NEWSLETTER

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Editorial Note

Bilthoven, 2 October 2020

Dear colleague,

I do hope that you are all still healthy and are not hit too hard by the second wave of the **SARS-CoV-2 virus**. The situation with the virus is still very unsure and unstable and may differ a lot per country, or even per region or city within a country. We understand very well that the COVID-19 pandemic may also influence your laboratory work, due to lack of resources and/or lack of staff. We are regularly facing similar problems. Still we will try to organise the **EURL-*Salmonella* Proficiency Tests** as scheduled. However, if you are facing problems in participation in these PTs, please inform us immediately and we will try to find a tailor-made solution for you, if possible.

As you know, we organised the first **PT on detection of *Salmonella* in bivalve molluscs (mussels)** during the first wave of COVID-19 in March of this year. Due to restrictions of the laboratory activities as a result of the COVID-19 pandemic, only 14 NRLs-*Salmonella* were able to perform this PT at the scheduled time. Therefore, we organised a second round of this PT for the remaining 9 NRLs-*Salmonella* in August, including a follow-up study for one NRL not performing well in the first round. All participants received their individual results in the first week of September (or earlier) and the first results of all participants were presented at the EURL-*Salmonella* workshop on 17 September (also see: link). We were happy to see that, as a final outcome, all NRLs-*Salmonella* investigating bivalve molluscs performed well in this PT. The interim summary of this PT will soon be shared with the participating NRLs as well as on the EURL-*Salmonella* website.

Earlier this week the samples were sent to the participants of the **PT on detection of *Salmonella* in samples from the Primary Production Stage (PPS) and in Food 2020.** The samples concern artificially contaminated hygiene sponges, and NRLs-*Salmonella* investigating PPS samples as well as NRLs-*Salmonella* investigating Food samples can participate. In total 37 NRLs-*Salmonella* analysing PPS samples and 29 NRLs-*Salmonella* analysing Food samples subscribed for this study. We do hope that all NRLs can participate without problems. The timetable for this study was published in the previous Newsletter and was shared with the participants. For your information, we include it again 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*, as well as a voluntary part on cluster analysis. The timetable for this study was included in the previous Newsletter as well as in this one.

On 17 and 18 September we organised **the 25th EURL-*Salmonella* workshop**, which was turned into an online workshop due to the COVID-19 pandemic. This was a new experience for us, as well as for the NRLs-*Salmonella*. Still it went, luckily, quite well despite the large number of approx. 72 participants. We received a lot of positive feed-back from the participants, which we highly appreciate. The presentations, which the presenters allowed us to make public, are available at the EURL-*Salmonella* website: link.

From 18 May until 16 August 2020 it was possible to vote for the launching of the New Work Item Proposal (NWIP) in ISO and CEN of '**Draft CEN 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 outcome of the voting in ISO and CEN was 100% approval

with only a few comments. The next steps will be to prepare the document in the CEN/ISO format and to review the comments. Next, this will be discussed in an online meeting of the Working group (WG)10 of ISO/TC34/SC9 in November 2020.

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.

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.

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From the Literature

Salmonella-related Literature from Scopus: July – September 2020

Wang, Y., Miao, X., Li, H., Su, P., Lin, L., Liu, L., Li, X.
The correlated expression of immune and energy metabolism related genes in the response to Salmonella enterica serovar Enteritidis inoculation in chicken
(2020) BMC Veterinary Research, 16 (1), art. no. 257, .
ABSTRACT: Abstract: Background: Salmonella enterica serovar Enteritidis (SE) is one of the food-borne pathogenic bacteria, which affects poultry production and poses severe threat to human health. The correlation of immune system and metabolism in chicken after SE inoculation is important but not clear. In the current study, we identified the expression of immune and energy metabolism related genes using quantitative PCR to evaluate the correlation between immune system and energy metabolism against SE inoculation in Jining Bairi chicken. Results: ATP5G1, ATP5G3 and ND2 were significantly up-regulated at 1 dpi (day post inoculation), and ATP5E, ATP5G1, ATP5G3 were significantly down-regulated at 7 dpi ($P < 0.05$). IL-8 and IL-1β were significantly down-regulated at 1 dpi, IL-8 and IL-18 were significantly down-regulated at 3 dpi, IL-8 and BCL10 were significantly up-regulated at 7 dpi ($P < 0.05$). Conclusions: These findings indicate that the correlation between immune and energy metabolism related genes gradually change with time points post SE inoculation, from one homeostasis to an opposite homeostasis with 3 dpi as a turning point. These results will pave the foundation for the relationship between immune system and energy metabolism in the response to SE inoculation in chicken. ISSN: 17466148

Jensen, A.N., Hansen, S.H., Baggesen, D.L.
Salmonella Typhimurium Level in Mealworms (Tenebrio molitor) After Exposure to Contaminated Substrate
(2020) Frontiers in Microbiology, 11, art. no. 1613, .
ABSTRACT: Findings of viable Salmonella spp., which are important foodborne pathogens, are seemingly not reported in mealworms (Tenebrio molitor) for feed and food. Still, the bacterial load of mealworms is naturally high and includes members of the Enterobacteriaceae family to which Salmonella belong. This indicates that Salmonella may be able to thrive in mealworms if introduced into the production. Therefore, this study aimed to assess the quantitative level of Salmonella enterica serovar Typhimurium (ST) in mealworms over a 14-day course after exposure to substrate contaminated with ST levels from 1.7 to 7.4 log CFU/g at start (i.e., day 0). The level of ST found in larvae was below the quantitative detection level (1 or 2 log CFU/g) on day 1 in larvae exposed to contamination levels of 1.7, 3.4, and 3.6 log CFU/g opposed to contamination levels of 5.4, 5.6, and 7.4 log CFU/g, respectively. The maximum level of ST detected in individual 1-g larvae samples was 5.8 log CFU/g, but the level varied among the triplicate samples from each sampling, and the highest average value was 5.3 ± 0.3. Beyond day 7, only larvae exposed to the contamination level of 7.4 log CFU/g were >1.0 log CFU/g in the triplicate samples. However, qualitative testing (10 g) showed the presence of ST in larvae until the end of the experiment on day 14 except for the lowest contamination level of 1.7 log CFU/g. Parallel testing of surface disinfected larvae indicated that some larvae may be ST-positive due to Salmonella residing on the surface only. Still, any detection of Salmonella is of concern from a food safety perspective. In substrate with contamination levels below 3.6 log CFU/g, the level of ST was below the quantitative detection limit within a few days. Still, ST was detected until the end of experiment on day 14 except for the lowest contamination level of 1.7 log CFU/g. This study indicates the importance of avoiding introduction of Salmonella into the production, e.g., via contaminated substrate in order to avoid Salmonella-positive larvae as they remained positive for at least 14 days (except at the lowest contamination level). ISSN: 1664302X

García-Soto, S., Abdel-Glil, M.Y., Tomaso, H., Linde, J., Methner, U.
Emergence of Multidrug-Resistant Salmonella enterica Subspecies enterica Serovar Infantis of Multilocus Sequence Type 2283 in German Broiler Farms
(2020) Frontiers in Microbiology, 11, art. no. 1741, .
ABSTRACT: During the last decade, Salmonella enterica subspecies enterica serovar Infantis (S. Infantis) has become more prevalent across Europe with an increased capability to persist in broiler farms. In this study, we aimed to identify potential genetic causes for the increased emergence and longer persistence of S. Infantis in German

poultry farms by high-throughput-sequencing. Broiler derived S. Infantis strains from two decades, the 1990s (n = 12) and the 2010s (n = 18), were examined phenotypically and genotypically to detect potential differences responsible for increased prevalence and persistence. S. Infantis organisms were characterized by serotyping and determining antimicrobial susceptibility using the microdilution method. Genotypic characteristics were analyzed by whole genome sequencing (WGS) to detect antimicrobial resistance and virulence genes as well as plasmids. To detect possible clonal relatedness within S. Infantis organisms, 17 accessible genomes from previous studies about emergent S. Infantis were downloaded and analyzed using complete genome sequence of SI119944 from Israel as reference. In contrast to the broiler derived antibiotic-sensitive S. Infantis strains from the 1990s, the majority of strains from the 2010s (15 out of 18) revealed a multidrug-resistance (MDR) phenotype that encodes for at least three antimicrobials families: aminoglycosides [ant(3")-Ia], sulfonamides (sul1), and tetracyclines [tet(A)]. Moreover, these MDR strains carry a virulence gene pattern missing in strains from the 1990s. It includes genes encoding for fimbriae clusters, the yersiniabactin siderophore, mercury and disinfectants resistance and toxin/antitoxin complexes. In depth genomic analysis confirmed that the 15 MDR strains from the 2010s carry a pESI-like megaplasmid with resistance and virulence gene patterns detected in the emerged S. Infantis strain SI119944 from Israel and clones inside and outside Europe. Genotyping analysis revealed two sequence types (STs) among the resistant strains from the 2010s, ST2283 (n = 13) and ST32 (n = 2). The sensitive strains from the 1990s, belong to sequence type ST32 (n = 10) and ST1032 (n = 2). Therefore, this study confirms the emergence of a MDR S. Infantis pESI-like clone of ST2283 in German broiler farms with presumably high tendency of dissemination. Further studies on the epidemiology and control of S. Infantis in broilers are needed to prevent the transfer from poultry into the human food chain.
ISSN: 1664302X

Li, Y, Yang, X, Zhang, H, Jia, H, Liu, X, Yu, B, Zeng, Y, Zhang, Y, Pei, X, Yang, D.
Prevalence and antimicrobial susceptibility of Salmonella in the commercial eggs in China (2020) International Journal of Food Microbiology, 325, art. no. 108623, .
ABSTRACT: Salmonellosis is a challenge to public health globally, and many infections have been principally linked to the consumption of contaminated eggs. The objective of this study is to estimate the prevalence of Salmonella in commercial eggs and susceptibilities of isolates to a panel of 14 antimicrobial agents which were determined according to Clinical and Laboratory Standards Institute (CSLI) procedures. A total of 33,288 eggs (5548 pooled samples of six eggs) were collected across China in 2016 and the prevalence of Salmonella was 0.5% (27/5548). The most predominant serotype was S. enteritidis. No significant differences were observed on the basis of the egg component tested, shell condition, packaging type, sampling site or sampling season. However, there were significant differences among provincial regions. About 64.3% (n = 18) isolates were resistant to nalidixic acid, followed by ampicillin (39.3%) and ampicillin/sulbactam (39.3%). All isolates were susceptible to ceftazidime, cefalothin, ciprofloxacin, cefepime, cefotaxime, imipenem and meropenem. Three Salmonella isolates exhibited resistance to multiple antibiotics. This study provides valuable baseline data of the occurrence of Salmonella in eggs, which will be used for risk assessments of possible human foodborne infections associated with the consumption of contaminated eggs. ISSN: 01681605

Lopes, I.G., Lalander, C., Vidotti, R.M., Vinnerås, B.
Reduction of Bacteria in Relation to Feeding Regimes When Treating Aquaculture Waste in Fly Larvae Composting
(2020) Frontiers in Microbiology, 11, art. no. 1616, .
ABSTRACT: This study evaluated the impact of feeding regimes on process performance and inactivation of microorganisms during treatment of aquaculture waste with black soldier fly (BSF) larvae. In three treatments (T1–T3), a blend of reclaimed bread and aquaculture waste was used as substrate for BSF larvae. In T1, the substrate was inoculated with four subtypes of Salmonella spp. and Escherichia coli (both at 1% w/w), and offered only once, at the beginning of the 14-day trial. In T2 and T3, the substrate was supplied on three different days, with contaminated substrate provided only the first event in T2 and in all three events in T3. Provision of a lump sum feeding (T1) proved unfavorable for larval growth and process efficiency, but did not affect the microbial reduction effect. The total reduction in Salmonella spp. was approximately 6 log10 in T1 and T2, and 3.3 log10 in T3, while the total reduction in E. coli was approximately 4 log10 in T1 and T2, and 1.9 log10 in T3. After removing the larvae, the treatment residues were re-inoculated with Salmonella spp. and E. coli. It was found that the inactivation in both organisms continued in all treatments that originally contained BSF larvae (T1–T3),

suggesting that antimicrobial substances may have been secreted by BSF larvae or by its associated microbiota. ISSN: 1664302X

Sodagari, H.R., Wang, P., Robertson, I., Habib, I., Sahibzada, S.
Non-typhoidal salmonella at the human-food-of-animal-origin interface in Australia
(2020) Animals, 10 (7), art. no. 1192, pp. 1-33.
ABSTRACT: Non-typhoidal Salmonella is a major zoonotic pathogen that plays a significant role in foodborne human salmonellosis worldwide through the consumption of contaminated foods, particularly those of animal origin. Despite a considerable reduction in human salmonellosis outbreaks in developed countries, Australia is experiencing a continuous rise of such outbreaks in humans. This review of the literature highlights the reported non-typhoidal Salmonella outbreaks in humans as well as the occurrence of the pathogen in foods from animal sources throughout Australia. Non-typhoidal Salmonella infections from food animals are more often associated with at-risk people, such as immunocompromised and aged people or children. Although several animal-sourced foods were recognised as the catalysts for salmonellosis outbreaks in Australia, egg and egg-based products remained the most implicated foods in the reported outbreaks. This review further highlights the antimicrobial resistance trends of non-typhoidal Salmonella isolates at the human–food interface, with a focus on clinically important antimicrobials in humans, by collating evidence from previous investigations in Australia. The rise in antimicrobial-resistant Salmonella, especially to antimicrobials commonly prescribed to treat human salmonellosis, has become a significant global public health concern. However, the overall prevalence of antimicrobial resistance in Australia is considerably lower than in other parts of the world, particularly in terms of critically important antimicrobials for the treatment of human salmonellosis. The present review adds to our understanding of the global epidemiology of non-typhoidal Salmonella with emphasis on the past few decades in Australia. ISSN: 20762615

Oblessuc, P.R., Melotto, M.
A Simple Assay to Assess Salmonella enterica Persistence in Lettuce Leaves After Low Inoculation Dose
(2020) Frontiers in Microbiology, 11, art. no. 1516, .
ABSTRACT: Salmonella enterica is an enterobacterium associated with numerous foodborne illnesses worldwide. Leafy greens have been a common vehicle for disease outbreaks caused by S. enterica. This human pathogen can be introduced into crop fields and potentially contaminate fresh produce. Several studies have shown that S. enterica can survive for long periods in the plant tissues. Often, S. enterica population does not reach high titers in leaves; however, it is still relevant for food safety due to the low infective dose of the pathogen. Thus, laboratory procedures to study the survival of S. enterica in fresh vegetables should be adjusted accordingly. Here, we describe a protocol to assess the population dynamics of S. enterica serovar Typhimurium 14028s in the leaf apoplast of three cultivars of lettuce (Lactuca sativa L.). By comparing a range of inoculum concentrations, we showed that vacuum infiltration of a bacterium inoculum level in the range of 3.4 Log CFU ml−1 (with a recovery of approximately 170 cells per gram of fresh leaves 2 h post inoculation) allows for a robust assessment of bacterial persistence in three lettuce cultivars using serial dilution plating and qPCR methods. We anticipate that this method can be applied to other leaf–human pathogen combinations in an attempt to standardize the procedure for future efforts to screen for plant phenotypic variability, which is useful for breeding programs. ISSN: 1664302X

