

NEWSLETTER

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Editorial Note

Bilthoven, 6 April 2018

Dear colleague,

The first quarter of this year we have been busy with several activities in relation to the EURL-*Salmonella* interlaboratory studies.

In January/February 2018, the analysis of the serotyping results of the **22nd EURL-*Salmonella* interlaboratory comparison study on typing of *Salmonella*** was performed. By mid-February the laboratories received their own results as well as the interim summary report containing the results of all participants. This interim summary report is also available at the EURL-*Salmonella* website:

<https://www.eurlsalmonella.eu/dsresource?type=pdf&disposition=inline&objectid=rivmp:338231&versionid=&subobjectname=> All participants scored a good performance with the serotyping of the different *Salmonella* serovars. The results of the PFGE typing part of the interlaboratory study are still under analysis and will soon be reported to the participants.

In February/March 2018, the **4th interlaboratory comparison study on the detection of *Salmonella* in animal feed** was organised. Unfortunately some unexpected results were found with this study, as the number of negative samples was higher than expected. Currently the results are under analysis and will be further investigated.

By the end of last year and earlier this year, we have sent information about the **EURL-*Salmonella* workshop of 2018**. With the kind help of the NRL-*Salmonella* in Sweden we are able to organise this years' workshop in Uppsala, Sweden. In the meantime NRLs have registered and our secretary, Jeanette van Essen, will soon start booking the flights after which the participants will be informed about the details. Currently we are preparing the draft program and as soon as this is worked out in more detail, the participants will be informed as well.

For the validation of **draft CEN ISO/TS 6579-4** on 'Identification of monophasic *Salmonella* Typhimurium (1,4,[5],12,i:-) by Polymerase chain reaction (PCR)', a call for test strains among the NRLs-*Salmonella* was made by end-2016. In total we have received approximately 400 strains early 2017, which is a great result but too much to test all. In 2017 we have confirmed all strains and informed all senders about these results by February 2018. Early 2018 we have made a further selection of 172 strains (target and non-target strains) which will be tested in the coming months with the PCR protocols by the NRL-*Salmonella* in Germany and by the EURL-*Salmonella*.

By mid-April 2018 we have to report the **annual technical and financial report of the activities of EURL-*Salmonella* performed in 2017** to EC DG-Sante. The draft report is ready and the final report will soon be sent to DG-Sante and will be included in the next newsletter.

By mid-2017 DG-SANTE has established a **working group (WG)** with the aim to promote the use of **Next Generation Sequencing (NGS)** across the EURLs' networks, to build capacity towards the use of this analytical technology within the EU and ensure liaison with the work of EFSA and ECDC on the Whole Genome Sequencing (WGS) mandate sent by the Commission. The WG includes all the EURLs for microbiological hazards and also involves the Commission and the agencies EFSA and ECDC as observers. One of the first actions of this WG is

to get a more precise knowledge of the status of the capacity towards the use of NGS in order to better define the activities of the WG and to target the actions on the actual needs of the NRLs. For this purpose, the WG has prepared a survey which was sent to all NRLs by the end of March 2018, and each NRL is kindly requested to participate and fill in the questions related to the status of using NGS in its NRL. Each of the EURLs composing the WG is sending out the same survey to their reference network, therefore it is possible that an institute will receive more than one request to fill in the questionnaire if this institute is at the same time NRL for more than one hazard. The NRLs are kindly requested to complete the relevant survey for each NRL. EURL-*Salmonella* sent the survey to the NRLs-*Salmonella* on 29 March 2018. In case you missed the email or did not receive it, please find here the link to access the **survey for the use of NGS** as sent by EURL-*Salmonella*:

<https://www.formdesk.com/rivm/SurveyofEURLSalmonella>

Thank you very much for completing the survey before 20 April 2018.

Report published in February 2018:

Kuijpers A.F.A. and Mooijman, K.A. EURL-*Salmonella* 8th interlaboratory comparison study Food 2016 - Detection of *Salmonella* in minced chicken meat. RIVM report 2017-0081. Available at:

<https://www.rivm.nl/bibliotheek/rapporten/2017-0081.pdf>

Best wishes,
Kirsten Mooijman
Coordinator EURL-*Salmonella*

From the Literature

Salmonella-related Literature from Scopus: January – March 2018

Pan, H., Paudyal, N., Li, X., Fang, W., Yue, M.

Multiple food-animal-borne route in transmission of antibiotic-resistant salmonella newport to humans

(2018) *Frontiers in Microbiology*, 9 (JAN), art. no. 23, .

ABSTRACT: Characterization of transmission routes of *Salmonella* among various food-animal reservoirs and their antibiogram is crucial for appropriate intervention and medical treatment. Here, we analyzed 3728 *Salmonella enterica* serovar Newport (*S. Newport*) isolates collected from various food-animals, retail meats and humans in the United States between 1996 and 2015, based on their minimum inhibitory concentration (MIC) toward 27 antibiotics. Random Forest and Hierarchical Clustering statistic was used to group the isolates according to their MICs. Classification and Regression Tree (CART) analysis was used to identify the appropriate antibiotic and its cut-off value between human- and animal-population. Two distinct populations were revealed based on the MICs of individual strain by both methods, with the animal population having significantly higher MICs which correlates to antibiotic-resistance (AR) phenotype. Only ~9.7% (267/2763) human isolates could be attributed to food-animal origins. Furthermore, the isolates of animal origin had less diverse antibiogram than human isolates ($P < 0.001$), suggesting multiple sources involved in human infections. CART identified trimethoprim-sulfamethoxazole to be the best classifier for differentiating the animal and human isolates. Additionally, two typical AR patterns, MDR-Amp and Tet-SDR dominant in bovine- or turkey-population, were identified, indicating that distinct food-animal sources could be involved in human infections. The AR analysis suggested fluoroquinolones (i.e., ciprofloxacin), but not extended-spectrum cephalosporins (i.e., ceftriaxone, cefoxitin), is the adaptive choice for empirical therapy. Antibiotic-resistant *S. Newport* from humans has multiple origins, with distinct food-animal-borne route contributing to a significant proportion of heterogeneous isolates. ISSN: 1664302X

Silva, N.F.D., Magalhães, J.M.C.S., Freire, C., Delerue-Matos, C.

Electrochemical biosensors for Salmonella: State of the art and challenges in food safety assessment

(2018) *Biosensors and Bioelectronics*, 99, pp. 667-682.

ABSTRACT: According to the recent statistics, *Salmonella* is still an important public health issue in the whole world. Legislated reference methods, based on counting plate methods, are sensitive enough but are inadequate as an effective emergency response tool, and are far from a rapid device, simple to use out of lab. An overview of the commercially available rapid methods for *Salmonella* detection is provided along with a critical discussion of their limitations, benefits and potential use in a real context. The distinguished potentialities of electrochemical biosensors for the development of rapid devices are highlighted. The state-of-art and the newest technologic approaches in electrochemical biosensors for *Salmonella* detection are presented and a critical analysis of the literature is made in an attempt to identify the current challenges towards a complete solution for *Salmonella* detection in microbial food control based on electrochemical biosensors. ISSN: 09565663

Soobhany, N.

Preliminary evaluation of pathogenic bacteria loading on organic Municipal Solid Waste compost and vermicompost

(2018) *Journal of Environmental Management*, 206, pp. 763-767.

ABSTRACT: The use of composts or vermicomposts derived from organic fraction of Municipal Solid Waste (OFMSW) brought about certain disagreement in terms

of high level of bacterial pathogens, thereby surpassing the legal restrictions. This preliminary study was undertaken to compare the evolution of pathogenic bacteria on OFMSW compost against vermicompost (generated by *Eudrilus eugeniae*) with promises of achieving sanitation goals. Analysis to quality data showed that OFMSW vermicomposting caused a moderately higher reduction in total coliforms in contrast to composting. *E. coli* in OFMSW composts was found to be in the range of 4.72–4.96 log₁₀ CFU g⁻¹ whilst on a clear contrary, *E. coli* was undetectable in the final vermicomposts (6.01–6.14 logs of reduction) which might be explained by the involvement of the digestive processes in worms' guts. Both OFMSW composts and vermicomposts generated Salmonella-free products which were acceptable for agricultural usage and soil improvement. In comparison to compost, the analysis of this research indicated that earthworm activity can effectively destroy bacterial pathogenic load in OFMSW vermicomposts. But still, this study necessitates extra research in order to comprehend the factors that direct pathogenic bacteria in vermicomposting and earthworm-free decomposition systems. ISSN: 03014797

van Bree, F.P.J., Bokken, G.C.A.M., Mineur, R., Franssen, F., Opsteegh, M., van der Giessen, J.W.B., Lipman, L.J.A., Overgaauw, P.A.M.

Zoonotic bacteria and parasites found in raw meat-based diets for cats and dogs (2018) The Veterinary record, 182 (2), p. 50.

ABSTRACT: Feeding raw meat-based diets (RMBDs) to companion animals has become increasingly popular. Since these diets may be contaminated with bacteria and parasites, they may pose a risk to both animal and human health. The purpose of this study was to test for the presence of zoonotic bacterial and parasitic pathogens in Dutch commercial RMBDs. We analysed 35 commercial frozen RMBDs from eight different brands. *Escherichia coli* serotype O157:H7 was isolated from eight products (23 per cent) and extended-spectrum beta-lactamases-producing *E. coli* was found in 28 products (80 per cent). *Listeria monocytogenes* was present in 19 products (54 per cent), other *Listeria* species in 15 products (43 per cent) and *Salmonella* species in seven products (20 per cent). Concerning parasites, four products (11 per cent) contained *Sarcocystis cruzi* and another four (11 per cent) *S. tenella*. In two products (6 per cent) *Toxoplasma gondii* was found. The results of this study demonstrate the presence of potential zoonotic pathogens in frozen RMBDs that may be a possible source of bacterial infections in pet animals and if transmitted pose a risk for human beings. If non-frozen meat is fed, parasitic infections are also possible. Pet owners should therefore be informed about the risks associated with feeding their animals RMBDs. ISSN: 20427670

Thung, T.Y., Radu, S., Mahyudin, N.A., Rukayadi, Y., Zakaria, Z., Mazlan, N., Tan, B.H., Lee, E., Yeoh, S.L., Chin, Y.Z., Tan, C.W., Kuan, C.H., Basri, D.F., Wan Mohamed Radzi, C.W.J.

Prevalence, virulence genes and antimicrobial resistance profiles of Salmonella serovars from retail beef in Selangor, Malaysia (2018) Frontiers in Microbiology, 8 (JAN), art. no. 2697, .

ABSTRACT: The aim of the present study was to investigate the prevalence of *Salmonella* spp., *Salmonella* Enteritidis and *Salmonella* Typhimurium in retail beef from different retail markets of Selangor area, as well as, to assess their pathogenic potential and antimicrobial resistance. A total of 240 retail beef meat samples (chuck = 60; rib = 60; round = 60; sirloin = 60) were randomly collected. The multiplex polymerase chain reaction (mPCR) in combination with the most probable number (MPN) method was employed to detect *Salmonella* spp., *S. Enteritidis* and *S. Typhimurium* in the meat samples. The prevalence of *Salmonella* spp., *S. Enteritidis* and *S. Typhimurium* in 240 beef meat samples were 7.50, 1.25, and 0.83%, respectively. The microbial loads of total *Salmonella* was found in the range of < 3 to 15 MPN/g. Eight different serovars of *Salmonella* were identified among the 23 isolates, and *S. Agona* was the predominant serovar (26.09%). Interestingly, all the *Salmonella* isolates were resistant to penicillin,

erythromycin and vancomycin, but the sensitivity was observed for tetracycline, gentamicin and amoxicillin/clavulanic acid. All 23 isolates were resistant to at least three antibiotics. Two *S. Typhimurium* isolates (8.70%) exhibited the highest multiple antibiotic resistance (MAR) index value of 0.56 which shown resistance to nine antibiotics. PCR analysis of virulence genes showed that all *Salmonella* isolates (100%) were positive for the *invA* gene. Meanwhile, *pefA* was only identified in *S. Enteritidis* and *S. Typhimurium*. The findings in this study indicate that retail beef products tested were widely contaminated with multi-drug resistant (MDR) *Salmonella* and various virulence genes are present among the isolated *Salmonella* serovars. ISSN: 1664302X

Jourdan-da Silva, N., Fabre, L., Robinson, E., Fournet, N., Nisavanh, A., Bruyand, M., Mailles, A., Serre, E., Ravel, M., Guibert, V., Issenhuth-Jeanjean, S., Renaudat, C., Tourdjman, M., Septfons, A., de Valk, H., Le Hello, S.

Ongoing nationwide outbreak of salmonella agona associated with internationally distributed infant milk products, France, December 2017
(2018) *Eurosurveillance*, 23 (2), art. no. 17-00852, 5 p.

ABSTRACT: On 1 December 2017, an outbreak of *Salmonella Agona* infections among infants was identified in France. To date, 37 cases (median age: 4 months) and two further international cases have been confirmed. Five different infant milk products manufactured at one facility were implicated. On 2 and 10 December, the company recalled the implicated products; on 22 December, all products processed at the facility since February 2017. Trace-forward investigations indicated product distribution to 66 countries. ISSN: 1025496X

Brandwagt, D., van den Wijngaard, C., Tulen, A.D., Mulder, A.C., Hofhuis, A., Jacobs, R., Heck, M., Verbruggen, A., van den Kerkhof, H., Slegers-Fitz-James, I., Mughini-Gras, L., Franz, E.

Outbreak of Salmonella Bovismorbificans associated with the consumption of uncooked ham products, the Netherlands, 2016 to 2017
(2018) *Eurosurveillance*, 23 (1), art. no. 17-00335, 6 p.

ABSTRACT: In January 2017, an increase in reported *Salmonella* enterica serotype *Bovismorbificans* cases in the Netherlands was observed since October 2016. We implemented a case-control study to identify the source, including all cases after December 2016. Adjusted odds ratios were calculated using logistic regression analysis. We traced back the distribution chain of suspected food items and sampled them for microbiological analysis. Human and food isolates were sequenced using whole genome sequencing (WGS). From October 2016 to March 2017, 54 *S. Bovismorbificans* cases were identified. Sequencing indicated that all were infected with identical strains. Twenty-four cases and 37 controls participated in the study. Cases were more likely to have consumed ham products than controls (aOR = 13; 95% CI: 2.0-77) and to have shopped at a supermarket chain (aOR = 7; 95% CI: 1.3-38). Traceback investigations led to a Belgian meat processor: one retail ham sample originating from this processor tested positive for *S. Bovismorbificans* and matched the outbreak strain by WGS. All ham products related to the same batch were removed from the market to prevent further cases. This investigation illustrates the importance of laboratory surveillance for all *Salmonella* serotypes and the usefulness of WGS in an outbreak investigation. ISSN: 1025496X

Domesle, K.J., Yang, Q., Hammack, T.S., Ge, B.

Validation of a Salmonella loop-mediated isothermal amplification assay in animal food

(2018) *International Journal of Food Microbiology*, 264, pp. 63-76.

ABSTRACT: Loop-mediated isothermal amplification (LAMP) has emerged as a promising alternative to PCR for pathogen detection in food testing and clinical diagnostics. This study aimed to validate a *Salmonella* LAMP method run on both turbidimetry (LAMP I) and fluorescence (LAMP II) platforms in representative

animal food commodities. The U.S. Food and Drug Administration (FDA)'s culture-based Bacteriological Analytical Manual (BAM) method was used as the reference method and a real-time quantitative PCR (qPCR) assay was also performed. The method comparison study followed the FDA's microbiological methods validation guidelines, which align well with those from the AOAC International and ISO. Both LAMP assays were 100% specific among 300 strains (247 *Salmonella* of 185 serovars and 53 non-*Salmonella*) tested. The detection limits ranged from 1.3 to 28 cells for six *Salmonella* strains of various serovars. Six commodities consisting of four animal feed items (cattle feed, chicken feed, horse feed, and swine feed) and two pet food items (dry cat food and dry dog food) all yielded satisfactory results. Compared to the BAM method, the relative levels of detection (RLODs) for LAMP I ranged from 0.317 to 1 with a combined value of 0.610, while those for LAMP II ranged from 0.394 to 1.152 with a combined value of 0.783, which all fell within the acceptability limit (2.5) for an unpaired study. This also suggests that LAMP was more sensitive than the BAM method at detecting low-level *Salmonella* contamination in animal food and results were available 3 days sooner. The performance of LAMP on both platforms was comparable to that of qPCR but notably faster, particularly LAMP II. Given the importance of *Salmonella* in animal food safety, the LAMP assays validated in this study holds great promise as a rapid, reliable, and robust method for routine screening of *Salmonella* in these commodities. ISSN: 01681605

Estrada-Acosta, M.D., Ramirez, K., Medrano-Félix, J.A., Castro-Del Campo, N., López-Moreno, H.S., Jimenez Edeza, M., Martínez-Urtaza, J., Chaidez, C.

