23-28.
ABSTRACT: Egg borne Salmonella Enteritidis is still a major cause of human food poisoning. Eggs can become internally contaminated following colonization of the hen's oviduct. In this paper we aimed to analyze the role of flagella of Salmonella Enteritidis in colonization of the hen's oviduct. Using a transposon library screen we showed that mutants lacking functional flagella are significantly more efficient in colonizing the hen's oviduct in vivo. A micro-array analysis proved that transcription of a number of flagellar genes is down-regulated inside chicken oviduct cells. Flagella contain flagellin, a pathogen associated molecular pattern known to bind to Toll-like receptor 5, activating a pro-inflammatory cascade. In vitro tests using primary oviduct cells showed that flagellin is not involved in invasion. Using a ligated loop model, a diminished inflammatory reaction was seen in the oviduct resulting from injection of an aflagellated mutant compared to the wild-type. It is hypothesized that Salmonella Enteritidis downregulates flagellar gene expression in the oviduct and consequently prevents a flagellin-induced inflammatory response, thereby increasing its oviduct colonization efficiency. ISSN: 01479571

Walther, B., Tedin, K., Lübke-Becker, A.
Multidrug-resistant opportunistic pathogens challenging veterinary infection control (2017) Veterinary Microbiology, 200, pp. 71-78.
ABSTRACT: Although the problems associated with healthcare-associated infections (HAI) and the emergence of zoonotic and multidrug-resistant pathogens in companion animal (dogs, cats and horses) medicine have been well-known for decades, current progress with respect to practical implementation of infection control programs in veterinary clinics has been limited. Clinical outbreak events reported for methicillin-resistant Staphylooccus aureus (MRSA) and Staphylococcus pseudintermedius (MRSP), extended spectrum beta-lactamase (ESBL)-producing Escherichia coli and multidrug-resistant (MDR) Salmonella Serovars indicate the necessity of infection control strategies for protecting animal patients at risk as well as veterinary personnel. The close bond between humans and their companion animals provides opportunities for exchange of microorganisms, including MDR pathogens. This particular aspect of the “One Health” idea requires more representative surveillance efforts and infection control strategies with respect to animal-species specific characters. ISSN: 03781135

Yun, O., Zeng, X.-A., Brennan, C.S., Zhi-wei, L.
Temperature alters the structure of membrane lipids and pulsed electric field (PEF) resistance of Salmonella Typhimurium
(2017) International Journal of Food Science and Technology, 52 (2), pp. 424-430.
ABSTRACT: The effect of culture temperature on the conformation of membrane lipid of Salmonella Typhimurium as well as their pulsed electric field (PEF) resistance was investigated. Results revealed that S. Typhimurium cells cultured at relatively lower temperature (10 and 25 °C) were more easily inactivated by PEF treatment than those cultured at relatively higher temperature (37 and 45 °C). Using a Weibull model, it was determined that the PEF treatment time to inactivate 90% S. Typhimurium cells cultured at 45 °C was almost fourfold compared to those cultured at 10 °C. Results of micro-Raman spectroscopy indicated that as the culture temperature increased, the order degree of C–C and lateral packing order of membrane lipid chain also increased, resulting in a drop in the membrane fluidity. These results are important in considering the use of heat and PEF to inactivate microbe contaminants in food.
ISSN: 09505423

Witkowska, E., Korsak, D., Kowalska, A., Księżopolska-Gocalska, M., Niedziółka-Jönsson, J., Roźniecka, E., Michałowicz, W., Albrycht, P., Podrażka, M., Hołyst, R., Waluk, J., Kamińska, A.
Surface-enhanced Raman spectroscopy introduced into the International Standard Organization (ISO) regulations as an alternative method for detection and identification of pathogens in the food industry
(2017) Analytical and Bioanalytical Chemistry, 409 (6), pp. 1555-1567.
ABSTRACT: We show that surface-enhanced Raman spectroscopy (SERS) coupled with principal component analysis (PCA) can serve as a fast, reliable, and easy method for detection and identification of food-borne bacteria, namely Salmonella spp., Listeria monocytogenes, and Cronobacter spp., in different types of food matrices (salmon, eggs, powdered infant formula milk, mixed herbs, respectively). The main aim of this work was to introduce the SERS technique into three ISO (6579:2002; 11290–1:1996/A1:2004; 22964:2006) standard procedures required for detection of these bacteria in food. Our study demonstrates that the SERS technique is effective in distinguishing very closely related bacteria within a genus grown on solid and liquid media. The advantages of the proposed ISO-SERS method for bacteria identification include simplicity and reduced time of analysis, from almost 144 h required by standard methods to 48 h for the SERS-based approach. Additionally, PCA allows one to perform statistical classification of studied bacteria and to identify the spectrum of an unknown sample. Calculated first and second principal components (PC-1, PC-2) account for 96, 98, and 90% of total variance in the spectra and enable one to identify the Salmonella spp., L. monocytogenes, and Cronobacter spp., respectively. Moreover, the presented study demonstrates the excellent possibility for simultaneous detection of analyzed food-borne bacteria in one sample test (98% of PC-1 and PC-2) with a goal of splitting the data set into three separated clusters corresponding to the three studied bacteria species. The studies described in this paper suggest that SERS represents an alternative to standard microorganism diagnostic procedures. [Figure not available: see fulltext.] ISSN: 16182642

Williams, K., Valencia, L., Gokulan, K., Trbojevich, R., Khare, S.
Assessment of antimicrobial effects of food contact materials containing silver on growth of Salmonella Typhimurium
(2017) Food and Chemical Toxicology, 100, pp. 197-206.
ABSTRACT: Food contact materials containing antibacterial properties are progressively appearing in the market. Items intended to provide antimicrobial effects such as increased shelf life and food safety are incorporating silver materials during the manufacture of such products. This study examined the total silver content, release capacity, and antibacterial activity of three different silver-containing food contact materials: plastic food storage containers, a plastic cutting board, and food wrapping paper. Silver content and release were determined by Inductively Coupled Plasma Mass Spectrometry, and the results showed that, although the amount of silver in each product was similar, migration varied considerably with kind of material and simulant choice. Antimicrobial effect was tested by measuring the growth of Salmonella Typhimurium during or after exposure to the different food contact materials. The results showed that the food storage containers and wrapping paper delayed the growth of S. Typhimurium under certain conditions, but that these effects were short-lived. The strain of S. Typhimurium used in this study was found to be negative for the presence of tested silver resistance genes. The results of this study suggest that a thorough investigation should be required to show/claim the efficacy of silver-containing food contact materials for food safety purposes. ISSN: 02786915

