NEWSLETTER

European Union Reference Laboratory for *Salmonella*

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European Union Reference Laboratory for *Salmonella*

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Editorial Note

Bilthoven, 10 July 2020

Dear colleague,

Unfortunately the **SARS-CoV-2 virus** is still among us and in the last few months we all had to adapt our lives to this situation to protect ourselves and others. Luckily the situation improved in many countries so that the very stringent measures could be loosened. On the other hand local outbreaks are seen, resulting again in some regional lock-downs. This shows, unfortunately, the instability of the situation. I do hope that the virus did not hit you too hard and that you are all healthy. From what we have heard, most NRLs-*Salmonella* were still able to continue their vital activities during the COVID-19 pandemic, which was probably not always easy. Hopefully, more activities have been started again at the NRLs, working in the 'new normal'. Something this pandemic has learned us is that a lot of our work can be done online from home (as long as it is non-laboratory work) and that online meetings seem to be a reasonable alternative, also saving a lot of traveling. Although all this online work seems technically perfectly possible, it can not replace the personal contact with colleagues which is also so very important.

One of the activities we had to change into an online activity is the **EURL-*Salmonella* workshop**. As a result of the COVID-19 pandemic we moved the workshop from 28-29 May to 17-18 September 2020, still hoping to be able to organise it as a physical meeting by then. However, we realise that a physical meeting is currently not possible. Not only traveling is still unsure, but also the space in the meeting room is too limited to maintain sufficient distance from each other as required according to the COVID-19 rules. Therefore we decided to change the workshop into an online workshop. The advantage of this is that we can host more participants (currently we have 70 registrations), but the disadvantage is that there is no real personal contact possible. For us it is the first time that we will organise the workshop as an online meeting and it is quite a challenge to get it all well-organised. Currently we are working hard on the (draft) program of the workshop and in addition we will draft a guidance for the participants in an attempt to organise this online meeting as smoothly as possible. We will inform the registered participants as soon as possible with more details.

Another meeting which has been changed into an online activity is the **conference 'Modern technologies to enable response to crises: Next Generation Sequencing to tackle food-borne diseases in the EU'**. This One-health conference was moved from 10 March to 25 September 2020, also with the hope that physical meetings would be possible again by then. This conference has also been transformed into an online conference. If you registered already for the conference of 25 September, you do not have to register again. If you did not yet register, but still want to participate, please register at https://w3.iss.it/site/sanvevent. The amended program for this conference can be found in this Newsletter.

Off course the laboratory work can not be changed into an online activity and I can inform you that we still try the utmost to continue with the organisation of the planned EURL-*Salmonella* Proficiency Tests.

In fall 2019, before the COVID-19 pandemic, we organised the **PT on typing of *Salmonella* 2019.** The results of the serotyping part of this study was already reported to the participants in February 2020. By mid-June

we also reported the results of the (optional) cluster analysis part of this study. The interim summary reports of both parts of this PT are available at the EURL-*Salmonella* website: https://www.eurlsalmonella.eu/publications/interlaboratory-comparison-study-reports. The cluster analysis of the molecular part of this Proficiency Test showed some interesting differences between laboratories and deviations from what we would have expected to find. The results will be further discussed internally with the EURL-*Salmonella* staff, to decide what additional tests we can perform to find explanations for the different results.

In March 2020 we planned to organise the first **PT on detection of *Salmonella* in bivalve molluscs (mussels)**. Although all parcels arrived safely and on time at the participants, several NRLs indicated not to be able to perform the analysis of this PT due to restrictions of the laboratory activities as a result of the COVID-19 pandemic. From the 14 NRLs-*Salmonella* that were able to perform this PT, we received their results well on time before mid-April 2020. The individual results, together with the intended results were reported to each participant by the end of April 2020. The results of all participants are not yet disclosed as we plan to organise a second round of this PT in August 2020, to give the remaining 9 NRLs-*Salmonella* the chance to still participate.

Currently we are also preparing the **PT on detection of *Salmonella* in samples from the primary production stage (PPS)**. Due to the nature of the samples, which will be hygienic swabs, and the absence of an EURL-*Salmonella* PT on detection of *Salmonella* in a food product this year, we have decided to make this PT also available for the NRLs **Food** in our EURL-*Salmonella* network. This may result in participation of more than one NRL per country (maximum 2). The NRLs-*Salmonella* were informed about the organisation of this study by early July 2020. When subscribing for this PT, each NRL has to indicate if they will participate as NRL for food analysis or as NRL for PPS analysis, or both. This combined Proficiency Test (**PPS-Food**) will be organised in the last week of September 2020. The timetable for this study is included in this Newsletter.

The last PT planned for this year is the **PT on typing of *Salmonella***, which will be organised in November 2020. The study will contain an obligatory part on serotyping of *Salmonella*, and we plan to include again a voluntary part on cluster analysis. The timetable for this study is also included in this Newsletter.

Another activity of which we think it to be difficult to turn it into an online activity is the **training on NGS**. We planned to organise this training, together with other EURL-NRL networks, at the premises of the EURL-*E. coli* in Rome in July of this year. However, due to the well-known measures against the COVID-19 we decided to postpone this training to 2021. We will keep you informed about the new planning.

By the end of May 2020 we informed you that the **New Work Item Proposal (NWIP) of draft ISO/TS 6579-4** was launched in ISO/TC34/SC9. The document is entitled: '(EN) ISO/TS 6579-4 Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 4: Identification of monophasic *Salmonella* Typhimurium (1,4,[5],12:i:-) by polymerase chain reaction (PCR)'. The members of ISO/TC34/SC9 are requested to indicate whether they approve, disapprove or abstain on this NWIP and whether they have comments to the document. When sufficient members of ISO/TC34/SC9 support the NWIP (at least 5 countries), the further work for this document (like discussing comments, drafting new versions of the draft ISO document) will be carried out in Working Group (WG) 10 of ISO/TC34/SC9. As many of the NRLs-*Salmonella* have expertise in the field of identification of monophasic *Salmonella* Typhimurium, we want to give the NRLs the opportunity to comment on draft ISO/TS 6579-4 and/or to become member of ISO/TC34/SC9/WG10. The deadline for comments/application for membership of ISO/TC34/SC9/WG10 is 31 July 2020. In case you missed the e-mail with the information on ISO/TS NP 6579-4 and you are still interested, please contact Kirsten Mooijman (Kirsten.mooijman@rivm.nl).

Please be reminded that you can still report your findings of *Salmonella* Mikawasima through the link at the EURL-*Salmonella* website: https://www.eurlsalmonella.eu/about-eurl. The aim of this monitoring is to follow cases throughout the year, as there seems to be a yearly trend with peaks in human cases across EU/EEA member states in autumn each year. By monitoring the events during the year, EFSA and ECDC could be prepared to react more rapidly when outbreaks are reported.

Reports published in the second quarter of 2020:

Jacobs-Reitsma, W.F., Verbruggen, A., Bouw, E., Mooijman, K.A. EURL-*Salmonella* Proficiency Test Typing 2018. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM report no.: 2019-0136 (April 2020); https://www.rivm.nl/bibliotheek/rapporten/2019-0136.pdf

Pol-Hofstad, I.E. and Mooijman, K.A. EURL-*Salmonella* Proficiency Test Primary Production, 2019 - Detection of *Salmonella* in chicken faeces samples. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM Report no.: 2020-0137 (June 2020); https://www.rivm.nl/bibliotheek/rapporten/2019-0137.pdf

Best wishes,
Kirsten Mooijman
Coordinator EURL-*Salmonella*

Contribution of the EURL-*Salmonella*

Timetable PT PPS-FOOD 2020

EURL- *Salmonella* Proficiency Test
Primary Production Stage – Food 2020
Detection of *Salmonella* in hygiene sponges

Week	Date	Subject
27	Week of 29 June	E-mailing of the link to the registration form for the Proficiency Test. Please **register by 30 august** at the latest.
39	Week of 21 September	E-mailing the link for the result form to the participants. E-mailing of the protocol and instructions for the result form to the NRLs. Preparation of media by the NRLs.
40	Week of 28 September	Shipment of the parcels to the participants as Biological Substance Category B (UN 3373).
41	Monday 5 October	Start performance of the Proficiency Test.
44	31 October 2020	Deadline for completing the result form: 31 October 2020 (23:59h CET). After this deadline the result form will be closed.

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

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Timetable PT Typing 2020

EURL-*Salmonella* Proficiency Test Typing 2020
Serotyping and optional part PFGE and/or MLVA and/or WGS Cluster Analysis

Week	Date	Subject
39	Week of 21 September	Emailing of the link to the registration form for the typing study. Please **register by 16 October 2020** at the latest.
43	Week of 19 October	Emailing of the protocol 2020.
45	2 November	Shipment of the parcels to the participants as Biological Substance Category B (UN 3373).
45	Week of 2 November	*Upon receipt*: Starting the identification of the strains, according to the usual practice of the laboratory. Sending the link for the result form on serotyping to the participants. Sending the link for the result form on PFGE and/or MLVA and/or WGS Cluster Analysis to the participants in a separate email.
50	11 December 2020 at the latest	Deadline for completing the electronic submission of **serotyping** results: **11 December 2020** After this deadline, the result form for serotyping will be closed.
	29 January 2021 at the latest	Deadline for completing the electronic submission of **PFGE/MLVA/WGS Cluster Analysis** results: **29 January 2021**
	February 2021	Serotyping: Evaluation of individual laboratory results and Interim Summary Report.
	April/May 2021	PFGE/MLVA/WGS Cluster Analysis: Evaluation of individual laboratory results and Summary Report.
	Summer 2021	Final report.