Effect of river water exposition on adhesion and invasion abilities of Salmonella Oranienburg and Saintpaul

(2018) *International Journal of Environmental Health Research*, 28 (1), pp. 43-54.

ABSTRACT: This study was performed to evaluate in vitro the adherence and invasiveness capacity of *Salmonella* Oranienburg and Saintpaul (isolated from river water) exposed to laboratory and river water growth conditions and inoculated into epithelial HEp-2 cell. Results showed that *Salmonella* Oranienburg and *Salmonella* Saintpaul showed lower ability to adhere and invade epithelial HEp-2 cells under both growth conditions as compared to *Salmonella* Typhimurium reference strain. *S.* Oranienburg adhesion capacity was not affected by the growth conditions, while *S.* Saintpaul exposed to river water significantly ($p < 0.05$) decreased its adhesion capacity by 75.7 %. On the contrary, *S.* Oranienburg exposed to river water reduced its invasion efficiency by 80 %, whereas *S.* Saintpaul showed no differences between growth conditions. In conclusion, this study suggests that the exposure to non-host conditions, such as river water, adversely affects the adhesion and invasiveness of *Salmonella* serotypes differently, impacting on their ability to re-enter a new host. ISSN: 09603123

Umesha, S., Manukumar, H.M.

Advanced molecular diagnostic techniques for detection of food-borne pathogens: Current applications and future challenges

(2018) *Critical Reviews in Food Science and Nutrition*, 58 (1), pp. 84-104.

ABSTRACT: The elimination of disease-causing microbes from the food supply is a primary goal and this review deals with the overall techniques available for detection of food-borne pathogens. Now-a-days conventional methods are replaced by advanced methods like Biosensors, Nucleic Acid-based Tests (NAT), and different PCR-based techniques used in molecular biology to identify specific pathogens. *Bacillus cereus*, *Staphylococcus aureus*, *Proteus vulgaris*, *Escherichia coli*, *Campylobacter*, *Listeria monocytogenes*, *Salmonella* spp., *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp., and pathogens are detected in contaminated food items that cause always diseases in human in any one or the other way. Identification of food-borne pathogens in a short period of time is still a challenge

to the scientific field in general and food technology in particular. The low level of food contamination by major pathogens requires specific sensitive detection platforms and the present area of hot research looking forward to new nanomolecular techniques for nanomaterials, make them suitable for the development of assays with high sensitivity, response time, and portability. With the sound of these, we attempt to highlight a comprehensive overview about food-borne pathogen detection by rapid, sensitive, accurate, and cost affordable in situ analytical methods from conventional methods to recent molecular approaches for advanced food and microbiology research. ISSN: 10408398

Smith, R.P., Andres, V., Martelli, F., Gosling, B., Marco-Jimenez, F., Vaughan, K., Tchorzewska, M., Davies, R.

Maternal vaccination as a Salmonella Typhimurium reduction strategy on pig farms

(2018) *Journal of Applied Microbiology*, 124 (1), pp. 274-285.

ABSTRACT: Aims: The control of Salmonella in pig production is necessary for public and animal health, and vaccination was evaluated as a strategy to decrease pig prevalence. Methods and Results: The study examined the efficacy of a live Salmonella Typhimurium vaccine, administered to sows on eight commercial farrow-to-finish herds experiencing clinical salmonellosis or Salmonella carriage associated with S. Typhimurium or its monophasic variants. Results of longitudinal Salmonella sampling were compared against eight similarly selected and studied control farms. At the last visit (~14 months after the start of vaccination), when all finishing stock had been born to vaccinated sows, both faecal shedding and environmental prevalence of Salmonella substantially declined on the majority of vaccinated farms in comparison to the controls. A higher proportion of vaccine farms resolved clinical salmonellosis than controls. However, Salmonella counts in positive faeces samples were similar between nonvaccinated and vaccinated herds. Conclusions: The results suggest that maternal vaccination is a suitable option for a Salmonella Typhimurium reduction strategy in farrow-to-finish pig herds. Significance and Impact of the Study: Salmonella vaccines have the potential to reduce the prevalence of Salmonella in pigs and result in a reduction of human cases attributed to pork. © 2017 Crown copyright. *Journal of Applied Microbiology* ISSN: 13645072

Santiago, P., Jiménez-Belenguer, A., García-Hernández, J., Estellés, R.M., Hernández Pérez, M., Castillo López, M.A., Ferrús, M.A., Moreno, Y.

High prevalence of Salmonella spp. in wastewater reused for irrigation assessed by molecular methods

(2018) *International Journal of Hygiene and Environmental Health*, 221 (1), pp. 95-101.

ABSTRACT: Salmonella spp. is one of the most important causal agents of food-borne illness in developed countries and its presence in irrigation water poses a risk to public health. Its detection in environmental samples is not easy when culture methods are used, and molecular techniques such as PCR or ribosomal rRNA probe hybridization (Fluorescent in situ Hybridization, FISH) are outstanding alternatives. The aim of this work was to determine the environmental risk due to the presence of Salmonella spp. in wastewater by culture, PCR and FISH. A new specific rDNA probe for Salmonella was designed and its efficiency was compared with the rest of methods Serotype and antibiotic resistance of isolated strains were determined. Forty-five wastewater samples (collected from two secondary wastewater treatment plants) were analysed. Salmonella strains were isolated in 24 wastewater samples (53%), two of them after disinfection treatment. Twenty-three Salmonella strains exhibited resistance to one or more antimicrobial agent. Analysis of wastewater samples yielded PCR positive results for Salmonella in 28 out of the 45 wastewater samples (62%). FISH analysis allowed for the detection of Salmonella in 27 (60%) samples. By using molecular methods, Salmonella was detected in four samples after disinfection treatment. These results show the prevalence of Salmonella in reclaimed wastewater even after U.V. disinfection,

what is a matter of public health concern, the high rates of resistance to antibiotics and the adequacy of molecular methods for its rapid detection. FISH method, with SA23 probe developed and assayed in this work provides a tool for detecting Salmonella in water within few hours, with a high rate of effectiveness. ISSN: 14384639

He, H., Arsenault, R.J., Genovese, K.J., Johnson, C., Kogut, M.H.

Chicken macrophages infected with Salmonella (S.) Enteritidis or S. Heidelberg produce differential responses in immune and metabolic signaling pathways (2018) Veterinary Immunology and Immunopathology, 195, pp. 46-55.

ABSTRACT: Protein kinases act in coordination with phosphatases to control protein phosphorylation and regulate signaling pathways and cellular processes involved in nearly every functions of cell life. Salmonella are known to manipulate the host kinase network to gain entrance and survive inside host cells. The effect of Salmonella infection on the host kinase network has been studied in mammalian cells, but information is largely lacking in chicken immune cells. Our previous study indicated that chicken macrophage cells respond differentially to different Salmonella strains. In order to better understand the interaction between chicken macrophages and Salmonella, we used a peptide array-based kinome analysis to identify cellular process and signaling pathways that may play a critical role in the outcome of Salmonella infection. The kinome assay was performed on chicken HD11 macrophages collected at 1.5, 3, and 7 h post-infection (hpi) with either S. Heidelberg or S. Enteritidis. A large number of peptides show significantly changed phosphorylation ($p \leq 0.05$) during the infection: 390, 449, and 575 peptides for S. Enteritidis and 185, 470, and 442 for S. Heidelberg at 1.5, 3, and 7 hpi, respectively. Many pathways involved in immunity, signal transduction, cellular process, and metabolism were significantly altered, in some case differentially, during the infection by the two Salmonella strains. Particularly, effects on lysosome process, iNOS, CARD9, NLRP3, and MAPK pathway provide significant insight to the inter play between pathogens and chicken macrophage cells during the infection. ISSN: 01652427

Kim, W.-J., Jeong, K.-O., Kang, D.-H.

Inhibition of initial attachment of injured salmonella typhimurium onto abiotic surfaces

(2018) Journal of Food Protection, 81 (1), pp. 37-42.

ABSTRACT: Following sanitation interventions in food processing facilities, sublethally injured bacterial cells can remain on food contact surfaces. We investigated whether injured Salmonella Typhimurium cells can attach onto abiotic surfaces, which is the initial stage for further biofilm development. We utilized heat, UV, hydrogen peroxide, and lactic acid treatments, which are widely utilized by the food industry. Our results showed that heat, UV, and hydrogen peroxide did not effectively change populations of attached Salmonella Typhimurium. Cells treated with hydrogen peroxide had a slightly higher tendency to adhere to abiotic surfaces, although there was no significant difference between the populations of control and hydrogen peroxide-treated cells. However, lactic acid effectively reduced the number of Salmonella Typhimurium cells attached to stainless steel. We also compared physicochemical changes of Salmonella Typhimurium after application of lactic acid and used hydrogen peroxide as a positive control because only lactic acid showed a decreased tendency for attachment and hydrogen peroxide induced slightly higher numbers of attached bacteria cells. Extracellular polymeric substance produced by Salmonella Typhimurium was not detected in any treatment. Significant differences in hydrophobicity were not observed. Surface charges of cell membranes did not show relevant correlation with numbers of attached cells, whereas autoaggregation showed a positive correlation with attachment to stainless steel. Our results highlight that when lactic acid is applied in a food processing facility, it can effectively interfere with adhesion of injured Salmonella Typhimurium cells onto food contact surfaces. ISSN: 0362028X

Ramirez-Hernandez, A., Inestroza, B., Parks, A., Brashears, M.M., Sanchez-Plata, M.X., Echeverry, A.

Thermal inactivation of salmonella in high-fat rendering meat products (2018) Journal of Food Protection, 81 (1), pp. 54-58.

ABSTRACT: Thermal inactivation of Salmonella is a critical component of the calculated thermal process to ensure the safety of cooked human and animal products. However, lethality performance standards for meat processing by-products that may harbor Salmonella have not been properly set under the actual conditions of rendering processes. The goal of this study was to evaluate the thermal inactivation parameters for Salmonella in high-fat beef trimmings as a model system for animal food products treated under simulated "worst-case scenario" commercial rendering conditions. Ground high-fat beef trimmings (50% fat) were artificially inoculated with a 10⁸ CFU/g Salmonella cocktail containing human outbreak strains including the highly thermotolerant serotype Salmonella Senftenberg. The meat samples were packaged and immersed in either water or silicon oil at predetermined temperatures ranging from 60 to 121°C (from 140 to 250°F). D-values of Salmonella at each temperature were calculated from the negative inverse slope of the log CFU per gram versus time plot. The z-values were determined from the negative inverse slope of the log D versus temperature plot. The D-values in thermal death curves for low-fat (20%) content materials (between 60 and 95°C) were 2.175, 0.658, 0.237, 1.563, 0.356, 0.284, 0.264, and 0.201 min, whereas materials with 50% fat (between 100 to 121°C) were 0.277, 0.286, 0.159, 0.143, 0.137, and 0.087 min. The z-values for low- and high-temperature schedules were 43.7 and 42.98°C, respectively. Thermal lethality data for Salmonella inactivation in high-fat rendering raw materials will help animal food processors design adequate thermal processing schedules and support critical control points to ensure the safety of final beef-based rendered products. ISSN: 0362028X

Campos, J., Mourão, J., Silveira, L., Saraiva, M., Correia, C.B., Maçãs, A.P., Peixe, L., Antunes, P.

Imported poultry meat as a source of extended-spectrum cephalosporin-resistant CMY-2-producing Salmonella Heidelberg and Salmonella Minnesota in the European Union, 2014–2015

(2018) International Journal of Antimicrobial Agents, 51 (1), pp. 151-154.

ABSTRACT: Extended-spectrum cephalosporin (ESC)-resistant Salmonella have been described at a low level in the EU, nevertheless the increasing importation of poultry meat could be an important source of epidemic strains carrying ESC resistance genes. This study evaluated ESC resistance and its genetic platform among Salmonella isolates from poultry meat products imported into Portugal as well as clonal relatedness of the isolates. All Salmonella isolates recovered from samples of fresh meat destined for import into the EU in the scope of Portuguese official border control (2014–2015) were studied. Antibiotic susceptibility and β -lactamase production was determined by disk diffusion/microdilution. Molecular studies included detection of genes encoding acquired AmpC and extended-spectrum β -lactamases, plasmid-mediated quinolone resistance and other antibiotic resistance genes by PCR/sequencing, and clonality by MLST and XbaI-PFGE. Plasmid characterisation was assessed by conjugation assays, replicon typing (PCR-PBRT/pMLST) and hybridisation experiments (I-CeuI/S1-PFGE nuclease). Isolates belonged to Salmonella Heidelberg (n = 6; ST15/eBG26) and Salmonella Minnesota (n = 1; ST548/eBG77) and presented multidrug-resistant profiles, including to ESCs and/or fluoroquinolones. All but one carried bla_{CMY-2}, located on two epidemic plasmids, IncA/C (ST2, n = 5) or transferable IncI1 (ST12, n = 1). Salmonella Heidelberg was associated with five PFGE types, including one similar to an American epidemic clone. This study reveals imported poultry products as a source of uncommon and/or invasive ESC-resistant Salmonella strains in the EU. The increase of clinically relevant poultry-related

serotypes in Europe must be taken into account in the current monitoring of antibiotic resistance trends and in re-evaluation of food regulations.

ISSN: 09248579

Lins, P.

Detection of Salmonella spp. in spices and herbs

(2018) *Food Control*, 83, pp. 61-68.

ABSTRACT: The detection of microbial contaminations in spices and herbs is a challenging task due to their strong antimicrobial effects, which potentially increase the risk for false-negative results. Therefore, the present study mainly focuses on the detection of *Salmonella* spiked to cinnamon and oregano. Both condiments completely inhibited the proliferation of *Salmonella* at a 1:10 (w/w) dilution. Consequently, the supplementation of the buffered peptone water with K₂SO₃ as well as the application of higher initial dilutions was investigated. While no detrimental effect of K₂SO₃ was observed during the growth of 14 different *Salmonella* isolates, it even improved their detection in condiments. An effect, which was also determined with increased dilution ratios. For detection, a quantitative approach via enumeration of the colony-forming units (CFUs), and qualitative approaches via the culture-based detection according to ISO 6579 and via the nucleic acid-based detection with the 3M Molecular Detection System (MDS) were performed. Subsequently, the limit of detection (LOD) was determined, which was ≤ 5 CFU 25 g⁻¹ for both qualitative approaches.

Furthermore, the persistence of *Salmonella* DNA spiked to parsley was determined with the MDS. Despite of the modifications, the LOD for *Salmonella* spiked to oregano was significantly lower prior than after enrichment, pointing to the requirement for further improvements. Last but not least, a ring trial was performed, which emphasized the importance for a reliable detection.

ISSN: 09567135

Das, G., Das, S., Dutta, S., Ghosh, I.

In silico identification and characterization of stress and virulence associated repeats in Salmonella

(2018) *Genomics*, 110 (1), pp. 23-34.

ABSTRACT: So much genomic similarities yet causing different diseases, is like a paradox in *Salmonella* biology. Repeat is one of the probes that can explain such differences. Here, a comparative genomics approach is followed to identify and characterize repeats that might play role in adaptation and pathogenesis. Repeats are non-randomly distributed in the genomes except few typhoid causing strains. Perfect long repeats are rare compare to polymorphic ones and both are statistically consistent. Significant differences in repeat densities in stress related genes manifest its probable participation in survival and virulence. 573 and 1053 repeat loci have been identified which are exclusively associated with stress and virulent genes respectively. In *Salmonella* Typhi, an octameric VNTR locus is found in between *acrD* and *yffB* genes having more than 25 perfect copies across *Salmonella* Typhi but possesses only single copy in other serovars. This repeat can be used as a diagnostic probe for typhoid. ISSN: 08887543

Underthun, K., De, J., Gutierrez, A., Silverberg, R., Schneider, K.R.

Survival of salmonella and Escherichia coli in two different soil types at various moisture levels and temperatures

(2018) *Journal of Food Protection*, 81 (1), pp. 150-157.