Wang, T., Kim, S., An, J.H.
A novel CMOS image sensor system for quantitative loop-mediated isothermal amplification assays to detect food-borne pathogens
(2017) Journal of Microbiological Methods, 133, pp. 1-7.
ABSTRACT: Loop-mediated isothermal amplification (LAMP) is considered as one of the alternatives to the conventional PCR and it is an inexpensive portable diagnostic system with minimal power consumption. The present work describes the application of LAMP in real-time photon detection and quantitative analysis of nucleic acids integrated with a disposable complementary-metal-oxide semiconductor (CMOS) image sensor. This novel system works as an amplification-coupled detection platform, relying on a CMOS image sensor, with the aid of a computerized circuitry controller for the temperature and light sources. The CMOS image sensor captures the light which is passing through the sensor surface and converts into digital units using an analog-to-digital converter (ADC). This new system monitors the real-time photon variation, caused by the color changes during amplification. Escherichia coli O157 was used as a proof-of-concept target for quantitative analysis, and compared with the results for Staphylococcus aureus and Salmonella enterica

to confirm the efficiency of the system. The system detected various DNA concentrations of E. coli O157 in a short time (45 min), with a detection limit of 10 fg/μL. The low-cost, simple, and compact design, with low power consumption, represents a significant advance in the development of a portable, sensitive, user-friendly, real-time, and quantitative analytic tools for point-of-care diagnosis. ISSN: 01677012

Maffei, D.F., Sant'Ana, A.S., Franco, B.D.G.M., Schaffner, D.W.
Quantitative assessment of the impact of cross-contamination during the washing step of ready-to-eat leafy greens on the risk of illness caused by Salmonella
(2017) Food Research International, 92, pp. 106-112.
ABSTRACT: The aim of this study was to develop a quantitative microbial risk assessment (QMRA) model to estimate the risk of illness caused by Salmonella in ready-to-eat (RTE) leafy greens, based on common practices in Brazilian processing plants. The risk assessment model considered five modules: in field, washing step, retail storage, home storage and dose-response. Fifty thousand iterations of a @Risk model built in Excel were run for each of sixty scenarios. These scenarios considered different initial pathogen concentrations, fractions of contaminated produce and chlorine concentrations. For chlorine, seven pre-set concentrations (0, 5, 10, 25, 50, 150 and 250 mg/L) and three triangular distributions were considered [RiskTriang(0,5,10 mg/L), RiskTriang(0,80,250 mg/L) and RiskTriang(10,120,250 mg/L)]. The outputs were risk of infection, estimated number of illnesses and estimated percent of illnesses arising from cross-contamination. The QMRA model indicated quantitatively that higher chlorine concentrations resulted in lower risk of illness. When simulation was done with < 5 mg/L of chlorine, most (> 96%) of the illnesses arose from cross-contamination, but when a triangular distribution with 10, 120 and 250 mg/L of chlorine was simulated, no illnesses arising from cross-contamination were predicted. Proper control of the sanitizer in the washing step is essential to reduce initial contamination and avoid cross-contamination. ISSN: 09639969

Chaves, B.D., Ruiz, H., Garcia, L.G., Echeverry, A., Thompson, L., Miller, M.F., Brashears, M.M.
High prevalence of Salmonella in lymph nodes and tonsils of swine presented for slaughter in Mexico
(2017) Food Protection Trends, 37 (1), pp. 25-29.
ABSTRACT: This study reports the prevalence of culturepositive Salmonella in lymphoid tissues of swine presented for slaughter in two municipal abattoirs in Mexico. Fifty tonsils, and 110 mandibular, 90 mesenteric, and 115 subiliac lymph nodes (LNs), were recovered from 115 pork carcasses sampled across four days during a six-month period in a Merida harvest facility. Additionally, 10 mandibular LNs, 10 subiliac LNs, and 10 tonsils were recovered from 10 pork carcasses in a Cancun facility. The prevalence of Salmonella in the Merida facility was 18.0 [9/50], 12.7 [14/110], 44.4 [40/90], and 10.2% [12/115] for tonsils, mandibular, mesenteric, and subiliac LNs, respectively. In the Cancun abattoir, the prevalence was 40.0 [4/10], 20.0 [2/10], and 20.0% [2/10] for tonsils, mandibular, and mesenteric LNs, respectively. In Merida, values varied significantly across sampling days for all three LN types. These results verify that swine carry Salmonella systemically, posing a potential risk of cross-contamination of pork products for human consumption into which lymphoid tissues may be incorporated. ISSN: 15419576

Wadamori, Y., Gooneratne, R., Hussain, M.A.
Outbreaks and factors influencing microbiological contamination of fresh produce
(2017) Journal of the Science of Food and Agriculture, 97 (5), pp. 1396-1403.
ABSTRACT: Fresh fruits and vegetables are nutritionally well-recognised as healthy components in diets. The microbiological foodborne outbreaks associated with the consumption of fresh produce have been increasing. Salmonella spp., Escherichia coli O157:H7, Staphylococcus aureus, Campylobacter spp. and Listeria monocytogenes are the most common pathogens that contaminate fresh produce. This review discusses recent foodborne outbreaks linked to fresh produce, factors that affect microbiological contamination and measures that could be adopted to reduce the foodborne illnesses. ISSN: 00225142

Moritz, M., Wiacek, C., Koethe, M., Braun, P.G.
Atmospheric pressure plasma jet treatment of Salmonella Enteritidis inoculated eggshells
(2017) International Journal of Food Microbiology, 245, pp. 22-28.
ABSTRACT: Contamination of eggshells with Salmonella Enteritidis remains a food safety concern. In many cases human salmonellosis within the EU can be traced back to raw or undercooked eggs and egg products. Atmospheric pressure plasma is a novel

decontamination method that can reduce a wide range of pathogens. The aim of this work was to evaluate the possibility of using an effective short time cold plasma treatment to inactivate Salmonella Enteritidis on the eggshell. Therefore, artificially contaminated eggshells were treated with an atmospheric pressure plasma jet under different experimental settings with various exposure times (15–300 s), distances from the plasma jet nozzle to the eggshell surface (5, 8 or 12 mm), feed gas compositions (Ar, Ar with 0.2, 0.5 or 1.0% O2), gas flow rates (5 and 7 slm) and different inoculations of Salmonella Enteritidis (101–106 CFU/cm2). Atmospheric pressure plasma could reduce Salmonella Enteritidis on eggshells significantly. Reduction factors ranged between 0.22 and 2.27 log CFU (colony-forming units). Exposure time and, particularly at 104 CFU/cm2 inoculation, feed gas had a major impact on Salmonella reduction. Precisely, longer exposure times led to higher reductions and Ar as feed gas was more effective than Ar[sbnd]O2 mixtures.
ISSN: 01681605