Contact for the EURL- *Salmonella* Proficiency Test Typing 2020:

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For Information

"Science meets Policy" online conference 25 September 2020
Modern technologies to enable response to crises: Next Generation Sequencing to tackle food-borne diseases in the EU

09:30	**Welcome and Introduction**	Silvio Brusaferro ISS President
09:45	**Greetings and event outline**	Stefano Morabito Chair of the organization committee, EURL for *E. coli*, ISS, Rome Italy
Morning Session: Building the capacity in Europe		
10:10	EU food safety policy and NGS	Martial Plantady, European Commission G4
10:30	EFSA State of play on NGS and future perspectives in the food safety area	Valentina Rizzi European Food Safety Authority
10:50	ECDC State of play on NGS and future perspectives in public health area	Saara Kotila, European Centre for Disease prevention and Control
11:10	Need for a legal framework for NGS data supporting action by the CAs	George Haringhuizen RIVM, The Netherlands
11:30	**Question time**	
12:00	**End of Morning Session**	
Afternoon Session: Building the pathway		
14.00	The Use of Genomics in One Health AMR Surveillance: The Experience of the US National Antimicrobial Resistance Monitoring System	Patrick McDermott FDA, USA
14:20	**Open discussion** **Chair:** Stefano Morabito **Panel:** Valentina Rizzi, Saara Kotila, George Haringhuizen, Eelco Franz, Annemarie Kaesbohrer, Martial Plantady	
16:30	**Concluding remarks and closure**	

From the Literature

Salmonella-related Literature from Scopus: April – June 2020

Onrust, L., Baeyen, S., Haesebrouck, F., Ducatelle, R., Van Immerseel, F.
Effect of in feed administration of different butyrate formulations on Salmonella Enteritidis colonization and cecal microbiota in broilers
(2020) Veterinary Research, 51 (1), art. no. 56, .
ABSTRACT: Butyrate has been used extensively as a feed additive to improve gut health and to decrease Salmonella colonization in poultry. Salmonella mainly colonizes the ceca so butyrate concentrations should be increased in this gut segment. Discrepancies on the effects of butyrate on Salmonella colonization, described in the scientific literature, could thus be due to butyrate release location effects. In this study, newly developed butyrate formulations were evaluated for their effect on cecal butyrate concentrations and on colonization by Salmonella Enteritidis. In a first trial, broilers were randomly allocated to 7 dietary treatment groups with formulations based on different approaches to modify the butyrate release profile: release from wax matrices based on diffusion/erosion; micropellets supposedly release butyrate around pH 7 in the colon; tributyrin is based on the hydrolysis of esters in the small intestine. Fat-protected butyrate was included as a reference, because of its known effect on reduction of Salmonella colonization. Four days after infection, the number of cfu Salmonella per g cecal content and spleen were determined. Butyrate formulations in a wax matrix significantly reduced the Salmonella colonization in cecal content. In a second trial, wax and fat-protected butyrate treatments were replicated and results from the first trial were confirmed. Compared to the control group a higher proportion of butyrate concentration was observed in ceca for those groups with reduced Salmonella colonization. This was associated with a beneficial shift in the cecal microbiota. In conclusion, formulations that increase cecal butyrate concentrations are superior in protecting against Salmonella Enteritidis colonization. ISSN: 09284249

Ogunremi, D., Dupras, A.A., Naushad, S., Gao, R., Duceppe, M.-O., Omidi, K., Márquez, I.G., Huang, H., Goodridge, L., Lévesque, R.C., Hasan, N.A., Dadlani, M., Dixon, B., Magierowski, S., Masson, L.
A New Whole Genome Culture-Independent Diagnostic Test (WG-CIDT) for Rapid Detection of Salmonella in Lettuce
(2020) Frontiers in Microbiology, 11, art. no. 602, .
ABSTRACT: The rapid detection of foodborne microbial pathogens contaminating fresh fruits and vegetables during the intervening period between harvest and consumption could revolutionize microbial quality assurance of food usually consumed raw and those with a limited shelf life. We have developed a sensitive, shotgun whole genome sequencing protocol capable of detecting as few as 1 colony forming unit (cfu) of Salmonella enterica serovar Typhimurium spiked on 25 g of lettuce. The Ion Torrent sequencing platform was used to generate reads of globally amplified DNA from microbes recovered from the surface of lettuce followed by bioinformatic analyses of the nucleotide sequences to detect the presence of Salmonella. The test is rapid and sensitive, and appropriate for testing perishable foods, and those consumed raw, for Salmonella contamination. The test has the potential to be universally applicable to any microbial contaminant on lettuce as long as a suitable bioinformatics pipeline is available and validated. A universal test is expected to pave the way for preventive and precision food safety and the re-shaping of the entire spectrum of food safety investigations from the current disease-limiting, reactive procedure to a proactive, disease prevention process. ISSN: 1664302X

Bogomazova, A.N., Gordeeva, V.D., Krylova, E.V., Soltynskaya, I.V., Davydova, E.E., Ivanova, O.E., Komarov, A.A.
Mega-plasmid found worldwide confers multiple antimicrobial resistance in Salmonella Infantis of broiler origin in Russia
(2020) International Journal of Food Microbiology, 319, art. no. 108497, .
ABSTRACT: Plasmids which are the mobile part of the bacterial genome can acquire and carry over genes conferring antimicrobial resistance, thus contributing to rapid adaptation of bacterial community to human-defined environment. In 2014, Israeli scientists have reported a large conjugative mega-plasmid pESI (plasmid for emerging S. Infantis) that provides multiple drug resistance (MDR) of Salmonella Infantis isolated from broilers. Later, very similar pESI-like plasmids have been found in Salmonella isolated from poultry in the United States, Italy, Switzerland, Hungary, and Japan. Here we report detection of

pESI-like plasmids in Salmonella Infantis isolated from chicken food products in Russia. Whole genome sequencing of three MDR isolates revealed pESI-like plasmids in all three cases. These plasmids have such typical pESI features as a locus for siderophore yersiniabactin, a cluster of IncI1 conjugative genes, a cluster of type IV pilus genes, and three toxin-antitoxin modules. The pESI-like plasmids carry from two to five resistance genes in each isolate. In total, we observed six antimicrobial resistance genes associated with pESI-like plasmids (aadA1, blaCTX-M-14, dfrA14, sul1, tetA/tetR, tetM). Besides plasmid genes of antimicrobial resistance, all three MDR isolates of S. Infantis harbor a mutation in chromosomal gene gyrA (p.S83Y or p.D87Y) that is associated with resistance to fluoroquinolones. In addition, we performed a comparative bioinformatics meta-analysis of 25 pESI-like plasmids hosted by S. Infantis from the USA, Europe, Latin America, Israel, and Japan. This analysis identified a 173 kB sequence that is common for all pESI-like plasmids and carries virulence operons and toxin-antitoxin modules. ISSN: 01681605

Trudeau, S., Thibodeau, A., Côté, J.-C., Gaucher, M.-L., Fravalo, P.
Contribution of the Broiler Breeders' Fecal Microbiota to the Establishment of the Eggshell Microbiota
(2020) Frontiers in Microbiology, 11, art. no. 666, .
ABSTRACT: In broiler chicken production, microbial populations on the eggshell surface following oviposition are still poorly characterized, though they may significantly impact both poultry and public health. The aim of this study was to describe the microbiota of both broiler breeder hens' feces and the surface of their eggs to assess the contribution of the parental fecal microbiota to the eggshell microbiota. A total of twelve breeder flocks in Quebec, Canada, were sampled at two different times, and a total of 940 feces and 16,400 egg surface samples were recovered. Using 16S rRNA gene sequencing, we showed that even if the microbiota of both feces and eggshells were mainly composed of the phyla Firmicutes, Actinobacteria, Proteobacteria, and Bacteroidetes, the bacterial community compositions and structures differed between both types of samples. Our results also showed that both the sampling time and the flock identity significantly influenced the alpha- and the beta-diversities of the studied microbiomes. Using a Venn diagram, we showed that 1790 operational taxonomic units (OTUs) were shared between feces and eggshell samples. Sequences associated with genera of potentially pathogenic and spoilage bacteria, Acinetobacter, Campylobacter, Escherichia/Shigella, Helicobacter, Listeria, Proteus, Pseudomonas, Salmonella, and Staphylococcus, were shared between sample types. Some OTUs highly represented in the fecal microbiota and associated with Lactobacillus and Streptococcus genera, were absent from eggshells, suggesting a selection during the microbiota transfer and/or the potential role of environmental contamination. To the best of our knowledge, this is the first study using 16S rRNA sequencing to describe the contribution of the transfer from the fecal microbial ecosystem of laying breeder hens to the establishment of the microbiota on the surface of laid eggs, as well as the bacterial communities at both the broiler breeder feces and the eggshell levels. ISSN: 1664302X