ABSTRACT: With the increased consumption of fresh produce, a proportional increase in numbers of produce-related foodborne illness has been observed. An estimate of foodborne illness during 1998 to 2008 attributed ~46% of the incidences to produce. Any foodborne illness associated with produce can have devastating consequences to the industry. The most recent data from the Centers for Disease Control and Prevention implicate leafy vegetables, vine-stalk vegetables, root vegetables, and sprouts as the most common cause of produce-related foodborne outbreaks. Excess rainfall or flooding, mainly by altering levels

of soil moisture and oxygen content, affects the microbial community in soil. The goal of this research was to determine the survivability of a three-serovar *Escherichia coli* and a five-serovar *Salmonella enterica* cocktail in microcosms prepared with Candler sand (CS) and Orangeburg sandy loam (OSL) soils. Microcosms were prepared with low, medium, and high volumetric water contents and were incubated at 20 and 30°C. Serotyping was used to determine which *E. coli* or *Salmonella* serovar(s) from each cocktail persisted. Microcosm inoculation levels were ~ 7.0 log CFU/g. Sampling for CS and OSL microcosms incubated at 20°C ended on day 364 and 357, respectively. The reduction of *Salmonella* and *E. coli* to below the limit of detection (extinction) in CS microcosms (incubated at 30°C at all volumetric water content [VWC] levels) was reached on day 168 and 56, respectively. Extinction of *Salmonella* and *E. coli* in OSL microcosms (incubated at 30°C at all VWCs) was reached on day 168 and 224, respectively. Of the *Salmonella* and *E. coli* serovars analyzed, *Salmonella* Javiana persisted the longest in both soil types, whereas *E. coli* O104:H4 and *E. coli* O145 persisted the longest in CS and OSL microcosms, respectively. Results from the current study suggest that soil type and temperature influenced pathogen persistence in CS and OSL soils more than moisture level and pathogen type. ISSN: 0362028X

Al-Habsi, K., Yang, R., Abraham, S., Ryan, U., Miller, D., Jacobson, C.

Molecular characterisation of Salmonella enterica serovar Typhimurium and Campylobacter jejuni faecal carriage by captured rangeland goats (2018) Small Ruminant Research, 158, pp. 48-53.

ABSTRACT: Western Australian rangeland goats were surveyed for faecal carriage of *Salmonella enterica* and *Campylobacter* spp. Faecal samples were collected from 125 goats on four occasions. The first sample was collected immediately upon arrival at a commercial goat depot (feedlot). Subsequent samples were collected at one month intervals thereafter. Frequency of detection and faecal carriage intensity were determined using qPCR targeting the *S. enterica* outer membrane protein (*ompF*) and *Campylobacter* spp. purine biosynthesis gene (*purA*). *Salmonella enterica* were identified in 40/500 of faecal samples, with *S. enterica* faecal carriage detected in 30% (38/125) goats over the duration of the study. *Campylobacter* spp. were identified in 12/500 of samples, with *Campylobacter* spp. detected in 10% (12/125) goats over duration of the study. Frequency of detection was highest at the first sample collection for both *S. enterica* (26%) and *Campylobacter* spp. (8%). Repeat detection of *Salmonella* was observed for only a single goat (0.8%). *Salmonella* qPCR positive samples were characterised at *ompF* and *invA* genes as *S. enterica*. Further characterisation at STM2755 and STM4497 genes confirmed the isolates were *S. enterica* serovar Typhimurium. Characterization at the 16S rRNA and hippuricase (*hipO*) genes revealed all *Campylobacter* spp. positive samples were *C. jejuni*. This study demonstrates that qPCR can be used for rapid identification of faecal carriage in goat faecal samples and showed evidence of carriage of zoonotic *S. Typhimurium* and *C. jejuni* by captured rangeland goats. The findings have implications for management of goats at abattoirs and in confined feeding facilities. ISSN: 09214488

Lins, P.

Antimicrobial activities of spices and herbs against Salmonella Oranienburg (2018) Food Control, 83, pp. 123-130.

ABSTRACT: The detection of *Salmonella* spp. within dried spices and herbs is challenging due to their potent antimicrobial activity. In the present study nine condiments were investigated whereof oregano and cinnamon led to a complete inhibition of *Salmonella* Oranienburg at a 1:10 dilution in buffered peptone water, while towards allspice and thyme an adaptation was apparent. At a next step, a tenacity study was set up where the survival of *S. Oranienburg* in dry condiment samples was followed qualitatively and quantitatively during storage at 25 °C for 365 days. Generally, a higher susceptibility of *S. Oranienburg* to spices than herbs was determined. Furthermore, to the best of the author's knowledge, this is

the first study presenting a significant antimicrobial effect of paprika/chilli against *Salmonella*. Nevertheless, *S. Oranienburg* was able to persist during storage in several condiment samples with a low reduction of log₁₀ < 1.5 colony-forming units g⁻¹. Thus, pointing to the outstanding ability of *S. Oranienburg* to survive dry storage conditions even spiked to condiments, known for their high antimicrobial activity. ISSN: 09567135

López-Romero, J.C., Valenzuela-Melendres, M., Juneja, V.K., García-Dávila, J., Camou, J.P., Peña-Ramos, A., González-Ríos, H.

Effects and interactions of gallic acid, eugenol and temperature on thermal inactivation of Salmonella spp. in ground chicken

(2018) *Food Research International*, 103, pp. 289-294.

ABSTRACT: The combined effects of heating temperature (55 to 65 °C), gallic acid (0 to 2.0%), and eugenol (0 to 2.0%) on thermal inactivation of *Salmonella* in ground chicken were assessed. Thermal death times were determined in bags submerged in a heated water bath maintained at various set temperatures, following a central composite design. The recovery medium was tryptic soy agar supplemented with 0.6% yeast extract and 1% sodium pyruvate. D-values were analyzed by second-order response surface regression for temperature, gallic acid, and eugenol. The observed D-values for chicken with no gallic acid or eugenol at 55, 57.5, 60, 62.5, and 65 °C were 21.85, 5.43, 2.83, 0.58, and 0.26 min, respectively. A second-order polynomial model developed to inactivate *Salmonella* was found to be significant ($p < 0.0001$) with a $R^2 = 0.95$ and a non-significant lack of fit ($p > 0.1073$). Efficacy of the additives in increasing the sensitivity of the pathogen to heat was concentration dependent. The model developed in this study can be used by processors to design appropriate thermal process to inactivate *Salmonella* in chicken products used in the study and thereby, ensuring an adequate degree of protection against risks associated with the pathogen. ISSN: 09639969

Abdullah, W.Z.W., Mackey, B.M., Karatzas, K.A.G.

High phenotypic variability among representative strains of common salmonella enterica serovars with possible implications for food safety

(2018) *Journal of Food Protection*, 81 (1), pp. 93-104.

ABSTRACT: *Salmonella* is an important foodborne pathogen, whose ability to resist stress and survive can vary among strains. This variability is normally not taken into account when predictions are made about survival in foods with negative consequences. Therefore, we examined the contribution of variable phenotypic properties to survival under stress in 10 *Salmonella* serovars. One strain (Typhimurium 10) was intentionally RpoS-negative; however, another strain (Heidelberg) showed an rpoS mutation, rendering it inactive. We assessed an array of characteristics (motility, biofilm formation, bile resistance, acid resistance, and colony morphology) that show major variability among strains associated with a 10- to 19-fold difference between the highest and the lowest strain for most characteristics. The RpoS status of isolates did not affect variability in the characteristics, with the exception of resistance to NaCl, acetic acid, lactic acid, and the combination of acetic acid and salt, where the variability between the highest and the lowest strain was reduced to 3.1-fold, 1.7-fold, 2-fold, and 1.7-fold, respectively, showing that variability was significant among RpoS-positive strains. Furthermore, we also found a good correlation between acid resistance and lysine decarboxylase activity, showing its importance for acid resistance, and demonstrated a possible role of RpoS in the lysine decarboxylase activity in *Salmonella*. ISSN: 0362028X

Minarovičová, J., Cabicarová, T., Kaclíková, E., Mader, A., Lopašovská, J., Siekel, P., Kuchta, T.

Culture-independent quantification of pathogenic bacteria in spices and herbs using real-time polymerase chain reaction

(2018) *Food Control*, 83, pp. 85-89.

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85008239910&doi=10.1016%2fj.foodcont.2016.12.025&partnerID=40&md5=da09cf3e47bc2fe4d2e8cb22afeb1a9>

ABSTRACT: A culture-independent method was developed for quantification of pathogenic bacteria in spices and herbs. The method is based on DNA extraction using cetyltrimethylammonium bromide (CTAB) and on real-time polymerase chain reaction (PCR). When evaluated with spices (black pepper, paprika) and herbs (oregano, parsley) artificially contaminated with *Staphylococcus aureus*, *Salmonella enterica* or *Escherichia coli*, the method demonstrated quantitative response with linear calibration lines and quantification limits of 10²–10⁴ CFU/g. The developed method is suitable for rapid microbiological analysis of spices and herbs, taking 8–9 h. ISSN: 09567135

Chakroun, I., Mahdhi, A., Morcillo, P., Cordero, H., Cuesta, A., Bakhrouf, A., Mahdouani, K., Esteban, M.Á.

Motility, biofilm formation, apoptotic effect and virulence gene expression of atypical Salmonella Typhimurium outside and inside Caco-2 cells (2018) Microbial Pathogenesis, 114, pp. 153-162.

ABSTRACT: Disease outbreaks related to waterborne pathogen contamination throughout the world as well as challenges that lie ahead for addressing persistent infection are of renewed interest. In this research, we studied the effects of prolonged exposure of *Salmonella enterica* serovar Typhimurium to the cues encountered in the extracellular environment particularly in seawater microcosm on bacterial virulence and subsequent infection in Caco-2 cells. Our data show a significant difference in biofilm formation, swimming and swarming motilities between normal and stressed cells of *S. Typhimurium* under differing NaCl conditions ($P < 0.05$). Interestingly, adhesion, invasion and apoptotic activity to Caco-2 epithelial cells were determined during infection with normal and stressed *Salmonella*. Furthermore, we compared the expression of SPI-1 virulence genes (*sopA*, *sopB*, *sopD*, *sopE2* and *hilA*) of normal and stressed *S. Typhimurium* in response to salt conditions encountered in the extracellular environment in LB broth and after epithelial cell exposure. The interest of the present study is due to the fact that to investigate the bacterial survival strategies during its movement from the natural surroundings to the host cell is fundamental to our understanding of the infection process during the host-pathogen interactions. ISSN: 08824010

Robertson, J., Yoshida, C., Gurnik, S., Nash, J.H.E.

Completed genome sequences of strains from 36 serotypes of Salmonella (2018) Genome Announcements, 6 (3), art. no. e01472-17, .

ABSTRACT: We report here the completed closed genome sequences of strains representing 36 serotypes of *Salmonella*. These genome sequences will provide useful references for understanding the genetic variation between serotypes, particularly as references for mapping of raw reads or to create assemblies of higher quality, as well as to aid in studies of comparative genomics of *Salmonella*. ISSN: 21698287

Lamas, A., Miranda, J.M., Regal, P., Vázquez, B., Franco, C.M., Cepeda, A.

A comprehensive review of non-enterica subspecies of Salmonella enterica (2018) Microbiological Research, 206, pp. 60-73.

ABSTRACT: *Salmonella* is a major foodborne pathogen with a complex nomenclature. This genus is composed of two species, *S. enterica* and *S. bongori*. *S. enterica* is divided into six subspecies. *S. enterica* subspecies *enterica* is composed of more than 1500 serotypes with some of great importance, such as *S. Typhimurium* and *S. Enteritidis*. *S. enterica* subsp. *enterica* is responsible of more than 99% of human salmonellosis and therefore it is widely studied. However, the non-enterica subspecies of *S. enterica* have been little studied. These subspecies are considered to be related to cold-blooded animals and their pathogenicity is very limited. Phenotype and genotype information generated

from different studies of non-enterica subspecies reveal poor ability to invade host cells and the absence or modification of important virulence factors. Also, the great majority of human infections due to non-enterica subspecies are related to a previous depressed immune system. Therefore, we propose to treat these subspecies only as opportunistic pathogens. For establish this premise, the present review evaluated, among other things, the genomic characteristics, prevalence, antimicrobial resistance and reported human cases of the non-enterica subspecies. ISSN: 09445013

Nakao, J.H., Talkington, D., Bopp, C.A., Besser, J., Sanchez, M.L., Guarisco, J., Davidson, S.L., Warner, C., McIntyre, M.G., Group, J.P., Comstock, N., Xavier, K., Pinsent, T.S., Brown, J., Douglas, J.M., Gomez, G.A., Garrett, N.M., Carleton, H.A., Tolar, B., Wise, M.E.

Unusually high illness severity and short incubation periods in two foodborne outbreaks of Salmonella Heidelberg infections with potential coincident Staphylococcus aureus intoxication

(2018) *Epidemiology and Infection*, 146 (1), pp. 19-27.

ABSTRACT: We describe the investigation of two temporally coincident illness clusters involving salmonella and Staphylococcus aureus in two states. Cases were defined as gastrointestinal illness following two meal events. Investigators interviewed ill persons. Stool, food and environmental samples underwent pathogen testing. Alabama: Eighty cases were identified. Median time from meal to illness was 5.8 h. Salmonella Heidelberg was identified from 27 of 28 stool specimens tested, and coagulase-positive S. aureus was isolated from three of 16 ill persons. Environmental investigation indicated that food handling deficiencies occurred. Colorado: Seven cases were identified. Median time from meal to illness was 4.5 h. Five persons were hospitalised, four of whom were admitted to the intensive care unit. Salmonella Heidelberg was identified in six of seven stool specimens and coagulase-positive S. aureus in three of six tested. No single food item was implicated in either outbreak. These two outbreaks were linked to infection with Salmonella Heidelberg, but additional factors, such as dual aetiology that included S. aureus or the dose of salmonella ingested may have contributed to the short incubation periods and high illness severity. The outbreaks underscore the importance of measures to prevent foodborne illness through appropriate washing, handling, preparation and storage of food. ISSN: 09502688

An, R., Lin, P., Bougouffa, S., Essack, M., Boxrud, D., Bajic, V.B., Vidovic, S.

Draft genome sequences of four Salmonella enterica subsp. enterica serovar Enteritidis strains implicated in infections of avian and human hosts

(2018) *Genome Announcements*, 6 (4), art. no. e01550-17, .

ABSTRACT: Salmonella enterica subsp. enterica serovar Enteritidis is a wide-host-range pathogen. Occasionally, it is involved in invasive infections, leading to a high mortality rate. Here, we present the draft genome sequences of four S. Enteritidis strains obtained from human and avian hosts that had been involved in bacteremia, gastroenteritis, and primary infections. ISSN: 21698287

Tadesse, D.A., Hoffmann, M., Sarria, S., Lam, C., Brown, E., Allard, M., McDermott, P.F.

Complete genome sequences of 14 Salmonella enterica serovar Enteritidis strains recovered from human clinical cases between 1949 and 1995 in the United States

(2018) *Genome Announcements*, 6 (1), art. no. e01406-17, .

ABSTRACT: Salmonella enterica serovar Enteritidis is one of the most commonly isolated foodborne pathogens and is transmitted primarily to humans through consumption of contaminated poultry and poultry products. We are reporting completely closed genome and plasmid sequences of historical S. Enteritidis isolates recovered from humans between 1949 and 1995 in the United States. ISSN: 21698287

**Mandilara, G., Vassalos, C.M., Chrisostomou, A., Karadimas, K.,
Mathioudaki, E., Georgakopoulou, T., Tsiodras, S., Mellou, K.**

A severe gastroenteritis outbreak of Salmonella enterica serovar Enteritidis PT8, with PFGE profile XbaI.0024 and MLVA profile 2-9-7-3-2 following a christening reception, Greece, 2016

(2018) Epidemiology and Infection, 146 (1), pp. 28-36.

ABSTRACT: In June 2016, a Salmonella enterica serovar Enteritidis outbreak (n = 56) occurred after a christening reception in Central Greece, mainly affecting previously healthy adults; one related death caused media attention. Patients suffered from profuse diarrhoea, fever and frequent vomiting episodes requiring prolonged hospitalisation and sick leave from work, with a 54% hospital admission rate. The majority of cases experienced serious illness within <12 h of attending the party. We investigated the outbreak to identify the source(s) of infection and contributing factors to the disease severity. From the retrospective cohort study, the cheesy penne pasta was the most likely vehicle of infection (relative risk 7.8; 95% confidence interval 3.6-16.8), explaining 79% of the cases. S. enterica ser. Enteritidis isolates were typed as phage-type PT8, pulsed-field gel electrophoresis type XbaI.0024, multiple locus variable-number tandem repeat analysis-type 2-9-7-3-2. The strain did not share the single-nucleotide polymorphism address of the concurrent European S. enterica ser. Enteritidis PT8 outbreak clusters. Following five consecutive years with no documented S. enterica ser. Enteritidis outbreaks in Greece, this outbreak, likely associated with a virulent strain, prompted actions towards the enhancement of the national Salmonella molecular surveillance and control programmes including the intensification of training of food handlers for preventing similar outbreaks in the future. Advanced molecular techniques were useful in distinguishing unrelated outbreak strains. ISSN: 09502688

Paramithiotis, S., Drosinos, E.H.