Um, M.M., Castonguay, M.H., Mahamad Amine, K., Giguère, J., Morin, I., Dufour, S.
Repeatability of a Commercially Available ELISA Test for Determining the Herd-Level Salmonella enterica subsp. enterica Serovar Dublin Status in Dairy Herds Using Bulk Milk
(2020) Frontiers in Veterinary Science, 7, art. no. 401, .
ABSTRACT: An Enzyme-Linked Immunosorbent Assay (ELISA) is currently available for detection of antibodies against Salmonella Dublin in bovine milk. However, when used in a surveillance program, samples may undergo various storage conditions. The objective of this study was to estimate the repeatability of an ELISA test when used on fresh and frozen samples. Each of 845 bulk milk collected samples was subdivided into 3 aliquots and analyzed using PrioCHECK™ Salmonella Ab Bovine Dublin. ELISA percent positivity results (PP%) were compared between aliquots submitted to the initial analysis and a second analysis conducted 24 h later. The third aliquots were either preserved for 13–14 days (n = 413) or 25–28 days (n = 432) at −20°C prior to analysis and results were compared to the initial analysis. There was excellent concordance between the two initial values and with values obtained after 13–14 and 25–28 days-freezing. The corresponding concordance correlation coefficients were 0.96, 0.97, and 0.94, respectively. Bland-Altman

plots showed differences of PP% of 0.1 percentage points on average between the initial and second fresh samples. Freezing for 13–14 and 25–28 days led to overestimation of the initial values by 0.1, and 0.4 percentage points, respectively. Regarding the classification of samples, greater disagreement was observed between 25 and 28 days-frozen and initial samples when using the cut-off 15% (kappa = 0.76) compared to 35% (kappa = 0.90). Our study showed that PrioCHECK™ has good repeatability and that frozen bulk milk samples could generate reliable results. However, the larger variability at lower PP% should be considered when setting up a threshold. ISSN: 22971769

Atterbury, R.J., Gigante, A.M., Rubio Lozano, M.D.L.S., Méndez Medina, R.D., Robinson, G., Alloush, H., Barrow, P.A., Allen, V.M.
Reduction of Salmonella contamination on the surface of chicken skin using bacteriophage (2020) Virology Journal, 17 (1), art. no. 98, .
ABSTRACT: Background: Enteric infections caused by Salmonella spp. remain a major public health burden worldwide. Chickens are known to be a major reservoir for this zoonotic pathogen. The presence of Salmonella in poultry farms and abattoirs is associated with financial costs of treatment and a serious risk to human health. The use of bacteriophages as a biocontrol is one possible intervention by which Salmonella colonization of chickens could be reduced. In a prior study, phages Eφ151 and Tφ7 significantly reduced broiler chicken caecal colonization by S. Enteritidis and S. Typhimurium respectively. Methods: Salmonella-free Ross broiler chickens were orally infected with S. Enteritidis P125109 or S. Typhimurium 4/74. After 7 days of infection, the animals were euthanased, and 25cm2 sections of skin were collected. The skin samples were sprayed with a phage suspension of either Eφ151 (S. Enteritidis), Tφ7 phage suspension (S. Typhimurium) or SM buffer (Control). After incubation, the number of surviving Salmonellas was determined by direct plating and Most Probable Number (MPN). To determine the rate of reduction of Salmonella numbers on the skin surface, a bioluminescent S. Typhimurium DT104 strain was cultured, spread on sections of chicken breast skin, and after spraying with a Tφ11 phage suspension, skin samples were monitored using photon counting for up to 24 h. Results: The median levels of Salmonella reduction following phage treatment were 1.38 log10 MPN (Enteritidis) and 1.83 log10 MPN (Typhimurium) per skin section. Treatment reductions were significant when compared with Salmonella recovery from control skin sections treated with buffer ($p < 0.0001$). Additionally, significant reduction in light intensity was observed within 1 min of phage Tφ11 spraying onto the skin contaminated with a bioluminescent Salmonella recombinant strain, compared with buffer-treated controls ($p < 0.01$), implying that some lysis of Salmonella was occurring on the skin surface. Conclusions: The results of this study suggest that phages may be used on the surface of chicken skin as biocontrol agents against Salmonella infected broiler chicken carcasses. The rate of bioluminescence reduction shown by the recombinant Salmonella strain used supported the hypothesis that at least some of the reduction observed was due to lysis occurred on the skin surface. ISSN: 1743422X

Wang, S., Liu, N., Zheng, L., Cai, G., Lin, J.
A lab-on-chip device for the sample-in-result-out detection of viable: Salmonella using loop-mediated isothermal amplification and real-time turbidity monitoring (2020) Lab on a Chip, 20 (13), pp. 2296-2305.
ABSTRACT: Rapid screening of foodborne pathogens is key to prevent food poisoning. In this study, a lab-on-chip device was developed for rapid, automatic and sensitive detection of viable Salmonella typhimurium using loop-mediated isothermal amplification (LAMP) and smartphone real-time turbidity monitoring. First, magnetic nanoparticles (MNPs) coated with anti-Salmonella capture antibodies in propidium monoazide (PMA) were fully mixed with bacterial samples using two active magnetic stirring mixers at reverse rotating directions, and incubated in the serpentine channel with 470 nm blue light exposure, allowing specific formation of magnetic bacteria and sufficient PMA pretreatment of the DNA of dead bacteria. Then, the PMA-treated magnetic bacteria were separated in the separation chamber using the magnetic field and their genomic DNA templates were extracted using lysis buffer at 70 °C. Finally, the viable bacteria's DNA was amplified using LAMP in the detection chamber preloaded with the lyophilized LAMP reagents at 67.5 °C after blocking with paraffin oil to avoid aerosol cross contamination. Finally, the turbidity of the LAMP reaction system was monitored in a real-time manner for the quantitative detection of viable bacteria. The experimental results demonstrated that this device was able to automatically detect viable Salmonella as low as 14 CFU mL-1 in spiked chicken meat supernatants within 1.5 h. This device is very promising to provide a sample-in-result-out solution for the in-field detection of Salmonella and could be easily extended for other foodborne pathogens. ISSN: 14730197

Villamil, C., Calderon, M.N., Arias, M.M., Leguizamon, J.E.
Validation of Droplet Digital Polymerase Chain Reaction for Salmonella spp. Quantification (2020) Frontiers in Microbiology, 11, art. no. 1512, .
ABSTRACT: Salmonellosis is a foodborne disease caused by Salmonella spp. Although cell culture is the gold standard for its identification, validated molecular methods are becoming an alternative, because of their rapidity, selectivity, and specificity. A simplex and duplex droplet digital polymerase chain reaction (ddPCR)-based method for the identification and quantification of Salmonella using ttr, invA, hilA, spaQ, and siiA gene sequences was validated. The method has high specificity, working interval between 8 and 8,000 cp/μL in ddPCR reaction, a limit of detection of 0.5 copies/μL, and precision ranging between 5 and 10% measured as a repeatability standard deviation. The relative standard measurement uncertainty was between 2 and 12%. This tool will improve food safety in national consumption products and will increase the competitiveness in agricultural product trade. ISSN: 1664302X

Zhou, A., Li, J., Xu, Z., Ni, J., Guo, J., Yao, Y.-F., Wu, W.
Whole-Genome Comparative and Pathogenicity Analysis of Salmonella enterica subsp. enterica Serovar Rissen
(2020) G3 (Bethesda, Md.), 10 (7), pp. 2159-2170.
ABSTRACT: Salmonella are a type of bacteria known to cause food-borne illness. Their host range varies widely, and their susceptibility to the host determines its pathogenicity. Salmonella enterica serovar Rissen (S Rissen) is a widely distributed serotype; however, its virulence and pathogenicity are poorly understood. In this study, the pathogenicity and antibiotic resistance of a representative S Rissen isolate were investigated. The cell model results showed that S Rissen preferred to replicate in human macrophage cells U937 compared to murine macrophage cells RAW264.7, suggesting that it has a level of host adaptability. Genome sequencing and comparison analysis revealed that the distribution and nonsynonymous single nucleotide polymorphisms of virulence factors in S Rissen were similar to those in S Typhi rather than to those in S Typhimurium. Taken together, our results suggest that although S Rissen is a common serotype distributed in swine herds, pork and chicken products, it has strong ability to infect humans. ISSN: 21601836

Ríos-Castillo, A.G., Ripolles-Avila, C., Rodríguez-Jerez, J.J.
Detection of Salmonella Typhimurium and Listeria monocytogenes biofilm cells exposed to different drying and pre-enrichment times using conventional and rapid methods (2020) International Journal of Food Microbiology, 324, art. no. 108611, .
ABSTRACT: The capacity of real-time PCR (RT-PCR), the VIDAS immunoassay system, and the conventional count method for detecting Salmonella enterica serovar Typhimurium and Listeria monocytogenes biofilm cells was evaluated in this study. After biofilm formation, tests were performed under different drying times (0, 6, 12, 24, and 72 h) and pre-enrichment times (0, 6, 18, and 25 h). The direct epifluorescence microscopic results demonstrated that Salmonella Typhimurium and L. monocytogenes biofilm cells can remain viable for 72 h under drying conditions. Pre-enrichment time and type of medium played an essential role in the detection of both microorganisms after drying. Furthermore, RT-PCR was more sensitive than VIDAS and the conventional method for detecting Salmonella Typhimurium and L. monocytogenes cells at different drying times and without pre-enrichment (0 h), with a detection range between 102 and 107 CFU/ml. TSBYE-T80 used as a pre-enrichment medium was effective for detecting both bacteria and was more effective than Demi Fraser-T80 medium for detecting L. monocytogenes. Therefore, pre-enrichment is recommended to avoid false positives and false negatives due to the presence of dead cells or a very low initial concentration of cells after drying.
ISSN: 01681605

Peruzy, M.F., Capuano, F., Proroga, Y.T.R., Cristiano, D., Carullo, M.R., Murru, N.
Antimicrobial susceptibility testing for salmonella serovars isolated from food samples: Five-year monitoring (2015–2019)
(2020) Antibiotics, 9 (7), art. no. 365, pp. 1-17.
ABSTRACT: The continuous collection and analysis of updated data on the antimicrobic resistance among bacterial strains represent the essential core for the surveillance of this problem. The present work aimed to investigate the occurrence of antimicrobial resistance among Salmonella serovars isolated in foods in 2015–2019. A total of 178 Salmonella strains belonging to 39 serovars were tested against 10 antimicrobials. High proportions of Salmonella isolates were resistant to tetracycline (n = 53.9%), ciprofloxacin (n = 47.2%), ampicillin (n = 44.4%), nalidixic acid (n = 42.7%), and trimethoprim-sulfamethoxazole (n = 38.8%). Different resistance rates were recorded among the different serotypes of Salmonella, and S. Infantis, exhibited the highest resistance to antibiotics. A high

percentage of strains isolated from poultry, pork, and bovine were resistant to at least one or two antimicrobials. Resistant and multidrug-resistant (MDR) strains were also recorded among the isolates from molluscan shellfish; however, the occurrence of resistant Salmonella strains isolated from this source was significantly lower compared with those reported for poultry, pork, and bovine. The high levels of resistance reported in the present study indicate a potential public health risk. Consequently, additional hygiene and antibiotic stewardship practices should be considered for the food industry to prevent the prevalence of Salmonella in foods. ISSN: 20796382

Jin, Y., Tang, J., Zhu, M.-J.
Water activity influence on the thermal resistance of Salmonella in soy protein powder at elevated temperatures
(2020) Food Control, 113, art. no. 107160, .
ABSTRACT: Salmonella is a leading cause of foodborne illness associated with low-moisture foods. In addition to being able to survive in low-moisture environments during long storage, Salmonella has shown sharply increased thermal tolerance making it difficult to control in low-moisture foods. This research utilized soy protein powder as a food career to study the thermal resistance of Salmonella under a wide range of temperatures and aw. Salmonella inoculated soy protein powder samples were pre-equilibrated to aw from 0.13 to 0.82 at room temperature, then subjected to heat treatment (60–99 °C) under isothermal conditions. The aw of soy protein powders at 25 to 99 °C were measured using high-temperature aw cells with humidity sensors. The D-values as a function of high-temperature aw from 0.25 to 0.70 showed a semi-log relationship under each treatment temperature level from 70 to 99 °C. Slightly downward trends were observed when the high-temperature aw was above 0.70, showing increased effectiveness of thermal inactivation. Results from this study provide insights to assist the design of thermal treatments for control of Salmonella in intermediate- and low-moisture foods.
ISSN: 09567135

Ferreira, V., Cardoso, M.J., Magalhães, R., Maia, R., Neagu, C., Dumitraşcu, L., Nicolau, A.I., Teixeira, P.
Occurrence of Salmonella spp. in eggs from backyard chicken flocks in Portugal and Romania - Results of a preliminary study
(2020) Food Control, 113, art. no. 107180, .
ABSTRACT: The aim of this study was to conduct a preliminary investigation on the occurrence of Salmonella spp. in eggs from chickens raised in backyards in Portugal and Romania. A lack of compliance with safety practices by chicken owners, was demonstrated, especially in Portugal, as 96% of the eggs were visibly dirty and 92.5% were stored at room temperature. In Romania the 202 analysed eggs were Salmonella free, whereas in Portugal six of the 200 eggs sampled were positive for Salmonella spp. (3%). A positive egg for Salmonella spp. was found in 10.7% of the 56 backyard flocks sampled in Portugal. One egg exhibited contamination both in the shell-membrane mixture and in its content, while in the remaining eggs, the pathogen was found either in the shell-membrane (n = 2) or in the yolk and white mixture (n = 3). The serotypes S. Typhimurium (with identical PFGE patterns) and S. Enteritidis were isolated from five eggs and one egg, respectively. Whilst S. Enteritidis was sensitive to the 14 antibiotics tested, S. Typhimurium isolates presented divergent antimicrobial resistant phenotypes and three were classified as multi-drug resistant. ISSN: 09567135

Clark, C.G., Landgraff, C., Robertson, J., Pollari, F., Parker, S., Nadon, C., Gannon, V.P.J., Johnson, R., Nash, J.
Distribution of heavy metal resistance elements in Canadian Salmonella 4,[5],12:I:- populations and association with the monophasic genotypes and phenotype
(2020) PLoS ONE, 15 (7 7), art. no. e0236436, .
ABSTRACT: Salmonella 4,[5],12:i:- are monophasic S. Typhimurium variants incapable of producing the second-phase flagellar antigen. They have emerged since the mid-1990s to become one of the most prevalent Salmonella serotypes causing human disease world-wide. Multiple genetic events associated with different genetic elements can result in the monophasic phenotype. Several jurisdictions have reported the emergence of a Salmonella 4,[5],12:i:- clone with SGI-4 and a genetic element (MREL) encoding a mercury resistance operon and antibiotic resistance loci that disrupts the second phase antigen region near the iroB locus in the Salmonella genome. We have sequenced 810 human and animal Canadian Salmonella 4,[5],12:i:- isolates and determined that isolates with SGI-4 and the mercury resistance element (MREL; also known as RR1&RR2) constitute several global clades containing various proportions of Canadian, US, and European isolates. Detailed analysis of the data provides a clearer picture of how these heavy metal elements interact

with bacteria within the Salmonella population to produce the monophasic phenotype. Insertion of the MREL near iroB is associated with several deletions and rearrangements of the adjacent flaAB hin region, which may be useful for defining human case clusters that could represent outbreaks. Plasmids carrying genes encoding silver, copper, mercury, and antimicrobial resistance appear to be derived from IS26 mediated acquisition of these genes from genomes carrying SGI-4 and the MREL. Animal isolates with the mercury and As/Cu/Ag resistance elements are strongly associated with porcine sources in Canada as has been shown previously for other jurisdictions. The data acquired in these investigations, as well as from the extensive literature on the subject, may aid source attribution in outbreaks of the organism and interventions to decrease the prevalence of this clone and reduce its impact on human disease. ISSN: 19326203