Nagy, T., Szmolka, A., Wilk, T., Kiss, J., Szabó, M., Pászti, J., Nagy, B., Olasz, F.
Comparative Genome Analysis of Hungarian and Global Strains of Salmonella Infantis
(2020) Frontiers in Microbiology, 11, art. no. 539, .
ABSTRACT: Introduction: The emergence and spread of new strains of zoonotic bacteria, such as multidrug resistant (MDR) Salmonella Infantis, represent a growing health risk for humans in and outside Europe due to foodborne infections of poultry meat origin. Objectives: In order to understand genome relations of S. Infantis strains from Hungary and from different geographic regions, we performed a comprehensive genome analysis of nine Hungarian and 67 globally selected strains of S. Infantis and 26 Salmonella strains representing 13 non-Infantis serovars. Results: Analyses of whole-, and accessory genomes, showed that almost all S. Infantis strains were separated from the non-Infantis serovars. S. Infantis strains from Hungary formed subclusters based on their time of isolation. In whole genome sequence analysis, the Swiss strains of S. Infantis were closely related to each other and clustered together with subclusters of strains from Hungary, Japan, Italy, United States, and Israel. The accessory genome analysis revealed that the Swiss strains were distinct from most of the strains investigated, including the Hungarian ones. Analysis of the cloud genes offered the most detailed insight into the genetic distance and relationship of S. Infantis strains confirming that the Swiss and Hungarian strains belonged to different lineages. As expected, core genome analysis provided the least discriminatory power for analysis of S. Infantis. Genomic sequences of nine strains from Brazil, Israel, Mexico, Nigeria, and Senegal (deposited as S. Infantis) proved to be outliers from the S. Infantis clade. They were predicted to be Salmonella Rissen, Salmonella Ouakarm, Salmonella Kentucky, Salmonella Thompson, and Salmonella

enterica subsp. diarizonae. Conclusion: Accessory genome of S. Infantis showed the highest diversity suggesting a faster evolution than that of the whole genomes contributing to the emergence of multiple genetic variants of S. Infantis worldwide. Accordingly, in spite of the comprehensive analysis of several genomic characteristics, no epidemiologic links between these S. Infantis strains from different countries could be established. It is also concluded that several strains originally designated as S. Infantis need in databanks reclassification. ISSN: 1664302X

Cooper, A.L., Low, A.J., Koziol, A.G., Thomas, M.C., Leclair, D., Tamber, S., Wong, A., Blais, B.W., Carrillo, C.D.
Systematic Evaluation of Whole Genome Sequence-Based Predictions of Salmonella Serotype and Antimicrobial Resistance
(2020) Frontiers in Microbiology, 11, art. no. 549, .
ABSTRACT: Whole-genome sequencing (WGS) is used increasingly in public-health laboratories for typing and characterizing foodborne pathogens. To evaluate the performance of existing bioinformatic tools for in silico prediction of antimicrobial resistance (AMR) and serotypes of Salmonella enterica, WGS-based genotype predictions were compared with the results of traditional phenotyping assays. A total of 111 S. enterica isolates recovered from a Canadian baseline study on broiler chicken conducted in 2012-2013 were selected based on phenotypic resistance to 15 different antibiotics and isolates were subjected to WGS. Both SeqSero2 and SISTR accurately determined S. enterica serotypes, with full matches to laboratory results for 87.4 and 89.2% of isolates, respectively, and partial matches for the remaining isolates. Antimicrobial resistance genes (ARGs) were identified using several bioinformatics tools including the Comprehensive Antibiotic Resistance Database – Resistance Gene Identifier (CARD-RGI), Center for Genomic Epidemiology (CGE) ResFinder web tool, Short Read Sequence Typing for Bacterial Pathogens (SRST2 v 0.2.0), and k-mer alignment method (KMA v 1.17). All ARG identification tools had ≥ 99% accuracy for predicting resistance to all antibiotics tested except streptomycin (accuracy 94.6%). Evaluation of ARG detection in assembled versus raw-read WGS data found minimal observable differences that were gene- and coverage-dependent. Where initial phenotypic results indicated isolates were sensitive, yet ARGs were detected, repeat AMR testing corrected discrepancies. All tools failed to find resistance-determining genes for one gentamicin- and two streptomycin-resistant isolates. Further investigation found a single nucleotide polymorphism (SNP) in the nuoF coding region of one of the isolates which may be responsible for the observed streptomycin-resistant phenotype. Overall, WGS-based predictions of AMR and serotype were highly concordant with phenotype determination regardless of computational approach used. ISSN: 1664302X

Massacci, F.R., Morelli, A., Cucco, L., Castinel, A., Ortenzi, R., Tofani, S., Pezzotti, G., Estellé, J., Paniccià, M., Magistrali, C.F.
Transport to the slaughterhouse affects the salmonella shedding and modifies the fecal microbiota of finishing pigs
(2020) Animals, 10 (4), art. no. 676, .
ABSTRACT: Contaminated pork is a significant source of foodborne Salmonellosis. Pork is contaminated at the slaughterhouse and the intestinal content is the predominant source of Salmonella for carcass contamination. The prevalence of Salmonella-positive pigs increases significantly when the time of transport to the slaughterhouse is longer than two hours. The hypothesis behind this study is that transport to the slaughterhouse increases the load of Salmonella in feces and determines a shift of the fecal microbiota in finishing pigs. Fecal samples were collected in a pig herd positive for Salmonella spp., the day before the transport and at the slaughterhouse. Salmonella loads were estimated by the most probable number (MPN) technique, according to the ISO/TS 6579-2:2012/A1. Moreover, the fecal bacteria composition was assessed by sequencing the V3-V4 hypervariable regions of the 16S rRNA gene. Our study showed that the load of Salmonella increases after transport, confirming that this phase of the production chain is a critical point for the control of Salmonella contamination. A lower richness and an increased beta-diversity characterized the fecal microbiota composition of Salmonella-positive animals after transport. In this stage, a natural Salmonella infection causes a disruption of the fecal microbiota as observed in challenge studies. ISSN: 20762615

Wu, Y., Hu, Q., Dehinwal, R., Rakov, A.V., Grams, N., Clemens, E.C., Hofmann, J., Okeke, I.N., Schifferli, D.M.
The not so good, the bad and the ugly: Differential bacterial adhesion and invasion mediated by salmonella pagn allelic variants
(2020) Microorganisms, 8 (4), art. no. 489, .

ABSTRACT: While advances in genomic sequencing have highlighted significant strain variability between and within Salmonella serovars, only a few protein variants have been directly related to evolutionary adaptation for survival, such as host specificity or differential virulence. The current study investigated whether allelic variation of the Salmonella adhesin/invasin PagN influences bacterial interaction with their receptors. The Salmonella enterica, subspecies enterica serovar Typhi (S. Typhi) allelic variant of PagN was found to bind significantly better to different enterocytes as well as to the extracellular matrix protein laminin than did the major Salmonella enterica, subspecies enterica serovar Typhimurium (S. Typhimurium) allele. The two alleles differed at amino acid residues 49 and 109 in two of the four predicted PagN surface loops, and residue substitution analysis revealed that a glutamic acid at residue 49 increased the adhesive and invasive properties of S. Typhi PagN. PagN sequence comparisons from 542 Salmonella strains for six representative S. enterica serovars and S. diarizonae further supported the role of glutamic acid at residues 49 and 109 in optimizing adhesion to cells and laminin, as well as for cell invasion. In summary, this study characterized unique residues in allelic variants of a virulence factor that participates in the colonization and invasive properties of different Salmonella stains, subspecies and serovars. ISSN: 20762607

Cuggino, S.G., Bascón-Villegas, I., Rincón, F., Pérez, M.A., Posada-Izquierdo, G., Marugán, J., Pablos Carro, C., Pérez-Rodríguez, F.
Modelling the combined effect of chlorine, benzyl isothiocyanate, exposure time and cut size on the reduction of Salmonella in fresh-cut lettuce during washing process (2020) Food Microbiology, 86, art. no. 103346, .
ABSTRACT: This work aimed to study the effect of the combination of Sodium hypochlorite, the most used disinfectant by the vegetable industry, with a natural antimicrobial, benzyl-isothiocyanate (BITC), considering cutting surface and contact time, on the reduction of Salmonella in fresh-cut produce in washing operations under typical industrial conditions. Overall, the combinations of disinfectant and process parameters resulted in a mean reduction of Salmonella of 2.5 log CFU/g. According to statistical analysis, free chlorine and BITC concentrations, contact time and cut size exerted a significant effect on the Salmonella reduction ($p \leq 0.05$). The optimum combination of process parameter values yielding the highest Salmonella reduction was a lettuce cut size of 15 cm2 washed for 110 s in industrial water containing 160 mg/L free chlorine and 40 mg/L BITC. A predictive model was also derived, which, as illustrated, could be applied to optimize industrial disinfection and develop probabilistic Exposure Assessments considering the effect of washing process parameters on the levels of Salmonella contamination in leafy green products. The present study demonstrated the efficacy of chlorine to reduce Salmonella populations in fresh-cut lettuce while highlighting the importance of controlling the washing process parameters, such as, contact time, cut size and concentration of the disinfectant to increase disinfectant efficacy and improve food safety. ISSN: 07400020