Molecular tools for epidemiological assessment of foodborne salmonellae (2018) Salmonella Enterica: Molecular Characterization, Role in Infectious Diseases and Emerging Research, pp. 25-48.

ABSTRACT: A series of molecular tools have been developed for accurate epidemiological assessment of Salmonella. Pulsed-Field Gel Electrophoresis (PFGE), the most widely applied approach, is characterized by the dependence upon clonality and epidemiological data. The former restricts its use, especially in the case of Salmonella, the clonality of which is serovar-dependent. Multi-Locus Variable number of tandem repeats Analysis (MLVA) and Multi Locus Sequence Typing (MLST) have the potential to become the ultimate approach for such type of studies and address the basic epidemiological need, i.e., to integrate instead of verify the epidemiological data. Such integration would allow accurate identification of both infection source and transmission route that would lead to implementation of effective control measures. However, there are certain drawbacks that limit their use and the need for further study is still apparent. In this chapter, the strengths and limitations of the available molecular tools for effective epidemiological assessment of foodborne salmonellae are collected and critically discussed. ISBN: 9781536130850; 9781536130843

Tamber, S.

Population-wide survey of Salmonella enterica response to highpressure processing reveals a diversity of responses and tolerance mechanisms (2018) Applied and Environmental Microbiology, 84 (2), art. no. e01673-17, .

ABSTRACT: High-pressure processing is a nonthermal method of food preservation that uses pressure to inactivate microorganisms. To ensure the effective validation of process parameters, it is important that the design of challenge protocols consider the potential for resistance in a particular species. Herein, the responses of 99 diverse Salmonella enterica strains to high pressure are reported. Members of this population belonged to 24 serovars and were

isolated from various Canadian sources over a period of 26 years. When cells were exposed to 600 MPa for 3 min, the average reduction in cell numbers for this population was 5.6 log₁₀ CFU/ml, with a range of 0.9 log₁₀ CFU/ml to 6 log₁₀ CFU/ml. Eleven strains, from 5 serovars, with variable levels of pressure resistance were selected for further study. The membrane characteristics (propidium iodide uptake during and after pressure treatment, sensitivity to membrane-active agents, and membrane fatty acid composition) and responses to stressors (heat, nutrient deprivation, desiccation, and acid) for this panel suggested potential roles for the cell membrane and the RpoS regulon in mediating pressure resistance in *S. enterica*. The data indicate heterogeneous and multifactorial responses to high pressure that cannot be predicted for individual *S. enterica* strains. ISSN: 00992240

Chifanzwa, R., Nayduch, D.

Dose-Dependent Effects on Replication and Persistence of Salmonella enterica serovar Typhimurium in House Flies (Diptera: Muscidae)
(2018) *Journal of Medical Entomology*, 55 (1), pp. 225-229.

ABSTRACT: Adult house flies (*Musca domestica* L.) ingest variable numbers of bacteria when they encounter microbe-rich substrates. Bacterial abundance may affect replication within the fly gut, which subsequently impacts vector potential. This study investigated the dose-dependent replication of GFP-expressing *Salmonella enterica* serovar Typhimurium (ex Kauffmann and Edwards1952) Le Minor and Popoff 1987, (Enterobacteriales: Enterobacteriaceae) (GFP *S. Typhimurium*) within the fly alimentary canal. Adult house flies were fed two doses (colony forming units, CFU) of GFP *S. Typhimurium* (high, ~10⁵ CFU and low, ~10⁴ CFU). Bacteria were examined at 2-, 4-, 6-, 12-, and 24-h postingestion (PI) in situ in the gut via epifluorescence microscopy and enumerated by culture on selective media. In both treatment groups, GFP *S. Typhimurium* proliferated and persisted in flies for 24 h. In the high-dose group, proliferation peaked at 6 h PI (>500% increase). In the low-dose group, proliferation peaked at both 4 and 6 h PI (>900% increase). Dose significantly affected bacterial replication within the house fly alimentary canal, particularly at 4-, 6-, and 12-h PI. The ability of *S. Typhimurium* to proliferate and persist in the alimentary canal demonstrates that house flies may serve as significant reservoirs and probable disseminators of this pathogen. Our results show that bacterial abundance should be considered when assessing the potential of house flies to harbor and transmit pathogens. ISSN: 00222585

Hayashi, R.M., Lourenço, M.C., Kraieski, A.L., Araujo, R.B., Gonzalez-Esquerria, R., Leonardecz, E., da Cunha, A.F., Carazzolle, M.F., Monzani, P.S., Santin, E.

Effect of feeding bacillus subtilis spores to broilers challenged with Salmonella enterica serovar Heidelberg Brazilian strain UFPR1 on performance, immune response, and gut health

(2018) *Frontiers in Veterinary Science*, 5 (FEB), art. no. 13, .

ABSTRACT: Salmonellosis is a poultry industry and public health concern worldwide. Recently, *Salmonella enterica* serovar Heidelberg (SH) has been reported in broilers in Brazil. The effect of feeding a blend of three strains of *Bacillus subtilis* (PRO) was studied in broilers orally challenged (10⁷ CFU/chick) or not with a SH isolated in south of Brazil (UFPR1 strain). Twelve male Cobb 500 broilers per pen were randomly assigned to six treatments in a 3 × 2 factorial experiment where PRO was added at 0, 250, or 500 g/ton of broiler feed and fed to either SH-challenged (SH Control, SH + PRO 250, and SH + PRO 500) or non-challenged birds (Control, PRO 250, and PRO 500). Broiler performance, histologic alterations in intestinal morphology, *Salmonella* quantification and immune cells counts in liver (macrophages, T CD4+ and T CD8+) were analyzed. Changes in the intestinal microbiota of broilers were also studied by metagenomics for Control, SH Control, SH + PRO 250, and SH + PRO 500 only. Feeding PRO at 250 or 500 g/ton reduced SH counts and incidence in liver and

cecum at 21 days of age. It was observed that PRO groups increased the macrophage mobilization to the liver in SH-challenged birds ($P < 0.05$) but reduced these cells in the liver of non-challenged birds, showing an interesting immune cell dynamics effect. PRO at 250 g/ton did not affect gut histology, but improved animal performance ($P < 0.05$) while PRO at 500 g/ton did not affect animal performance but increased histologic alteration related to activation of the defense response in the ileum in SH challenged birds compared to control birds ($P < 0.05$). SH + PRO 500 group presented a more diverse cecal microbiota (Shannon-Wiener index; $P < 0.05$) compared to Control and SH Control groups; while SH + PRO 250 had greater ileal richness (Jackknife index) compared to Control ($P < 0.05$). PRO was effective in reducing Salmonella colonization in liver and cecum when fed at 250 or 500 g/ton to broilers inoculated with SH strain UFPR1. PRO promotes positive alterations in performance (at 250 g/ton), immune modulatory effect in the gastrointestinal tract, SH reduction, and intestinal microbiota modulation. ISSN: 22971769

Das, C., Mokashi, C., Mande, S.S., Saini, S.

Dynamics and control of flagella assembly in Salmonella typhimurium

(2018) *Frontiers in Cellular and Infection Microbiology*, 8 (FEB), art. no. 36, .

ABSTRACT: The food-borne pathogen Salmonella typhimurium is a common cause of infections and diseases in a wide range of hosts. One of the major virulence factors associated to the infection process is flagella, which helps the bacterium swim to its preferred site of infection inside the host, the M-cells (Microfold cells) lining the lumen of the small intestine. The expression of flagellar genes is controlled by an intricate regulatory network. In this work, we investigate two aspects of flagella regulation and assembly: (a) distribution of the number of flagella in an isogenic population of bacteria and (b) dynamics of gene expression post cell division. More precisely, in a population of bacteria, we note a normal distribution of number of flagella assembled per cell. How is this distribution controlled, and what are the key regulators in the network which help the cell achieve this? In the second question, we explore the role of protein secretion in dictating gene expression dynamics post cell-division (when the number of hook basal bodies on the cell surface is reduced by a factor of two). We develop a mathematical model and perform stochastic simulations to address these questions. Simulations of the model predict that two accessory regulators of flagella gene expression, FliZ and FliT, have significant roles in maintaining population level distribution of flagella. In addition, FliT and FlgM were predicted to control the level and temporal order of flagellar gene expression when the cell adapts to post cell division consequences. Further, the model predicts that, the FliZ and FliT dependent feedback loops function under certain thresholds, alterations in which can substantially affect kinetics of flagellar genes. Thus, based on our results we propose that, the proteins FlgM, FliZ, and FliT, thought to have accessory roles in regulation of flagella, likely play a critical role controlling gene expression during cell division, and frequency distribution of flagella. ISSN: 22352988

Bruce, H.L., Barrow, P.A., Rycroft, A.N.

Zoonotic potential of Salmonella enterica carried by pet tortoises

(2018) *The Veterinary record*, 182 (5), p. 141.

ABSTRACT: The prevalence of Salmonella in chelonians is not known in the UK and it is not clear whether such Salmonella strains would be pathogenic for human beings. Some strains, such as members of the Arizonae subgroup, may be unable to cause anything more than very mild disease. To determine the carriage of Salmonella in pet tortoises, cloacal swabs were taken for culture. Salmonella enterica Group D was isolated from 5 of the 89 samples. All five were from the same household of seven tortoises. Salmonella isolates were shown by PCR to carry the *invA* and *spiC* genes associated with pathogenicity islands 1 and 2. Each isolate carried both genes indicating they had the genetic basis for disease and enterocyte invasion in human beings. The study indicates a low rate of

asymptomatic carriage among the general population of pet tortoises. However, it does suggest that those *Salmonella* strains colonising the tortoise can carry *Salmonella* pathogenicity island (SPI)-1 and SPI-2 conferring the potential to cause disease in human beings and other animals. ISSN: 20427670

Wang, M., Yang, J., Gai, Z., Huo, S., Zhu, J., Li, J., Wang, R., Xing, S., Shi, G., Shi, F., Zhang, L.

Comparison between digital PCR and real-time PCR in detection of Salmonella typhimurium in milk

(2018) *International Journal of Food Microbiology*, 266, pp. 251-256.

ABSTRACT: As a kind of zero-tolerance foodborne pathogens, *Salmonella typhimurium* poses a great threat to quality of food products and public health. Hence, rapid and efficient approaches to identify *Salmonella typhimurium* are urgently needed. Combined with PCR and fluorescence technique, real-time PCR (qPCR) and digital PCR (ddPCR) are regarded as suitable tools for detecting foodborne pathogens. To compare the effect between qPCR and ddPCR in detecting *Salmonella typhimurium*, a series of nucleic acid, pure strain culture and spiking milk samples were applied and the resistance to inhibitors referred in this article as well. Compared with qPCR, ddPCR exhibited more sensitive (10– 4 ng/μl or 102 cfu/ml) and less pre-culturing time (saving 2 h). Moreover, ddPCR had stronger resistance to inhibitors than qPCR, yet absolute quantification hardly performed when target's concentration over 1 ng/μl or 106 cfu/ml. This study provides an alternative strategy in detecting foodborne *Salmonella typhimurium*. ISSN: 01681605

Mahmoud, M., Askora, A., Barakat, A.B., Rabie, O.E.-F., Hassan, S.E.

Isolation and characterization of polyvalent bacteriophages infecting multi drug resistant Salmonella serovars isolated from broilers in Egypt

(2018) *International Journal of Food Microbiology*, 266, pp. 8-13.

ABSTRACT: In this study, we isolated and characterized three phages named as Salmacey1, Salmacey2 and Salmacey3, infecting multi drug resistant *Salmonella* serovars isolated from broilers in Egypt. The most prevalent *Salmonella* serovars were *S. typhimurium*, *S. enteritidis*, and *S. kentucky*. All these *Salmonella* serovars were found to be resistant to more than two of the ten antimicrobial agents tested. Only *S. kentucky* was found to be resistant to seven antimicrobial agents. Examination of these phage particles by transmission electron microscopy (TEM), demonstrated that two phages (Salmacey1, Salmacey2) were found to belong to family Siphoviridae, and Salmacey3 was assigned to the family Myoviridae. The results of host range assay revealed that these bacteriophages were polyvalent and thus capable of infecting four strains of *Salmonella* serovars and *Citrobacter freundii*. Moreover, the two phages (Salmacey1, Salmacey2) had a lytic effect on *Enterobacter cloacae* and Salmacey3 was able to infect *E. coli*. All phages could not infect *S. para Typhi*, *Staphylococcus aureus* and *Bacillus cereus*. One-step growth curves of bacteriophages revealed that siphovirus phages (Salmacey1, Salmacey2) have burst size (80 and 90 pfu per infected cell with latent period 35 min and 40 min respectively), and for the myovirus Salmacey3 had a burst size 110 pfu per infected cell with latent period 60 min. Molecular analyses indicated that these phages contained double-stranded DNA genomes. The lytic activity of the phages against the most multidrug resistant serovars *S. kentucky* as host strain was evaluated. The result showed that these bacteriophages were able to completely stop the growth of *S. kentucky* in vitro. These results suggest that phages have a high potential for phage application to control *Salmonella* serovars isolated from broilers in Egypt. ISSN: 01681605

Li, Q., Wang, X., Yin, K., Hu, Y., Xu, H., Xie, X., Xu, L., Fei, X., Chen, X., Jiao, X.

Genetic analysis and CRISPR typing of Salmonella enterica serovar Enteritidis from different sources revealed potential transmission from poultry and pig to human

(2018) *International Journal of Food Microbiology*, 266, pp. 119-125.

ABSTRACT: *Salmonella enterica* serovar Enteritidis (*S. Enteritidis*) is one of the most prevalent serotypes in *Salmonella* isolated from poultry and the most commonly reported cause of human salmonellosis. In this study, we aimed to assess the genetic diversity of 329 *S. Enteritidis* strains isolated from different sources from 2009 to 2016 in China. Clustered regularly interspaced short palindromic repeat (CRISPR) typing was used to characterize these 262 chicken clinical isolates, 38 human isolates, 18 pig isolates, six duck isolates, three goose isolates and two isolates of unknown source. A total of 18 Enteritidis CRISPR types (ECTs) were identified, with ECT2, ECT8 and ECT4 as the top three ECTs. CRISPR typing identified ECT2 as the most prevalent ECT, which accounted for 41% of *S. Enteritidis* strains from all the sources except duck. ECT9 and ECT13 were identified in both pig and human isolates and revealed potential transmission from pig to human. A cluster analysis distributed 18 ECTs, including the top three ECTs, into four lineages with LI as the predominant lineage. Forty-eight out of 329 isolates were subjected to whole genome sequence typing, which divided them into four clusters, with Cluster I as the predominant cluster. Cluster I included 92% (34/37) of strains located in LI identified from the CRISPR typing, confirming the good correspondence between both typing methods. In addition, the CRISPR typing also revealed the close relationship between ECTs and isolated areas, confirming that CRISPR spacers might be obtained by bacteria from the unique phage or plasmid pools in the environment. However, further analysis is needed to determine the function of CRISPR-Cas systems in *Salmonella* and the relationship between spacers and the environment. ISSN: 01681605

Rivera, D., Toledo, V., Pillo, F.D.I., Dueñas, F., Tardone, R., Hamilton-West, C., Vongkamjan, K., Wiedmann, M., Moreno Switt, A.I.

Backyard Farms Represent a Source of Wide Host Range Salmonella Phages That Lysed the Most Common Salmonella Serovars

(2018) *Journal of Food Protection*, 81 (2), pp. 272-278.

ABSTRACT: The genus *Salmonella* has more than 2,600 serovars, and this trait is important when considering interventions for *Salmonella* control. Bacteriophages that are used for biocontrol must have an exclusively lytic cycle and the ability to lyse several *Salmonella* serovars under a wide range of environmental conditions. *Salmonella* phages were isolated and characterized from 34 backyard production systems (BPSs) with a history of *Salmonella* infections. BPSs were visited once, and cloacal or fecal samples were processed for phage isolation. Four hosts, *Salmonella* serovars Enteritidis, Heidelberg, Infantis, and Typhimurium, were used for phage isolation. The host range of the phages was later characterized with a panel of 23 *Salmonella* serovars (serovar diversity set) and 31 isolates obtained from the same farms (native set). Genetic relatedness for 10 phages with a wide host range was characterized by restriction fragment length polymorphism, and phages clustered based on the host range. We purified 63 phages, and 36 phage isolates were obtained on *Salmonella* Enteritidis, 16 on *Salmonella* Heidelberg, and 11 on *Salmonella* Infantis. Phages were classified in three clusters: (i) phages with a wide host range (cluster I), (ii) phages that lysed the most susceptible *Salmonella* serovars (serogroup D) and other isolates (cluster II), and (iii) phages that lysed only isolates of serogroup D (cluster III). The most susceptible *Salmonella* serovars were Enteritidis, Javiana, and Dublin. Seven of 34 farms yielded phages with a wide host range, and these phages had low levels of genetic relatedness. Our study showed an adaptation of the phages in the sampled BPSs to serogroup D *Salmonella* isolates and indicated that isolation of *Salmonella* phages with wide host range differs by farm. A better understanding of the factors driving the *Salmonella* phage host range could be useful when designing risk-based sampling strategies to obtain phages with a wide lytic host range for biocontrol purposes. ISSN: 0362028X

Nascimento, M.S., Carminati, J.A., Morishita, K.N., Amorim Neto, D.P., Pinheiro, H.P., Maia, R.P.