Bergšpica, I., Ozola, A., Miltiņa, E., Alksne, L., Meistere, I., Cibrovska, A., Grantiņa-Ieviņa, L.
Occurrence of Pathogenic and Potentially Pathogenic Bacteria in Microgreens, Sprouts, and Sprouted Seeds on Retail Market in Riga, Latvia
(2020) Foodborne pathogens and disease, 17 (7), pp. 420-428.
ABSTRACT: Microgreens and sprouts have been used for raw consumption for a long time and are generally viewed as a healthy food. However, several serious outbreaks of foodborne illness have been recorded in European countries, Japan, and North America. Many companies in Latvia nowadays are producing this type of products. The aim of this study was to characterize the incidence of Shiga toxin-producing Escherichia coli (STEC), Salmonella spp., and Listeria spp. in microgreens, sprouts, and seeds intended for domestic production of microgreens on retail market in Riga, Latvia, from January to April 2019. The background microflora was identified as well. A total of 45 samples were purchased, including fresh and processed sprouts, microgreens, baby greens, as well as seeds intended for domestic production of microgreens and sprouts. The samples were processed according to the methods set by the International Organization for Standardization (ISO)-ISO/TS 13136:2012 for STEC, ISO 6579-1:2017 for Salmonella spp., and ISO 11290-1:2017 for Listeria spp. Molecular detection of Salmonella spp. was also performed using real-time polymerase chain reaction. The typical and atypical colonies isolated from selective plates were identified with matrix-assisted laser desorption and ionization time-of-flight mass spectrometry. Listeria monocytogenes was not detected in any of the tested samples. However, the presence of Listeria innocua was detected in two (4.4%) of the samples. Three (6.7%) samples of dried sprouts were positive for the STEC virulence genes. Salmonella spp. was detected in one (2.2%) sample of common sunflower seeds. Altogether, 46 different background bacterial species were identified. The majority were environmental bacteria characteristic to soil, water, and plants, including coliform bacteria. The results provide evidence that microgreens and seeds available for Latvian consumers are generally safe, however, attention has to be paid to dried sprouts.
ISSN: 15567125

Cho, S., Jackson, C.R., Frye, J.G.
The prevalence and antimicrobial resistance phenotypes of Salmonella, Escherichia coli and Enterococcus sp. in surface water
(2020) Letters in Applied Microbiology, 71 (1), pp. 3-25.
ABSTRACT: Surface water is prone to bacterial contamination as it receives wastes and pollutants from human and animal sources, and contaminated water may expose local populations to health risks. This review provides a brief overview on the prevalence and antimicrobial resistance (AR) phenotypes of Salmonella, Escherichia coli and Enterococcus, found in natural freshwaters. These bacteria are frequently detected in surface waters, sometimes as etiological agents of waterborne infections, and AR strains are not uncommonly identified in both developed and developing countries. Data relating to Salmonella, E. coli and Enterococcus present in environmental water are lacking, and in order to understand their development and dissemination using the One Health approach, understanding the prevalence, distribution and characteristics of the bacteria present in surface water as well as their potential sources is important. Furthermore, AR bacteria in natural watersheds are not well investigated and their impacts on human health and food safety are not well understood. As surface water is a receptacle for AR bacteria from human and animal sources and a vehicle for their dissemination, this is a crucial data gap in understanding AR and minimizing its spread. For this review, Salmonella, E. coli and Enterococcus were chosen to evaluate the presence of primary pathogens and opportunistic pathogens as well as to monitor AR trends in the environmental water. Studies around the world have demonstrated the widespread distribution of pathogenic and AR bacteria in surface waters of both developing and developed countries, confirming

the importance of environmental waters as a reservoir for these bacteria and the need for more attention on the environmental bacteria for emerging AR. ISSN: 02668254

Bucher, M.G., Zwirzitz, B., Oladeinde, A., Cook, K., Plymel, C., Zock, G., Lakin, S., Aggrey, S.E., Ritz, C., Looft, T., Lipp, E., Agga, G.E., Abdo, Z., Sistani, K.R.
Reused poultry litter microbiome with competitive exclusion potential against Salmonella Heidelberg
(2020) Journal of Environmental Quality, 49 (4), pp. 869-881.
ABSTRACT: The success of poultry litter reuse in U.S. poultry production can be attributed to the efficient treatment methods used by producers during downtimes (the time lapse between consecutive flocks, during which the broiler house is empty). During this period, reused litter may be decaked, tilled/windrowed, or treated with acid-based amendments to reduce ammonia and bacteria levels. Competitive exclusion, pH, and temperature are proposed factors that influence the level of pathogens and the overall litter microbiome during downtimes. We previously reported on the bacterial genetic factors associated with the fitness of two strains of Salmonella enterica serovar Heidelberg (SH) incubated for 14 d in reused litter. Here, we investigated the physicochemical parameters and the microbiome of the litter correlating with SH abundance during this period. We used 16S ribosomal RNA gene sequencing to determine the litter microbiome and whole genome sequencing to characterize bacteria with competitive exclusion potential against SH. The β diversity of the litter microbiome was significantly affected by the duration of incubation, microcosm, and microcosm plus Heidelberg strain combinations. In addition, β diversity was significantly affected by litter parameters, including NH4, pH, moisture, water activity, and aluminum. The major phyla observed in the reused litter throughout the 14-d incubation experiment were Firmicutes and Actinobacteria, although their abundance differed by microcosm and time. Amplicon-specific variants homologous to the members of the genera Nocardiopsis and Lentibacillus and the family Bacillaceae_2 were found to significantly correlate with the abundance of Salmonella. A consortium of Bacillus subtilis strains isolated from the litter microcosms reduced the growth of SH in vitro. ISSN: 00472425

Mangmee, S., Reamtong, O., Kalambaheti, T., Roytrakul, S., Sonthayanon, P.
MALDI-TOF mass spectrometry typing for predominant serovars of non-typhoidal Salmonella in a Thai broiler industry
(2020) Food Control, 113, art. no. 107188, .
ABSTRACT: Rapid and reliable detection of non-typhoidal Salmonella (NTS) is essential for effective monitoring and controlling in broiler industries. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been reported as a sensitive and accurate method for microbial investigations at genus and species level while subspecies level is still obscure. Here, we developed a MALDI-TOF MS-based method to improve the simultaneous identification of species, subspecies, and serovars of NTS isolated from broiler samples in a Thai slaughtering and processing factory. Whole-cell peptide patterns from 142 NTS isolates were integrated with the commercial database for species and subspecies identification based on weighted pattern (subtyping MSP) matching. Serovar-specific peaks were searched and determined using the machine-learning analysis. The classification tree was created for detection of the five predominant NTS serovars (i.e., Albany, Agona, Typhimurium/I 4,[5],12:i:-, Altona, and Enteritidis). One hundred and forty-five NTS isolates were evaluated and yielded all accurate identification at species and subspecies level corresponding to conventional methods. Besides, the serovar classification was achieved with 99.3% accuracy when compared with serotyping. This method would be useful for large scale screening of NTS serovars in food industries where cost-effectiveness, rapid and highly accurate methods are required. ISSN: 09567135

Burnett, J., Wu, S.T., den Bakker, H.C., Cook, P.W., Veenhuizen, D.R., Hammons, S.R., Singh, M., Oliver, H.F.
Listeria monocytogenes is prevalent in retail produce environments but Salmonella enterica is rare
(2020) Food Control, 113, art. no. 107173, .
ABSTRACT: The purpose of this study was to determine the prevalence of Listeria monocytogenes and Salmonella enterica in retail produce environments and to elucidate possible ecological niches. Thirty environmental samples per store were collected during daily operations monthly for six months in 30 retail produce departments across seven states. Selected samples were serially diluted and plated to determine aerobic plate count. Each sample was tested for L. monocytogenes and S. enterica using ROKA Atlas LmG2 and SEN assays, respectively. A total of 5,112 samples were tested for each pathogen. S. enterica was found during one sampling event in a single store; less than 0.1% of samples

were positive overall. A total of 4.4% environmental samples tested positive for L. monocytogenes. L. monocytogenes was present on 8.1% of non-food contact surfaces and 1.6% of food contact surfaces tested; L. monocytogenes prevalence was highly variable among stores. Most of the positive L. monocytogenes samples were found in drains, floors, squeegees, or standing water. Genetically similar L. monocytogenes clones were found in multiple stores across multiple states. The odds of detecting L. monocytogenes increased 1.8-fold for every 1-log increase in APC ($p < 0.0001$). This is the first study to investigate prevalence and persistence of L. monocytogenes and S. enterica in retail produce environments. Our data suggest that some, but not all retail produce environments have high L. monocytogenes prevalence, which may cross-contaminate produce. Sites heavily contaminated with L. monocytogenes indicate potential targets for sanitation operating procedures and food safety management strategies. However, S. enterica prevalence is very low and likely due to transient contamination. Further, APC may be a cost-effective environmental monitoring tool that could indicate an environment capable of harboring L. monocytogenes. ISSN: 09567135

Khan, S., Moore, R.J., Stanley, D., Chousalkar, K.K.
The gut microbiota of laying hens and its manipulation with prebiotics and probiotics to enhance gut health and food safety
(2020) Applied and Environmental Microbiology, 86 (13), art. no. e00600-20, .
ABSTRACT: The microbiota plays a vital role in maintaining gut health and influences the overall performance of chickens. Most gut microbiota-related studies have been performed in broilers, which have different microbial communities compared to those of layers. The normal gut microbiota of laying chickens is dominated by Proteobacteria, Firmicutes, Bacteroidetes, Fusobacteria, and Actinobacteria at the phylum level. The composition of the gut microbiota changes with chicken age, genotype, and production system. The metabolites of gut microbiota, such as shortchain fatty acids, indole, tryptamine, vitamins, and bacteriocins, are involved in hostmicrobiota cross talk, maintenance of barrier function, and immune homeostasis. Resident gut microbiota members also limit and control the colonization of foodborne pathogens. In-feed supplementations of prebiotics and probiotics strengthen the gut microbiota for improved host performance and colonization resistance to gut pathogens, such as Salmonella and Campylobacter. The mechanisms of action of prebiotics and probiotics come through the production of organic acids, activation of the host immune system, and production of antimicrobial agents. Probiotic candidates, including Lactobacillus, Bifidobacterium, Bacillus, Saccharomyces, and Faecalibacterium isolates, have shown promising results toward enhancing food safety and gut health. Additionally, a range of complex carbohydrates, including mannose oligosaccharides, fructo-oligosaccharides, and galacto-oligosaccharides, and inulin are promising candidates for improving gut health. Here, we review the potential roles of prebiotics and probiotics in the reshaping of the gut microbiota of layer chickens to enhance gut health and food safety. ISSN: 00992240

Yu, J., Xing, J., Zhan, X., Yang, Z., Qi, J., Wei, Y., Liu, Y.
Improvement of Loop-Mediated Isothermal Amplification Combined with Chromatographic Flow Dipstick Assay for Salmonella in Food Samples
(2020) Food Analytical Methods, 13 (7), pp. 1398-1408.
ABSTRACT: Loop-mediated isothermal amplification (LAMP) had been employed as a powerful tool to facilitate genetic tests for various food pathogens, as it is easy to perform. Recently, various methods of detecting the LAMP amplicon were developed. In this study, we improved two LAMP assays by combining LAMP with chromatographic flow dipstick (LFD) assays for Salmonella (targeting phoP and invA, respectively). We evaluated different labeled primer sets, then selected the optimal sets to perform the LFD assays. We compared the optimal LFD and LAMP assays with the ISO standard method. The results showed that LFD was more sensitive and quicker than LAMP. Furthermore, enrichment broths of 225 food samples were tested. The sensitivity of two LFD assays was 100%. The specificity of LFD assay targeting phoP was 99.1%, and LFD assay targeting invA was 99.5%. For the LFD assay targeting phoP, the estimate of limit of detection (LOD) 50% was 0.061 CFU/g and the estimate of LOD 95% was 0.265 CFU/g. For the LFD assay targeting invA, the estimate of LOD 50% was 0.040 CFU/g and the estimate of LOD 95% was 0.172 CFU/g. We validated this method in a primary laboratory, where we accomplished the assay only using an incubator and a heating block. It suggested that the LFD assay had the potential to become a suitable diagnostic method in field test and primary labs. ISSN: 19369751

Maio, R., García-Díez, J., Saraiva, C.
Microbiological quality of foodstuffs sold on expiry date at retail in Portugal: A preliminary study
(2020) Foods, 9 (7), art. no. A26, .
ABSTRACT: Currently, food waste represents an important issue due to its negative economic, social and environmental impact. To reduce the food waste levels, some retailers' brands implement discounting based on the proximity to expiry. Since this practice may involve potential food poisoning, a total of 94 food products from animal origin, purchased in two supermarkets in North Portugal on the expiry date, were analyzed for selected foodborne and spoilage microorganisms. Moreover, the samples were classified as satisfactory and not satisfactory according to their microbiological quality. The results showed that none of the samples presented counts for Salmonella spp., S. aureus, B. cereus. L. monocytogenes was detected in one sample over the limit of 2 log cfu/g as defined by Regulation 2073/2005. The evaluation of food hygiene and spoilage indicators showed that the processed foods displayed lower counts than raw products (beef, pork, chicken and fish). Regarding Enterobacteriaceae, raw products presented on average over 2 log cfu/g than processed foods, with the exception of beef samples that accounted over 3 log cfu/g more than processed foods. In addition, E. coli was mainly detected in fresh meat of which chicken and pork displayed the highest counts. Regarding the qualitative classification, 51.06% of the samples were not satisfactory for the total mesophilic counts, while 62.76% and 58.51% displayed positive results for Enterobacteriaceae and molds and yeasts (M&Y) criteria, respectively. In all, 70.21% of the samples analyzed at the expiry date failed, at least, in one microbiological criterion. The results indicate that the foods available at the end of the shelf life in supermarkets do not represent a risk for food poisoning due to the absence of foodborne pathogens. Since the microbiological indicators of storage/handling of raw products were mainly unsatisfactory, this indicates that the sale of these perishable foods at the end of the shelf life may not be recommended. On the other hand, processed products subjected to food conservation procedures (i.e., thermal processing) could be sold at the end of their shelf life or donated beyond the best-before date, due to its physical, chemical and microbiological stability. However, evidences of foodborne outbreaks associated to this kind of foodstuffs indicated the need of a proper risk assessment. Moreover, it is important to remark that other factors such as small sample size, the absence of the evaluation of the handling, and storage conditions along the food chain or organoleptic alterations must be assessed in further studies.
ISSN: 23048158

Casanova, L.M., Hill, V.R., Sobsey, M.D.
Antibiotic-resistant Salmonella in swine wastes and farm surface waters
(2020) Letters in Applied Microbiology, 71 (1), pp. 117-123.
ABSTRACT: Hog production takes place mostly in large concentrated animal feeding operations (CAFOs) where waste is managed by storing in lagoons prior to land application of lagoon liquid. Salmonella, including antibiotic-resistant Salmonella, have been found in the farm environment and lagoons. The objective of this research was to determine whether Salmonella resistant to clinically relevant antibiotics were present in wastewaters and surface waters from hog CAFOs. Samples of hog waste and on farm environmental waters were analysed for Salmonella, which were tested for antimicrobial susceptibility. The highest percentage of resistant isolates were found in raw waste flushed from hog houses and in lagoon wastewater; few resistant isolates were found in on-farm surface water. Resistance to sulphamethoxazole was most common, mostly in waste samples and less commonly in surface water, followed by chloramphenicol and ampicillin. No resistance to cephalosporin or fluoroquinolones was found. Resistance to clinically relevant antibiotics was commonly found in Salmonella from hog waste but was less extensive in farm surface waters. Management of wastes from hog CAFOs should be designed to further reduce the risk of human exposures resulting from environmental contamination with Salmonella. Significance and Impact of the Study: This study suggests antibiotic-resistant Salmonella were common in hog wastes and present in environmental waters associated with hog CAFOs. Low levels of antibiotic-resistant Salmonella in on-farm stream waters suggest surface waters could have been contaminated, potentially serving as a mechanism of off-farm transport. Since the study, there have been multiple economic, regulatory and practice changes at the federal, state and industry level. These include regulation of antibiotic use and animal waste treatment, vertical integration in the industry and changes in antibiotic use practice. This study is a useful historical baseline against which current antibiotic resistance trends can be measured. ISSN: 02668254