Tomás Fornés, D., McMahon, W., Moulin, J., Klijn, A.
Validation of test portion pooling for Salmonella spp. detection in foods
(2017) International Journal of Food Microbiology, 245, pp. 13-21.
ABSTRACT: Pathogen monitoring programs play a crucial role in the verification of the effectiveness of implemented hygiene control measures. Sampling and testing procedures included in pathogen monitoring involve the analysis of multiple test portions where all samples must be negative for the presence of pathogens for a certain test portion size. Many food safety programs require increased testing due to the risks that a pathogen may be present. Analyzing more than one test portion could prove to be expensive and labor intensive. When more than one test portion for a specified food item is to be tested, the test portions could be combined to form a pooled test portion to reduce laboratory workload, costs of reagents and further confirmatory steps, but only when evidence is available that pooling does not affect on the number of false negative results for different matrices. This study has been performed to demonstrate the equivalence of test portion pooling for Salmonella detection with five different methods using cultural, ELISA and Real Time PCR technologies. Twenty-three (23) different food items including confectionary products, meal components, infant formula, pet food and powdered beverages were validated. Other complementary parameters like impact of minimum and maximum incubation time for pre-enrichment, temperature profile, pH and Salmonella concentration after the pre-enrichment and background flora have also been considered in the study. The results showed that pooling test portions up to 375 g for Salmonella detection is valid for the methods that were tested. Relative level of detection (RLOD50) values for 22 of the food items tested were acceptable (i.e. lower than 2.5) when comparing the reference sample size (25 g) against the alternative pooled sample size (375 g), provided the enrichment broth was pre-warmed and maximum incubation time is respected.
ISSN: 01681605

Shah, M.K., Asa, G., Sherwood, J., Graber, K., Bergholz, T.M.
Efficacy of vacuum steam pasteurization for inactivation of Salmonella PT 30, Escherichia coli O157:H7 and Enterococcus faecium on low moisture foods
(2017) International Journal of Food Microbiology, 244, pp. 111-118.
ABSTRACT: Low moisture foods such as nuts, spices, and seeds have been implicated in several outbreaks due to Salmonella or E. coli O157:H7 contamination. Such foods may be consumed raw, and can be used as ingredients in other food products. While numerous thermal inactivation studies have been conducted for Salmonella on nuts, studies on other seeds and grains are minimal. Product water activity can influence the thermal resistance of pathogens, where thermal resistance increases as water activity decreases, leading to a requirement for higher temperatures and longer exposure times to achieve significant reduction of pathogen numbers. Vacuum steam pasteurization uses steam under vacuum, which can be operated at temperatures above and below 100 °C. The objective of this study was to determine the efficacy of vacuum steam pasteurization for inactivation of pathogens on whole flaxseed, quinoa, sunflower kernels, milled flaxseed and whole black peppercorns. The use of E. faecium as a potential surrogate for Salmonella and E. coli O157:H7 in vacuum steam pasteurization was also evaluated. Pasteurization for 1 min at 75 °C yielded average log reductions of 5.48 ± 1.22, 5.71 ± 0.40 and 5.23 ± 0.61 on flaxseed, 4.29 ± 0.92, 5.89 ± 0.26 and 2.39 ± 0.83 on quinoa, and 4.01 ± 0.74, 5.40 ± 0.83 and 2.99 ± 0.92 on sunflower kernels for Salmonella PT 30, E. coli O157:H7 and E. faecium, respectively. Similarly, on milled flaxseed and black peppercorns average log reductions of 3.02 ± 0.79 and 6.10 ± 0.64 CFU/g were observed for Salmonella PT 30 after 1 min of treatment at 75 °C but, on average, > 6.0 log reductions were observed after pasteurization at 85 °C. Our data demonstrate that vacuum steam pasteurization can be effectively used to reduce pathogens on these low moisture foods at temperature as low

as 75 and 85 °C, and that E. faecium may be used as a potential surrogate for Salmonella PT 30 and E. coli O157:H7. ISSN: 01681605

Martelli, F., Gosling, R.J., Callaby, R., Davies, R.
Observations on Salmonella contamination of commercial duck farms before and after cleaning and disinfection
(2017) Avian Pathology, 46 (2), pp. 131-137.
ABSTRACT: In the European Union, statutory control of Salmonella is in place in the chicken and turkey sectors, but not in the duck sector. In this study, 14 Salmonella-positive duck farms were sampled before and after cleaning and disinfection, and once the houses had been restocked with a new flock. The cleaning and disinfection programmes used were subdivided into two main categories: ones in which a final formaldehyde disinfection step was included (1) and ones in which it was not included (2). Several types of samples were collected during the study, and faecal samples were those more frequently positive (62% of faecal samples were positive for Salmonella in comparison to 2–23% of samples from all the other sample categories) ($P < 0.001$). Independently of the cleaning and disinfection programme used, there was a statistically significant ($P < 0.001$) reduction in the percentage of Salmonella-positive samples between before cleaning and disinfection (41.1%) and after cleaning and disinfection (3.1%). After restocking, the number of Salmonella-positive samples increased significantly ($P < 0.001$), with 65.3% of the samples tested being positive for Salmonella. Farms in which disinfection programme 1 was used were 5.34 times less likely to have samples positive for Salmonella after cleaning and disinfection than farms which implemented programme 2. Formaldehyde acts effectively against Salmonella even in the presence of some residual organic matter. Limited residual contamination on farms after cleaning and disinfection represents a risk of infection for young ducklings, and thorough cleaning and disinfection procedures should be implemented to reduce the carry-over of infection between flocks. ISSN: 03079457

Wigley, P.
Salmonella enterica serovar Gallinarum: addressing fundamental questions in bacteriology sixty years on from the 9R vaccine
(2017) Avian Pathology, 46 (2), pp. 119-124.
ABSTRACT: Sixty years on from Smith's seminal work on Fowl Typhoid vaccines, there is renewed interest in experimental avian salmonellosis and in particular the use of Salmonella enterica serovar Gallinarum as a tool to understand key features of bacterial evolution and host adaptation. In this short review we outline some of the recent advances in avian salmonellosis research that have coupled both the power of whole genome analysis and new tools to understand the host response to existing experimental infection models. These approaches are underpinning a fundamental understanding of Salmonella biology relevant to both the chicken and other avian and mammalian species.
ISSN: 03079457