Long-term kinetics of Salmonella Typhimurium ATCC 14028 survival on peanuts and peanut confectionery products

(2018) *PLoS ONE*, 13 (2), art. no. e0192457, .

ABSTRACT: Due to recent large outbreaks, peanuts have been considered a product of potential risk for *Salmonella*. Usually, peanut products show a low water activity (a_w) and high fat content, which contribute to increasing the thermal resistance and survival of *Salmonella*. This study evaluated the long-term kinetics of *Salmonella* survival on different peanut products under storage at 28°C for 420 days. Samples of raw in-shell peanuts ($a_w = 0.29$), roasted peanuts ($a_w = 0.39$), unblanched peanut kernel ($a_w = 0.54$), peanut brittle ($a_w = 0.30$), paçoca ($a_w = 0.40$) and pé-de-moça ($a_w = 0.68$) were inoculated with *Salmonella* Typhimurium ATCC 14028 at two inoculum levels (3 and 6 log cfu/ g). The *Salmonella* behavior was influenced ($p < 0.05$) by a_w , lipid, carbohydrate and protein content. In most cases for both inoculum levels, the greatest reductions were seen after the first two weeks of storage, followed by a slower decline phase. The lowest reductions were verified in paçoca and roasted peanuts, with counts of 1.01 and 0.87 log cfu/ g at low inoculum level and 2.53 and 3.82 log cfu/ g at high inoculum level at the end of the storage time. The highest loss of viability was observed in pé-de-moça, with absence of *Salmonella* in 10-g after 180 days at low inoculum level. The Weibull model provided a suitable fit to the data ($R^2 = 0.81$), with δ value ranging from 0.06 to 49.75 days. Therefore, the results demonstrated that *Salmonella* survives longer in peanut products, beyond the shelf life (>420 days), especially in products with a_w around 0.40. ISSN: 19326203

Fernández Márquez, M.L., Grande Burgos, M.J., Pulido, R.P., Gálvez, A., Lucas López, R.

Correlations among Resistances to Different Antimicrobial Compounds in Salmonella Strains from Hen Eggshells

(2018) *Journal of Food Protection*, 81 (2), pp. 178-185.

ABSTRACT: Persistence of antibiotic-resistant *Salmonella* in the food chain may depend on strain tolerance to other antimicrobials and also on biofilm formation capacity. Yet, there is limited information on sensitivity of antibiotic-resistant *Salmonella* to other antimicrobials, such as phenolic compounds, chemical preservatives, or antimicrobial peptides. This study aimed at correlating antimicrobial resistance and biofilm formation capacity in antibiotic-resistant, biocide-tolerant *Salmonella* strains from hen eggshells. A collection of 21 strains previously selected according to their antibiotic resistance and biocide tolerance phenotypes were used for the present study. Strains were inspected for their biofilm formation capacity and for their sensitivity to (i) phenolic compounds (carvacrol, thymol), (ii) chemical preservatives (sodium lactate, trisodium phosphate), and (iii) cationic antimicrobials (polymyxin B, lysozyme-EDTA). Biofilm formation capacity was not correlated with antimicrobial resistances of the planktonic *Salmonella*. Polymyxin B and the lysozyme-EDTA combinations showed significant ($P, 0.05$) positive correlations to each other and to sodium lactate. Significant ($P, 0.05$) positive correlations were also observed for benzalkonium chloride and cetrimide with carvacrol, thymol, and trisodium phosphate, or between hexadecylpyridinium chloride and carvacrol. Antibiotic resistance also correlated positively with a higher tolerance to other antimicrobials (cefotaxime, ceftazidime, and ciprofloxacin with carvacrol, thymol, and trisodium phosphate; netilmicin with thymol and trisodium phosphate; tetracycline with carvacrol and thymol). These results must be taken into consideration to ensure a proper use of antimicrobials in the poultry industry, at concentrations that do not allow coselection of biocide-tolerant, antibiotic-resistant *Salmonella*. ISSN: 0362028X

Kljujev, I., Raicevic, V., Vujovic, B., Rothballer, M., Schmid, M.

Salmonella as an endophytic colonizer of plants - A risk for health safety vegetable production

(2018) *Microbial Pathogenesis*, 115, pp. 199-207.

ABSTRACT: Contamination of vegetables and fruits is the result of presence of human pathogen bacteria which can contaminate products in any part of production chain. There is an evidence of presence of: *Salmonella* spp. on the fresh vegetables and Salmonellosis is connected with tomato, sprouts, cantaloupe etc. The goal of this research is transmission of pathogen bacteria from irrigation water to plants and studying/monitoring the ability of the *Salmonella* spp. to colonize the surface and interior (endophytic colonization) of root at different vegetable species. Transmission of three *Salmonella* spp. strains from irrigation water to plants, as well as colonization of plants by these bacteria was investigated by using Fluorescence In Situ Hybridization (FISH) in combination with confocal laser scanning microscopy (CLSM). All tested *Salmonella* spp. strains showed ability to more or less colonize the surface and interior niches of the root, stem and leaf of the investigated plant species. These bacteria also were found in plant cells cytoplasm, although the mechanism of their entrance has not been clarified yet. ISSN: 08824010

Tozzo, K., Neto, A.F.G., Spencoski, K.M., Ronnau, M., Soares, V.M., Bersot, L.S.

Migration of Salmonella serotypes Heidelberg and Enteritidis in previously frozen chicken breast meat

(2018) *Food Microbiology*, 69, pp. 204-211.

ABSTRACT: *Salmonella* spp. have been shown to migrate to the internal regions of meat cuts. Storage conditions and the presence of proteolytic microbiota can influence this process. Our study assessed the impact of storage time, temperature, and the presence of proteolytic psychrotrophic bacteria on migration. Samples of previously frozen chicken breast with skin and bone were then sterilized using gamma ray irradiation and a cobalt-60 source (11 KGy) and them were inoculated with cultures of *S. Enteritidis*, *S. Enteritidis* and psychrotrophs, *S. Heidelberg*, or *S. Heidelberg* and psychrotrophs. Inoculated samples were stored for 6, 12, 24, 48, or 168 h at 2, 7, or -30 °C. After treatment, samples were divided into similar-sized segments and bacterial counts were determined in different regions (A – superface, B – intermediate region, and C – internal region). *S. Heidelberg* and *S. Enteritidis* both demonstrated successful internal migration for each time, temperature, and bacterial combination ($p < 0.05$). Our data revealed that *Salmonella* migration proceeded for 24 h, but slowed at 48 h ($p < 0.05$). *S. Enteritidis* with psychrotrophs showed a low amount of internal migration ($p < 0.05$). We therefore conclude that *Salmonella* spp. are able to migrate into the internal regions of meat cuts in a short period of time, even at low temperatures. The presence of proteolytic psychrotrophs inhibits the migration of *S. Enteritidis*. ISSN: 07400020

Dantas, S.T.A., Rossi, B.F., Bonsaglia, E.C.R., Castilho, I.G., Hernandes, R.T., Fernandes, A., Rall, V.L.M.

Cross-Contamination and Biofilm Formation by Salmonella enterica Serovar Enteritidis on Various Cutting Boards

(2018) *Foodborne Pathogens and Disease*, 15 (2), pp. 81-85.

ABSTRACT: Cross-contamination is one of the main factors related to foodborne outbreaks. This study aimed to analyze the cross-contamination process of *Salmonella enterica* serovar *Enteritidis* from poultry to cucumbers, on various cutting board surfaces (plastic, wood, and glass) before and after washing and in the presence and absence of biofilm. Thus, 10 strains of *Salmonella Enteritidis* were used to test cross-contamination from poultry to the cutting boards and from thereon to cucumbers. Moreover, these strains were evaluated as to their capacity to form biofilm on hydrophobic (wood and plastic) and hydrophilic materials (glass). We recovered the 10 isolates from all unwashed boards and from all cucumbers that had contacted them. After washing, the recovery ranged from 10% to 100%, depending on the board material. In the presence of biofilm, the recovery of salmonellae was 100%, even after washing. Biofilm formation occurred more on wood (60%) and plastic (40%) than glass (10%) boards,

demonstrating that bacteria adhered more to a hydrophobic material. It was concluded that the cutting boards represent a critical point in cross-contamination, particularly in the presence of biofilm. Salmonella Enteritidis was able to form a biofilm on these three types of cutting boards but glass showed the least formation. ISSN: 15353141

Rubini, S., Galletti, G., D'Incau, M., Govoni, G., Boschetti, L., Berardelli, C., Barbieri, S., Meriardi, G., Formaglio, A., Guidi, E., Bergamini, M., Piva, S., Serraino, A., Giacometti, F.

Occurrence of Salmonella enterica subsp. enterica in bivalve molluscs and associations with Escherichia coli in molluscs and faecal coliforms in seawater (2018) Food Control, 84, pp. 429-435.

ABSTRACT: The objectives of this study were to present data on the presence of Salmonella enterica subsp. enterica and on the enumeration of Escherichia coli and faecal coliforms respectively in different species of bivalve molluscs and seawater and to conduct a retrospective evaluation to assess the capacity of E. coli in molluscs and faecal coliforms and S. enterica subsp. enterica in sea and brackish water to predict the presence of S. enterica subsp. enterica in bivalve molluscs, and therefore, the risk of exposure for consumers. Data were collected from 4972 seawater samples and 5785 live bivalve molluscs samples (2877 Ruditapes philippinarum, 2177 Mytilus galloprovincialis, 256 Chamelae gallina and 475 C. gigas and O. edulis) collected in the molluscs production area of Ferrara, Northern Italy, from 1997 to 2015. An overall S. enterica subsp. enterica occurrence of 2.2% was reported in water and molluscs, with percentages varying depending on the type of sample and on the classification areas. All the 237 Salmonella strains were identified as genus Salmonella and a total of 53 different serovars were observed. Significant associations between the fecal indicators and presence of S. enterica subsp. enterica were observed both applying EU and USA criteria, but, it should be noted that the EU approach seems to be more stringent achieving the goal of identifying the most critical batches (94 out of the 100) whereas, following the USA approach, a not negligible and higher number of batches compliant for faecal coliforms but contaminated by S. enterica subsp. enterica has to be mentioned. In any case, the faecal indicators E. coli in molluscs and faecal coliforms in seawaters reflect only in part the presence of S. enterica subsp. enterica in molluscs and the consequent potential risk for consumers. Microbiological evaluation of seawaters seems to have a minor impact into the prediction of S. enterica subsp. enterica presence in molluscs. ISSN: 09567135

Allard, M.W., Bell, R., Ferreira, C.M., Gonzalez-Escalona, N., Hoffmann, M., Muruvanda, T., Ottesen, A., Ramachandran, P., Reed, E., Sharma, S., Stevens, E., Timme, R., Zheng, J., Brown, E.W.

Genomics of foodborne pathogens for microbial food safety (2018) Current Opinion in Biotechnology, 49, pp. 224-229.

ABSTRACT: Whole genome sequencing (WGS) has been broadly used to provide detailed characterization of foodborne pathogens. These genomes for diverse species including Salmonella, Escherichia coli, Listeria, Campylobacter and Vibrio have provided great insight into the genetic make-up of these pathogens. Numerous government agencies, industry and academia have developed new applications in food safety using WGS approaches such as outbreak detection and characterization, source tracking, determining the root cause of a contamination event, profiling of virulence and pathogenicity attributes, antimicrobial resistance monitoring, quality assurance for microbiology testing, as well as many others. The future looks bright for additional applications that come with the new technologies and tools in genomics and metagenomics. ISSN: 09581669

Usongo, V., Berry, C., Yousfi, K., Doualla-Bell, F., Labbé, G., Johnson, R., Fournier, E., Nadon, C., Goodridge, L., Bekal, S.

Impact of the choice of reference genome on the ability of the core genome snv methodology to distinguish strains of salmonella enterica serovar heidelberg

(2018) *PLoS ONE*, 13 (2), art. no. e0192233, .

ABSTRACT: *Salmonella enterica* serovar Heidelberg (S. Heidelberg) is one of the top serovars causing human salmonellosis. The core genome single nucleotide variant pipeline (cgSNV) is one of several whole genome based sequence typing methods used for the laboratory investigation of foodborne pathogens. SNV detection using this method requires a reference genome. The purpose of this study was to investigate the impact of the choice of the reference genome on the cgSNV-informed phylogenetic clustering and inferred isolate relationships. We found that using a draft or closed genome of S. Heidelberg as reference did not impact the ability of the cgSNV methodology to differentiate among 145 S. Heidelberg isolates involved in foodborne outbreaks. We also found that using a distantly related genome such as S. Dublin as choice of reference led to a loss in resolution since some sporadic isolates were found to cluster together with outbreak isolates. In addition, the genetic distances between outbreak isolates as well as between outbreak and sporadic isolates were overall reduced when S. Dublin was used as the reference genome as opposed to S. Heidelberg. ISSN: 19326203

Kim, S.U., Batule, B.S., Mun, H., Shim, W.-B., Kim, M.-G.

Ultrasensitive colorimetric detection of Salmonella enterica Typhimurium on lettuce leaves by HRPzyme-Integrated polymerase chain reaction (2018) Food Control, 84, pp. 522-528.

ABSTRACT: *Salmonella enterica* is one of the most encountered causative pathogens of food-borne illnesses. Outbreaks of such diseases are commonly associated with the consumption of fresh fruits and vegetables. In this study, we report a simple colorimetric strategy for the detection of S. enterica Serovar Typhimurium in fresh-cut lettuce leaves based on polymerase chain reaction (PCR) products generated by gene-specific primers integrated with the horseradish peroxidase-mimicking DNAzyme (HRPzyme). The HRPzyme sequence was integrated at the 5' end of the forward and reverse primers specific to 16S rRNA of S. enterica Typhimurium. At the end of the PCR reaction, unamplified HRPzyme-integrated primers were folded into G-quadruplex structure in the presence of hemin and then, they catalyzed the oxidation of 2,2'-azinobis (3-ethylbenzothiazolinesulfonic acid) (ABTS) with H₂O₂. The intensity of oxidized ABTS colorimetric signal was linearly and inversely related to S. enterica Typhimurium concentration. The latter relationship demonstrated diagnostic potential of this rapid, simple, highly sensitive, and selective colorimetric platform for S. enterica Typhimurium detection. ISSN: 09567135

Jansen, W., Woudstra, S., Müller, A., Grabowski, N., Schoo, G., Gerulat, B., Klein, G., Kehrenberg, C.

The safety and quality of pork and poultry meat imports for the common European market received at border inspection post Hamburg Harbour between 2014 and 2015

(2018) *PLoS ONE*, 13 (2), art. no. e0192550, .

ABSTRACT: Though imports of products of animal origin into the European Union (EU) have to comply with legal requirements and quality standards of the community, food consignment rejections at external EU borders have been increasing in recent years. This study explored microbiological metrics according to national target and critical values valid for samples at consumer level of 498 fresh poultry meat and 136 fresh pork filets from consignments subjected to physical checks during clearing at the border inspection post Hamburg harbour between January 2014 and December 2015 with ISO standard methods. Quantitative results indicated that critical thresholds for aerobic counts, Enterobacteriaceae, and E. coli were never surpassed. Merely for staphylococci, one poultry sample (0.2%) and 10 pork samples (9.3%) exceeded the critical limit (3.7 log cfu/g). However, qualitative analyses revealed that, *Staphylococcus aureus* was present in 16% and 10% of all poultry and pork samples, respectively, though no methicillin-resistant *Staphylococcus aureus* could be

confirmed. Moreover, E. coli was present in 50% and 67% of all pork and poultry samples, respectively, and thereof 33 isolates were confirmed as extended-spectrum β -lactamase-producing E. coli. Only 1.2% of the poultry samples were unacceptable due to the presence of Salmonella spp., whereas they were not detected in any pork sample. Campylobacter spp. were not detected in any sample. Though imported pork and poultry meat complies mostly with national market requirements, it might pose a potential risk to public health, especially for a direct or indirect foodborne transmission of imported, uncommon strains of zoonotic bacteria. ISSN: 19326203

Saltykova, A., Wuyts, V., Mattheus, W., Bertrand, S., Roosens, N.H.C., Marchal, K., De Keersmaecker, S.C.J.