Tarlak, F., Johannessen, G., Bascón Villegas, I., Bolívar, A., Posada-Izquierdo, G.D., Pérez-Rodríguez, F.
Modelling of the behaviour of salmonella enterica serovar reading on commercial fresh-cut iceberg lettuce stored at different temperatures
(2020) Foods, 9 (7), art. no. 946, .
ABSTRACT: The aim of this study was to model the growth and survival behaviour of Salmonella Reading and endogenous lactic acid bacteria on fresh pre-cut iceberg lettuce stored under modified atmosphere packaging for 10 days at different temperatures (4, 8 and 15 °C). The Baranyi and Weibull models were satisfactorily fitted to describe microbial growth and survival behaviour, respectively. Results indicated that lactic acid bacteria (LAB) could grow at all storage temperatures, while S. Reading grew only at 15 °C. Specific growth rate values (μmax) for LAB ranged between 0.080 and 0.168 h-1 corresponding to the temperatures 4 and 15 °C while for S. Reading at 15 °C, μmax = 0.056 h-1. This result was compared with published predictive microbiology models for other Salmonella serovars in leafy greens, revealing that predictions from specific models could be valid for such a temperature, provided they were developed specifically in lettuce regardless of the type of serovars inoculated. The parameter delta obtained from theWeibull model for the pathogen was found to be 16.03 and 18.81 for 4 and 8 °C, respectively, indicating that the pathogen underwent larger reduction levels at lower temperatures (2.8 log10 decrease at 4 °C). These data suggest that this Salmonella serovar is especially sensitive to low temperatures, under the assayed conditions, while showcasing that a correct refrigeration could be an effective measure to control microbial risk in commercial packaged lettuce. Finally, the microbiological data and models from this study will be useful to consider more specifically the behaviour of S. Reading during transport and storage of fresh-cut lettuce, elucidating the contribution of this serovar to the risk by Salmonella in leafy green products. ISSN: 23048158

Kotian, A., Aditya, V., Jazeela, K., Karunasagar, I., Karunasagar, I., Deekshit, V.K.
Effect of bile on growth and biofilm formation of non-typhoidal salmonella serovars isolated from seafood and poultry
(2020) Research in Microbiology, 171 (5-6), pp. 165-173.
ABSTRACT: Bacterial cells adopt various strategies to adapt themselves in diverse environmental conditions. Salmonella is one such bacteria with diverse mechanisms to survive, replicate and infect in wide host range. This study aims at investigating the biofilm-forming ability of multidrug-resistant and sensitive Salmonella serovars on exposure to bile. Antibiogram of all the isolates was determined by disk diffusion method and their biofilm-forming ability in the presence or absence of bile was assessed by microtiter plate assay. Biofilm results were validated by calcofluor, Congo red plate and test tube method. Few isolates were selected for further study of their expression of biofilm related genes on exposure to bile using real time PCR. Among the 59 isolates of Salmonella isolated from seafood and poultry, 30 isolates were multi-drug resistant (MDR). Under control conditions, 57% (n = 25) of the serovars were able to form biofilm. While, 86% (n = 51) of the serovars produced biofilm in the presence of bile. The relative gene expression study of the selected serovars for 8 different genes showed a striking difference in the expression levels, supporting the hypothesis that the presence of bile triggers biofilm formation in food associated strains of non-typhoidal Salmonella by upregulation of genes involved in biofilm production. ISSN: 09232508

Machado Junior, P.C., Chung, C., Hagerman, A.
Modeling Salmonella Spread in Broiler Production: Identifying Determinants and Control Strategies
(2020) Frontiers in Veterinary Science, 7, art. no. 564, .
ABSTRACT: The presence of Salmonella spp. in broiler production is a food safety concern as the bacterium can be transmitted to humans via contaminated meat and derived products. Salmonella detection in litter at the pre-slaughter period has been linked to increased odds of contaminated broiler carcasses and meat derived products. To determine risk factors related to farm and broiler house characteristics and management practices, this study uses a unique longitudinal data set from a Brazilian integrated broiler enterprise, which contains official results of Salmonella spp. isolation from drag swabs collected at the end of the grow-out period. A Bayesian hierarchical spatio-temporal model found significant spatial and time influence on the odds of isolating Salmonella spp. from litter as well as significant effects from the size of a broiler house, total housing area per farm, type of broiler house, and number of litter recycles. Results indicate that recycling litter beyond 6 rearing cycles significantly increased the odds of isolating Salmonella before slaughter, and the bacterium was more likely to persist in conventional broiler houses, compared to broiler houses with controlled environment. Evidence of a potential principal-agent problem

was also found in setting strategies to control the bacterium from litter, which suggests strong incentives to adopt the strategies aiming to reduce prevalence of the bacterium in the integrated enterprise. Our findings could be used to develop alternative measures to reduce the risk of persistence of the bacterium in the broiler production chain. ISSN: 22971769

Li, Y, Huang, T, Bai, C, Fu, J, Chen, L, Liang, Y, Wang, K, Liu, J, Gong, X, Liu, J.
Reduction, Prevention, and Control of Salmonella enterica Viable but Non-culturable Cells in Flour Food
(2020) Frontiers in Microbiology, 11, art. no. 1859, .
ABSTRACT: The processing and storage conditions of flour food inevitably pose environmental stress, which promote bacteria to enter a viable but non-culturable (VBNC) state. The existence of VBNC cells causes false-negative detection in traditional culture-based detection methods, resulting in food quality and safety issues. This study aimed at investigating the influence factors including nutrition, acid, salt, and temperature for the entry into a VBNC state of Salmonella enterica and an efficient detection method. During induction with multi-stress conditions, nutrition starvation antagonizes with low-level acidity. Besides, high-level acidity was considered as an inhibitor for VBNC induction. Four inducers including nutrition starvation, salt stress, low-level acidity, and low temperature were concluded for a VBNC state. In addition, the keynote conditions for S. enterica entering a VBNC state included (i) nutrient-rich acidic environment, (ii) oligotrophic low-acidity environment, and (iii) oligotrophic refrigerated environment. Based on the keynote conditions, the environmental conditions of high acidity (1.0% v/v acetate) with low temperature (−20°C) could successfully eliminate the formation of S. enterica VBNC cells in flour food. In addition, combining with propidium monoazide pretreatment, PCR technology was applied to detect S. enterica VBNC cells. The sensitivity of the PMA–PCR technology was 105 CFU/ml in an artificially simulated food system. The results derived from this study might aid in the detection and control of VBNC state S. enterica in flour food products. ISSN: 1664302X

Bataller, E., García-Romero, E., Llobat, L., Lizana, V., Jiménez-Trigos, E.
Dogs as a source of Salmonella spp. in apparently healthy dogs in the Valencia Region. Could it be related with intestinal lactic acid bacteria?
(2020) BMC veterinary research, 16 (1), p. 268.
ABSTRACT: BACKGROUND: Although salmonellosis is considered one of the most important food-borne zoonotic diseases in Europe, close contact between dogs and their owners can also be a potential source of Salmonella spp. for humans. This study assessed the prevalence and antimicrobial resistance of Salmonella spp. in apparently healthy dogs in the Valencian Region, eastern Spain. Moreover, a macroscopic comparison of lactic acid bacteria in both Salmonella-positive and Salmonella-negative dogs was carried out. RESULTS: Of a total of 325 dogs sampled, 6 (1.85%) were positive for Salmonella spp. with 3 different serotypes, Havana (3), Mikawasima (2) and monophasic Typhimurium (1). All isolates were susceptible to all antimicrobials tested except monophasic S. Typhimurium, which was resistant to ampicillin. Finally, macroscopic results revealed that lactic acid bacteria had higher heterogeneity in the Salmonella-negative dogs than in the Salmonella-positive dogs. Although the results in our study showed a low prevalence of Salmonella spp., raw food has been suggested as a risk factor for bacteria in dog faeces. CONCLUSIONS: Public awareness campaigns on good hygiene practices, especially after handling canine faeces or raw food, are necessary. Furthermore, to reduce the potential transmission of bacteria, dogs should be fed food that has been properly cooked, as raw or undercooked food can be a source of zoonotic pathogens. Moreover, further studies must be performed to determine the relationship between lactic acid bacteria and Salmonella spp. in dog faeces. ISSN: 17466148

Aung, K.T., Khor, W.C., Octavia, S., Ye, A., Leo, J., Chan, P.P., Lim, G., Wong, W.K., Tan, B.Z.Y., Schlundt, J., Dalsgaard, A., Ng, L.C., Lin, Y.N.
Distribution of salmonella serovars in humans, foods, farm animals and environment, companion and wildlife animals in Singapore
(2020) International Journal of Environmental Research and Public Health, 17 (16), art. no. 5774, pp. 1-13.
ABSTRACT: We analyzed the epidemiological distribution of Salmonella serovars in humans, foods, animals and the environment as a One-Health step towards identifying risk factors for human salmonellosis. Throughout the 2012–2016 period, Salmonella ser. Enteritidis was consistently the predominating serovar attributing to >20.0% of isolates in humans. Other most common serovars in humans include Salmonella ser. Stanley, Salmonella ser. Weltevreden, Salmonella ser. Typhimurium and Salmonella ser. 4,5,12:b:-

(dT+). S. Enteritidis was also the most frequent serovar found among the isolates from chicken/chicken products (28.5%) and eggs/egg products (61.5%) during the same period. In contrast, S. Typhimurium (35.2%) and Salmonella ser. Derby (18.8%) were prevalent in pork/pork products. S. Weltevreden was more frequent in seafood (19.2%) than others (≤3.0%). Most isolates (>80.0%) from farms, companion and wildlife animals belonged to serovars other than S. Enteritidis or S. Typhimurium. Findings demonstrate the significance of a One-Health investigative approach to understand the epidemiology Salmonella for more effective and integrated surveillance systems. ISSN: 16617827

Kumar, G.D., Patel, J., Ravishankar, S.
Contamination of spinach at germination: A route to persistence and environmental reintroduction by Salmonella
(2020) International Journal of Food Microbiology, 326, art. no. 108646, .
ABSTRACT: The effects of using contaminated seed and water on the persistence and internalization of Salmonella Newport in organic spinach cultivars- Lazio, Space, Emilia and Waitiki were studied. Seeds were contaminated by either immersing in a suspension of Salmonella and then sprouted or were sprouted in Salmonella contaminated water in the dark at 25 °C. After 5 days, germinated sprouts were analyzed for S. Newport population and internalization. Germinated sprouts were potted in soil and grown in a plant incubator for 4 weeks. Leaves, stems and roots were sampled for Salmonella population by plating on CHROMagar™. Plants surface-sterilized with chlorine were analyzed for internalized pathogen. Potting soil and water runoff were sampled for Salmonella after 4 weeks of plant growth. Contaminated seeds and irrigation water had S. Newport populations of 7.64±0.43 log CFU/g and 7.12±0.04 log CFU/ml, respectively. Sprouts germinated using contaminated water or seeds had S. Newport populations of 8.09±0.04 and 8.08±0.03 log CFU/g, respectively and had a Salmonella population that was significantly higher than other spinach tissues (P<0.05). Populations of S. Newport in leaves, stem and roots of spinach plants were as follows: contaminated seed- 2.82±1.69, 1.69±0.86, and 4.41±0.62 log CFU/ml; contaminated water- 3.56±0.90, 3.04±0.31, and 4.03±0.42 log CFU/ml of macerated tissue suspension, respectively. Internalization was observed in plants developing from contaminated seeds and in sprouts germinated using contaminated water. S. Newport populations of 2.82±0.70 log CFU/g and 1.76±0.46 log CFU/ml were recovered from soil and water runoff, respectively. The results indicate that contamination of spinach during germination can result in persistence, internalization and environmental reintroduction of Salmonella. ISSN: 01681605

Sekhon, A.S., Singh, A., Michael, M.
Short communication: Decimal log reductions of Salmonella Senftenberg 775 W and other Salmonella serovars in nonfat milk and powder
(2020) Journal of Dairy Science, 103 (8), pp. 6894-6899.
ABSTRACT: This study aimed to compare the thermal resistance of Salmonella Senftenberg 775 W with other serovars of Salmonella in nonfat dry milk (NDM) and hydrated NDM. The scientific literature suggests that Salmonella Senftenberg 775 W is the most heat-resistant serovar in high-water-activity foods such as milk, but little is known about the heat resistance of Salmonella Senftenberg 775 W compared with other Salmonella serovars in low-water-activity foods such as NDM. The 5 serovars of Salmonella used in this study were Enteritidis, Montevideo, Newport, Senftenberg, and Typhimurium. The hydration of NDM was conducted at 13% (wt/vol) total solids. The NDM was inoculated with the 5 individual serovars of Salmonella and dried again to its original pre-inoculation water activity. Hydrated NDM was prepared from individually inoculated NDM. The surviving Salmonella population at predetermined time-temperature intervals were enumerated using injury-recovery medium, and the average log reductions for the individual serovars were calculated. As expected, Salmonella Senftenberg 775 W was the most heat-resistant serovar in hydrated NDM at 59°C and 65°C. However, the heat resistance of Salmonella Senftenberg 775 W was found to be lower than or comparable to that of other serovars in low-water-activity NDM at 80°C and 90°C. ISSN: 00220302

Muckey, M., Huss, A.R., Yoder, A., Jones, C.
Research Note: Evaluating the roles of surface sanitation and feed sequencing on mitigating Salmonella Enteritidis contamination on animal food manufacturing equipment
(2020) Poultry Science, 99 (8), pp. 3841-3845.
ABSTRACT: The objective of this study was to evaluate the efficacy of flushing surfaces with untreated feed vs. the use of 2 different dry chemical sanitizers on residual surface and feed Salmonella Enteritidis contamination. First, a Salmonella-negative batch of poultry feed was mixed in 9 laboratory-scale paddle mixers. A feed sample was collected, and targeted locations on surfaces within the mixer were swabbed to confirm Salmonella-

negative. Next, a Salmonella-positive batch of poultry feed was mixed, sampled, and mixer surfaces swabbed. Mean Salmonella Enteritidis contamination across all 9 mixers were 3.63 cfu/g for sampled feed and 1.27 cfu/cm^2 for surface contamination. Next, the mixers manufactured one of the following treatments (3 mixers/treatment): 1) none (control); 2) a commercially available essential oil blend; or 3) rice hulls treated with a 10% concentration of a propriety blend of medium-chain fatty acids (MCFA). After each treatment, each mixer manufactured another 2 batches of Salmonella-free feed (sequence 1 and sequence 2). Feed samples were collected, and surfaces were swabbed between each batch of feed. Manufacturing sequence (P < 0.0001) but not treatment (P > 0.05) impacted feed or surface contamination of Salmonella Enteritidis. There was Salmonella-positive residue in the batch of feed manufactured immediately after the positive control batch. However, no Salmonella residue was detected in batches of feed treated with either the commercial essential oil blend or MCFA. Low levels of Salmonella residue were observed from either feed (0.7 cfu/g for commercial essential oil blend) or surfaces (0.1 cfu/cm^2 for MCFA) manufactured in sequence 1, but no residue was observed in sequence 2. These data suggest that sequencing of feed during manufacturing reduces Salmonella-positive contamination within animal food and on manufacturing surfaces, particularly after the second batch or with the use of chemical treatments. ISSN:00325791