Topalcengiz, Z., Spanninger, P.M., Jeamsripong, S., Persad, A.K., Buchanan, R.L., Saha, J., Lejeune, J., Jay-Russell, M.T., Kniel, K.E., Danyluk, M.D.
Survival of Salmonella in various wild animal feces that may contaminate produce (2020) Journal of Food Protection, 83 (4), pp. 651-660.
ABSTRACT: Heightened concerns about wildlife on produce farms and possible introduction of pathogens to the food supply have resulted in required actions following intrusion events. The purpose of this study was to evaluate the survival of Salmonella in feces from cattle and various wild animals (feral pigs, waterfowl, deer, and raccoons) in California, Delaware, Florida, and Ohio. Feces were inoculated with rifampin-resistant Salmonella enterica cocktails that included six serotypes: Typhimurium, Montevideo, Anatum, Javiana, Braenderup, and Newport (104 to 106 CFU/g). Fecal samples were stored at ambient temperature. Populations were enumerated for up to 1 year (364 days) by spread plating onto tryptic soy agar supplemented with rifampin. When no colonies were detected, samples were enriched. Colonies were banked on various sampling days based on availability of serotyping in each state. During the 364-day storage period, Salmonella populations decreased to ≤2.0 log CFU/g by day 84 in pig, waterfowl, and raccoon feces from all states. Salmonella populations in cattle and deer feces were 3.3 to 6.1 log CFU/g on day 336 or 364; however, in Ohio Salmonella was not detected after 120 days. Salmonella serotypes Anatum, Braenderup, and Javiana were the predominant serotypes throughout the storage period in all animal feces and states. Determination of appropriate risk mitigation strategies following animal intrusions can improve our understanding of pathogen survival in animal feces. ISSN: 0362028X

Lopes, S.M., Tondo, E.C.
Survival of Salmonella in spaghetti alla carbonara

(2020) LWT, 123, art. no. 109115, .
ABSTRACT: Spaghetti alla carbonara is a traditional Italian dish, which the sauce made of raw egg yolks is heated using only the heat of cooked pasta. Concerns about the safety of this preparation have been raised due the possibility of egg yolks be contaminated by Salmonella and the heat treatment may not be sufficient for total Salmonella inactivation. This study was undertaken to analyze the survival of Salmonella in spaghetti alla carbonara in which the only thermal processing of egg yolks was the heat transfer from the pasta. A pool of Salmonella was inoculated in egg yolks reaching 8.8 log10 CFU/g. Contaminated egg yolks were added to the cooked spaghetti, away from the heat source. Results indicated that immediately after cooking and draining, the pasta reached 86.0 °C. After 4.5 min of contact with the egg yolks, the mean temperature of spaghetti alla carbonara decreased to lower than 60 °C. The preparation method was able to inactivate approximately 4.7 log10 CFU/g of Salmonella and the spaghetti alla carbonara processed by this method had a creamy and silky sauce formed by yolks. Based on the results, it should be advisable the use of thermo-processed eggs to ensure the safety of this preparation. ISSN: 00236438

Lewis, E., Hudson, J.A., Cook, N., Barnes, J.D., Haynes, E.
Next-generation sequencing as a screening tool for foodborne pathogens in fresh produce (2020) Journal of Microbiological Methods, 171, art. no. 105840, .
ABSTRACT: Next generation sequencing (NGS) approaches are increasingly applied to tracing microbial contaminants entering the food chain due to NGS' untargeted nature and ability to investigate non-culturable (and/or difficult to culture) organisms while yielding genomic information about the microbiota. So far, a plethora of microbes has been shown to be associated with fresh produce, but few studies have utilised NGS to identify contamination with human pathogens. This study aims to establish the limit of detection (LoD) for Salmonella and phage MS2 (a Norovirus surrogate) contamination of fresh produce employing NGS approaches on the Illumina MiSeq: 16S amplicon-sequencing, and RNA-seq, using ScriptSeq (Illumina) and NEBNext (New England BioLabs) kits. ScriptSeq proved the most sensitive approach; delivering an LoD of 104 CFU reaction−1 (Colony Forming Units) for Salmonella and 105 PFU reaction−1 (Plaque Forming Units) for phage MS2. Use of the NEBNext kit resulted in detection of Salmonella at 106 CFU reaction−1 and phage MS2 at 107 PFU reaction−1. 16S amplicon-sequencing yielded a similar LoD of 105 CFU reaction−1 for Salmonella but could not detect MS2. The tested NGS methodologies, in combination with bioinformatics approaches applied, proved less sensitive than conventional microbial detection approaches. ISSN: 01677012

Cox, N.A., Berrang, M.E., House, S.L., Hinton, A., Jr., Eric Line, J., Wiggins, L.T.
Detection of multiple naturally occurring Salmonella serotypes from commercial broiler carcasses with conventional methods
(2020) Journal of Food Safety, 40 (2), art. no. e12761, .
ABSTRACT: Many laboratories sampling foods for Salmonella are interested only in presence or absence of Salmonella, so only one colony may be selected. The objectives of this study were to use two selective enrichment broths and two selective agar plating media for Salmonella recovery from naturally contaminated broiler carcass rinsates and evaluate these media combinations on Salmonella serotypes recovered from each carcass. Broiler carcasses (n = 52) from a commercial processing plant prior to chilling were rinsed with buffered peptone water and after incubation subcultured to gram-negative and tetrathionate, and after inoculation to Rappaport Vassiliadis broth which was incubated and then streaked onto plates of Brilliant Green Sulfa and Xylose-Lysine-Tergitol-4 agar. On 11/49 positive carcasses, both plating media yielded the same serotypes; for the other 38 positive samples different serotypes were found on the different plating media. Enrichment and plating media combinations influence the serotypes recovered and demonstrates bias even in a limited study such as this. ISSN: 01496085

Viegas, F.M., Ramos, C.P., Xavier, R.G.C., Lopes, E.O., Junior, C.A.O., Bagno, R.M., Diniz, A.N., Lobato, F.C.F., Silva, R.O.S.
Fecal shedding of Salmonella spp., Clostridium perfringens, and Clostridioides difficile in dogs fed raw meat-based diets in Brazil and their owners' motivation
(2020) PLoS ONE, 15 (4), art. no. e0231275, .
ABSTRACT: The present study aimed to explore the motivations of Brazilian dog owners and their knowledge about the risks related to raw meat-based diets (RMBD) as well as to evaluate important enteropathogens such as Salmonella spp., C. perfringens, and C. difficile, in feces of dogs fed different diets. The majority of the pet owners (69.3%) reported to have chosen this diet for their dogs, considering it to be more "natural". A large number of owners declared that RMBD do not pose health risks for their animals

(87.9%) or humans (98.8%), even though almost one third of the respondents (34.8%) declared having at least one individual at high risk of infection in contact with RMBD-fed dogs. Stool samples from 46 RMBD-fed dogs and 192 dogs fed commercial dry feed were collected. The present study revealed that dogs fed raw meat diets were almost 30 times more likely to be positive for Salmonella spp. than dogs on a conventional diet. Some of the serovars detected were commonly associated with human salmonellosis, such as S. Typhimurium and S. Saintpaul, and were multidrug resistant. RMBD-fed dogs were more likely to be positive for C. perfringens type A (p = 0.008) and one C. perfringens type F was isolated from these animals. Two toxigenic strains (4.3%) of C. difficile were isolated only from raw meat-fed dogs, all of which were under antibiotic therapy. These toxigenic C. difficile isolates were classified as RT106/ST54 and RT600/ST149, previously associated with infection in dogs and humans. The present work revealed that the owners have a tendency to ignore or are unaware of the risks associated with raw meat diets for dogs. Also, the higher fecal shedding of important enteropathogens in dogs fed RMBD suggests that this diet poses a risk for the animals and the people in contact with them.
ISSN: 19326203

Langsrud, S., Sørheim, O., Skuland, S.E., Almli, V.L., Jensen, M.R., Grøvlen, M.S., Ueland, Ø., Møretrø, T.
Cooking chicken at home: Common or recommended approaches to judge doneness may not assure sufficient inactivation of pathogens
(2020) PLoS ONE, 15 (4), art. no. e0230928, .
ABSTRACT: About one third of foodborne illness outbreaks in Europe are acquired in the home and eating undercooked poultry is among consumption practices associated with illness. The aim of this study was to investigate whether actual and recommended practices for monitoring chicken doneness are safe. Seventy-five European households from five European countries were interviewed and videoed while cooking chicken in their private kitchens, including young single men, families with infants/in pregnancy and elderly over seventy years. A cross-national web-survey collected cooking practices for chicken from 3969 households. In a laboratory kitchen, chicken breast fillets were injected with cocktails of Salmonella and Campylobacter and cooked to core temperatures between 55 and 70°C. Microbial survival in the core and surface of the meat were determined. In a parallel experiment, core colour, colour of juice and texture were recorded. Finally, a range of cooking thermometers from the consumer market were evaluated. The field study identified nine practical approaches for deciding if the chicken was properly cooked. Among these, checking the colour of the meat was commonly used and perceived as a way of mitigating risks among the consumers. Meanwhile, chicken was perceived as hedonically vulnerable to long cooking time. The quantitative survey revealed that households prevalently check cooking status from the inside colour (49.6%) and/or inside texture (39.2%) of the meat. Young men rely more often on the outside colour of the meat (34.7%) and less often on the juices (16.5%) than the elderly (>65 years old; 25.8% and 24.6%, respectively). The lab study showed that colour change of chicken meat happened below 60°C, corresponding to less than 3 log reduction of Salmonella and Campylobacter. At a core temperature of 70°C, pathogens survived on the fillet surface not in contact with the frying pan. No correlation between meat texture and microbial inactivation was found. A minority of respondents used a food thermometer, and a challenge with cooking thermometers for home use was long response time. In conclusion, the recommendations from the authorities on monitoring doneness of chicken and current consumer practices do not ensure reduction of pathogens to safe levels. For the domestic cook, determining doneness is both a question of avoiding potential harm and achieving a pleasurable meal. It is discussed how lack of an easy "rule-of-thumb" or tools to check safe cooking at consumer level, as well as national differences in contamination levels, food culture and economy make it difficult to develop international recommendations that are both safe and easily implemented. ISSN: 19326203