Funke, S., Anker, J.C.H., Ethelberg, S.
Salmonella Dublin patients in Denmark and their distance to cattle farms
(2017) Infectious Diseases, 49 (3), pp. 208-216.
ABSTRACT: Background: The Salmonella serotype Dublin is specifically adapted to cattle but may infect humans leading to severe disease. We described human S. Dublin cases and investigated a potential spatial relation between their addresses and cattle farms in Denmark. Methods: We extracted S. Dublin patient surveillance data, 2000–2014, and performed descriptive analyses. We geocoded residential and cattle farm addresses and mapped their incidence by region, province and municipality. We used linear correlation and spatial autocorrelation analysis at the municipality level and calculated the direct network distance from the nearest farm to the residential address of cases and 20,000 randomly selected citizens representing the background population. Results: We identified 484 S. Dublin cases, 57% were male, median age 65 years. Seven patients (1%) acquired their infection abroad. The 30 days all-cause mortality was 13%. Overall, cumulative incidence was 8.0 per 100,000 inhabitants. Cattle farms were located predominantly in the western part of the country. Neither visual inspection nor correlation analysis indicated a relationship between municipalities with high incidences of human cases and cattle farms. Global Moran's Index analysis showed municipalities with high incidence of cases to be randomly distributed. We found equal direct network distances between cattle farms and both addresses of S. Dublin cases and the background population. Conclusions: We found S. Dublin infections in Denmark to affect the elderly, be serious and acquired domestically. Our findings indicate that the risk of infection with S. Dublin in Denmark is independent of living in the proximity to cattle farms. ISSN: 23744235

Peters, T., Bertrand, S., Björkman, J.T., Brandal, L.T., Brown, D.J., Erdősi, T., Heck, M., Ibrahem, S., Johansson, K., Kornschober, C., Kotila, S.M., Le Hello, S., Lienemann, T., Mattheus, W., Nielsen, E.M., Ragimbeau, C., Rumore, J., Sabol, A., Torpdahl, M., Trees, E., Tuohy, A., De Pinna, E.
Multi-laboratory validation study of multilocus variablenumber tandem repeat analysis (MLVA) for Salmonella enterica serovar Enteritidis, 2015
(2017) Eurosurveillance, 22 (9), art. no. 2, .
ABSTRACT: Multilocus variable-number tandem repeat analysis (MLVA) is a rapid and reproducible typing method that is an important tool for investigation, as well as detection, of national and multinational outbreaks of a range of food-borne pathogens. Salmonella enterica serovar Enteritidis is the most common Salmonella serovar associated with human salmonellosis in the European Union/European Economic Area and North America. Fourteen laboratories from 13 countries in Europe and North America participated in a validation study for MLVA of S. Enteritidis targeting five loci. Following normalisation of fragment sizes using a set of reference strains, a blinded set of 24 strains with known allele sizes was analysed by each participant. The S. Enteritidis 5-loci MLVA protocol was shown to produce internationally comparable results as more than 90% of the participants reported less than 5% discrepant MLVA profiles. All 14 participating laboratories performed well, even those where experience with this typing method was limited. The raw fragment length data were consistent throughout, and the interlaboratory validation helped to standardise the conversion of raw data to repeat numbers with at least two countries updating their internal procedures. However, differences in assigned MLVA profiles remain between well-established protocols and should be taken into account when exchanging data. ISSN: 1025496X

Zhang, G., Hu, L., Melka, D., Wang, H., Laasri, A., Brown, E.W., Strain, E., Allard, M., Bunning, V.K., Musser, S.M., Johnson, R., Farakos, S.M.S., Scott, V.N., Pouillot, R., Van Doren, J.M., Hammack, T.S.
Prevalence of salmonella in cashews, hazelnuts, macadamia nuts, pecans, pine nuts, and walnuts in the United States
(2017) Journal of Food Protection, 80 (3), pp. 459-466.
ABSTRACT: Nuts have been identified as a vector for salmonellosis. The objective of this project was to estimate the prevalence and contamination level of Salmonella in raw tree nuts (cashews, pecans, hazelnuts, macadamia nuts, pine nuts, and walnuts) at retail markets in the United States. A total of 3,656 samples of six types of tree nuts were collected from different types of retail stores and markets nationwide between October 2014 and October 2015. These samples were analyzed using a modified version of the Salmonella culture method from the U.S. Food and Drug Administration's Bacteriological Analytical Manual. Of the 3,656 samples collected and tested, 32 were culturally confirmed as containing Salmonella. These isolates represented 25 serotypes. Salmonella was not detected in pecans and in-shell hazelnuts. Salmonella prevalence estimates (and 95% confidence intervals) in cashews, shelled hazelnuts, pine nuts, walnuts, and macadamia nuts were 0.55% [0.15, 1.40], 0.35% [0.04, 1.20], 0.48% [0.10, 1.40], 1.20% [0.53, 2.40], and 4.20% [2.40, 6.90], respectively. The rates of Salmonella isolation from major or big chain supermarkets, small chain supermarkets, discount, variety, or drug stores, and online were 0.64% [0.38, 1.00], 1.60% [0.80, 2.90], 0.00% [0.00, 2.40], and 13.64% [2.90, 35.00], respectively (Cochran-Mantel-Haenszel test: P = 0.02). The rates of Salmonella isolation for conventional and organic nuts were not significantly different. Of the samples containing Salmonella, 60.7% had levels less than 0.003 most probable number (MPN)/g. The highest contamination level observed was 0.092 MPN/g. The prevalence and levels of Salmonella in these tree nut samples were comparable to those previously reported for similar foods. ISSN: 0362028X