Tîrziu, E., Barbalan, G., Morar, A., Herman, V., Cristina, R.T., Imre, K.
Occurrence and Antimicrobial Susceptibility Profile of Salmonella spp. in Raw and Ready-To-Eat Foods and Campylobacter spp. in Retail Raw Chicken Meat in Transylvania, Romania
(2020) Foodborne Pathogens and Disease, 17 (8), pp. 479-484.
ABSTRACT: The survey was undertaken to investigate the presence and antimicrobial susceptibility profile of Salmonella spp. in raw and ready-to-eat (RTE) foods, and Campylobacter spp. in the retail raw chicken meat collected in two counties of Transylvania, Romania. A total of 13.1% (51/388) of the examined food samples were found to be Salmonella positive, with a distribution of 14.7% (48/326) in the raw food (i.e., pork, chicken carcass, and shell egg) and 4.8% (3/62) in the RTE samples (i.e., sausages, but not ham and salami), respectively. These differences were statistically significant (p=0.034). The isolates were serotyped as Salmonella Infantis (n=19), Salmonella Typhimurium (n=11) Salmonella Rissen (n=8), Salmonella Derby (n=3), Salmonella Enteritidis (n=3), Salmonella Bredeney (n=2), Salmonella Brandenburg (n=1), Salmonella Gloucester (n=1), Salmonella Goldcoast (n=1), Salmonella Kottbus (n=1), and Salmonella Ruzizi (n=1). Campylobacter strains were present in 29.4% (10/34) of the investigated chicken samples, and the identified species were Campylobacter coli (70%) and C. jejuni (30%). From the 14 tested antimicrobials, the Salmonella isolates were resistant against azithromycin (88.2%), tetracycline (54.9%), sulfamethoxazole (54.9%), ciprofloxacin (45.1%), nalidixic acid (43.1%), ampicillin (35.3%), chloramphenicol (33.3%), tigecycline (25.5%), cefotaxime (13.7%), colistin (13.7%), trimethoprim (7.8%), and gentamicin (2%), resulting in the expression of 21 multidrug-resistant (MDR) profiles. Of 10 Campylobacter isolates, 80% were resistant to ciprofloxacin and nalidixic acid, 40% to tetracycline, and 10% to streptomycin and erythromycin, respectively. Our findings indicate that Romanian isolates of Salmonella spp. and Campylobacter spp., contaminating animal-origin foods, can exhibit MDR patterns, representing a public health risk.
ISSN: 15353141

Manafi, L., Aliakbarlu, J., Dastmalchi Saei, H.
Antibiotic resistance and biofilm formation ability of Salmonella serotypes isolated from beef, mutton, and meat contact surfaces at retail
(2020) Journal of Food Science, 85 (8), pp. 2516-2522.
ABSTRACT: Abstract: In this study, Salmonella isolates recovered from meat (beef and mutton) and meat contact surfaces at retail were investigated to determine their serotype, antibiotic resistance, and biofilm formation ability. Salmonella was found in 29 (24.17%) samples out of 120 samples including 14/50 (28%) of beef, 10/40 (25%) of mutton, and 5/30 (16.67%) of meat contact surfaces. Seven isolates were identified as S. Enteritidis, three as S. Typhimurium, and two as S. Typhi, while the rest of the isolates were considered as other Salmonella spp. All of the isolates were resistant to at least one antimicrobial agent and 48.27% of them were identified as multidrug-resistant (MDR) Salmonella. All (100%) of meat contact surfaces isolates, 42.8% of beef isolates, and 30% of mutton isolates were found to be MDR Salmonella. Resistance to nalidixic acid (100%), tetracycline (79.3%), and sulphamethoxazole/trimethoprim (44.8%) were observed. The gyrA gene was detected in 19 of 29 isolates, but tetA was found in one isolate. All of the serotypes were able to form biofilm (75.86 % moderate and 24.14 % strong) and S. Enteritidis was the strongest biofilm producer. The findings indicated that the majority of

Salmonella isolates in this study were MDR and biofilm producer. Then, safety measures such as cleaning and disinfection must be taken to control Salmonella and promote public health. Practical Application: The present study provides useful information on the prevalence of Salmonella serotypes in meat and meat contact surfaces and their antibiotic resistance patterns as well as biofilm formation capacities. Improving hygiene practices in livestock, slaughterhouses, and at retails may reduce the risk of meat contamination to Salmonella. Meanwhile, high levels of antibiotic resistance in Salmonella isolates emphasized on the improper use of antibiotics. ISSN: 00221147

Richards, A.K., Hopkins, B.A., Shariat, N.W.
Conserved CRISPR arrays in Salmonella enterica serovar Infantis can serve as qPCR targets to detect Infantis in mixed serovar populations
(2020) Letters in Applied Microbiology, 71 (2), pp. 138-145.
ABSTRACT: Salmonellosis is a leading bacterial cause of foodborne illness, and numerous Salmonella enterica serovars have been responsible for foodborne outbreaks. In the United States outbreaks are often linked to poultry and poultry-related products. The prevalence of Salmonella serovar Infantis has been increasing in poultry processing facilities over the past few years and in 2018 was identified as the causative agent for a large multistate outbreak linked to raw chicken. CRISPR-typing is a subtyping approach based on PCR and the sequencing of two Salmonella loci, CRISPR1 and CRISPR2. CRISPR-typing was used to interrogate 138 recent (2018–2019) isolates and genomes of ser. Infantis. Results show that the CRISPR elements are remarkably conserved in this serovar. The most conserved spacers, and those also unique to ser. Infantis, were used as targets to develop a ser. Infantis-specific qPCR assay. This assay was able to detect ser. Infantis in mixed serovar cultures of Salmonella, down to 0·1% of the population, highlighting the utility of this molecular approach in improving surveillance sensitivity for this important food safety pathogen. Significance and Impact of the Study: The incidence of human salmonellosis cases caused by Salmonella enterica serovar Infantis (ser. Infantis) has been increasing, as has its prevalence in broiler chickens, which are a frequent reservoir of Salmonella. A cluster of ser. Infantis genetically linked to an outbreak strain have been identified in numerous processing facilities. A qPCR assay targeting CRISPR elements that are unique to ser. Infantis has been developed and can detect this serovar directly from mixed cultures. This assay is sensitive enough to reveal ser. Infantis within a mixed Salmonella population where it constitutes only 0·1% of the population. The rapid nature of qPCR lends this assay to high-throughput screening of poultry samples to detect this important pathogen. ISSN: 02668254

Wellawa, D.H., Allan, B., White, A., Köster, W.
Iron-uptake systems of chicken-associated salmonella serovars and their role in colonizing the avian host
(2020) Microorganisms, 8 (8), art. no. 1203, pp. 1-25.
ABSTRACT: Iron is an essential micronutrient for most bacteria. Salmonella enterica strains, representing human and animal pathogens, have adopted several mechanisms to sequester iron from the environment depending on availability and source. Chickens act as a major reservoir for Salmonella enterica strains which can lead to outbreaks of human salmonellosis. In this review article we summarize the current understanding of the contribution of iron-uptake systems to the virulence of non-typhoidal S. enterica strains in colonizing chickens. We aim to address the gap in knowledge in this field, to help understand and define the interactions between S. enterica and these important hosts, in comparison to mammalian models. ISSN: 20762607

Kurtz, J.R., Nieves, W., Bauer, D.L., Israel, K.E., Adcox, H.E., Gunn, J.S., Morici, L.A., McLachlan, J.B.
Salmonella persistence and host immunity are dictated by the anatomical microenvironment
(2020) Infection and Immunity, 88 (8), art. no. e00026-20, .
ABSTRACT: The intracellular bacterial pathogen Salmonella is able to evade the immune system and persist within the host. In some cases, these persistent infections are asymptomatic for long periods and represent a significant public health hazard because the hosts are potential chronic carriers, yet the mechanisms that control persistence are incompletely understood. Using a mouse model of chronic typhoid fever combined with major histocompatibility complex (MHC) class II tetramers to interrogate endogenous, Salmonella-specific CD4+ helper T cells, we show that certain host microenvironments may favorably contribute to a pathogen's ability to persist in vivo. We demonstrate that the environment in the hepatobiliary system may contribute to the persistence of Salmonella enterica subsp. enterica serovar Typhimurium through liver-resident

immunoregulatory CD4+ helper T cells, alternatively activated macrophages, and impaired bactericidal activity. This contrasts with lymphoid organs, such as the spleen and mesenteric lymph nodes, where these same cells appear to have a greater capacity for bacterial killing, which may contribute to control of bacteria in these organs. We also found that, following an extended period of infection of more than 2 years, the liver appeared to be the only site that harbored Salmonella bacteria. This work establishes a potential role for nonlymphoid organ immunity in regulating chronic bacterial infections and provides further evidence for the hepatobiliary system as the site of chronic Salmonella infection. ISSN: 00199567

Carlin, C.R., Lau, S.S., Cheng, R.A., Buehler, A.J., Kassaify, Z., Wiedmann, M.
Validation using diverse, difficult-to-detect salmonella strains and a dark chocolate matrix highlights the critical role of strain selection for evaluation of simplified, rapid PCR-based methods offering next-day time to results
(2020) Journal of Food Protection, 83 (8), pp. 1374-1386.
ABSTRACT: Modifications to pathogen detection kits to accomplish simplified protocols with reduced time to results may impact method performance, particularly when combining shortened enrichment times and simplified enrichment procedures. We used Salmonella detection in dark chocolate as a model to test the impact of different enrichment times (minimum and maximum validated times) and procedures on detection of low levels of difficult-to-detect Salmonella strains, for three PCR kits that were AOAC International Performance Tested Method certified for detection of Salmonella spp. in dark chocolate. Initial inclusivity studies with pure cultures showed that all three kits detected 70 of 70 Salmonella spp. strains at 1 log above the theoretical limit of detection, with some strains yielding later cycle threshold values or having variable detection among technical replicates, indicating reduced assay performance for these strains. Based on these data, we selected a S. enterica subsp. enterica serovar Poona strain as well as three non-subsp. enterica strains to test the ability of the three kits to detect Salmonella in dark chocolate inoculated at low levels (0.06 to 1.18 most probable number per 25 g). With primary enrichment in skim milk at 358C, detection frequency for all assays did not significantly differ from the reference method for both the minimum and maximum validated enrichment times. However, a pilot study that used primary enrichment in buffered peptone water at 428C yielded significantly fewer positive samples (13 of 80) than were obtained with the U.S. Food and Drug Administration Bacteriological Analytical Manual method using enrichment in skim milk at 358C (40 of 80 positive samples); strains representing subsp. houtenae and salamae were detected in significantly fewer chocolate samples than enrichment with skim milk. Our data indicate that continued efforts to simplify rapid pathogen detection kits may reduce kit performance in a way that can only be detected with stringent evaluation protocols that are designed to identify kit failure modes. ISSN: 0362028X

Phan-Thien, K., Metaferia, M.H., Bell, T.L., Bradbury, M.I., Sassi, H.P., van Ogtrop, F.F., Suslow, T.V., McConchie, R.
Effect of soil type and temperature on survival of Salmonella enterica in poultry manure-amended soils
(2020) Letters in Applied Microbiology, 71 (2), pp. 210-217.
ABSTRACT: The effects of soil type and temperature on the survival of a cocktail of five Salmonella enterica serotypes (Enteritidis, Infantis, Montevideo, Typhimurium and Zanzibar) in manure-amended soils under controlled laboratory conditions was assessed. Containers of clay loam or sandy soil, unaltered or amended with 2% (w/w) poultry manure, were inoculated with S. enterica (~5 log10 CFU per gram) and held at 5, 21 or 37°C for 6 weeks. Statistical analysis of the persistence of S. enterica identified a significant three-way interaction between soil type, manure amendment and temperature. Clay loam soils and lower temperatures tended to support S. enterica persistence over 6 weeks with only 1- and 2-log reductions respectively. In contrast, sand and higher temperatures resulted in a 4-log and either 3- to 4-log reductions respectively. Manure amendment had an overarching effect of reducing die-off of S. enterica in comparison with unamended soils. This study highlights that a large component of variation of the rate of S. enterica reduction in soils may be attributed to combinations of environmental factors, in particular, soil type and temperature. It further underscores the importance of risk management strategies and industry guidelines based on local data and that reflect the diversity of prevailing horticultural production environments. Significance and Impact of the Study: The persistence of Salmonella enterica in soil environments was shown to be significantly influenced by a range of individual and interacting environmental effects, including temperature, soil type and amendment addition. This indicates that current horticultural food safety management systems which employ a uniform prescribed

exclusion period between application of manure and time of harvest may be unfit for purpose under certain conditions by either underestimating or overestimating pathogen die-off. These findings support exclusion periods that account for a range of environmental factors including temperature, soil type and growing region that may be more appropriate to manage microbiological risks associated with soil which has been amended with manure. ISSN: 02668254

Ćwiek, K., Korzekwa, K., Tabiś, A., Bania, J., Bugla-Płoskońska, G., Wieliczko, A.
Antimicrobial resistance and biofilm formation capacity of salmonella enterica serovar enteritidis strains isolated from poultry and humans in Poland
(2020) Pathogens, 9 (8), art. no. 643, pp. 1-22.
ABSTRACT: Salmonella enterica ser. Enteritidis (S. enterica ser. Enteritidis) is the most frequently detected serovar in human salmonellosis, and its ability to produce a biofilm and the risk of transmission from animals and food of animal origin to humans are significant. The main aim of the present work was to compare S. enterica ser. Enteritidis strains isolated from poultry and human feces in terms of resistance profiles, prevalence of selected resistance genes, and their potential for biofilm formation, by assessing their biofilm growth intensity, the prevalence and expression of selected genes associated with this phenomenon, and the correlation between increased antimicrobial resistance and biofilm formation ability of the two tested groups of S. enterica ser. Enteritidis. This study showed a difference in antimicrobial resistance (minimal inhibitory concentration value) between S. enterica ser. Enteritidis groups; however, the majority of multidrug-resistant (MDR) strains were isolated from poultry (environmental samples from chicken broilers, turkey broilers, and laying hens). Differences in the prevalence of resistance genes were observed; the most common gene among poultry strains was floR, and that among strains from humans was blaTEM. S. enterica ser. Enteritidis strains isolated from poultry under the tested incubation conditions exhibited better biofilm growth than strains isolated from humans. A higher level of gene expression associated with the production of cellulose was only detected in the S48 strain isolated from poultry. On the other hand, increased expression of genes associated with quorum sensing was observed in two strains isolated from poultry farms and one strain isolated from human feces. ISSN: 20760817

Figueras, L., Ferrer, L.M., González, J.M., Bueso, J.P., Ramos, J.J., Rubira, I., Burian, E., Lacasta, D.
Prevalence of Salmonella enterica subsp. diarizonae serotype 61:k:1:5:(7) in nasal secretions and stool of sheep flocks with and without cases of chronic proliferative rhinitis
(2020) Veterinary Microbiology, 247, art. no. 108767, .
ABSTRACT: Salmonella enterica subsp. diarizonae serotype 61: k: 1,5, (7) (SED) is a microorganism well adapted to sheep; however, it has also been described producing chronic proliferative rhinitis (CPR) in ovine. CPR causes a proliferative inflammation of the ventral nasal turbinates that may totally obstruct the nasal cavity. The main objective of the present study was to investigate the prevalence of SED in nostrils and stool of sheep without CPR clinical signs in commercial sheep farms of Spain with and without previous clinical cases of CPR. Five samplings were performed in 10 commercial sheep farms for one year. Samples from nostrils and faeces were taken from four animals without CPR visible clinical signs that belonged to four different age ranges at each farm visit. The prevalence of positive animals was 45.3 %, and the number of positive samples in nostrils was higher than in faeces (38.5 % vs 22.5 %). Only on one farm was no positive result obtained in the entire study. In almost all positive farms, sheep belonging to the youngest age ranges accounted for more than 50 % of positive isolates. Finally, farms with a previous diagnosis of CPR were 1.784 times more likely to have an animal with positive isolation than farms without a previous diagnosis. This could suggest that the infection pressure in the farm might favour the occurrence of clinical cases of the disease. However, further studies will be necessary to unravel why this saprophytic bacterium is able to cross the epithelial barrier causing severe rhinitis in certain animals. ISSN: 03781135