Elpers, L., Kretzschmar, J., Nuccio, S.-P., Bäumler, A.J., Hensel, M.
Factors required for adhesion of salmonella enterica serovar typhimurium to corn salad (Valerianella locusta)
(2020) Applied and Environmental Microbiology, 86 (8), art. no. 2757, .
ABSTRACT: Salmonella enterica is a foodborne pathogen often leading to gastroenteritis and is commonly acquired by consumption of contaminated food of animal origin. However, frequency of outbreaks linked to the consumption of fresh or minimally processed food of nonanimal origin is increasing. New infection routes of S. enterica by vegetables, fruits, nuts, and herbs have to be considered. This leads to special interest in S. enterica interactions with leafy products, e.g., salads, that are mainly consumed in a minimally processed form. The attachment of S. enterica to salad is a crucial step in

contamination, but little is known about the bacterial factors required and mechanisms of adhesion. S. enterica possesses a complex set of adhesive structures whose functions are only partly understood. Potentially, S. enterica may deploy multiple adhesive strategies for adhering to various salad species and other vegetables. In this study, we systematically analyzed the contributions of the complete adhesiome, of lipopolysaccharide (LPS), and of flagellum-mediated motility of S. enterica serovar Typhimurium (STM) in adhesion to Valerianella locusta (corn salad). We deployed a reductionist, synthetic approach to identify factors involved in the surface binding of STM to leaves of corn salad, with particular regard to the expression of all known adhesive structures, using the Tet-on system. This work reveals the contribution of Saf fimbriae, type 1 secretion system-secreted BapA, an intact LPS, and flagellum-mediated motility of STM in adhesion to corn salad leaves. ISSN: 00992240

Camba, S.I., del Valle, F.P., Shirota, K., Sasai, K., Katoh, H.
Evaluation of 3-week-old layer chicks intratracheally challenged with Salmonella isolates from serogroup c1 (O:6,7) and Salmonella Enteritidis
(2020) Avian Pathology, 49 (3), pp. 305-310.
ABSTRACT: With the exception to Salmonella enterica serotype Typhimurium and S. Enteritidis (serogroups B [O:4] and D [O:9], respectively), there have been very few studies conducted on the respiratory tract as route of infection in chickens with salmonellas from serogroup C1 (O:6,7). Therefore, the purpose of this present study was to determine the potential organ invasion by Salmonella enterica serotype Potsdam (SP), S. Mbandaka (SM), and S. Infantis (SI) from serogroup C1 (O:6,7) and compare their characteristics with those of S. Enteritidis (SE) on intratracheally (IT) challenged 3-week-old layer chicks. A total of 360 one-day-old White Leghorn layer chicks were acquired from a commercial hatchery and randomly assigned into four treatment groups (SP, SM, SI, and SE, respectively), consisting of three independent trials. Chicks were grown up to 21 days (3 weeks) and IT-challenged thereafter with 106 CFU of respective salmonella organisms per group (n = 30). Chicks (n = 5) were humanely sacrificed every 24 h for 6 days post-IT infection and organs such as lung, heart, liver, spleen, kidney and caecal content were cultured for salmonella. All treatment groups exhibited colonization of lungs and caecal contents at 1 d (P = 0.475) and 4 d (P = 0.696) post-IT infection, respectively. There was no isolation of SP, SM, and SI in heart, liver, spleen, and kidney. In contrast, SE was obtained from heart, liver, and spleen of IT-infected chicks. The findings of this study contribute to a better understanding of the importance of the respiratory route in salmonella infection in poultry. ISSN: 03079457

McWhorter, A.R., Chousalkar, K.K.
Salmonella on Australian cage egg farms: Observations from hatching to end of lay
(2020) Food Microbiology, 87, art. no. 103384, .
ABSTRACT: Single-aged caged layer hen flocks were monitored for Salmonella over the course of their lifetime. Chicks from both flocks were Salmonella negative at hatch and remained negative during rearing. Pullets were transported to production farms at 15 weeks of age. Pre-population dust swabs collected from both production sheds had a high percentage of Salmonella positive samples (80 and 90%). Flocks were sampled at regular intervals until 70–72 weeks of age. The proportion of Salmonella positive samples and mean load detected on eggs was low on both farms. Analysis of dust samples revealed that Salmonella persisted in dust over 8 weeks. Dust total moisture content and water activity appears to influence bacterial persistence. On egg grading equipment, only suction cups prior to egg washing were Salmonella positive (mean proportion Salmonella positive samples 0.13 ± 0.07; mean load of 18.6 ± 12.31 MPN/ml). An egg washing experiment demonstrated that while washing reduced the total Salmonella load from eggshell surfaces, no effect was observed for shell pores. These results demonstrate that despite environmental contamination on farm, Salmonella contamination of eggs is low and is further minimized by washing. ISSN: 07400020

Domesle, K.J., Young, S.R., Yang, Q., Ge, B.
Loop-mediated isothermal amplification for screening salmonella in animal food and confirming salmonella from culture isolation
(2020) Journal of Visualized Experiments, 2020 (159), art. no. e61239, .
ABSTRACT: Loop-mediated isothermal amplification (LAMP) has emerged as a powerful nucleic acid amplification test for the rapid detection of numerous bacterial, fungal, parasitic, and viral agents. Salmonella is a bacterial pathogen of worldwide food safety concern, including food for animals. Presented here is a multi-laboratory-validated Salmonella LAMP protocol that can be used to rapidly screen animal food for the presence of Salmonella contamination and can also be used to confirm presumptive Salmonella

isolates recovered from all food categories. The LAMP assay specifically targets the Salmonella invasion gene (invA) and is rapid, sensitive, and highly specific. Template DNAs are prepared from enrichment broths of animal food or pure cultures of presumptive Salmonella isolates. The LAMP reagent mixture is prepared by combining an isothermal master mix, primers, DNA template, and water. The LAMP assay runs at a constant temperature of 65 °C for 30 min. Positive results are monitored via real-time fluorescence and can be detected as early as 5 min. The LAMP assay exhibits high tolerance to inhibitors in animal food or culture medium, serving as a rapid, reliable, robust, cost-effective, and user-friendly method for screening and confirming Salmonella. The LAMP method has recently been incorporated into the U.S. Food and Drug Administration's Bacteriological Analytical Manual (BAM) Chapter 5. ISSN: 1940087X

Gand, M., Mattheus, W., Roosens, N.H.C., Dierick, K., Marchal, K., De Keersmaecker, S.C.J., Bertrand, S.
A multiplex oligonucleotide ligation-PCR method for the genoserotyping of common Salmonella using a liquid bead suspension assay
(2020) Food Microbiology, 87, art. no. 103394, .
ABSTRACT: Salmonella is a major pathogen having a public health and economic impact in both humans and animals. Six serotypes of the Salmonella genus are mentioned in the Belgian and European regulation as to be rapidly excluded from the food chain (EU regulation N°2160/2003, Belgian royal decree 27/04/2017). The reference method for Salmonella serotyping, including slide-agglutination and biochemical tests, is time-consuming, expensive, not always objective, and therefore does not match the fast identification criteria required by the legislation. In this study, a molecular method, using genetic markers detected by Multiplex Oligonucleotide Ligation – PCR and Luminex technology, was developed for the identification of the 6 Salmonella serotypes and their variants subjected to an official control. The resulting method was validated with the analysis of 971 Salmonella isolated from different matrixes (human, animal, food or environment) and 33 non-Salmonella strains. The results were compared with the reference identifications, achieving an accuracy of 99.7%. The cost-effective high-throughput genoserotyping assay is performed in 1 day and generates objective results, thanks to the automatic interpretation of raw data using a barcode system. In conclusion, it is fully adapted to the implementation in first line laboratories and meets the requirements of the regulation. ISSN: 07400020