Dueñas, F., Rivera, D., Toledo, V., Tardone, R., Hervé-Claude, L.P., Hamilton-West, C., Switt, A.I.M.
Short communication: Characterization of Salmonella phages from dairy calves on farms with history of diarrhea
(2017) Journal of Dairy Science, 100 (3), pp. 2196-2200.
ABSTRACT: Salmonella enterica can cause disease and mortality in calves. This pathogen is also a zoonosis that can be transmitted by animal contact or by food. The prevalence of Salmonella in dairy farms has been reported to range from 0 to 64%, and, due to the diversity of Salmonella serovars that can be circulating, Salmonella is an important concern for dairy production. Bacteriophages that infect Salmonella have been documented to be abundant and widely distributed in the dairy environment. The current study investigated the diversity of Salmonella serovars and Salmonella phages in 8 dairy farms with a history of diarrhea in southern Chile. A total of 160 samples from sick calves,

healthy calves, and the environment were analyzed for Salmonella and phage. Isolated phages were characterized and classified by their host range using a panel of 26 Salmonella isolates representing 23 serovars. Host ranges were classified according to lysis profiles (LP) and their spatial distribution was mapped. Salmonella-infecting phages were identified, but none of the 160 samples were positive for Salmonella. A total of 45 phage isolates were obtained from sick calves (11), healthy calves (16), or the environment (18). According to their host range, 19 LP were identified, with LP1 being the most common on all 8 farms; LP1 represents phages that only lyse serogroup D Salmonella. The identification of Salmonella phages but not Salmonella in the same samples could suggest that these phages are controlling Salmonella in these farms. ISSN: 00220302

Zhang, G., Ali, L., Gill, V., Tatavarthy, A., Deng, X., Hu, L., Brown, E.W., Hammack, T.S.
Development and validation of a cultural method for the detection and isolation of salmonella in cloves
(2017) Journal of Food Protection, 80 (3), pp. 376-382.
ABSTRACT: Detection of Salmonella in some spices, such as cloves, remains a challenge due to their inherent antimicrobial properties. The purpose of this study was to develop an effective detection method for Salmonella from spices using cloves as a model. Two clove varieties, Ceylon and Madagascar, were used in the study. Cloves were inoculated with Salmonella enterica subsp. enterica serotypes Montevideo, Typhimurium, or Weltevreden at about 1, 3, or ± log CFU/25 g. Two test portion sizes, 10 and 25 g, were compared. After adding Trypticase soy broth (TSB) to the weighed cloves for preenrichment, three preenrichment methods were compared: cloves were left in the TSB for 24 h during preenrichment (PreE1), or the cloves-TSB mixture was shaken vigorously for 30 s (PreE2) or 60 s (PreE3), and the decanted material was transferred to a new bag for 24 h of preenrichment. The rest of the procedures were carried out according to the U.S. Food and Drug Administration Bacteriological Analytical Manual (BAM). At the low inoculation level (,1 log CFU/25 g), the detection rate was low across the three preenrichment methods, with the highest for PreE3 and lowest for PreE1. At the medium and high inoculation levels (3 and ± log CFU/25 g), all samples from PreE2 and PreE3 were positive for Salmonella, whereas PreE1 produced only 12 positive samples from the 48 samples at the medium inoculation level and 38 positive samples from the 48 samples at the high inoculation level. Therefore, PreE3 with 25 g of cloves per sample was more effective than the other two tested methods. This newly designed method was then validated by comparing with the BAM method in six trials, with each trial consisting of 40 test samples. The results showed that PreE3 detected Salmonella from 88 of 120 inoculated test samples compared with only 31 positive from 120 test samples with the BAM method. Thus, our newly designed method PreE3 was more sensitive and easier to operate than the current BAM method for detection of Salmonella in cloves. ISSN: 0362028X

Milazzo, A., Giles, L.C., Zhang, Y., Koehler, A.P., Hiller, J.E., Bi, P.
Factors Influencing Knowledge, Food Safety Practices and Food Preferences during Warm Weather of Salmonella and Campylobacter Cases in South Australia
(2017) Foodborne Pathogens and Disease, 14 (3), pp. 125-131.
ABSTRACT: Objective: To assess food safety practices, food shopping preferences, and eating behaviors of people diagnosed with Salmonella or Campylobacter infection in the warm seasons, and to identify socioeconomic factors associated with behavior and practices. Methods: A cross-sectional survey was conducted among Salmonella and Campylobacter cases with onset of illness from January 1 to March 31, 2013. Multivariable logistic regression analyses examined relationships between socioeconomic position and food safety knowledge and practices, shopping and food preferences, and preferences, perceptions, and knowledge about food safety information on warm days. Results: Respondents in our study engaged in unsafe personal and food hygiene practices. They also carried out unsafe food preparation practices, and had poor knowledge of foods associated with an increased risk of foodborne illness. Socioeconomic position did not influence food safety practices. We found that people's reported eating behaviors and food preferences were influenced by warm weather. Conclusions: Our study has explored preferences and practices related to food safety in the warm season months. This is important given that warmer ambient temperatures are projected to rise, both globally and in Australia, and will have a substantial effect on the burden of infectious gastroenteritis including foodborne disease. Our results provide information about modifiable behaviors for the prevention of foodborne illness in the household in the warm weather and the need for information to be disseminated across the general population. An understanding of the knowledge and factors associated with human behavior during warmer weather is critical for public health interventions on foodborne prevention. ISSN: 15353141

Singh, P., Silva, M.F., Ryser, E.T., Ha, S.D., Kang, I.
Recovery of associated and internalized Salmonella in broiler skin by stomaching and grinding
(2017) Food Control, 73, pp. 883-888.
ABSTRACT: The objective of this study was to evaluate the recovery of associated and internalized Salmonella by stomaching and grinding broiler skin during exposure at 4 °C and at room temperature, using a two-strain green fluorescent protein (GFP) labeled cocktail of Salmonella Enteritidis. In the first experiment, broiler skins were immediately taken from eviscerated carcasses and exposed to a Salmonella cocktail containing ~1 × 109 CFU/ml for 0.5, 6, 12, 24, and 48 h at 4 °C. After each exposure, two 1-min stomachings and subsequent grinding of the stomached skin were conducted to quantify loosely associated (from two stomachings) and tightly associated (from grinding) Salmonella on the skin, respectively. Broiler skins exposed to Salmonella for 24 and 48 h were also examined by confocal microscopy before and after the two stomachings. The 1st and 2nd stomachings recovered an average of 71 and 17% of the Salmonella population, respectively, with an additional 12% of the cells recovered after subsequent grinding, regardless of incubation time. Based on the confocal images, most Salmonella were removed after two stomachings, however a few cells further penetrated from 9 to 29 μm into the skin. In the second experiment, broiler skins were immersed in the same two-strain Salmonella cocktail (~1 × 108 cells/ml) and dip-inoculated for 2 min with/without stomaching at room temperature. Based on the confocal images, Salmonella penetrated the flat skin surfaces and crevices up to 10 and 68 μm without stomaching, respectively, and up to 62 and 132 μm with stomaching. The presence of free-floating Salmonella cells in the skin crevices indicates that entrapped water is important for bacterial translocation in poultry skin. These findings indicated that extent of observable Salmonella association, penetration, and subsequent recovery from poultry skin is related to both surface topography of poultry skin and method of sample processing. ISSN: 09567135