Comparison of SNP-based subtyping workflows for bacterial isolates using WGS data, applied to Salmonella enterica serotype Typhimurium and serotype 1,4,[5],12:i:-

(2018) PLoS ONE, 13 (2), art. no. e0192504, .

ABSTRACT: Whole genome sequencing represents a promising new technology for subtyping of bacterial pathogens. Besides the technological advances which have pushed the approach forward, the last years have been marked by considerable evolution of the whole genome sequencing data analysis methods. Prior to application of the technology as a routine epidemiological typing tool, however, reliable and efficient data analysis strategies need to be identified among the wide variety of the emerged methodologies. In this work, we have compared three existing SNP-based subtyping workflows using a benchmark dataset of 32 Salmonella enterica subsp. enterica serovar Typhimurium and serovar 1,4,[5],12:i:- isolates including five isolates from a confirmed outbreak and three isolates obtained from the same patient at different time points. The analysis was carried out using the original (high-coverage) and a down-sampled (low-coverage) datasets and two different reference genomes. All three tested workflows, namely CSI Phylogeny-based workflow, CFSAN-based workflow and PHEnix-based workflow, were able to correctly group the confirmed outbreak isolates and isolates from the same patient with all combinations of reference genomes and datasets. However, the workflows differed strongly with respect to the SNP distances between isolates and sensitivity towards sequencing coverage, which could be linked to the specific data analysis strategies used therein. To demonstrate the effect of particular data analysis steps, several modifications of the existing workflows were also tested. This allowed us to propose data analysis schemes most suitable for routine SNP-based subtyping applied to S. Typhimurium and S. 1,4,[5],12:i:-. Results presented in this study illustrate the importance of using correct data analysis strategies and to define benchmark and fine-tune parameters applied within routine data analysis pipelines to obtain optimal results. ISSN: 19326203

Richardson, K.E., Cox, N.A., Cosby, D.E., Berrang, M.E.

Impact of desiccation and heat exposure stress on Salmonella tolerance to acidic conditions

(2018) Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes, 53 (2), pp. 141-144.

ABSTRACT: In a recent study, the pH of commonly used Salmonella pre-enrichment media became acidic (pH 4.0 to 5.0) when feed or feed ingredients were incubated for 24 h. Acidic conditions have been reported to injure or kill Salmonella. In this study, cultures of four known feed isolates (S. montevideo, S. senftenberg, S. tennessee, and S. schwarzengrund) and four important processing plant isolates (S. typhimurium, S. enteritidis, S. infantis, and S. heidelberg) were grown on meat and bone meal and later subjected to desiccation and heat exposure to stress the microorganism. The impact of stress on the isolates ability to survive in acidic conditions ranging from pH 4.0 to 7.0 was compared to the non-stressed isolate. Cell injury was determined on xylose lysine tergitol 4 (XLT4) and cell death determined on nutrient agar (NA). When

measured by cell death in non-stressed Salmonella, *S. typhimurium* was the most acid tolerant and *S. heidelberg* was the most acid sensitive whereas in stressed Salmonella, *S. senftenberg* was the most acid tolerant and *S. tennessee* was the most acid sensitive. The pH required to cause cell injury varied among isolates. With some isolates, the pH required for 50% cell death and 50% cell injury was similar. In other isolates, cell injury occurred at a more neutral pH. These findings suggest that the pH of pre-enrichment media may influence the recovery and bias the serotype of Salmonella recovered from feed during pre-enrichment.
ISSN: 03601234

Vohra, P., Bugarel, M., Turner, F., Loneragan, G.H., Hope, J.C., Hopkins, J., Stevens, M.P.

Quantifying the survival of multiple Salmonella enterica serovars in vivo via massively parallel whole-genome sequencing to predict zoonotic risk (2018) *Applied and Environmental Microbiology*, 84 (4), art. no. e02262-17, .
ABSTRACT: Salmonella enterica is an animal and zoonotic pathogen of worldwide importance. Salmonella serovars that differ in their host and tissue tropisms exist. Cattle are an important reservoir of human nontyphoidal salmonellosis, and contaminated bovine peripheral lymph nodes enter the food chain via ground beef. The relative abilities of different serovars to survive within the bovine lymphatic system are poorly understood and constrain the development of control strategies. This problem was addressed by developing a massively parallel whole-genome sequencing method to study mixed-serovar infections in vivo. Salmonella serovars differ genetically by naturally occurring single nucleotide polymorphisms (SNPs) in certain genes. It was hypothesized that these SNPs could be used as markers to simultaneously identify serovars in mixed populations and quantify the abundance of each member in a population. The performance of the method was validated in vitro using simulated pools containing up to 11 serovars in various proportions. It was then applied to study serovar survival in vivo in cattle challenged orally with the same 11 serovars. All the serovars successfully colonized the bovine lymphatic system, including the peripheral lymph nodes, and thus pose similar risks of zoonosis. This method enables the fates of multiple genetically unmodified strains to be evaluated simultaneously in a single animal. It could be useful in reducing the number of animals required to study mixed-strain infections and in testing the cross-protective efficacy of vaccines and treatments. It also has the potential to be applied to diverse bacterial species which possess shared but polymorphic alleles. ISSN: 00992240

Liao, H., Jiang, L., Zhang, R.

Induction of a viable but non-culturable state in Salmonella Typhimurium by thermosonication and factors affecting resuscitation (2018) *FEMS Microbiology Letters*, 365 (2), art. no. fnx249, .
ABSTRACT: The objective of this work was to analyze the effects of thermosonication (TS) on induction of a viable but non-culturable (VBNC) state in Salmonella Typhimurium and to examine incubation factors affecting subsequent resuscitation of cells. A TS treatment of 380 W at 53°C for 30 min induced the VBNC state in *S. Typhimurium* cells in beef peptone yeast (BPY) broth, apple/carrot juice, physiological saline and phosphate buffer solution. The logarithmic and decline phases of growth were more sensitive to the TS treatment compared to stationary phase cells. Meanwhile, VBNC *S. Typhimurium* could be resuscitated back to culturable cells by using suitable incubation temperatures and media. Addition of Tween 20 hindered resuscitation compared to the use of BPY medium alone. The optimal growth temperature (i.e. 37°C) was the most suitable temperature to resuscitate cells from the VBNC state. The VBNC incidence index decreased with the addition of sodium pyruvate during TS treatment, as it accelerated resuscitation. The results demonstrated that free radicals produced during TS processing and the growth phase of cells affected induction of the VBNC state in *S. Typhimurium*. Hence, the kinds and amounts of

free radicals generated during TS treatment should be analyzed in the future.
ISSN: 03781097

Anany, H., Brovko, L., El Dougdoug, N.K., Sohar, J., Fenn, H., Alasiri, N., Jabrane, T., Mangin, P., Monsur Ali, M., Kannan, B., Filipe, C.D.M., Griffiths, M.W.

Print to detect: a rapid and ultrasensitive phage-based dipstick assay for foodborne pathogens

(2018) *Analytical and Bioanalytical Chemistry*, 410 (4), pp. 1217-1230.

ABSTRACT: Foodborne pathogens are a burden to the economy and a constant threat to public health. The ability to rapidly detect the presence of foodborne pathogens is a vital component of any strategy towards establishing a safe and secure food supply chain. Bacteriophages (phages) are viruses capable of infecting and replicating within bacteria in a strain-specific manner. The ubiquitous and selective nature of phages makes them ideal for the detection and biocontrol of bacteria. Therefore, the objective of this research was to develop and test a phage-based paper dipstick biosensor for the detection of various foodborne pathogens in food matrices. The first step was to identify the best method for immobilizing phages on paper such that their biological activity (infectivity) was preserved. It was found that piezoelectric inkjet printing resulted in lower loss of phage infectivity when compared with other printing methods (namely gravure and blade coating) and that ColorLok paper was ideally suited to create functional sensors. The phage-based bioactive papers developed with use of piezoelectric inkjet printing actively lysed their target bacteria and retained this antibacterial activity for up to 1 week when stored at room temperature and 80% relative humidity. These bioactive paper strips in combination with quantitative real-time PCR were used for quantitative determination of target bacteria in broth and food matrices. A phage dipstick was used to capture and infect *Escherichia coli* O157:H7, *E. coli* O45:H2, and *Salmonella* Newport in spinach, ground beef and chicken homogenates, respectively, and quantitative real-time PCR was used to detect the progeny phages. A detection limit of 10–50 colony-forming units per millilitre was demonstrated with a total assay time of 8 h, which was the duration of a typical work shift in an industrial setting. This detection method is rapid and cost-effective, and may potentially be applied to a broad range of bacterial foodborne pathogens. [Figure not available: see fulltext.]. ISSN: 16182642

Hruby, C.E., Soupier, M.L., Moorman, T.B., Pederson, C., Kanwar, R.
Salmonella and Fecal Indicator Bacteria Survival in Soils Amended with Poultry Manure

(2018) *Water, Air, and Soil Pollution*, 229 (2), art. no. 32, .

ABSTRACT: Minimizing the risks associated with manure-borne pathogenic microorganisms requires an understanding of microbial survival under realistic field conditions. The objective of this 3-year study was to assess the fate of *Salmonella* (SALM) and fecal indicator bacteria (FIB), *E. coli* (EC) and enterococci (ENT), in glacial till-derived soils, after application of poultry manure (PM) to cornfields under chisel-plowed (CP) or no-till (NT) management. From 2010 to 2012, soil samples were obtained each spring at 0–15- and 15–30-cm depths, to determine whether over-wintering of target bacteria had occurred. Sampling was followed by application of PM at low (PM1) and high (PM2) rates, based on nitrogen application goals. In 2012, soil samples were collected 21, 42, and 158 days after manure application (DAM), to assess the effects of time, application rates, and tillage on frequency of detection and concentrations of target bacteria. Despite dry conditions, all three target organisms were detected 158 DAM in 2012, and detection of these organisms in spring soil samples from manured plots in 2011 and 2012, nearly a full year after PM application, suggests that these organisms can persist in the soil environment long after application. The highest SALM concentration (790 cfu/g dry weight) and detection rate (25%) was found in PM2 plots 42 DAM. SALM were detected more frequently in CP plots (20%) compared to NT plots (5%). In contrast, tillage practices had no apparent

effect on EC or ENT survival, as indicated by both soil, and decay rates estimated from tile-water bacteria concentrations. Decay rate constants (μ) ranged from 0.044 to 0.065 day⁻¹ for EC and 0.010 to 0.054 day⁻¹ for ENT. ISSN: 00496979

Roschanski, N., Fischer, J., Falgenhauer, L., Pietsch, M., Guenther, S., Kreienbrock, L., Chakraborty, T., Pfeifer, Y., Guerra, B., Roesler, U.H.
Retrospective analysis of bacterial cultures sampled in German chicken-fattening farms during the years 2011-2012 revealed additional VIM-1 carbapenemase-producing Escherichia coli and a serologically rough Salmonella enterica serovar infantis

(2018) *Frontiers in Microbiology*, 9 (MAR), art. no. 538, .

ABSTRACT: Carbapenems are last-resort antibiotics used in human medicine. The increased detection of carbapenem-resistant Enterobacteriaceae (CRE) is therefore worrying. In 2011 we reported the first livestock-associated VIM-1-producing Salmonella (S.) enterica serovar Infantis (R3) isolate from dust, sampled in a German chicken fattening farm. Due to this observation we retrospectively investigated more than 536 stored bacterial cultures, isolated from 45 chicken fattening farms during the years 2011 and 2012. After a non-selective overnight incubation, the bacteria were transferred to selective media. Escherichia (E.) coli and Salmonella growing on these media were further investigated, including antibiotic susceptibility testing, carbapenemase gene screening and whole genome sequencing (WGS). In total, four CRE were found in three out of 45 investigated farms: Besides R3, one additional Salmonella (G-336-1a) as well as two E. coli isolates (G-336-2, G-268-2). All but G-268-2 harbored the blaVIM-1 gene. Salmonella isolates R3 and G-336-1 were closely related although derived from two different farms. All three blaVIM-1-encoding isolates possessed identical plasmids and the blaVIM-1- containing transposon showed mobility at least in vitro. In isolate G-268-2, the AmpC beta-lactamase gene blaCMY-2 but no known carbapenemase gene was identified. However, a transfer of the phenotypic resistance was possible. Furthermore, G-268-2 contained the mcr-1 gene, combining phenotypical carbapenem- as well as colistin resistance in one isolate. Carbapenem-resistant Enterobacteriaceae have been found in three out of 45 investigated chicken flocks. This finding is alarming and emphasizes the importance of intervention strategies to contain the environmental spread of resistant bacteria in animals and humans.

ISSN: 1664302X

Neuert, S., Nair, S., Day, M.R., Doumith, M., Ashton, P.M., Mellor, K.C., Jenkins, C., Hopkins, K.L., Woodford, N., de Pinna, E., Godbole, G., Dallman, T.J.

Prediction of phenotypic antimicrobial resistance profiles from whole genome sequences of non-typhoidal Salmonella enterica

(2018) *Frontiers in Microbiology*, 9 (MAR), art. no. 592, .

ABSTRACT: Surveillance of antimicrobial resistance (AMR) in non-typhoidal Salmonella enterica (NTS), is essential for monitoring transmission of resistance from the food chain to humans, and for establishing effective treatment protocols. We evaluated the prediction of phenotypic resistance in NTS from genotypic profiles derived from whole genome sequencing (WGS). Genes and chromosomal mutations responsible for phenotypic resistance were sought in WGS data from 3,491 NTS isolates received by Public Health England's Gastrointestinal Bacteria Reference Unit between April 2014 and March 2015. Inferred genotypic AMR profiles were compared with phenotypic susceptibilities determined for fifteen antimicrobials using EUCAST guidelines. Discrepancies between phenotypic and genotypic profiles for one or more antimicrobials were detected for 76 isolates (2.18%) although only 88/52,365 (0.17%) isolate/antimicrobial combinations were discordant. Of the discrepant results, the largest number were associated with streptomycin (67.05%, n = 59). Pan-susceptibility was observed in 2,190 isolates (62.73%). Overall, resistance to tetracyclines was most common (26.27% of isolates, n = 917) followed by sulphonamides (23.72%, n = 828) and

ampicillin (21.43%, n = 748). Multidrug resistance (MDR), i.e., resistance to three or more antimicrobial classes, was detected in 848 isolates (24.29%) with resistance to ampicillin, streptomycin, sulphonamides and tetracyclines being the most common MDR profile (n = 231; 27.24%). For isolates with this profile, all but one were *S. Typhimurium* and 94.81% (n = 219) had the resistance determinants blaTEM-1, strA-strB, sul2 and tet(A). Extended-spectrum β -lactamase genes were identified in 41 isolates (1.17%) and multiple mutations in chromosomal genes associated with ciprofloxacin resistance in 82 isolates (2.35%). This study showed that WGS is suitable as a rapid means of determining AMR patterns of NTS for public health surveillance. ISSN: 1664302X

Bradley, A.J., Leach, K.A., Green, M.J., Gibbons, J., Ohnstad, I.C., Black, D.H., Payne, B., Prout, V.E., Breen, J.E.

The impact of dairy cows' bedding material and its microbial content on the quality and safety of milk – A cross sectional study of UK farms
(2018) *International Journal of Food Microbiology*, 269, pp. 36-45.

ABSTRACT: The introduction of bedding dairy cows on recycled manure solids (RMS) in the UK led to concern by competent authorities that there could be an increased, unacceptable risk to animal and human health. A cross-sectional study was designed to evaluate the microbial content of different bedding materials, when used by dairy cows, and its impact on the microbial content of milk. Data were collected from farms bedding lactating cows on sand (n = 41), sawdust (n = 44) and RMS (n = 40). The mean duration of RMS use prior to sampling was 13 months. Total bacterial count, and counts of *Streptococcus/Enterococcus* spp., *Staphylococcus* spp., *Bacillus cereus*, thermophilic, thermotolerant and psychrotrophic bacteria were determined in used bedding and milk. Samples were evaluated for the presence/absence of *Listeria monocytogenes*, *Salmonella* spp. and *Yersinia enterocolitica*. Data on milking practices were collected to investigate their potential to reduce microbial transfer from bedding to milk. There were substantial differences in bacterial counts both within and between bedding materials. However, there were no significant differences between bedding groups in counts in milk for any of the organisms studied, and no significant correlations between bacterial load in used bedding and milk. Fore-milking was associated with a reduced total bacterial count in milk. Dipping teats with disinfectant and drying, prior to milking, was associated with lower numbers of *Streptococcus/Enterococcus* spp. in milk. Disinfecting clusters between milking different cows was associated with a reduction in thermophilic and psychrotrophic counts in milk. This study did not provide evidence that use of RMS bedding increased the risk of presence of *Y. enterocolitica*, *Salmonella* spp. or *L. monocytogenes* in milk. However, the strength of this conclusion should be tempered by the relatively small number of farms on which *Y. enterocolitica* and *Salmonella* spp. were isolated. It is concluded that, despite the higher bacterial load of RMS, its use as bedding for lactating dairy cows need not be associated with a higher bacterial load in milk than the use of sand or sawdust. However, this finding must be interpreted in the light of the relatively recent introduction of RMS as a bedding material on the farms studied. Teat preparation provides a control point for the potential transfer of microorganisms from bedding to milk. The detection of zoonotic pathogens in a small proportion of milk samples, independent of bedding type, indicates that pasteurisation of milk prior to human consumption remains an important control measure. ISSN: 01681605

Portmann, A.-C., Fournier, C., Gimonet, J., Ngom-Bru, C., Barretto, C., Baert, L.