Teichmann, J., Litt, P.K., Sharma, M., Nyarko, E., Kniel, K.E.
Influence of poultry litter amendment type and irrigation events on survival and persistence of salmonella newport
(2020) Journal of Food Protection, 83 (5), pp. 821-828.
ABSTRACT: Salmonella enterica subsp. enterica serovar Newport is a bacterial foodborne pathogen isolated from several environmental reservoirs on the Delmarva Peninsula and has been associated with several produce-related outbreaks. However, little is known about specific interactions between Salmonella Newport and soil amendments used as fertilizers. The purpose of this study was to determine Salmonella Newport persistence and resuscitation in raw poultry litter (PLR), a common biological soil amendment, and in soils containing poultry litter-based (heat-treated poultry pellets [HTPP]) or chemical fertilizer (urea [U]) amendments to provide equivalent levels of nitrogen to the soil. Inoculated samples were stored in a growth chamber and irrigated regularly over 4 weeks. Soil samples were collected every week for 4 weeks to determine moisture content and surviving Salmonella Newport populations (log CFU per gram dry weight). Data were analyzed by using a one-way analysis of variance and Student's t test. The PLR supported significantly higher (5.07 log CFU/g dry weight [gdw]) populations of Salmonella Newport than HTPP only (1.70 log CFU/gdw). However, PLR-amended (PLRA) soil (2.5 log CFU/gdw) samples had significantly ($P < 0.05$) lower Salmonella Newport populations compared with HTPP-amended (4.5 log CFU/gdw) and Uamended (4.0 log CFU/gdw) soil samples. The effect of irrigation on Salmonella Newport population levels in PLRA soils was significant, and in a comparative study, the overall increase in the pathogen levels in U-amended soil (mean = 1.12 log CFU/ gdw) was significantly greater than that in PLRA soil (mean = 0.54 log CFU/gdw), whereas that in HTPP-amended soil (0.80 log CFU/gdw) was not significantly different from PLRA soil. ISSN: 0362028X

Rosen, D.K., Gallardo, M., Vail, M., Hellberg, R.S.
Microplate immunocapture coupled with the 3M molecular detection system and selective plating for the rapid detection of Salmonella infantis in dry dog food and treats
(2020) Journal of Microbiological Methods, 172, art. no. 105881, .

ABSTRACT: The objective of this study was to use microplate immunocapture (IC) to reduce the enrichment time required for detection of Salmonella in pet food with the 3 M Molecular Detection System (MDS) or selective plating on XLD. Dog food and pig ear treats were inoculated with Salmonella Infantis at concentrations of 100–104 CFU/25 g, followed by a 3-h enrichment, then microplate IC and 3 M MDS or microplate IC and selective plating on XLD. Another set of samples underwent a traditional 24-h enrichment followed by 3 M MDS or selective plating. Based on the results of three independent trials, microplate IC followed by selective plating enabled detection of Salmonella in 100% of dog food and treat samples tested, including at levels as low as 100 CFU/25 g. Microplate IC coupled with 3 M MDS enabled detection of Salmonella in dog food and treat samples down to levels of 100 CFU/25 g, with an overall detection rate of 92%. These results indicate high potential for microplate IC to be used in place of the traditional 24-h enrichment step, enabling detection of Salmonella in complex matrices when coupled with 3 M MDS or selective plating. ISSN: 01677012

Pouillot, R., Schlosser, W., van Doren, J.M., Dennis, S.B., Kause, J.R.
Assessment of the risk of salmonellosis linked to the consumption of liquid egg products made from internally contaminated shell eggs initially stored at 65°F (18°C) compared with eggs stored at 45°F (7°C)
(2020) Journal of Food Protection, 83 (5), pp. 767-778.
ABSTRACT: According to the U.S. Food and Drug Administration's (FDA's) rule on "Prevention of Salmonella Enteritidis in Shell Eggs during Production, Storage, and Transportation," shell eggs intended for human consumption are required to be held or transported at or below 45°F (7.2°C) ambient temperature beginning 36 h after time of lay. Meanwhile, eggs in hatcheries are typically stored at a temperature of 65°F (18.3°C). Although most of those eggs are directed to incubators for hatching, excess eggs have the potential to be diverted for human consumption as egg products through the "breaker" market if these eggs are refrigerated in accordance with FDA's requirement. Combining risk assessment models developed by the U.S. Department of Agriculture's Food Safety and Inspection Service for shell eggs and for egg products, we quantified and compared Salmonella Enteritidis levels in eggs held at 65°F versus 45°F, Salmonella Enteritidis levels in the resulting egg products, and the risk of human salmonellosis from consumption of those egg products. For eggs stored 5 days at 65°F (following 36 h at 75°F [23.9°C] in the layer house), the mean level of Salmonella Enteritidis contamination is 30-fold higher than for eggs stored at 45°F. These increased levels of contamination lead to a 47-fold increase in the risk of salmonellosis from consumption of egg products made from these eggs, with some variation in the public health risk on the basis of the egg product type (e.g., whole egg versus whole egg with added sugar). Assuming that 7% of the liquid egg product supply originates from eggs stored at 65°F versus 45°F, this study estimates an additional burden of 3,562 cases of salmonellosis per year in the United States. A nominal range uncertainty analysis suggests that the relative increase in the risk linked to the storage of eggs at higher temperature estimated in this study is robust to the uncertainty surrounding the model parameters. The diversion of eggs from broiler production to human consumption under the current storage practices of 65°F (versus 45°F) would present a substantive overall increase in the risk of salmonellosis. ISSN: 0362028X

Pires, S.M., Jakobsen, L.S., Ellis-Iversen, J., Pessoa, J., Ethelberg, S.
Burden of Disease Estimates of Seven Pathogens Commonly Transmitted Through Foods in Denmark, 2017
(2020) Foodborne Pathogens and Disease, 17 (5), pp. 322-339.
ABSTRACT: Burden of disease metrics are increasingly established to prioritize food safety interventions. We estimated the burden of disease caused by seven foodborne pathogens in Denmark in 2017: Campylobacter, Salmonella, Shiga toxin-producing Escherichia coli, norovirus, Yersinia enterocolitica, Listeria monocytogenes, and Toxoplasma gondii. We used public health surveillance data and scientific literature to estimate incidence, mortality, and total disability-adjusted life year (DALY) of each, and linked results with estimates of the proportion of disease burden that is attributable to foods. Our estimates showed that Campylobacter caused the highest burden of disease, leading to a total burden of 1709 DALYs (95% uncertainty interval [UI] 1665-1755), more than threefold higher than the second highest ranked pathogen (Salmonella: 492 DALYs; 95% UI 481-504). Campylobacter still led the ranking when excluding DALYs attributable to nonfoodborne routes of exposure. The total estimated incidence was highest for norovirus, but this agent ranked sixth when focusing on foodborne burden. Salmonella ranked second in terms of foodborne burden of disease, followed by Listeria and Yersinia. Foodborne congenital toxoplasmosis was estimated to cause the loss of ~100 years of healthy life, a burden that was borne by a low number of cases in the population. The ranking of

foodborne pathogens varied substantially when based on reported cases, estimated incidence, and burden of disease estimates. Our results reinforce the need to continue food safety efforts throughout the food chain in Denmark, with a particular focus on reducing the incidence of Campylobacter infections. ISSN: 15353141

Eyler, A.B., M'ikanatha, N.M., Xiaoli, L., Dudley, E.G.
Whole-genome sequencing reveals resistome of highly drug-resistant retail meat and human Salmonella Dublin
(2020) Zoonoses and Public Health, 67 (3), pp. 251-262.
ABSTRACT: Non-typhoidal Salmonella (NTS) are a significant source of foodborne illness worldwide, with disease symptoms most often presenting as self-limiting gastroenteritis; however, occasionally the infection spreads and becomes invasive, frequently requiring anti-microbial treatment. The cattle-adapted Dublin serovar of NTS has commonly been associated with invasive illness and anti-microbial resistance (AMR). Here, the enhanced resolution conferred by whole-genome sequencing was utilized to elucidate and compare the resistome and genetic relatedness of 14 multidrug-resistant (MDR) and one pan-susceptible S. Dublin, isolated primarily in Pennsylvania, from fresh retail meat (one isolate) and humans (14 isolates). Twelve different genetic AMR determinants, including both acquired and chromosomal, were identified. Furthermore, comparative plasmid analysis indicated that AMR was primarily conferred by a putative IncA/C2 plasmid. A single pan-susceptible S. Dublin isolate, collected from the same timeframe and geographical region as the MDR isolates, did not carry an IncA/C2 replicon sequence within its genome. Moreover, the pan-susceptible isolate was genetically distinct from its MDR counterparts, as it was separated by ≥267 single nucleotide polymorphisms (SNPs), whereas there was a ≤38 SNP distance between the MDR isolates. Collectively, this data set advances our understanding of the genetic basis of the highly drug-resistant nature of S. Dublin, a serovar with significant public health implications. ISSN: 18631959

Merlotti, A., Manfreda, G., Munck, N., Hald, T., Litrup, E., Nielsen, E.M., Remondini, D., Pasquali, F.
Network Approach to Source Attribution of Salmonella enterica Serovar Typhimurium and Its Monophasic Variant
(2020) Frontiers in Microbiology, 11, art. no. 1205, .
ABSTRACT: Salmonella enterica subspecies enterica serovar Typhimurium and its monophasic variant are among the most common Salmonella serovars associated with human salmonellosis each year. Related infections are often due to consumption of contaminated meat of pig, cattle, and poultry origin. In order to evaluate novel microbial subtyping methods for source attribution, an approach based on weighted networks was applied on 141 human and 210 food and animal isolates of pigs, broilers, layers, ducks, and cattle collected in Denmark from 2013 to 2014. A whole-genome SNP calling was performed along with cgMLST and wgMLST. Based on these genomic input data, pairwise distance matrices were built and used as input for construction of a weighted network where nodes represent genomes and links to distances. Analyzing food and animal Typhimurium genomes, the coherence of source clustering ranged from 89 to 90% for animal source, from 84 to 85% for country, and from 63 to 65% for year of isolation and was equal to 82% for serotype, suggesting animal source as the first driver of clustering formation. Adding human isolate genomes to the network, a percentage between 93.6 and 97.2% clustered with the existing component and only a percentage between 2.8 and 6.4% appeared as not attributed to any animal sources. The majority of human genomes were attributed to pigs with probabilities ranging from 83.9 to 84.5%, followed by broilers, ducks, cattle, and layers in descending order. In conclusion, a weighted network approach based on pairwise SNPs, cgMLST, and wgMLST matrices showed promising results for source attribution studies. ISSN: 1664302X