Kloska, F., Casteel, M., Kump, F.W.-S., Klein, G.
Implementation of a Risk-Orientated Hygiene Analysis for the Control of Salmonella JAVA in the Broiler Production
(2017) Current Microbiology, 74 (3), pp. 356-364.
ABSTRACT: This field study aimed to establish a risk-orientated hygiene analysis on two broiler farms in Northwestern Germany for the practical use in broiler housing to evaluate the success of disinfection procedures for eradicating S. Java. The risk-orientated hygiene analysis enables fast, reproducible and cost-effective testing of broiler farms and in turn helps minimize the public health risk ensuing from S. Java. Farms were tested before and after cleaning as well as after disinfection according to a risk-orientated hygiene analysis for the presence of Salmonella DNA with qPCR. Positive PCR samples were confirmed by classical microbiology. Before cleaning, all checkpoints were tested positive for Salmonella DNA. Salmonella reduction of ca 66% of the sampled points could be achieved by intensive cleaning. A first disinfection on farm A and B failed to completely eradicate S. Java. A second disinfection followed and finally achieved a Salmonella-free status of the barns. During nine rearing periods, farms were tested weekly with boot swabs for Salmonella and at slaughter carcasses were tested for Salmonella status. No Salmonella were detected in these examinations. The two studied broiler farms have, to date, remained free of Salmonella. ISSN: 03438651

Bugarel, M., Tudor, A., Loneragan, G.H., Nightingale, K.K.
Molecular detection assay of five Salmonella serotypes of public interest: Typhimurium, Enteritidis, Newport, Heidelberg, and Hadar
(2017) Journal of Microbiological Methods, 134, pp. 14-20.
ABSTRACT: Foodborne illnesses due to Salmonella represent an important public-health concern worldwide. In the United States, a majority of Salmonella infections are associated with a small number of serotypes. Furthermore, some serotypes that are overrepresented among human disease are also associated with multi-drug resistance phenotypes. Rapid detection of serotypes of public-health concern might help reduce the burden of salmonellosis cases and limit exposure to multi-drug resistant Salmonella. We developed a two-step real-time PCR-based rapid method for the identification and detection of five Salmonella serotypes that are either overrepresented in human disease or frequently associated with multi-drug resistance, including serotypes Enteritidis, Typhimurium, Newport, Hadar, and Heidelberg. Two sets of four markers were developed to detect and differentiate the five serotypes. The first set of markers was developed as a screening step to detect the five serotypes; whereas, the second set was used to further distinguish serotypes Heidelberg, Newport and Hadar. The utilization of these markers on a two-step

investigation strategy provides a diagnostic specificity of 97% for the detection of Typhimurium, Enteritidis, Heidelberg, Infantis, Newport and Hadar. The diagnostic sensitivity of the detection makers is > 96%. The availability of this two-step rapid method will facilitate specific detection of Salmonella serotypes that contribute to a significant proportion of human disease and carry antimicrobial resistance. ISSN: 01677012

Brown, A.C., Grass, J.E., Richardson, L.C., Nisler, A.L., Bicknese, A.S., Gould, L.H.
Antimicrobial resistance in Salmonella that caused foodborne disease outbreaks: United States, 2003-2012
(2017) Epidemiology and Infection, 145 (4), pp. 766-774.
ABSTRACT: Although most non-typhoidal Salmonella illnesses are self-limiting, antimicrobial treatment is critical for invasive infections. To describe resistance in Salmonella that caused foodborne outbreaks in the United States, we linked outbreaks submitted to the Foodborne Disease Outbreak Surveillance System to isolate susceptibility data in the National Antimicrobial Resistance Monitoring System. Resistant outbreaks were defined as those linked to one or more isolates with resistance to at least one antimicrobial drug. Multidrug resistant (MDR) outbreaks had at least one isolate resistant to three or more antimicrobial classes. Twenty-one per cent (37/176) of linked outbreaks were resistant. In outbreaks attributed to a single food group, 73% (16/22) of resistant outbreaks and 46% (31/68) of non-resistant outbreaks were attributed to foods from land animals (P < 0.05). MDR Salmonella with clinically important resistance caused 29% (14/48) of outbreaks from land animals and 8% (3/40) of outbreaks from plant products (P < 0.01). In our study, resistant Salmonella infections were more common in outbreaks attributed to foods from land animals than outbreaks from foods from plants or aquatic animals. Antimicrobial susceptibility data on isolates from foodborne Salmonella outbreaks can help determine which foods are associated with resistant infections. ISSN: 09502688

Wan, Z., Chen, Y., Pankaj, S.K., Keener, K.M.
High voltage atmospheric cold plasma treatment of refrigerated chicken eggs for control of Salmonella Enteritidis contamination on egg shell
(2017) LWT - Food Science and Technology, 76, pp. 124-130.
ABSTRACT: Salmonella Enteritidis (SE) contamination is a major risk for U.S. consumers from chicken eggs. This study is primarily focused on evaluation of a novel High Voltage Atmospheric Cold Plasma (HVACP) technology for inactivation of Salmonella and its effect on the egg quality. Spot inoculated chicken eggs were treated with high voltage cold plasma (85 kV) under direct and indirect mode of exposure in dry air and modified atmospheric gas environment. A reduction of 5.53 log cfu/egg was observed for egg surfaces directly treated under modified atmospheric gas for 15 min. SE reductions was found dependent on treatment times, gas type, and mode of exposure of eggs to the plasma. No significant difference (p > 0.05) in direct and indirect mode of exposure was observed on the egg quality after plasma treatment. These results demonstrate the potential of HVACP to be used as a suitable non-thermal treatment for reducing Salmonella from packaged chicken eggs. ISSN: 00236438