*A validation approach of an end-to-end whole genome sequencing workflow for source tracking of *Listeria monocytogenes* and *Salmonella enterica**
(2018) *Frontiers in Microbiology*, 9 (MAR), art. no. 446, .

ABSTRACT: Whole genome sequencing (WGS), using high throughput sequencing technology, reveals the complete sequence of the bacterial genome in a few days. WGS is increasingly being used for source tracking, pathogen surveillance and

outbreak investigation due to its high discriminatory power. In the food industry, WGS used for source tracking is beneficial to support contamination investigations. Despite its increased use, no standards or guidelines are available today for the use of WGS in outbreak and/or trace-back investigations. Here we present a validation of our complete (end-to-end) WGS workflow for *Listeria monocytogenes* and *Salmonella enterica* including: subculture of isolates, DNA extraction, sequencing and bioinformatics analysis. This end-to-end WGS workflow was evaluated according to the following performance criteria: stability, repeatability, reproducibility, discriminatory power, and epidemiological concordance. The current study showed that few single nucleotide polymorphism (SNPs) were observed for *L. monocytogenes* and *S. enterica* when comparing genome sequences from five independent colonies from the first subculture and five independent colonies after the tenth subculture. Consequently, the stability of the WGS workflow for *L. monocytogenes* and *S. enterica* was demonstrated despite the few genomic variations that can occur during subculturing steps. Repeatability and reproducibility were also demonstrated. The WGS workflow was shown to have a high discriminatory power and has the ability to show genetic relatedness. Additionally, the WGS workflow was able to reproduce published outbreak investigation results, illustrating its capability of showing epidemiological concordance. The current study proposes a validation approach comprising all steps of a WGS workflow and demonstrates that the workflow can be applied to *L. monocytogenes* or *S. enterica*. ISSN: 1664302X

Elnekave, E., Hong, S., Mather, A.E., Boxrud, D., Taylor, A.J., Lappi, V., Johnson, T.J., Vannucci, F., Davies, P., Hedberg, C., Perez, A., Alvarez, J.
Salmonella enterica Serotype 4,[5],12:i:-in Swine in the United States Midwest: An Emerging Multidrug-Resistant Clade
(2018) *Clinical Infectious Diseases*, 66 (6), pp. 877-885.

ABSTRACT: Background. *Salmonella* 4,[5],12:i:-, a worldwide emerging pathogen that causes many food-borne outbreaks mostly attributed to pig and pig products, is expanding in the United States Methods. Whole-genome sequencing was applied to conduct multiple comparisons of 659 *S. 4,[5],12:i:-* and 325 *Salmonella* Typhimurium from different sources and locations (ie, the United States and Europe) to assess their genetic heterogeneity, with a focus on strains recovered from swine in the US Midwest. In addition, the presence of resistance genes and other virulence factors was detected and the antimicrobial resistance phenotypes of 50 and 22 isolates of livestock and human origin, respectively, was determined. Results. The *S. 4,5,12:i:-* strains formed two main clades regardless of their source and geographic origin. Most (84%) of the US isolates recovered in 2014-2016, including those (48 of 51) recovered from swine in the US Midwest, were part of an emerging clade. In this clade, multiple genotypic resistance determinants were predominant, including resistance against ampicillin, streptomycin, sulfonamides, and tetracyclines. Phenotypic resistance to enrofloxacin (11 of 50) and cefixime (9 of 50) was found in conjunction with the presence of plasmid-mediated resistance genes (*qnrB19/qnrB2/qnrS1* and *blaCMY-2/blaSHV-12*, respectively). Higher similarity was also found between *S. 4,[5],12:i:-* from the emerging clade and *S. Typhimurium* from Europe than with *S. Typhimurium* from the United States. Conclusions. *Salmonella 4,[5],12:i:-* currently circulating in swine in the US Midwest are likely to be part of an emerging multidrug-resistant clade first reported in Europe, and can carry plasmid-mediated resistance genes that may be transmitted horizontally to other bacteria, and thus may represent a public health concern. ISSN: 10584838

Pande, V., McWhorter, A.R., Chousalkar, K.K.

Anti-bacterial and anti-biofilm activity of commercial organic acid products against Salmonella enterica isolates recovered from an egg farm environment
(2018) *Avian Pathology*, 47 (2), pp. 189-196.

ABSTRACT: This study evaluated the antibacterial activity of commercially available organic acid water additives against *Salmonella enterica* isolates and

examined the susceptibility of *Salmonella* Typhimurium biofilms to these products. Three commercial organic acid products (A, B, and C) were evaluated for minimum inhibitory and bactericidal concentrations against isolates of *S. enterica* serovars. Three- and five-day-old *S. Typhimurium* biofilms were formed at $22 \pm 2^\circ\text{C}$ using an MBEC™ assay system and exposed for 30 min or 90 min at 0.2% and 0.4% concentrations. No significant difference among serovars for inhibitory and bactericidal concentrations was detected. Two products (A and C) significantly reduced viable cells from biofilms of both ages in a dose- and time-dependent manner. Increased biofilm age did not enhance resistance towards organic acid treatments. None of the products completely eliminated biofilm cells at any concentration or exposure time. Product composition, exposure time, and concentration of organic acid products were important factors in reducing viable biofilm cells. This study has expanded our understanding about the susceptibility of *Salmonella* biofilms to commercial organic acid products. These findings have implications in the usage, development, and optimization of organic acid products. ISSN: 03079457

Xu, Y., Tao, S., Hinkle, N., Harrison, M., Chen, J.

Salmonella, including antibiotic-resistant *Salmonella*, from flies captured from cattle farms in Georgia, U.S.A.

(2018) *Science of the Total Environment*, 616-617, pp. 90-96.

ABSTRACT: Flies can be transmission vehicles of *Salmonella* from cattle to humans. This study determined the prevalence of *Salmonella* in/on flies captured from 33 cattle farms, including 5 beef and 28 dairy farms, in Georgia, USA, and characterized antibiotic resistance profiles of the isolated *Salmonella*. Twenty-six out of the 33 cattle farms (79%) and 185 out of the 1650 flies (11%) tested positive for *Salmonella* in the study. The incidence of *Salmonella*-positive flies varied from farm to farm, ranging from 0 to 78%. Among the 185 *Salmonella* isolated from flies, 29% were resistant to ampicillin, 28% to tetracycline, 21% to amoxicillin/clavulanic acid, 20% to cefoxitin, and 12% to streptomycin. Incidences of resistance against other tested antibiotics were low, ranging from 0 to 3%. Furthermore, 28% of the *Salmonella* isolates were multidrug resistant, demonstrating resistance to 3 or more antibiotics. The minimal inhibitory concentrations of ampicillin, cefoxitin, streptomycin, and tetracycline against the *Salmonella* isolates ranged from 32 to > 2048, 64 to 2048, 128 to 1024, and 32 to 1024 $\mu\text{g/mL}$, respectively. These data suggest that flies could be effective vehicles of transmitting antibiotic resistant *Salmonella* and disseminating antibiotic resistance genes on cattle farms, posing risks to human and animal health. ISSN: 00489697

Harris, C.S., Tertuliano, M., Rajeev, S., Vellidis, G., Levy, K.

Impact of storm runoff on Salmonella and Escherichia coli prevalence in irrigation ponds of fresh produce farms in southern Georgia

(2018) *Journal of Applied Microbiology*, 124 (3), pp. 910-921.

ABSTRACT: Aims: To examine *Salmonella* and *Escherichia coli* in storm runoff and irrigation ponds used by fresh produce growers, and compare *Salmonella* serovars with those found in cases of human salmonellosis. Methods and Results: We collected water before and after rain events at two irrigation ponds on farms in southern Georgia, USA, and collected storm runoff/storm flow within the contributing watershed of each pond. *Salmonella* and *E. coli* concentrations were higher in ponds after rain events by an average of 0.46 ($P < 0.01$) and 0.61 ($P < 0.05$) log₁₀ most probable number (MPN) per 100 ml respectively. *Salmonella* concentrations in storm runoff from fields and forests were not significantly higher than in ponds before rain events, but concentrations in storm flow from streams and ditches were higher by an average of 1.22 log₁₀ MPN per 100 ml ($P < 0.001$). Eighteen *Salmonella* serovars were identified from 155 serotyped isolates, and eight serovars were shared between storm runoff/storm flow and ponds. Seven of the serovars, including five of the shared serovars, were present in cases of human illness in the study region in the same year. However,

several serovars most commonly associated with human illness in the study region (e.g. Javiana, Enteritidis, and Montevideo) were not found in any water samples. Conclusions: Salmonella and E. coli concentrations in irrigation ponds were higher, on average, after rain events, but concentrations of Salmonella were low, and the ponds met FDA water quality standards based on E. coli. Some similarities and notable differences were found between Salmonella serovars in water samples and in cases of human illness. Significance and Impact of the Study: This study directly examined storm runoff/storm flow into irrigation ponds and quantified increases in Salmonella and E. coli following rain events, with potential implications for irrigation pond management as well as human health. ISSN: 13645072

Nascimento, M.S., Carminati, J.A., Silva, I.C.R.N., Silva, D.L., Bernardi, A.O., Copetti, M.V.

Salmonella, Escherichia coli and Enterobacteriaceae in the peanut supply chain: From farm to table

(2018) *Food Research International*, 105, pp. 930-935.

ABSTRACT: Due to recent foodborne outbreaks, peanuts have been considered a potential risk for Salmonella transmission. For this reason, the aim of this study was to determine the prevalence and contamination load of Salmonella, Escherichia coli and Enterobacteriaceae throughout the peanut supply chain in Brazil. Samples of peanuts and peanut-containing processed products from post-harvest (n = 129), secondary processing (n = 185) and retail market (n = 100) were analyzed. The results showed high Enterobacteriaceae counts in the post-harvest samples. At the end of the secondary processing, 16% of the samples remained contaminated by this group of microorganisms. Six peanut samples from primary production and one sample of peanut butter were tested positive for E. coli while Salmonella was detected in nine samples (2.2%): six from post-harvest, two from the initial stage of the secondary processing and one from retail. The Salmonella counts ranged between 0.004 and 0.092 MPN/g and five serotypes were identified (Muenster, Miami, Javiana, Oranienburg, Glostrup). The results demonstrated a high prevalence of Enterobacteriaceae and low prevalence of E. coli throughout the peanut supply chain. Furthermore, it was verified that peanuts may become contaminated by Salmonella during different stages of the supply chain, especially at post-harvest. ISSN: 09639969

Zhang, G., Chen, Y., Hu, L., Melka, D., Wang, H., Laasri, A., Brown, E.W., Strain, E., Allard, M., Bunning, V.K., Parish, M., Musser, S.M., Hammack, T.S.

Survey of foodborne pathogens, aerobic plate counts, total coliform counts, and Escherichia coli counts in leafy greens, sprouts, and melons marketed in the United States

(2018) *Journal of Food Protection*, 81 (3), pp. 400-411.

ABSTRACT: The objective of this research was to assess the microbiological status of leafy greens, sprouts, and melons from U.S. markets. A total of 14,183 samples of leafy greens, 2,652 samples of sprouts, and 3,411 samples of melons were collected throughout the United States from 2009 to 2014. The samples were analyzed for aerobic plate counts, total coliform counts, Escherichia coli counts, and the presence and levels of Salmonella, Shigella, Listeria monocytogenes, and Shiga toxin-producing E. coli (STEC), depending on the year and type of produce. Among the leafy greens, no E. coli O157:H7 or non-O157 STEC were detected from iceberg lettuce samples. The overall prevalences of Salmonella, E. coli O157:H7, non-O157 STEC, and L. monocytogenes in the 14,183 samples of leafy greens were 0.05, 0.01, 0.07, and 0.11%, respectively. Among sprout samples, no Salmonella or E. coli O157:H7 was detected, and the overall prevalences of non-O157 STEC and L. monocytogenes were 0.04 and 0.11%, respectively. Among melon samples, no Salmonella was detected from cucumbers, no L. monocytogenes was detected from cantaloupes, and the overall prevalences of Salmonella and L. monocytogenes were 0.12 and 0.23%,

respectively. *L. monocytogenes* levels were 0.4 to 1,470 most probable number (MPN)/g in leafy greens, 0.36 to 1,100 MPN/g in sprouts, and 0.03 to 150 MPN/g in melons, and most positive samples had low levels of these pathogens. The isolates from these foods were very diverse genetically. Foodborne pathogens, including *Salmonella*, STEC, and *L. monocytogenes*, had relatively low prevalences in the produce surveyed. Because these foods are usually consumed raw, measures should be taken to significantly minimize the presence and levels of human pathogens. ISSN: 0362028X

Saucedo-Alderete, R.O., Eifert, J.D., Boyer, R.R., Williams, R.C., Welbaum, G.E.

Delmopinol hydrochloride reduces Salmonella on cantaloupe surfaces (2018) Food Science and Nutrition, 6 (2), pp. 373-380.

ABSTRACT: Since the surfaces of cantaloupes are highly rough or irregular, bacteria can easily attach and become difficult to remove. Appropriate postharvest washing and sanitizing procedures can help control *Salmonella* and other pathogens on cantaloupe or other melons during postharvest operations. Delmopinol hydrochloride (delmopinol) is a cationic surfactant that is effective for treating and preventing gingivitis and periodontitis. The application of delmopinol to two cantaloupe cultivars was evaluated for reducing the level of inoculated *Salmonella*. Athena and Hale's Best Jumbo (HBJ) cantaloupe rind plugs (2.5 cm. dia.) were inoculated with nalidixic acid-resistant *Salmonella* Michigan (approx. 1.0×10^9 CFU/ml). After 15 min, rind plugs were sprayed with 10 ml of a delmopinol spray solution (0% or 1.0% vol/vol) and held at 35°C for 1 hr or 24 hr. Rind plugs were diluted with Butterfield's phosphate buffer, shaken and sonicated, and solutions were enumerated on 50 ppm nalidixic acid-tryptic soy agar. The texture quality and color of additional cantaloupes were evaluated, after 1% delmopinol spray treatment, over 14-day storage at 4°C. A 1.0% application of delmopinol after 1 hr reduced *Salmonella* concentration by ~ 3.1 log CFU/ml for both "HBJ" skin rind plugs and "Athena" stem scar rind plugs in comparison to the control ($p < .05$). No differences were observed in the texture and color (L^* , a^* , b^* values) of 1% delmopinol-treated cantaloupes as compared to control. Storage of cantaloupes treated with 1.0% delmopinol solution for 1 hr had a greater effect on reducing concentration of *Salmonella* compared to 24-hr treatment. A surface spray application of 1% delmopinol on cantaloupes could be an alternative antimicrobial postharvest treatment that could make surface bacteria more susceptible to sanitizers or physical removal. ISSN: 20487177

Sabbatucci, M., Dionisi, A.M., Pezzotti, P., Lucarelli, C., Barco, L., Mancin, M., Luzzi, I.

Molecular and epidemiologic analysis of reemergent salmonella enterica serovar Napoli, Italy, 2011–2015

(2018) Emerging Infectious Diseases, 24 (3), pp. 562-565.

ABSTRACT: Human infections with *Salmonella enterica* serovar Napoli are uncommon in Europe. However, these infections represented 5.9% of salmonellosis cases in Italy during 2014–2015. The source of infection is unknown. We analyzed surveillance data and compared strain genetic similarities and found that contaminated vegetables and surface water are probable sources of human infection. ISSN: 10806040

Christieans, S., Picgirard, L., Parafita, E., Lebert, A., Gregori, T.

Impact of reducing nitrate/nitrite levels on the behavior of Salmonella Typhimurium and Listeria monocytogenes in French dry fermented sausages (2018) Meat Science, 137, pp. 160-167.