Esan, O.B., Perera, R., McCarthy, N., Violato, M., Fanshawe, T.R.
Incidence, risk factors, and health service burden of sequelae of campylobacter and non-typhoidal salmonella infections in England, 2000–2015: A retrospective cohort study using linked electronic health records
(2020) Journal of Infection, 81 (2), pp. 221-230.
ABSTRACT: Background: Reactive arthritis, irritable bowel syndrome (IBS), Guillain-Barré syndrome, ulcerative colitis, and Crohn's disease may be sequelae of Campylobacter or non-typhoidal Salmonella (NTS) infections. Proton pump inhibitors (PPI) and antibiotics may increase the risk of gastrointestinal infections (GII); however, their impact on sequelae onset is unclear. We investigated the incidence of sequelae, their association with

antibiotics and PPI prescription, and assessed the economic impact on the NHS. Methods: Data from the Clinical Practice Research Datalink for patients consulting their GP for Campylobacter or NTS infection, during 2000–2015, were linked to hospital, mortality, and Index of Multiple Deprivation data. We estimated the incidence of sequelae and deaths in the 12 months following GII. We conducted logistic regression modelling for the adjusted association with prescriptions. We compared differences in resource use and costs pre- and post-infection amongst patients with and without sequelae. Findings: Of 20,471 patients with GII (Campylobacter 17,838), less than 2% (347) developed sequelae, with IBS (268) most common. Amongst Campylobacter patients, those with prescriptions for PPI within 12 months before and cephalosporins within 7-days before/after infection had elevated risk of IBS (adjusted odds ratio [aOR] 2.1, 1.5–2.9) and (aOR 3.6, 1.1–11.7) respectively. Campylobacter sequelae led to ~ £1.3 million, (£750,000, £1.7 million) in additional annual NHS expenditure. Interpretation: Sequelae of Campylobacter and NTS infections are rare but associated with increased NHS costs. Prior prescription of PPI may be a modifiable risk factor. Incidence of sequelae, healthcare resource use and costs are essential parameters for future burden of disease studies. ISSN: 01634453

de Knegt, L.V., Kudirkiene, E., Rattenborg, E., Sørensen, G., Denwood, M.J., Olsen, J.E., Nielsen, L.R.
Combining Salmonella Dublin genome information and contact-tracing to substantiate a new approach for improved detection of infectious transmission routes in cattle populations (2020) Preventive Veterinary Medicine, 181, art. no. 104531, .
ABSTRACT: This study presents a new method for detection of between-herd livestock movements to facilitate disease tracing and more accurately describe network behaviour of relevance for spread of infectious diseases, including within-livestock business risk-carrying contacts that are not necessarily recorded anywhere. The study introduces and substantiates the concept of grouping livestock herds into business-units based on ownership and location in the tracing analysis of animal movement-based contact networks. To test the utility of this approach, whole core genome sequencing of 196 Salmonella Dublin isolates stored from previous surveillance and project activities was combined with information on cattle movements recorded in the Danish Cattle Database between 1997 and 2017. The aim was to investigate alternative explanations for S. Dublin circulation in groups of herds connected by ownership, but without complete records of livestock movements. The EpiContactTrace R-package was used to trace the contact networks between businesses and compare the network characteristics of businesses sharing strains of S. Dublin with different levels of genetic relatedness. The ownership-only definition proved to be an unreliable grouping approach for large businesses, which could have internal distances larger than 250 km and therefore do not represent useful epidemiological units. Therefore, the grouping was refined using spatial analysis. More than 90% of final business units formed were composed of one single cattle property, whereas multi-property businesses could reach up to eight properties in a given year, with up to 15 cattle herds having been part of the same business through the study period. Results showed markedly higher probabilities of introduction of infectious animals between proposed businesses from which the same clone of S. Dublin had been isolated, when compared to businesses with non-related strains, thus substantiating the business-unit as an important epidemiological feature to consider in contact network analysis and tracing of infection routes. However, this approach may overestimate real-life contacts between cattle properties and putatively overestimate the degree of risk-contacts within each business, since it is based solely on information about property ownership and location. This does not consider administrative and individual farmers behaviours that essentially keep two properties separated. Despite this, we conclude that defining epidemiological units based on businesses is a promising approach for future disease tracing tasks. ISSN: 01675877

Uelze, L., Borowiak, M., Bönn, M., Brinks, E., Deneke, C., Hankeln, T., Kleta, S., Murr, L., Stingl, K., Szabo, K., Tausch, S.H., Wöhlke, A., Malorny, B.
German-Wide Interlaboratory Study Compares Consistency, Accuracy and Reproducibility of Whole-Genome Short Read Sequencing
(2020) Frontiers in Microbiology, 11, art. no. 573972, .
ABSTRACT: We compared the consistency, accuracy and reproducibility of next-generation short read sequencing between ten laboratories involved in food safety (research institutes, state laboratories, universities and companies) from Germany and Austria. Participants were asked to sequence six DNA samples of three bacterial species (Campylobacter jejuni, Listeria monocytogenes and Salmonella enterica) in duplicate, according to their routine in-house sequencing protocol. Four different types of Illumina sequencing platforms (MiSeq, NextSeq, iSeq, NovaSeq) and one Ion Torrent sequencing

instrument (S5) were involved in the study. Sequence quality parameters were determined for all data sets and centrally compared between laboratories. SNP and cgMLST calling were performed to assess the reproducibility of sequence data collected for individual samples. Overall, we found Illumina short read data to be more accurate (higher base calling accuracy, fewer miss-assemblies) and consistent (little variability between independent sequencing runs within a laboratory) than Ion Torrent sequence data, with little variation between the different Illumina instruments. Two laboratories with Illumina instruments submitted sequence data with lower quality, probably due to the use of a library preparation kit, which shows difficulty in sequencing low GC genome regions. Differences in data quality were more evident after assembling short reads into genome assemblies, with Ion Torrent assemblies featuring a great number of allele differences to Illumina assemblies. Clonality of samples was confirmed through SNP calling, which proved to be a more suitable method for an integrated data analysis of Illumina and Ion Torrent data sets in this study. ISSN: 1664302X

Wang, F., Deng, L., Huang, F., Wang, Z., Lu, Q., Xu, C.
Flagellar Motility Is Critical for Salmonella enterica Serovar Typhimurium Biofilm Development
(2020) Frontiers in Microbiology, 11, art. no. 1695, .
ABSTRACT: The food-borne pathogen Salmonella enterica serovar Typhimurium (S. Typhimurium) causes self-limiting gastroenteritis in humans and is not easily eradicated because it often attaches to suitable surfaces to form biofilms that have high resistance to disinfectants and antimicrobials. To develop an alternative strategy for the treatment of biofilms, it is necessary to further explore the effects of flagellar motility on the development process of Salmonella biofilms. Here, we constructed flagella mutants (ΔflgE and ΔfliC) to systematically study this process. By comparing them with wild-type strains, we found that these mutants lacking flagellar motility form fewer biofilms in the early stage, and the formed mature biofilms contain more cells and extracellular polymeric substances (EPS). In addition, fewer mutant cells adhered to glass plates compared with wild-type cells even after 6 h of incubation, suggesting that flagellar motility plays a significant role in preliminary cell-surface interactions. More importantly, the motility of wild-type strain was greatly decreased when they were treated with carbonyl cyanide m-chlorophenylhydrazone, which inhibited flagellar motility and reduced biofilm formation, as in the case of the ΔflgE mutant. Overall, these findings suggest that flagellar motility plays an important role in Salmonella biofilm initiation and maturation, which can help us to counteract the mechanisms involved in biofilm formation and to develop more rational control strategies. ISSN: 1664302X

Luo, Y, Huang, C, Ye, J, Octavia, S, Wang, H, Dunbar, SA, Jin, D, Tang, YW, Lan, R
Comparison of xMAP Salmonella Serotyping Assay With Traditional Serotyping and Discordance Resolution by Whole Genome Sequencing
(2020) Frontiers in Cellular and Infection Microbiology, 10, art. no. 452, .
ABSTRACT: Salmonella spp. are a major cause of foodborne illness throughout the world. Traditional serotyping by antisera agglutination has been used as a standard identification method for many years but newer nucleic acid-based tests have become available that may provide advantages in workflow and test turnaround time. In this study, we evaluated the Luminex® xMAP® Salmonella Serotyping Assay (SSA), a multiplex nucleic acid test capable of identifying 85% of the most common Salmonella serotypes, in comparison to the traditional serum agglutination test (SAT) on 4 standard strains and 255 isolates from human (224), environmental, and food (31) samples. Of the total of 259 isolates, 256 could be typed by the SSA. Of these, 197 (77.0%) were fully typed and 59 (23.0%) were partially typed. By SAT, 246 of the 259 isolates (95%) were successfully typed. Sixty isolates had discrepant results between SAT and SSA and were resolved using whole genome sequencing (WGS). By SAT, 80.0% (48/60) of the isolates were consistent with WGS while by SSA 91.7% (55/60) were partially consistent with WGS. By serovar, all 30 serovars except one tested were fully or partially typable. The workflow comparison showed that SSA provided advantages over SAT with a hands-on time (HOT) of 3.5 min and total turnaround time (TAT) of 6 h, as compared to 1 h HOT and 2–6 days TAT for SAT. Overall, this study showed that molecular serotyping is promising as a rapid method for Salmonella serotyping with good accuracy for typing most common Salmonella serovars circulating in China.
ISSN: 22352988

Arafat, N., Abd El Rahman, S., Naguib, D., El-Shafei, R.A., Abdo, W., Eladl, A.H.
Co-infection of Salmonella enteritidis with H9N2 avian influenza virus in chickens
(2020) Avian Pathology, 49 (5), pp. 496-506.

ABSTRACT: Salmonella and avian influenza virus are important pathogens affecting the poultry industry and human health worldwide. In this experimental study, we evaluated the consequences of co-infection of Salmonella enteritidis (SE) with H9N2 avian influenza virus (H9N2-AIV) in chickens. Four groups were included: control group, H9N2-AIV group, H9N2-AIV + SE group, and SE group. Infected chickens were intranasally inoculated with H9N2-AIV at 21 days of age and then orally administered SE on the same day. The birds were monitored for clinical signs, mortality rates, and alterations in body weight. Sera, intestinal fluids, oropharyngeal, and cloacal swabs, and tissue samples were collected at 2, 6, 10, and 14 days post-infection (dpi). Significant increases in clinical signs and mortality rates were observed in the H9N2-AIV + SE group. Moreover, chickens with co-infection showed a significant change in body weight. SE faecal shedding and organ colonization were significantly higher in the H9N2-AIV + SE group than in the SE group. H9N2-AIV infection compromised the systemic and mucosal immunity against SE, as evidenced by a significant decrease in lymphoid organ indices as well as systemic antibody and intestinal immunoglobulin A (IgA) responses to SE and a significant increase in splenic and bursal lesion scores. Moreover, SE infection significantly increased shedding titres and duration of H9N2-AIV. In conclusion, this is the first report of co-infection of SE with H9N2-AIV in chickens, which leads to increased pathogenicity, SE faecal shedding and organ colonization, and H9N2-AIV shedding titre and duration, resulting in substantial economic losses and environmental contamination, ultimately leading to increased zoonoses.
ISSN: 03079457

Drauch, V., Ibesich, C., Vogl, C., Hess, M., Hess, C.
In-vitro testing of bacteriostatic and bactericidal efficacy of commercial disinfectants against Salmonella Infantis reveals substantial differences between products and bacterial strains
(2020) International Journal of Food Microbiology, 328, art. no. 108660, .
ABSTRACT: Salmonella (S.) Infantis is currently the most common serovar in broilers and boiler meat in the European Union. In the field, eradication of S. Infantis in affected poultry flocks is considered extremely difficult. Despite stringent cleaning and disinfection measures between the placement of flocks, recurrent infections are often reported. So far, the efficacy of disinfectants on S. Infantis has rarely been studied. Therefore, in the present in-vitro study the bacteriostatic and bactericidal efficacy of ten commercial disinfectants were tested against seven S. Infantis field isolates. Combinations of aldehyde and quarternary ammonium were the active compounds of five, peroxygen of three, cresol and alkylamines of one disinfectant, respectively. Investigations were performed according to standard protocols and regulations. Different concentrations of disinfectants were used to test the bacteriostatic efficacy. Different temperatures and low and high protein exposures were applied as variables to investigate the bactericidal efficacy. Following neutralization of the disinfectants an additional incubation step was introduced to investigate the revitalisation potential of S. Infantis. The bacteriostatic efficacy could be assessed for seven disinfectants. For three disinfectants a bacteriostatic effect was observed when the recommended concentration was used, whereas with four disinfectants only increased concentrations led to this effect. The bactericidal efficacy was not influenced by temperature, whereas high protein exposure decreased the efficacy of nine disinfectants. Furthermore, reactivation of S. Infantis was revealed after application of disinfectants for the majority of products. Interestingly, the strain of S. Infantis influenced the efficacy of the disinfectants. Overall, products based on aldehydes and quarternary ammonium compounds proved most efficient, followed by peroxgen, cresol and alkylamines. ISSN: 01681605

Peeters, L., Dewulf, J., Boyen, F., Brossé, C., Vandersmissen, T., Rasschaert, G., Heyndrickx, M., Cargnel, M., Mattheus, W., Pasmans, F., Haesebrouck, F., Maes, D.
Bacteriological evaluation of vaccination against Salmonella Typhimurium with an attenuated vaccine in subclinically infected pig herds
(2020) Preventive Veterinary Medicine, 182, art. no. 104687, .
ABSTRACT: Subclinical infections with Salmonella Typhimurium occur frequently in pigs. They constitute a risk for human salmonellosis and are difficult to control with currently available control measures. Vaccination against Salmonella Typhimurium in pigs can be an effective tool to control Salmonella infections at farm level. In the present study, the efficacy of an attenuated Salmonella Typhimurium vaccine (Salmoporc®, IDT Biologika) to control Salmonella infections in pigs was evaluated in three subclinically infected pig herds. The effect on Salmonella excretion and the number of pigs positive for Salmonella Typhimurium field and vaccine strains in ileocecal lymph nodes at slaughter were evaluated using five different vaccination strategies: 1. vaccination of sows, 2. vaccination of sows and piglets, 3. vaccination of sows and fattening pigs, 4. vaccination of piglets, 5.

vaccination of fattening pigs, which were all compared to a non-vaccinated control group (experimental group 6). Each vaccination strategy was implemented in each farm, during two consecutive production cycles of the same sows. The prevalence of Salmonella Typhimurium field strain excretion was low; in total, 4% of the fecal and overshoe samples collected in the non-vaccinated control group were Salmonella Typhimurium field strain positive. The excretion of Salmonella Typhimurium field strain did not significantly differ between farms, production cycles and experimental groups. Applying vaccination in either sows and piglets, sows and fattening pigs, or in piglets only, resulted in a significantly reduced number of Salmonella Typhimurium field strain positive lymph nodes of slaughter pigs in the second production cycle, but not in the first production cycle. Vaccination of sows and piglets resulted in the most consistent reduction of Salmonella Typhimurium field strain positive lymph nodes at slaughter. The vaccine strain was detected in the lymph nodes of 13 pigs at slaughter, indicating the possible persistence of the vaccine strain until slaughter. Because of limitations in the study design, and the variability between farms and production cycles, the results of the current observational study should be extrapolated with care. Nevertheless, the results provide evidence that applying vaccination against Salmonella Typhimurium in sows and piglets (preferred), sows and fattening pigs, and piglets only can support the control of Salmonella Typhimurium infections by decreasing the prevalence of Salmonella Typhimurium field strain positive lymph nodes at slaughter. ISSN: 01675877