Crouse, A., Schramm, C., Emond-Rheault, J.-G., Herod, A., Kerhoas, M., Rohde, J., Gruenheid, S., Kukavica-Ibrulj, I., Boyle, B., Greenwood, C.M.T., Goodridge, L.D., Garduno, R., Levesque, R.C., Malo, D., Daigle, F.
Combining Whole-Genome Sequencing and Multimodel Phenotyping To Identify Genetic Predictors of Salmonella Virulence
(2020) mSphere, 5 (3), .
ABSTRACT: Salmonella comprises more than 2,600 serovars. Very few environmental and uncommon serovars have been characterized for their potential role in virulence and human infections. A complementary in vitro and in vivo systematic high-throughput analysis of virulence was used to elucidate the association between genetic and phenotypic variations across Salmonella isolates. The goal was to develop a strategy for the classification of isolates as a benchmark and predict virulence levels of isolates. Thirty-five

phylogenetically distant strains of unknown virulence were selected from the Salmonella Foodborne Syst-OMICS (SalFoS) collection, representing 34 different serovars isolated from various sources. Isolates were evaluated for virulence in 4 complementary models of infection to compare virulence traits with the genomics data, including interactions with human intestinal epithelial cells, human macrophages, and amoeba. In vivo testing was conducted using the mouse model of Salmonella systemic infection. Significant correlations were identified between the different models. We identified a collection of novel hypothetical and conserved proteins associated with isolates that generate a high burden. We also showed that blind prediction of virulence of 33 additional strains based on the pan-genome was high in the mouse model of systemic infection (82% agreement) and in the human epithelial cell model (74% agreement). These complementary approaches enabled us to define virulence potential in different isolates and present a novel strategy for risk assessment of specific strains and for better monitoring and source tracking during outbreaks.IMPORTANCESalmonella species are bacteria that are a major source of foodborne disease through contamination of a diversity of foods, including meat, eggs, fruits, nuts, and vegetables. More than 2,600 different Salmonella enterica serovars have been identified, and only a few of them are associated with illness in humans. Despite the fact that they are genetically closely related, there is enormous variation in the virulence of different isolates of Salmonella enterica Identification of foodborne pathogens is a lengthy process based on microbiological, biochemical, and immunological methods. Here, we worked toward new ways of integrating whole-genome sequencing (WGS) approaches into food safety practices. We used WGS to build associations between virulence and genetic diversity within 83 Salmonella isolates representing 77 different Salmonella serovars. Our work demonstrates the potential of combining a genomics approach and virulence tests to improve the diagnostics and assess risk of human illness associated with specific Salmonella isolates. ISSN: 23795042

Jones, D.R., Gast, R.K., Regmi, P., Ward, G.E., Anderson, K.E., Karcher, D.M.
Pooling of laying hen environmental swabs and efficacy of salmonella detection (2020) Journal of Food Protection, 83 (6), pp. 943-950.
ABSTRACT: Environmental testing for Salmonella Enteritidis is required for U.S. shell egg producers with 3,000 hens on a farm. The egg producer assumes all costs for the mandatory testing. According to the U.S. Food and Drug Administration (FDA) Egg Rule, either manure scraper or drag swabs can be collected according to published guidelines and requirements. The present study was undertaken to determine the efficacy of Salmonella detection with one-, two-, and four-swab pools of either manure scraper or drag swabs. Resistant isolates of Salmonella serovars Enteritidis (1,000 ppm of streptomycin), Heidelberg (200 ppm of nalidixic acid [NA]), Typhimurium (200 ppm of NA), and Kentucky (200 ppm of NA) were utilized. Low (approximately 8.4 CFU) and high (approximately 84 CFU) levels of inocula were introduced onto a single swab within a pool. Single flocks from each conventional cage (manure scraper swabs) and cage-free barn (drag swabs) were monitored throughout the study at the ages required under the FDA Egg Rule. The highest and most consistent recovery of inoculum was found in single swab samples. For low dose inocula, recovery of isolates was low from single manure scraper swabs (57.9 to 29.2%) and decreased as more swabs were added to the pool. Recovery of isolates from manure scraper swabs was higher for high dose inocula, although Salmonella Heidelberg was outcompeted by the naturally occurring flora and had the lowest rate of recovery among the isolates tested. One- and two-swab pools of drag swabs had similar rates of recovery at both low and high doses for Salmonella Enteritidis, Salmonella Heidelberg, and Salmonella Typhimurium. When Salmonella Enteritidis and Salmonella Kentucky were combined in an inoculum, Salmonella Enteritidis was recovered at a much higher rate than was Salmonella Kentucky for all types of swabs and doses of inocula. Pooling of two drag swabs allowed for similar detection of low and high dose Salmonella, but the pooling of manure scraper swabs decreased detection of low dose Salmonella. ISSN: 0362028X

Guillén, S., Marcén, M., Mañas, P., Cebrián, G.
Differences in resistance to different environmental stresses and non-thermal food preservation technologies among Salmonella enterica subsp. enterica strains (2020) Food Research International, 132, art. no. 109042, .
ABSTRACT: In this work the resistance of 15 strains belonging to 11 serovars of Salmonella enterica subsp. enterica to several different environmental stresses (acid, hydrogen peroxide, NaCl and heat) and non-thermal food preservation technologies (HHP, PEF, UV) was determined and compared. Results obtained showed that differences in resistance among strains, quantified as 2D-values, varied less than 2.4-fold for all agents, including heat if S. senftenberg 775W is excluded from the analysis. These results also

indicate that variability in resistance among strains of the same serovar was comparable to inter-serovar variability. Salmonella strains that were the most resistant to a given stress were not more resistant to other types of stress. Nevertheless, a positive correlation was observed between the resistance of Salmonella strains to oxidative and osmotic stress, as well as between UV and PEF resistance. These results would be especially helpful in defining safe food preservation processes and might be very useful for improving quantitative microbiological risk assessments of Salmonella in food products.
ISSN: 09639969

Sodagari, H.R., Habib, I., Shahabi, M.P., Dybing, N.A., Wang, P., Bruce, M.
A review of the public health challenges of salmonella and turtles
(2020) Veterinary Sciences, 7 (2), art. no. 56, .
ABSTRACT: Non-typhoidal Salmonella serovars are recognized as zoonotic pathogens. Although human salmonellosis is frequently associated with ingestion of contaminated foods of animal origin, contact with animals may also be a significant source of Salmonella infection, especially contact with turtles, which have shown to be an important reservoir of Salmonella, specifically through their intestinal tracts. Turtles are among the most common reptiles kept as house pets that may pose a public health risk associated with Salmonella exposure, especially among infants and young children. This review discusses the literature reporting the link between turtles and Salmonella as well as turtle-associated human salmonellosis in the last ten years. In most outbreaks, a high proportion of patients are children under five years of age, which indicates that children are at the greatest risk of turtle-associated salmonellosis. Therefore, turtles should not be preferred as recommended pets for children under five years of age. Reducing turtle stress to minimise Salmonella shedding as well as providing client education handouts at the points of sale of these animals may reduce the risk of transmitting such significant pathogen to humans. Further studies are required to investigate the role of both direct contact with turtles as well as indirect contact through cross-contamination in the transmission of turtles-associated Salmonella to humans. ISSN: 23067381

Sullivan, G., Guo, X., Tokman, J.I., Roof, S., Trmcic, A., Baker, R.C., Tang, S., Markwell, P., Wiedmann, M., Kovac, J.
Extended enrichment procedures can be used to define false-negative probabilities for cultural gold standard methods for salmonella detection, facilitating comparisons between gold standard and alternative methods
(2020) Journal of Food Protection, 83 (6), pp. 1030-1037.
ABSTRACT: Evaluation of alternative detection methods for foodborne pathogens typically involves comparisons against a "gold standard" culture method, which may produce false-negative (FN) results, particularly under worst-case scenarios such as low contamination levels, difficult-to-detect strains, and challenging food matrices (e.g., matrices with a water activity of ,0.6). We used extended enrichment times (up to 72 h for both primary and secondary enrichments) to evaluate a gold standard method for Salmonella detection (the U.S. Food and Drug Administration Bacteriological Analytical Manual [BAM] method) in two lowwater- activity foods (dry pet food and chocolate) inoculated at low contamination levels (most probable number ca. 1/25 g) with five Salmonella strains. Strains were selected to include those with a poor ability to grow in enrichment media. Among the 100 pet food and 100 chocolate samples tested, 53 and 50, respectively, were positive with the standard BAM method, and 57 and 59, respectively, were positive with the extended BAM method. Thus, the FN probabilities for the standard BAM method were 7% for pet food and 15% for chocolate. An alternative enzyme immunoassay method for detection of Salmonella in chocolate produced FN probabilities of 6 and 20% when compared against the standard and extended BAM methods, respectively. Detection of Salmonella Mississippi was significantly reduced with the alternative method ($P = 0.023$) compared with the extended BAM method. We calculated a composite reference standard to further define FN probabilities based on variable results from multiple assays (the standard BAM, extended BAM, and alternative methods). Based on this standard, the enzyme immunoassay for Salmonella detection in chocolate had a 28% FN probability and the standard and extended BAM methods had 23 and 9% FN probabilities, respectively. These results provide a framework for how inclusion of extended enrichment times can facilitate evaluation of alternative detection methods. ISSN: 0362028X

Ren, J., Man, Y., Li, A., Liang, G., Jin, X., Pan, L.
Detection of Salmonella enteritidis and Salmonella typhimurium in foods using a rapid, multiplex real-time recombinase polymerase amplification assay
(2020) Journal of Food Safety, 40 (3), art. no. e12784, .