Ho, Y.-N., Chou, M.-Y., Tsai, H.-C., Huang, T.-Y., Fan, C.-W., Hsu, B.-M.
Empirical testing of modified Salmonella MLST in aquatic environmental samples by in silico analysis
(2017) Science of the Total Environment, 581-582, pp. 378-385.
ABSTRACT: Multilocus sequence typing (MLST) is an approach for prediction of Salmonella servoar and eBRUST groups (eBGs) based on seven typing scheme of housekeeping genes. Up to date, > 220.000 allelic profiles and 65,973 Salmonella strains have been established in the MLST database. Several studies have modified MLST method with fewer targeted housekeeping genes for the purpose of economy and efficiency. Nevertheless, no study has conducted systematically to evaluate the correlation between the numbers of housekeeping genes targeted and the accuracy of prediction rate. In this study, we aimed to tackle this problem by extracting data from the MLST database as a whole using the software RStudio. Our results indicated that as the numbers of genes in MLST scheme increased, the accuracy of the eBGs prediction rate increased and reached 100% when the gene numbers are greater than or equal to 5. To examine the applicability of the approach, 395 environmental water samples were subjected to this study. A set of 52 Salmonella enterica isolates was initially used to develop MLST targeting seven housekeeping genes. A total of 29 sequence types, including 11 new sequence types were found among the 52 sequenced isolates that differentiated into 19 serotypes. Moreover, two novel sequence types did not belong to current classification. Our results show that the outcome in the three-gene sequence typing (aroC, hisD, and purE) was as accurate as in the seven-gene sequence typing for prediction of environmental Salmonella isolates. Our data suggested

that this five-gene and reduced gene-number sequence-typing scheme can serve as an alternative modified MLST when effectiveness and financial management were the concerns. ISSN: 00489697

Li, F., Li, F., Chen, B., Zhou, B., Yu, P., Yu, S., Lai, W., Xu, H.
Sextuplex PCR combined with immunomagnetic separation and PMA treatment for rapid detection and specific identification of viable Salmonella spp., Salmonella enterica serovars Paratyphi B, Salmonella Typhimurium, and Salmonella Enteritidis in raw meat
(2017) Food Control, 73, pp. 587-594.
ABSTRACT: Salmonella is one of the most common pathogens that cause foodborne diseases in humans, resulting in high medical and economical costs worldwide. The aim of this study was to develop a rapid and accurate approach for simultaneous detection of viable Salmonella spp. in raw meat, including Salmonella enterica serovars Paratyphi B, S. Typhimurium, and S. Enteritidis. To reduce detection time and improve sensitivity, immunomagnetic separation (IMS) was used as a pre-concentration and separation method. In addition, false positive and false negative results were removed by combining propidium monoazide (PMA) treatment with internal amplification control. Results showed that the detection limit of IMS-PMA combined with sextuplex polymerase chain reaction (PCR) assay reached as low as 101 CFU/mL in pure culture and 102 CFU/g in spiked raw meat (ground pork and ground beef), and the total assay time took less than 6 h. Thus, the novel IMS-PMA-mPCR assay developed in this study holds promise for routine screening of viable Salmonella serovars in meat and other samples. ISSN: 09567135

Zheng, H., Hu, Y., Li, Q., Tao, J., Cai, Y., Wang, Y., Li, J., Zhou, Z., Pan, Z., Jiao, X.
Subtyping Salmonella enterica serovar Derby with multilocus sequence typing (MLST) and clustered regularly interspaced short palindromic repeats (CRISPRs)
(2017) Food Control, 73, pp. 474-484.
ABSTRACT: Salmonella Derby is one of the most prevalent serovars in pork and the most common serotype isolated from infants and toddlers. However, Salmonella Derby is also poorly understood, so we used Multilocus sequence typing (MLST) and Clustered regularly interspaced short palindromic repeats (CRISPRs) to subtype 100 Salmonella Derby isolates from pig farms, pig slaughterhouses, retail markets and humans that were collected during different years in Yangzhou, Jiangsu Province, China, in respect to the transmission of clonal groups of the serovar along the food chain. MLST analysis showed that two sequence type (ST) patterns (ST40 and ST71) were shared, and ST40 was the most common sequence type among isolates from four different sources. CRISPRs typing identified 32 different Derby CRISPR types (DCTs); ST40 and ST71 strains had 21 and 11 DCTs respectively, demonstrating the distinctiveness of the CRISPR regions among the isolates from the four sources during a seven-year period. It demonstrated that Salmonella Derby clones persisted in the same places and spread along the pork production chain. Overall, 100 spacers were analysed, including 61 for CRISPR1 (18 new) and 39 for CRISPR2 (17 new). Interestingly, we also found that the spacer arrangements were distinct between ST40 and ST71 strains, except for strain 13-S1. This analysis revealed that CRISPR genes are highly polymorphic even in the same serotype, which could be tremendously useful for bacterial subtyping during molecular epidemiological investigations. ISSN: 09567135

Ashayerizadeh, A., Dastar, B., Shams Shargh, M., Sadeghi Mahoonak, A., Zerehdaran, S.
Fermented rapeseed meal is effective in controlling Salmonella enterica serovar Typhimurium infection and improving growth performance in broiler chicks
(2017) Veterinary Microbiology, 201, pp. 93-102.
ABSTRACT: The aim of present experiment was to assess the effects of fermented rapeseed meal (FRSM) on Salmonella enterica serovar Typhimurium (S. Typhimurium) colonization and growth performance in broiler chicks. Two hundred forty day-old male Cobb 500 broiler chicks were divided into six experimental treatments with four replicates and 10 birds per each. The treatments were including two positive and negative controls which birds received a basal corn-soybean diet as well as four others which birds received the diets that rapeseed meal (RSM) or FRSM was replaced with soybean meal at 50 and 100% levels. All chicks except the negative control birds were challenged orally with 105 CFU of S. Typhimurium at 3 days of age. Results showed that birds were fed FRSM had significantly greater lactic acid bacteria populations and lesser S. Typhimurium colonization in ileal and cecal sections compared to others (P < 0.05). The less percentage of liver and bursa of fabricius was belonged to negative control group. At 10 day, feeding chicks with diet containing FRSM, but not RSM, significantly decreased the organ invasion by S. Typhimurium (P < 0.05). Heterophil to lymphocyte ratio was significantly lesser in chicks

were fed FRSM compared to those fed RSM or positive control (P < 0.05). Birds were fed FRSM had significantly higher weight gain and better feed conversion ratio compared to those birds were fed RSM (P < 0.05). The findings of present experiment concerning positive effects of feeding FRSM on reducing S. Typhimurium and improving growth performance show that this processed protein source can be considered as a nutritional effective strategy to control Salmonella contamination in broiler chicks. ISSN: 03781135