Obe, T., Nannapaneni, R., Schilling, W., Zhang, L., McDaniel, C., Kiess, A.
Prevalence of Salmonella enterica on poultry processing equipment after completion of sanitization procedures
(2020) Poultry Science, 99 (9), pp. 4539-4548.
ABSTRACT: Salmonella is a poultry-borne pathogen that causes illness throughout the world. Consequently, it is critical to control Salmonella during the process of converting broilers to poultry meat. Sanitization of a poultry processing facility, including processing equipment, is a crucial control measure that is utilized by poultry integrators. However, prevalence of Salmonella on equipment after sanitization and its potential risk to food safety has not been evaluated thoroughly. Therefore, the objective of this study was to evaluate the persistence of Salmonella on poultry processing equipment before and following cleaning and sanitization procedure. A total of 15 locations within 6 commercial processing plants were sampled at 3 time points: (A) after processing; (B) after cleaning; and (C) after sanitization, on 3 separate visits for a total of 135 samples per plant. Salmonella-positive isolates were recovered from samples using the United States Department of Agriculture MLG 4.09 conventional method. Presumptive Salmonella colonies were subjected to biochemical tests for confirmation. Salmonella isolates recovered after sanitization were serotyped and tested for the presence of specific virulence genes. A completely randomized design with a 6 × 3 × 15 factorial arrangement was utilized to analyze the results for Salmonella prevalence between processing plants. Means were separated using Fishers protected least significant difference when $P \leq 0.05$. For Salmonella prevalence between processing plants, differences ($P < 0.0001$) were observed in the 6 plants tested where the maximum and minimum prevalence was 29.6 and 7.4%, respectively. As expected, there was a difference ($P < 0.0001$) in the recovery of Salmonella because of sampling time. Salmonella prevalence at time A (36%) was significantly higher, whereas there was no difference between time B (12%) and C (9%). There was a location effect ($P < 0.0001$) for the prevalence of Salmonella with the head puller, picker, cropper, and scalder having a significantly higher prevalence when compared with several other locations. At sampling time C, a trend toward a difference ($P = 0.0899$) was observed for Salmonella prevalence between the 6 plants, whereas significant differences were observed because of location ($P = 0.0031$). Five prominent Salmonella enterica serovars were identified, including Kentucky, Schwarzengrund, Enteritidis, Liverpool, and Typhimurium with S. Kentucky being the most prevalent. PCR analysis of 8 Salmonella virulence genes showed that the invA, sipB, spiA, sseC, and fimA were detected in all isolates, whereas genes carried on plasmids and/or fimbriae varied remarkably among all isolates. This study established Salmonella prevalence and persistence in poultry processing facilities after antimicrobial application through sanitization procedures which could result in contamination of poultry carcasses and food safety risks because of poultry meat. ISSN: 00325791

Sevilla-Navarro, S., Catalá-Gregori, P., Marin, C.
Salmonella bacteriophage diversity according to most prevalent salmonella serovars in layer and broiler poultry farms from Eastern Spain
(2020) Animals, 10 (9), art. no. 1456, pp. 1-11.

ABSTRACT: The exploration of novel nonantibiotic interventions in the field, such as the use of bacteriophages, is necessary to avoid the presence of Salmonella. Bacteriophages are a group of viruses widely distributed in nature, strictly associated with the prokaryotic cell. Researchers have demonstrated the success of phage therapy in reducing Salmonella counts in poultry products. However, the impact that phage concentration in the environment may have against certain Salmonella serovars is not well understood. Therefore, the aim of this study was to assess Salmonella phage prevalence in commercial poultry farms in terms of the production type: layers or broilers. The most prevalent Salmonella serovars isolated in poultry production were used for phage isolation. Salmonella specific phages were isolated from 141 layer and broiler farms located in the Valencia region during 2019. Analysis of the samples revealed that 100% presented Salmonella phages, the most prevalent being against serovar S. Enteritidis (93%), followed by S. Virchow (59%), S. Typhimurium (55%), S. Infantis (52%) and S. Ohio (51%). These results indicate that poultry farms could represent an important source of Salmonella phages. Nevertheless, further studies are needed to assess the epidemiology of phages against other serovars present in other countries and their diversity from the point of view of molecular studies. ISSN: 20762615

Siemionek, J., Przywara, K., Szczerba-Turek, A.
The prevalence of salmonella spp. In two arctic fox (alopex lagopus) farms in Poland (2020) Animals, 10 (9), art. no. 1688, pp. 1-7.
ABSTRACT: The objective of the study was to determine the occurrence of Salmonella spp. infections in two Arctic fox (Alopex lagopus) farms in Poland, and to analyse the correlations between animals that tested positive for Salmonella spp and breeding results. Faecal samples were taken from 1094 clinically healthy blue foxes from the basic stock of farms A and B. Salmonella spp. were detected in 18.06% (56/310) of the samples collected in farm A and in 15.94% (125/784) of the samples collected in farm B. All isolated strains belonged to S. enterica subsp. enterica serotypes Salmonella Saintpaul (S. Saintpaul), Salmonella Reading (S. Reading), and Salmonella Heidelberg (S. Heidelberg). All three serotypes are typically isolated from commercial poultry flocks. Salmonella spp. infections significantly increased the risk of female infertility, but further research is needed to confirm the results. This is the first report on the prevalence of S. Heidelberg, S. Saintpaul, and S. Reading in faecal samples collected from Arctic fox (Alopex lagopus) farms in Poland. ISSN: 20762615

Mourão, J., Rebelo, A., Ribeiro, S., Peixe, L., Novais, C., Antunes, P.
Atypical non-h2s-producing monophasic salmonella typhimurium st3478 strains from chicken meat at processing stage are adapted to diverse stresses (2020) Pathogens, 9 (9), art. no. 701, pp. 1-18.
ABSTRACT: Poultry products are still an important cause of Salmonella infections worldwide, with an increasingly reported expansion of less-frequent serotypes or atypical strains that are frequently multidrug-resistant. Nevertheless, the ability of Salmonella to survive antimicrobials promoted in the context of antibiotic reducing/replacing and farming rethinking (e.g., organic acids and copper in feed/biocides) has been scarcely explored. We investigated Salmonella occurrence (conventional and molecular assays) among chicken meat at the processing stage (n = 53 batches/29 farms) and characterized their tolerance to diverse stress factors (antibiotics, copper, acid pH, and peracetic acid). Whole-genome sequencing was used to assess adaptive features and to perform comparative analysis. We found a low Salmonella occurrence (4%) and identified S. Enteritidis/ST11 plus atypical non-H2S-producing S. 1,4,[5],12:i:-/ST3478. The ST3478 presented the ability to grow under diverse stresses (antibiotics, copper, and acid-pH). Comparative genomics among ST3478 isolates showed similar antibiotic/metal resistance gene repertoires and identical nonsense phsA thiosulfate reductase mutations (related to H2S-negative phenotype), besides their close phylogenetic relationship by cgMLST and SNPs. This study alerts for the ongoing national and international spread of an emerging monophasic Salmonella Typhimurium clonal lineage with an enlarged ability to survive to antimicrobials/biocides commonly used in poultry production, being unnoticed by conventional Salmonella detection approaches due to an atypical non-H2S-producing phenotype. ISSN: 20760817

Peeters, L., Dewulf, J., Boyen, F., Brossé, C., Vandersmissen, T., Rasschaert, G., Heyndrickx, M., Cargnel, M., Mattheus, W., Pasmans, F., Haesebrouck, F., Maes, D.
Evaluation of group vaccination of sows and gilts against Salmonella Typhimurium with an attenuated vaccine in subclinically infected pig herds (2020) Preventive Veterinary Medicine, 182, art. no. 104884, .
ABSTRACT: Subclinical Salmonella Typhimurium infections occur frequently in pigs and constitute a major risk for human salmonellosis. With the currently available control

measures, Salmonella Typhimurium infections in pigs remain difficult to control. Vaccination has been proposed to be an effective tool to control infections at farm level. In the current study, the effect of group vaccination of sows and gilts against Salmonella Typhimurium is evaluated on Salmonella prevalence in fecal and overshoe samples and ileocecal lymph nodes, and on serology in the sows and their offspring in three subclinically infected pig farms. In each farm, all sows and gilts were vaccinated twice, three weeks apart, with an attenuated histidine-adenine auxotrophic vaccine (Salmoporc®, IDT Biologika). From three months after the group vaccination onwards, all sows were given a booster dose three weeks before every farrowing. The farms were monitored bacteriologically and serologically from 12 months before until 15 months after the group vaccination. After group vaccination, no significant effect was detected in the prevalence of Salmonella Typhimurium in the fecal and overshoe samples collected in the sows (before: 2 %, after: 0 %) and their offspring at 18 weeks (before: 17 %, after: 11 %) and at 26 weeks of age (before: 15 %, after: 7 %), and when combining the results of the offspring at 18 and 26 weeks of age (before: 16 %, after: 9 %). Also, no significant effect was detected in the prevalence of Salmonella Typhimurium positive lymph nodes of sows (before and after: 0 %) and their offspring (before: 4 %, after: 7 %). Regarding serology, the mean S/P-ratios of the sows were significantly higher after the group vaccination, compared to before group vaccination (before: 1.50, after: 2.32, $p < 0.001$). The mean S/P-ratios of the offspring at slaughter age were significantly lower after the group vaccination, compared to before group vaccination (before: 1.71, after: 1.04, $p = 0.001$). In conclusion, group vaccination of sows and gilts resulted in a more beneficial serological status of the offspring, but did not significantly decrease Salmonella Typhimurium excretion and lymph node contamination. ISSN: 01675877

Rockett, R.J., Arnott, A., Wang, Q., Howard, P., Sintchenko, V., Rockett, R.J.
Genomic surveillance enables suitability assessment of salmonella gene targets used for culture-independent diagnostic testing
(2020) Journal of Clinical Microbiology, 58 (9), art. no. e00038-20, .
ABSTRACT: Salmonella is a highly diverse genus consisting of over 2,600 serovars responsible for high-burden food- and waterborne gastroenteritis worldwide. Sensitivity and specificity of PCR-based culture-independent diagnostic testing (CIDT) systems for Salmonella, which depend on a highly conserved gene target, can be affected by single nucleotide polymorphisms (SNPs), indels, and genomic rearrangements within primer and probe sequences. This report demonstrates the value of prospectively collected genomic data for verifying CIDT targets. We utilized the genomes of 3,165 Salmonella isolates prospectively collected and sequenced in Australia. The sequences of Salmonella CIDT PCR gene targets (ttrA, spaO, and invA) were systematically interrogated to measure nucleotide dissimilarity. Analysis of 52 different serovars and 79 multilocus sequencing types (MLST) demonstrated dissimilarity within and between PCR gene targets ranging between 0 and 81.3 SNP/kbp (0 and 141 SNPs). The lowest average dissimilarity was observed in the ttrA target gene used by the Roche LightMix at 2.0 SNP/kbp (range, 0 to 46.7); however, entropy across the gene demonstrates that it may not be the most stable CIDT target. While debate continues over the benefits and pitfalls of replacing bacterial culture with molecular assays, the growing volumes of genomic surveillance data enable periodic regional reassessment and validation of CIDT targets against both prevalent and emerging serovars. If PCR systems are to become the primary screening and diagnostic tool for laboratory diagnosis of salmonellosis, ongoing monitoring of the genomic diversity in PCR target regions is warranted, as is the potential inclusion of two Salmonella PCR targets in frontline diagnostic systems. ISSN: 00951137

Sabry, M.A., Abdel-Moein, K.A., Abdel-Kader, F., Hamza, E.
Extended-spectrum β-lactamase-producing Salmonella serovars among healthy and diseased chickens and their public health implication
(2020) Journal of Global Antimicrobial Resistance, 22, pp. 742-748.
ABSTRACT: Objectives: This study investigated the occurrence of extended-spectrum β-lactamase (ESBL)-producing Salmonella and the associated virulence genes among farmed chickens. Methods: Cloacal swab samples were collected from apparently healthy and diseased chickens and were cultured for Salmonella using conventional methods. The isolates were serotyped using slide agglutination tests and were examined by polymerase chain reaction (PCR) for the virulence genes invA, stn, svpC and pefA and the outer membrane protein-encoding genes ompA and ompF. Screening for ESBL resistance was performed using the disk-diffusion test, the combinational-disk test with clavulanic acid, and multiplex PCR for blaTEM, blaSHV, blaCTX-M and blaOXA. The presence of the AmpC blaCMy-2 was tested among the ESBL-negative isolates by uniplex PCR. The resistant isolates were partially sequenced based on the stn gene. Results: The Salmonella isolation

rate was 3.4% (6/175) from healthy and 11.1% (14/126) from diseased chickens. The 20 isolates belong to serotypes with public health significance like Typhimurium, Kentucky and Infantis. All the isolates possess invA, stn, svpC and ompF genes; 16 isolates harboured ompA, and one carried pefA. Of the 20 isolates, 19 were resistant to more than one antibiotic. Of these 19 isolates, 16 were ESBL-producing with the majority carrying blaTEM and blaSHV genes. The four ESBL-negative isolates carried blaCMY-2. Partial-stn-sequencing of the isolates revealed a high genetic relatedness to Salmonella strains from patients in Egypt and Asia. Conclusions: Virulent ESBL-producing Salmonella was isolated from healthy and diseased chickens; the strains have a close relationship to human strains, posing a public health threat. ISSN: 22137165

Guerrero, T., Calderón, D., Zapata, S., Trueba, G.
Salmonella grows massively and aerobically in chicken faecal matter
(2020) Microbial Biotechnology, 13 (5), pp. 1678-1684.
ABSTRACT: The use of wastewater for irrigation and animal manure as fertilizer can cause transmission of intestinal pathogens, conditions frequently observed in low- and middle-income countries (LMICs). Here, we tested the ability of Salmonella to grow in the faecal matter. We inoculated freshly isolated Salmonella strains (from chickens) in chicken faecal matter and incubated for 1 to 12 days, under aerobic and anaerobic conditions. We found that both Salmonella and Escherichia coli multiplied massively in faecal matter outside a host and significantly higher in aerobic conditions. Our results have critical implications in waste management, as we demonstrate that aerobic treatments may not be the best to reduce the number of Salmonella in the environment. ISSN: 17517907

Lee, K.-H., Lee, J.-Y., Roy, P.K., Mizan, M.F.R., Hossain, M.I., Park, S.H., Ha, S.-D.
Viability of Salmonella Typhimurium biofilms on major food-contact surfaces and eggshell treated during 35 days with and without water storage at room temperature
(2020) Poultry Science, 99 (9), pp. 4558-4565.
ABSTRACT: Salmonella is one of the main foodborne pathogens that affect humans and farm animals. The Salmonella genus comprises a group of food-transmitted pathogens that cause highly prevalent foodborne diseases throughout the world. The aim of this study was to appraise the viability of Salmonella Typhimurium biofilm under water treatment at room temperature on different surfaces, specifically stainless steel (SS), plastic (PLA), rubber (RB), and eggshell (ES). After 35 D, the reduction of biofilm on SS, PLA, RB, and ES was 3.35, 3.57, 3.22, and 2.55 log CFU/coupon without water treatment and 4.31, 4.49, 3.50, and 1.49 log CFU/coupon with water treatment, respectively. The dR value (time required to reduce bacterial biofilm by 99% via Weibull modeling) of S. Typhimurium without and with water treatment was the lowest on PLA (176.86 and 112.17 h, respectively) and the highest on ES (485.37 and 2,436.52 h, respectively). The viability of the S. Typhimurium on ES and the 3 food-contact surfaces was monitored for 5 wk (35 D). The results of this study provide valuable information for the control of S. Typhimurium on different surfaces in the food industry, which could reduce the risk to consumers. ISSN: 00325791