ABSTRACT: *Salmonella* and *Listeria monocytogenes* are two pathogenic bacteria that most frequently contaminate pork meat. In dry fermented sausages, several hurdles are used for controlling bacterial growth such as nitrite and salt addition. In Europe, practices consist of adding potassium nitrate (250 ppm expressed as

NaNO₃) or a combination of nitrate/nitrite (150/150 ppm expressed as NaNO₃/NaNO₂ respectively). However, involvement of these additives in nitrosamine formation is a matter of concern. Consequently, a decrease in nitrite/nitrate amounts is proposed. The aim of this study was to evaluate the impact of reducing levels of these additives on *Listeria* and *Salmonella* behavior. Using challenge-tests, five trials were carried out by varying the concentration of nitrate and nitrate/nitrite. Results shown that nitrite is a relevant hurdle for control *Salmonella* and *Listeria*. At the end of drying, the most significant reductions of pathogens are obtained in sausages with nitrite added at the both tested concentrations (120 or 80 ppm NaNO₂). ISSN: 03091740

Seixas, R., Nunes, T., Machado, J., Tavares, L., Owen, S.P., Bernardo, F., Oliveira, M.

Demographic characterization and spatial cluster analysis of human Salmonella 1,4,[5],12:i:- infections in Portugal: A 10 year study
(2018) *Journal of Infection and Public Health*, 11 (2), pp. 178-182.

ABSTRACT: *Salmonella* 1,4,[5],12:i:- is presently considered one of the major serovars responsible for human salmonellosis worldwide. Due to its recent emergence, studies assessing the demographic characterization and spatial epidemiology of salmonellosis 1,4,[5],12:i:- at local- or country-level are lacking. In this study, a analysis was conducted over a 10 year period, from 2000 to the first quarter of 2011 at the Portuguese National Laboratory in Portugal mainland, with a total of 215 *Salmonella* 1,4,[5],12:i:- serotyped isolates obtained from human infections by a passive surveillance system. Data regarding source, year and month of sampling, gender, age, district and municipality of the patients were registered. Descriptive statistical analysis and a spatial scan statistic combined with a geographic information system were employed to characterize the epidemiology and identify spatial clusters. Results showed that most districts have reports of *Salmonella* 1,4,[5],12:i:-, with a higher number of cases at the Portuguese coastland, including districts like Porto (n = 60, 27.9%), Lisboa (n = 29, 13.5%) and Aveiro (n = 28, 13.0%). An increased incidence was observed in the period from 2004 to 2011 and most infections occurred during May and October. Spatial analysis revealed 4 clusters of higher than expected infection rates. Three were located in the north of Portugal, including two at the coastland (Cluster 1 [RR = 3.58, p ≤ 0.001] and 4 [RR = 10.42 p ≤ 0.230]), and one at the countryside (Cluster 3 [RR = 17.76, p ≤ 0.001]). A larger cluster was detected involving the center and south of Portugal (Cluster 2 [RR = 4.85, p ≤ 0.001]). The present study was elaborated with data provided by a passive surveillance system, which may originate an underestimation of disease burden. However, this is the first report describing the incidence and the distribution of areas with higher risk of infection in Portugal, revealing that *Salmonella* 1,4,[5],12:i:- displayed a significant geographic clustering and these areas should be further evaluated to identify risk factors in order to establish prevention programs. ISSN: 18760341

Akyol, I.

Development and application of RTi-PCR method for common food pathogen presence and quantity in beef, sheep and chicken meat
(2018) *Meat Science*, 137, pp. 9-15.

ABSTRACT: Two different RTi-PCR protocol were designed and quantifications were validated by using various amounts of DNA. Multiplex AGR1 and AGR2 RTi-PCR amplification reactions quantified successfully for *Clostridium perfringens*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli* O157:H7 and *Salmonella enterica*. Using the validated multiplex RTi-PCR reactions, the presence and quantification of pathogens were investigated in 40 beef, 41 sheep and 30 chicken meat samples. Results showed that the existence of *C. perfringens*, *E. faecalis* and *S. aureus* was 79%, 86% and 94%, respectively in the samples. Presence of *E. coli* O157:H7 and *S. enterica* were 90% and 91% in meat samples. The results showed that many meat samples were contaminated

by examined five pathogens. Therefore, it is considered that these samples may pose a potential risk to the human health since same equipment are used for different animals in the slaughterhouse. This neglect increases the amount of pathogenic contamination. ISSN: 03091740

Purevdorj-Gage, L., Nixon, B., Bodine, K., Xu, Q., Doerler, W.T.

Differential effect of food sanitizers on formation of viable but nonculturable salmonella enterica in poultry

(2018) *Journal of Food Protection*, 81 (3), pp. 386-393.

ABSTRACT: A method for microscopic enumeration of viable *Salmonella enterica* in meat samples was developed by using the LIVE/ DEAD BacLight kit technology. A two-step centrifugation and wash process was developed to clean the samples from food and chemical impurities that might otherwise interfere with the appropriate staining reactions. The accuracy of the BacLight kit-based viability assessments was confirmed with various validation tests that were conducted by following the manufacturer's instructions. For the biocide challenge tests, chicken parts each bearing around 8.5 log of *S. enterica* were sprayed with common food sanitizers such as 1,3-dibromo-5,5-dimethylhydantoin (DBDMH), lactic acid (LA), and peracetic acid (PAA). The log reduction (LR) of *S. enterica* for each test biocide was evaluated by microscopic and conventional culture plate methods. The results show that both LA and PAA treatments generated a greater number of microscopic counts compared with the corresponding plate counts with differences being around half a log. This discrepancy is believed to occur when cells enter a so-called viable but nonculturable (VBNC) state, and to our knowledge, this is the first report documenting the presence of VBNC in PAA- and LA-treated food samples. In contrast, the BacLight-based viable counts were comparable to the culture-based enumerations of all DBDMH-treated samples. Therefore, we concluded that DBDMH-treated meat did not contain significant VBNC populations of *S. enterica*. A detailed description of our spray system, the dye validation, and the treatment reproducibility are also provided in this work. Copyright ISSN: 0362028X

Black, E.P., Hinrichs, G.J., Barcay, S.J., Gardner, D.B.

Fruit flies as potential vectors of foodborne illness

(2018) *Journal of Food Protection*, 81 (3), pp. 509-514.

ABSTRACT: Fruit flies are a familiar sight in many food service facilities. Although they have been long considered as "nuisance pests," some of their typical daily activities suggest they may pose a potential public health threat. The aim of this study was to provide evidence of the ability of small flies to transfer bacteria from a contaminated source, food, or waste to surfaces or ready-to-eat food. Laboratory experiments were conducted by using purpose-built fly enclosures to assess the bacterial transfer capability of fruit flies. *Drosophila repleta* were capable of transferring *Escherichia coli* O157:H7, *Salmonella* Saint Paul, and *Listeria innocua* from an inoculated food source to the surface of laboratory enclosures. In addition, using an inoculated doughnut and noncontaminated lettuce and doughnut surfaces, fly-mediated cross-contamination of ready-to-eat food was demonstrated. Fruit flies were shown to be capable of accumulating approximately 2.9×10^3 log CFU of *E. coli* per fly within 2 h of exposure to a contaminated food source. These levels of bacteria did not decrease over an observation period of 48 h. Scanning electron micrographs were taken of bacteria associated with fly food and contact body parts and hairs during a selection of these experiments. These data, coupled with the feeding and breeding behavior of fruit flies in unsanitary areas of the kitchen and their propensity to land and rest on food preparation surfaces and equipment, indicate a possible role for fruit flies in the spread of foodborne pathogens. ISSN: 0362028X

Mueller-Doblies, D., Speed, K.C.R., Kidd, S., Davies, R.H.

Salmonella Typhimurium in livestock in Great Britain - Trends observed over a 32-year period

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ABSTRACT: In this retrospective study, we describe and analyse Salmonella data from four livestock species in Great Britain between 1983 and 2014, focusing on Salmonella Typhimurium. A total of 96 044 Salmonella isolates were obtained during the study period. S. Typhimurium was the predominant serovar isolated from cattle and pigs and represented 40.7% (18 455/45 336) and 58.3% (4495/7709) of isolates from these species respectively, while it only accounted for 6.7% (2114/31 492) of chicken isolates and 8.1% (926/11 507) of Turkey isolates. Over the study period, DT104 was the most common phage type in all four species; however, DT104 peaked in occurrence between 1995 and 1999, but is currently rare. Monophasic strains of S. Typhimurium represented less than 3% of all Salmonella isolates in cattle and chickens in 2014, but accounted for 10.4% of all Turkey isolates and 39.0% of all pig isolates in the same year. Salmonella isolates were tested for their in vitro susceptibility to 16 antimicrobials. Antimicrobial resistance of S. Typhimurium isolates is largely influenced by the dominance of specific phage types at a certain time, which are commonly associated with particular resistance patterns. Changes in resistance patterns over time were analysed and compared between species. ISSN: 09502688

Cox, N.A., Cosby, D.E., Berrang, M.E., Richardson, K.E., Holcombe, N., Weller, C.

The effect of environmental poultry samples on the pH of typical Salmonella pre-enrichment and enrichment media following incubation
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ABSTRACT: The first step to detect Salmonella in feed and other dry contaminated samples is a preenrichment broth that can assist in the recovery of small numbers of stressed Salmonella cells. A previous study demonstrated that incubation of feed and feed ingredients in commonly used pre-enrichment media resulted in a low pH that injured or killed the Salmonella. The objective of this study was to determine which environmental samples and pre-enrichment and selective broths could interfere with the accuracy of Salmonella detection by allowing pH to drop. Samples were collected from commercial poultry operations. Triplicate 10-g subunits were dispensed into sterile 18-oz Whirl Pak bags and 90 mL of each of the media [lactose broth (LB), buffered peptone water (BPW), Universal Pre-enrichment (UP), minimal salts (M-9), tetrathionate broth (TT) and Rappaport-Vassiliadis broth (RV)] were added to the bags and incubated 24 h at 37°C (or 42°C for RV and TT). The pH was measured after incubation. With Turkey litter, fluff, and eggshells, regardless of media, the pH never went below 5.7. Regardless of sample type, the lowest pH was 6.1, 6.2, and 6.4 for UP, M-9, and BPW, respectively. For the pre-enrichment medium commonly used in the US, (LB) the pH dropped to 4.7 to 4.9 with broiler litter and 4.2 for boot covers used in Turkey or broiler houses. Many researchers testing these sample types are unaware of this potential for pH change during pre-enrichment and may be underestimating the presence of Salmonella. ISSN: 10566171

Savran, D., Pérez-Rodríguez, F., Halkman, A.K.

Modeling the survival of Salmonella Enteritidis and Salmonella Typhimurium during the fermentation of yogurt
(2018) *Food Science and Technology International*, 24 (2), pp. 110-116.

ABSTRACT: The objective of this study was to evaluate the behavior of Salmonella Enteritidis and Salmonella Typhimurium, the two most important serovars of salmonellosis, during the fermentation of yogurt. The microorganisms were enumerated in milk throughout the fermentation process at three initial inoculum levels (3, 5 and 7 log CFU/mL). DMFit software was used in the fitting procedure of the data (IFR, Norwich, UK, Version 3.5). The data provided sigmoidal curves that were successfully displayed with the Baranyi model. The results showed that the initial inoculum level did not affect the growth for both pathogens; thus, the μ_{max} values (maximum specific growth rate) did not significantly differ across all the contamination levels, ranging from 0.26 to 0.38

for *S. Enteritidis* and from 0.50 to 0.56 log CFU/g/h for *S. Typhimurium* ($P > 0.05$). However, the μ_{max} values significantly differed between the two serovars ($P < 0.05$). The λ values (lag time) did not have a clear trend in either of the pathogens. The present study showed that *Salmonella* can survive the fermentation process of milk even at a low contamination level. In addition, the models presented in this study can be used in quantitative risk assessment studies to estimate the threat to consumers. ISSN: 10820132

Lang, E., Guyot, S., Peltier, C., Alvarez-Martin, P., Perrier-Cornet, J.-M., Gervais, P.

Cellular injuries in Cronobacter sakazakii CIP 103183T and Salmonella enterica exposed to drying and subsequent heat treatment in milk powder (2018) Frontiers in Microbiology, 9 (MAR), art. no. 475, .

ABSTRACT: Because of the ability of foodborne pathogens to survive in low-moisture foods, their decontamination is an important issue in food protection. This study aimed to clarify some of the cellular mechanisms involved in inactivation of foodborne pathogens after drying and subsequent heating. Individual strains of *Salmonella Typhimurium*, *Salmonella Senftenberg*, and *Cronobacter sakazakii* were mixed into whole milk powder and dried to different water activity levels (0.25 and 0.58); the number of surviving cells was determined after drying and subsequent thermal treatments in closed vessels at 90 and 100°C, for 30 and 120 s. For each condition, the percentage of unculturable cells was estimated and, in parallel, membrane permeability and respiratory activity were estimated by flow cytometry using fluorescent probes. After drying, it was clearly observable that the percentage of unculturable cells was correlated with the percentage of permeabilized cells (responsible for 20-40% of the total inactivated bacteria after drying), and to a lesser degree with the percentage of cells presenting with loss of respiratory activity. In contrast, the percentages of unculturable cells observed after heat treatment were strongly correlated with the loss of respiratory activity and weakly with membrane permeability (for 70-80% of the total inactivated bacteria after heat treatment). We conclude that cell inactivation during drying is closely linked to membrane permeabilization and that heat treatment of dried cells affects principally their respiratory activity. These results legitimize the use of time-temperature scales and allow better understanding of the cellular mechanisms of bacterial death during drying and subsequent heat treatment. These results may also allow better optimization of the decontamination process to ensure food safety by targeting the most deleterious conditions for bacterial cells without denaturing the food product. ISSN: 1664302X

Hutchinson, J.A., Wheeler, C., Mohle-Boetani, J.C.

Outbreak epidemiologically linked with a composite product of beef, mechanically separated chicken and textured vegetable protein contaminated with multiple serotypes of Salmonella enterica including multidrug-resistant Infantis, California 2016

(2018) Epidemiology and Infection, 146 (4), pp. 430-436.

ABSTRACT: A salmonellosis outbreak occurred at a California prison in April and May 2016. In a cohort study of 371 inmates, persons who consumed dishes from the prison kitchen made from ground meat had a higher attack rate (15%) than those who did not (4%) (risk ratio 3.4, 95% CI 1.1-10.6). The ground meat product was composed exclusively of beef, mechanically separated chicken (MSC) and textured vegetable protein; eight of eight lots of the product collected from the prison and processing facility were contaminated with *Salmonella enterica* of eight serotypes and 17 distinct PFGE patterns, including multidrug-resistant *S. Infantis*. Either the MSC or the beef could have been the source of the particular strains of *S. enterica* isolated from patients or the product. The microbiological evidence is most consistent with MSC as the source of the high levels of *S. enterica* in the epidemiologically linked meat product. Our findings contribute to

the growing body of evidence about the hazard posed by the use of products containing raw mechanically separated poultry in kitchens in institutions.
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Clavijo, V., Flórez, M.J.V.

The gastrointestinal microbiome and its association with the control of pathogens in broiler chicken production: A review
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ABSTRACT: The microbiome of the broiler chicken gastrointestinal tract (GIT) has been extensively studied, and it has been amply demonstrated that it plays an important role in the health of the host, as it has a positive impact on the immune system, the physiology of the GIT, and productivity. Also, the microbiota is involved in reducing and preventing colonization by enteric pathogens through the process of competitive exclusion and the production of bacteriostatic and bactericidal substances. The taxonomic composition of the microbiota is affected by different factors, such as the organ, the age of the animal, diet and the use of antimicrobials. Different kinds of additives that regulate the microbial community in feed include probiotics (live microorganisms that when administered in adequate amounts confer a health benefit on the host), prebiotics (ingredients that stimulate increased beneficial microbial activity in the digestive system in order to improve the health of the host) and phytobiotics (primary or secondary components of plants that contain bioactive compounds that exert a positive effect on the growth and health of animals). Phages may potentially provide an integrated solution to modulate the intestinal microbiome of chicken intestines, as they reduce specific pathogenic microbial populations, permitting the proliferation of beneficial microbiota. Studies have shown that the use of cocktails of phages, especially in high concentrations and with short lapses of time between exposure to the bacteria and treatment with phages, optimize the reduction of *Salmonella* in chickens. Each of these technologies has demonstrable positive effects on the health of the host and the reduction of the pathogen load in controlled assays. This paper presents a comprehensive summary of the role of the microbiota in the broiler chicken gastrointestinal tract, and discusses the usefulness of different strategies for its modulation to control pathogens, with a particular emphasis on bacteriophages. ISSN: 00325791