Das, Q., Islam, M.R., Marcone, M.F., Warriner, K., Diarra, M.S.
Potential of berry extracts to control foodborne pathogens
(2017) Food Control, 73, pp. 650-662.
ABSTRACT: Globally, foodborne diseases continues to be a serious public health problem. Among the infectious bacteria implicated in these diseases, non-typhoidal Salmonella enterica (NTS) serovars are the major cause of hospitalization and death, followed by Campylobacter, Clostridium perfringens, Escherichia coli O157:H7, and Listeria monocytogenes. In addition, the emergence and spread of antibiotic-resistance strains among these bacteria is becoming a worldwide food safety issue. This rise of resistance led to the restriction of antibiotics use in animal productions in the European Union and application of a possible similar action in the North America. To limit the use of antibiotics in agricultures while satisfying the consumer demands, effective alternative approaches to maintain the animal health and productivity as well as to preserve food need to be explored. In this context, the plant-derived antibacterial compounds could provide novel approaches to control pathogenic bacteria in food industry. In this paper, we review the potential of three different berries (cranberries, blueberries and strawberries) extracts, as alternative antibacterial products against foodborne pathogens. These extracts show various antimicrobial activities against Gram positive (Listeria, Staphylococcus aureus, and Clostridium perfringens) and Gram negative (Salmonella enterica, E. coli and Campylobacter spp.) bacteria. Berry extracts seem to have a pleotropic mode of actions against foodborne bacteria. Several studies on proanthocyanidins from cranberry demonstrated its bactericidal action through anti-adhesion activities and free iron sequestration. Blueberry phenolics were reported to decrease cell auto-aggregation, motility and affect the cellular hydrophobicity. Similar action was observed with strawberry extracts due to their immobilizing capacity. Key research gaps include the effects of processing, bioavailability and detail mechanisms of action of berry compounds.
ISSN: 09567135

Lobete, M.M., Noriega, E., Batalha, M.A., de Beurme, S., Van de Voorde, I., Van Impe, J.F.
Effect of tagatose on growth dynamics of Salmonella Typhimurium and Listeria monocytogenes in media with different levels of structural complexity and in UHT skimmed milk
(2017) Food Control, 73, pp. 31-42.
ABSTRACT: Tagatose is a novel low-calorie sweetener which addition changes food product properties, e.g., aw, and formula, that may influence food-microbial growth dynamics in both liquid and solid products. The aim of this work was to study growth dynamics of Salmonella Typhimurium and Listeria monocytogenes in liquid and solid media enriched with tagatose (1.5, 4.5 and 7.5% (w/v)), at 4, 8 and 20 °C. More specifically, liquid media were chemically defined minimal medium, general medium and UHT skimmed milk; gelatine and a gelatine-dextran mixture were the gelling agents used, leading respectively a homogeneous and heterogeneous solid system. At regular intervals, cell concentration was determined and the Baranyi and Roberts (1994) model was fitted for growth parameter estimation. Results show a reduced and even no growth of S. Typhimurium with increasing tagatose concentrations, especially at low temperatures and when increasing media complexity. The behaviour of L. monocytogenes is not affected by tagatose, except in liquid at 4 °C, where tagatose facilitates the growth in general culture media. Varying responses of the studied bacteria to changes in media formulation should be considered for future product design. ISSN: 09567135

Gabriel, A.A., Vera, D.D., Lazo, O.M.Y., Azarcon, V.B., De Ocampo, C.G., Marasigan, J.C., Sandel, G.T.
Ultraviolet-C inactivation of Escherichia coli O157:H7, Listeria monocytogenes, Pseudomonas aeruginosa, and Salmonella enterica in liquid egg white
(2017) Food Control, 73, pp. 1303-1309.
ABSTRACT: The study determined and compared the ultraviolet-C (UV-C) resistance of selected foodborne microorganisms including Escherichia coli O157:H7, Listeria monocytogenes, Pseudomonas aeruginosa and Salmonella enterica in turbulent flowing liquid egg white. Results showed that all test organisms exhibited first-order inactivation

kinetics, characterized by logarithmic-linear inactivation behavior (R2 = 0.91–0.98). The decimal reduction times (D) of the organisms ranged from 26.44 to 37.22 min; with corresponding UV-C energy dose (DUV-C) of 170.71–240.33 mJ/cm2. P. aeruginosa had the highest resistance while E. coli O157:H7 had the least. The differences between the D and DUV-C values of the test organisms were however not statistically significant. The inactivation rate of P. aeruginosa was used to establish UV-C processes capable of reducing the reference organism by 99.9 (3D) and 99.999% (5D). Quality evaluations of UV-C processed liquid egg whites showed that both process schedules resulted in significant alterations in color and sensory quality attributes. Increasing the number of UV-C lamp, or combining UV-C treatment with mild heating to achieve the target reference organism reduction without the unacceptable change in quality was recommended for future studies. The results reported in this work may serve as baseline information for further improving the UV-C processing technique for liquid egg whites and similar commodities. ISSN: 09567135

Chardon, J.E., Evers, E.G.
Improved swift Quantitative Microbiological Risk Assessment (sQMRA) methodology (2017) Food Control, 73, pp. 1285-1297.
ABSTRACT: We developed an improved simplified Quantitative Microbiological Risk Assessment (QMRA) model and tool with reduced data need, applicable to any pathogen - food product combination and in addition suitable for basic QMRA education. The swift QMRA (sQMRA2) – model follows pathogen numbers through part of the food chain, starting at the retail phase, and ends with the estimated number of human cases of illness. The accompanying tool was implemented in Excel/@Risk. Relative risk (compared to other pathogen-food product combinations) rather than absolute risk was considered the most useful model output. The model includes storage at home (categories: room/fridge/freezer), cross-contamination (yes/no) and heating (done/undercooked/raw) during preparation in the kitchen and a dose response relationship (Binomial/Beta-Binomial). The model also includes variability, e.g. of pathogen concentration and food product heating (time, temperature) in the kitchen. The general setup of the sQMRA2 tool consists of 14 consecutive (sets of) questions for values of parameters and per phase detailed intermediate model output broken down into categories. On a separate sheet, attribution of storage, cross-contamination and heating transmission routes in terms of exposure (probability of a contaminated portion, number of cfu) and number of human cases are presented. Further, intermediate exposures (number of contaminated portions, number of cfu) and final risks (number of human cases, DALYs, cost of illness), relative as well as absolute, are given. sQMRA2 is useful for quickly obtaining relative public health risk QMRA estimates of multiple pathogen - food combinations, which can be directly useful for risk management in terms of attribution or for the selection of high risk candidates for the application of extensive QMRA. It is also useful for educational purposes because of the insightful presentation of intermediate and final model output. As an example, sQMRA2 calculations were given for Campylobacter and Salmonella in chicken fillet, filet americain and table eggs. ISSN: 09567135