ABSTRACT: Salmonella has been recognized as a major foodborne pathogen for humans and animals. In this study, a multiplex real-time recombinase polymerase amplification (RPA) was developed for simultaneous detection of Salmonella enterica serovars, Salmonella enteritidis and Salmonella typhimurium, from chicken, eggs, lettuce, and papaya. The reaction was performed for 20 min at 35°C, and the detection limit of the assay was 102 CFU/ml for pure culture. In food application, the limit of detection (LOD) of S. enteritidis and S. typhimurium using multiplex real-time RPA without enrichment procedure was 102 CFU/25 g, respectively. After enrichment, the LOD of S. enteritidis and S. typhimurium was 10 CFU/25 g. Moreover, the result for Salmonella spp. was not significantly different from those obtained using a culture-based method. Additionally, the assay has a lower cross-reactivity with other pathogenic microorganisms and a good stability performance. Thus, the developed multiplex RPA assay could be used as a rapid tool for the detection of S. enteritidis and S. typhimurium in food. ISSN: 01496085

Assaf, A., Grangé, E., Cordella, C.B.Y., Rutledge, D.N., Lees, M., Lahmar, A., Thouand, G.
Evaluation of the impact of buffered peptone water composition on the discrimination between Salmonella enterica and Escherichia coli by Raman spectroscopy
(2020) Analytical and Bioanalytical Chemistry, 412 (15), pp. 3595-3604.
ABSTRACT: The detection of Salmonella spp. in food samples is regulated by the ISO 6579:2002 standard, which requires that precise procedures are followed to ensure the reliability of the detection process. This standard requires buffered peptone water as a rich medium for the enrichment of bacteria. However, the effects of different brands of buffered peptone water on the identification of microorganisms by Raman spectroscopy are unknown. In this regard, our study evaluated the discrimination between two bacterial species, Salmonella enterica and Escherichia coli, inoculated and analyzed with six of the most commonly used buffered peptone water brands. The results showed that bacterial cells behaved differently according to the brand used in terms of biomass production and the spectral fingerprint. The identification accuracy of the analyzed strains was between 85% and 100% depending on the given brand. Several batches of two brands were studied to evaluate the classification rates between the analyzed bacterial species. The chemical analysis performed on these brands showed that the nutrient content was slightly different and probably explained the observed effects. On the basis of these results, Raman spectroscopy operators are encouraged to select an adequate culture medium and continue its use throughout the identification process to guarantee optimal recognition of the microorganism of interest. ISSN: 16182642

Smith, O.M., Snyder, W.E., Owen, J.P.
Are we overestimating risk of enteric pathogen spillover from wild birds to humans?
(2020) Biological Reviews, 95 (3), pp. 652-679.
ABSTRACT: Enteric illnesses remain the second largest source of communicable diseases worldwide, and wild birds are suspected sources for human infection. This has led to efforts to reduce pathogen spillover through deterrence of wildlife and removal of wildlife habitat, particularly within farming systems, which can compromise conservation efforts and the ecosystem services wild birds provide. Further, Salmonella spp. are a significant cause of avian mortality, leading to additional conservation concerns. Despite numerous studies of enteric bacteria in wild birds and policies to discourage birds from food systems, we lack a comprehensive understanding of wild bird involvement in transmission of enteric bacteria to humans. Here, we propose a framework for understanding spillover of enteric pathogens from wild birds to humans, which includes pathogen acquisition, reservoir competence and bacterial shedding, contact with people and food, and pathogen survival in the environment. We place the literature into this framework to identify important knowledge gaps. Second, we conduct a meta-analysis of prevalence data for three human enteric pathogens, Campylobacter spp., E. coli, and Salmonella spp., in 431 North American breeding bird species. Our literature review revealed that only 3% of studies addressed the complete system of pathogen transmission. In our meta-analysis, we found a Campylobacter spp. prevalence of 27% across wild birds, while prevalence estimates of pathogenic E. coli (20%) and Salmonella spp. (6.4%) were lower. There was significant bias in which bird species have been tested, with most studies focusing on a small number of taxa that are common near people (e.g. European starlings Sturnus vulgaris and rock pigeons Columba livia) or commonly in contact with human waste (e.g. gulls). No pathogen prevalence data were available for 65% of North American breeding bird species, including many commonly in contact with humans (e.g. black-billed magpie Pica hudsonia and great blue heron Ardea herodias), and our metadata suggest that some under-studied species, taxonomic groups, and guilds may represent equivalent or greater risk to human infection than heavily studied species. We conclude that current data do not provide

sufficient information to determine the likelihood of enteric pathogen spillover from wild birds to humans and thus preclude management solutions. The primary focus in the literature on pathogen prevalence likely overestimates the probability of enteric pathogen spillover from wild birds to humans because a pathogen must survive long enough at an infectious dose and be a strain that is able to colonize humans to cause infection. We propose that future research should focus on the large number of under-studied species commonly in contact with people and food production and demonstrate shedding of bacterial strains pathogenic to humans into the environment where people may contact them. Finally, studies assessing the duration and intensity of bacterial shedding and survival of bacteria in the environment in bird faeces will help provide crucial missing information necessary to calculate spillover probability. Addressing these essential knowledge gaps will support policy to reduce enteric pathogen spillover to humans and enhance bird conservation efforts that are currently undermined by unsupported fears of pathogen spillover from wild birds. ISSN: 14647931

Fikiin, K., Akterian, S., Stankov, B.
Do raw eggs need to be refrigerated along the food chain?: Is the current EU regulation ensuring high-quality shell eggs for the European consumers?
(2020) Trends in Food Science and Technology, 100, pp. 359-362.
ABSTRACT: Background: EC Regulation No. 589/2008 for egg handling contains a number of incongruities and incompleteness, which confuse the EU food chain operators and consumers. The major inconsistencies, challenged in this comment article, result from: (i) overemphasis on the possibility of eggshell condensation and Salmonella-related safety risks, while overlooking other substantial safety and quality hazards, and (ii) obscure or missing temperature and humidity control requirements, which inspires fear from chilled storage but tolerates handling at high temperatures. Scope and approach: The regulation's performance is deliberated as regards its geographical coverage and applicability under natural and artificial conditions in various climatic regions across Europe. A brief outline of published scientific evidence clearly demonstrates that a continuous and ubiquitous cold chain, along with a proper humidity control and anti-condensation measures (where necessary), dramatically improve egg safety, quality and shelf-life. Several alternative legislations around the world are also recapped in this context. Key findings and conclusions: The applicable EU egg control regulation needs to be carefully reconsidered and updated by introducing temperature and humidity conditions correlated with resulting safety, quality and shelf life. Substantial efforts should also be made to harmonise the huge discrepancies between relevant codes and practices in Europe, USA and the rest of the world. ISSN: 09242244

Castellanos, L.R., van der Graaf-van Bloois, L., Donado-Godoy, P., Veldman, K., Duarte, F., Acuña, M.T., Jarquín, C., Weill, F.-X., Mevius, D.J., Wagenaar, J.A., Hordijk, J., Zomer, A.L.
Antimicrobial Resistance in Salmonella enterica Serovar Paratyphi B Variant Java in Poultry from Europe and Latin America
(2020) Emerging infectious diseases, 26 (6), pp. 1164-1173.
ABSTRACT: Salmonella enterica serovar Paratyphi B variant Java sequence type 28 is prevalent in poultry and poultry meat. We investigated the evolutionary relatedness between sequence type 28 strains from Europe and Latin America using time-resolved phylogeny and principal component analysis. We sequenced isolates from Colombia, Guatemala, Costa Rica, and the Netherlands and complemented them with publicly available genomes from Europe, Africa, and the Middle East. Phylogenetic time trees and effective population sizes (Ne) showed separate clustering of strains from Latin America and Europe. The separation is estimated to have occurred during the 1980s. Ne of strains increased sharply in Europe around 1995 and in Latin America around 2005. Principal component analysis on noncore genes showed a clear distinction between strains from Europe and Latin America, whereas the plasmid gene content was similar. Regardless of the evolutionary separation, similar features of resistance to β-lactams and quinolones/fluoroquinolones indicated parallel evolution of antimicrobial resistance in both regions. ISSN: 10806059