Müştak, İ.B., Müştak, H.K., Sarıçam, S.
Molecular characterisation of hydrogen sulfide negative Salmonella enterica serovar Havana
(2020) Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology, 113 (9), pp. 1241-1246.
ABSTRACT: Hydrogen sulfide (H2S) detection is a screening method for distinguishing and identifying Salmonella strains from other bacteria in the intestine. Incidences of H2S-negative Salmonella have recently been reported in different countries. Although a high resistance rate against antimicrobial agents has been reported for H2S-positive Salmonella in many regions of the world, there is increasing evidence that high resistance to antibiotics has also increased in many H2S-negative Salmonella isolates. In this study, molecular characterisation of three H2S-negative Salmonella Havana, isolated from cloacal swab samples of broiler chickens, was performed. The phsA, phsB and phsC genes of the phs operon, which is responsible for hydrogen sulfide production, were amplified. Sequence analysis was then performed to identify mutations in the gene cluster. The antimicrobial resistance profiles of the isolates were determined by disc diffusion. Molecular characterisation was performed by multilocus sequence typing (MLST) and pulsed field gel electrophoresis (PFGE). The sequence analysis showed identified five point mutations in the phsA gene and one point mutation in the phsC gene in all isolates. The antibiotic resistance profile showed that the strains were resistant to cefoxitin and ceftazidime. MLST analysis showed that all strains belonged to sequence type (ST) 1621. This study is the first to report the H2S-negative S. Havana serotype. ISSN: 00036072

Belias, A.M., Sbodio, A., Truchado, P., Weller, D., Pinzon, J., Skots, M., Allende, A., Munther, D., Suslow, T., Wiedmann, M., Ivanek, R.
Effect of weather on the die-off of escherichia coli and attenuated salmonella enterica serovar typhimurium on preharvest leafy greens following irrigation with contaminated water
(2020) Applied and Environmental Microbiology, 86 (17), art. no. e00899, .
ABSTRACT: The Food Safety Modernization Act (FSMA) includes a time-to-harvest interval following the application of noncompliant water to preharvest produce to allow for microbial die-off. However, additional scientific evidence is needed to support this rule. This study aimed to determine the impact of weather on the die-off rate of Escherichia coli and Salmonella on spinach and lettuce under field conditions. Standardized, replicated field trials were conducted in California, New York, and Spain over 2 years. Baby spinach and lettuce were grown and inoculated with an ~104-CFU/ml cocktail of E. coli and attenuated Salmonella. Leaf samples were collected at 7 time points (0 to 96 h) following inoculation; E. coli and Salmonella were enumerated. The associations of die-off with study design factors (location, produce type, and bacteria) and weather were assessed using log-linear and biphasic segmented log-linear regression. A segmented loglinear model best fit die-off on inoculated leaves in most cases, with a greater variation in the segment 1 die-off rate across trials (-0.46 [95% confidence interval [95% CI],-0.52,-0.41] to-6.99 [95% CI,-7.38,-6.59] log10 die-off/day) than in the segment 2 die-off rate (0.28 [95% CI,-0.20, 0.77] to-1.00 [95% CI,-1.16,-0.85] log10 die-off/ day). A lower relative humidity was associated with a faster segment 1 die-off and an earlier breakpoint (the time when segment 1 die-off rate switches to the segment 2 rate). Relative humidity was also found to be associated with whether die-off would comply with FSMA's specified die-off rate of-0.5 log10 die-off/day. IMPORTANCE The log-linear die-off rate proposed by FSMA is not always appropriate, as the die-off rates of foodborne bacterial pathogens and specified agricultural water quality indicator organisms appear to commonly follow a biphasic pattern with an initial rapid decline followed by a period of tailing. While we observed substantial variation in the net culturable population levels of Salmonella and E. coli at each time point, die-off rate and FSMA compliance (i.e., at least a 2 log10 die-off over 4 days) appear to be impacted by produce type, bacteria, and weather; die-off on lettuce tended to be faster than that on spinach, die-off of E. coli tended to be faster than that of attenuated Salmonella, and die-off tended to become faster as relative humidity decreased. Thus, the use of a single die-off rate for estimating time-to-harvest intervals across different weather conditions, produce types, and bacteria should be revised.
ISSN: 00992240

Salive, A.F.V., Prudêncio, C.V., Baglinière, F., Oliveira, L.L., Ferreira, S.O., Vanetti, M.C.D.
Comparison of stress conditions to induce viable but non-cultivable state in Salmonella (2020) Brazilian Journal of Microbiology, 51 (3), pp. 1269-1277.
ABSTRACT: Salmonella can enter on the viable but non-culturable state (VBNC), characterized by the loss of ability to grow in routine culture media hindering detection by conventional methods and underestimation of the pathogen. Despite advances in research done so far, studies comparing conditions that lead Salmonella into the VBNC state are scarce. The main objective of this study was to evaluate different stresses to induce Salmonella to the VNBC state. Osmotic (1.2 M NaCl), acid (peracetic acid, 5.66 mg/mL) and oxidative (hydrogen peroxide, 1.20 mg/mL) stress were used at 4 °C to induce Salmonella enterica serovars Enteritidis and Typhimurium to the VBNC state. The culturability loss was monitored in the brain heart infusion (BHI) broth and agar, and the viability was determined by fluorescence microscopy, using the Live/Dead® kit, and by flow cytometry. Besides, the morphological characterization by atomic force microscopy (AFM) was performed. Storage in 1.2 M NaCl at 4 °C induced the VBNC state in Salmonella cells for periods longer than 121 days, and the percentage of viable cells has reached above 80.9%. More aggressive stress conditions promoted by peracetic acid and hydrogen peroxide induced the VBNC state in periods of, at most 0.14 day, and resulted in percentages of 8.5% to 45.5% viable cells, respectively. The counts of viable cells in the flow cytometer corroborate the results obtained by microscopic counts. The VBNC cells obtained in 1.2 M NaCl at 4 °C showed morphological changes, reducing the size and changing the morphology from bacillary to coccoid. No morphological change was observed on the cells stressed by acid or oxidant compounds. ISSN: 15178382

Shah, D.H., Board, M.M., Crespo, R., Guard, J., Paul, N.C., Faux, C.
The occurrence of Salmonella, extended-spectrum β-lactamase producing Escherichia coli and carbapenem resistant non-fermenting Gram-negative bacteria in a backyard poultry flock environment

(2020) Zoonoses and Public Health, 67 (6), pp. 742-753.
ABSTRACT: Increase in the number of small-scale backyard poultry flocks in the USA has substantially increased human-to-live poultry contact, leading to increased public health risks of the transmission of multi-drug resistant (MDR) zoonotic and food-borne bacteria. The objective of this study was to detect the occurrence of Salmonella and MDR Gram-negative bacteria (GNB) in the backyard poultry flock environment. A total of 34 backyard poultry flocks in Washington State (WA) were sampled. From each flock, one composite coop sample and three drag swabs from nest floor, waterer-feeder, and a random site with visible faecal smearing, respectively, were collected. The samples were processed for isolation of Salmonella and other fermenting and non-fermenting GNB under ceftiofur selection. Each isolate was identified to species level using MALDI-TOFF and tested for resistance against 16 antibiotics belonging to eight antibiotic classes. Salmonella serovar 1,4,[5],12:i:- was isolated from one (3%) out of 34 flocks. Additionally, a total of 133 ceftiofur resistant (CefR) GNB including Escherichia coli (53), Acinetobacter spp. (45), Pseudomonas spp. (22), Achromobacter spp. (8), Bordetella trematum (1), Hafnia alvei (1), Ochrobactrum intermedium (1), Raoultella ornithinolytica (1), and Stenotrophomonas maltophilia (1) were isolated. Of these, 110 (82%) isolates displayed MDR. Each flock was found positive for the presence of one or more CefR GNB. Several MDR E. coli (n = 15) were identified as extended-spectrum β-lactamase (ESBL) positive. Carbapenem resistance was detected in non-fermenting GNB including Acinetobacter spp. (n = 20), Pseudomonas spp. (n = 11) and Stenotrophomonas maltophila (n = 1). ESBL positive E. coli and carbapenem resistant non-fermenting GNB are widespread in the backyard poultry flock environment in WA State. These GNB are known to cause opportunistic infections, especially in immunocompromised hosts. Better understanding of the ecology and epidemiology of these GNB in the backyard poultry flock settings is needed to identify potential risks of transmission to people in proximity. ISSN: 18631959

Zhang, J.-F., Wei, B., Cha, S.-Y., Shang, K., Jang, H.-K., Kang, M.
The use of embryonic chicken eggs as an alternative model to evaluate the virulence of Salmonella enterica serovar Gallinarum
(2020) PLoS ONE, 15 (9 September), art. no. e0238630, .
ABSTRACT: Salmonella enterica serovar Gallinarum (S. Gallinarum) can cause fowl typhoid, a severe systemic disease responsible for considerable economic losses. Chicken pathogenicity test is the traditional method for assessing the virulence of S. Gallinarum. However, this method is limited by several factors, including ethical considerations, costs, and the need for specialized facilities. Hence, we established a chicken embryo lethality assay (ELA) model to determine the virulence of S. Gallinarum. Three virulent and three avirulent representative strains, which were confirmed by the chicken pathogenicity test, were used to perform the ELA. The most significant difference between the virulent and avirulent strains could be observed when 13-day-old embryos were inoculated via the AC route and incubated for 5 days. Based on a 50% embryo lethal dose (ELD50), isolates considered to be virulent had a Log10ELD50 of ≦ 4.0, moderately virulent strains had a Log10ELD50 of 4.0−6.1, and avirulent isolates had a Log10ELD50 of ≥ 6.1. Different abilities to invade the liver of embryos were found between the virulent and avirulent strains by a growth curve experiment in vitro. The maximum colony-forming units (CFU) of the virulent strain was about 10,000 times higher than that of the avirulent strain in the liver at 5 days post infection. The ELA results of 42 field strains showed that thirty-two strains (76.2%) were virulent, nine were moderately virulent (21.4%), and one strain was avirulent (2.4%). In conclusion, these results suggest that the ELA can be used as an alternative method to assess the virulence of S. Gallinarum, which will contribute to the study of virulence genes, virulence evolution, pathogenic mechanisms and vaccine development. ISSN: 19326203

Mao, C., Xue, C., Wang, X., He, S., Wu, L., Yan, X.
Rapid quantification of pathogenic Salmonella Typhimurium and total bacteria in eggs by nano-flow cytometry
(2020) Talanta, 217, art. no. 121020, .
ABSTRACT: Rapid quantification of pathogenic Salmonella Typhimurium (S. Typhimurium) and total bacteria in eggs is highly desired for food safety control. However, the complexity of egg matrix presents a significant challenge for sensitive detection of bacteria. In this study, a sample pretreatment protocol, including dilution, fat dissolution, protein degradation, filtration, and washing was developed to circumvent this challenge. A laboratory-built nano-flow cytometer (nFCM) that is hundreds of fold more sensitive than the conventional flow cytometer was employed to analyze individual bacteria upon nucleic acid and immunofluorescent staining. Eggs spiked with pathogenic S. Typhimurium and harmless Escherichia coli K12 (E. coli K12) were used as the model system to optimize the

sample pretreatment protocol. S. Typhimurium and total bacteria in eggs can be quantified without cultural enrichment, and the whole process of sample pretreatment, staining, and instrument analysis can be accomplished within 1.5 h. The bacterial recovery rate upon sample pretreatment, detection limit, and dynamic range for S. Typhimurium in eggs were 92%, 2 × 103 cells/mL, and from 2 × 103 to 4 × 108 cells/mL, respectively. The as-developed approach can specifically distinguish S. Typhimurium from other bacteria and successful application to bacterial detection in eggs freshly purchased from supermarket and spoiled eggs upon inappropriate storage was demonstrated. ISSN: 00399140

Munck, N., Njage, P.M.K., Leekitcharoenphon, P., Litrup, E., Hald, T.
Application of Whole-Genome Sequences and Machine Learning in Source Attribution of Salmonella Typhimurium
(2020) Risk Analysis, 40 (9), pp. 1693-1705.
ABSTRACT: Prevention of the emergence and spread of foodborne diseases is an important prerequisite for the improvement of public health. Source attribution models link sporadic human cases of a specific illness to food sources and animal reservoirs. With the next generation sequencing technology, it is possible to develop novel source attribution models. We investigated the potential of machine learning to predict the animal reservoir from which a bacterial strain isolated from a human salmonellosis case originated based on whole-genome sequencing. Machine learning methods recognize patterns in large and complex data sets and use this knowledge to build models. The model learns patterns associated with genetic variations in bacteria isolated from the different animal reservoirs. We selected different machine learning algorithms to predict sources of human salmonellosis cases and trained the model with Danish Salmonella Typhimurium isolates sampled from broilers (n = 34), cattle (n = 2), ducks (n = 11), layers (n = 4), and pigs (n = 159). Using cgMLST as input features, the model yielded an average accuracy of 0.783 (95% CI: 0.77–0.80) in the source prediction for the random forest and 0.933 (95% CI: 0.92–0.94) for the logit boost algorithm. Logit boost algorithm was most accurate (valid accuracy: 92%, CI: 0.8706–0.9579) and predicted the origin of 81% of the domestic sporadic human salmonellosis cases. The most important source was Danish produced pigs (53%) followed by imported pigs (16%), imported broilers (6%), imported ducks (2%), Danish produced layers (2%), Danish produced cattle and imported cattle (<1%) while 18% was not predicted. Machine learning has potential for improving source attribution modeling based on sequence data. Results of such models can inform risk managers to identify and prioritize food safety interventions. ISSN: 02724332

Mizzi, L., Maniscalco, D., Gaspari, S., Chatzitzika, C., Gatt, R., Valdramidis, V.P.
Assessing the individual microbial inhibitory capacity of different sugars against pathogens commonly found in food systems
(2020) Letters in Applied Microbiology, 71 (3), pp. 251-258.
ABSTRACT: Highly concentrated sugar solutions are known to be effective antimicrobial agents. However, it is unknown whether this effect is solely the result of the collective osmotic effect imparted by a mixture of sugars or whether the type of carbohydrate used also has an individual chemical effect on bacterial responses, that is, inhibition/growth. In view of this, in this work, the antimicrobial properties of four sugars, namely, glucose, fructose, sucrose and maltose against three common food pathogens; Staphylococcus aureus, Escherichia coli and Salmonella enterica, were investigated using a turbidimetric approach. The results obtained indicate that the type of sugar used has a significant effect on the extent of bacterial inhibition which is not solely dependent on the water activity of the individual sugar solution. In addition, while it was shown that high sugar concentrations inhibit bacterial growth, very low concentrations show the opposite effect, that is, they stimulate bacterial growth, indicating that there is a threshold concentration upon which sugars cease to act as antimicrobial agents and become media instead. Significance and Impact of the Study: In this work, an analysis on the antimicrobial properties of glucose, fructose, sucrose and maltose in solution was conducted using a turbidimetric approach. Our findings indicate that while, as expected, all of these sugars exhibit significant antimicrobial effects at high concentrations, at low concentrations they appear to act as substrates for the bacteria which results in enhanced microbial growth instead of inhibition. In addition, the results obtained also suggest that the resultant osmotic stress imparted by the sugar solutions is not the only factor which determines their antimicrobial activity and that other chemical factors may be playing a significant role. ISSN: 02668254