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Contents

Editorial Note

Bilthoven, 2 April 2019

Dear colleague,

It may sound trivial (and it probably is) that we have had an internal discussion about the 'correct' expression for our interlaboratory comparison studies. This discussion was triggered by the training about 'Implementing ISO/IEC 17043 - General requirements for proficiency testing', given by the EC Joint Research Centre last year and earlier this year. In this ISO document the following definition for Proficiency Testing is given: 'evaluation of participant performance against pre-established criteria by means of interlaboratory comparisons'. Following this definition, the expression 'interlaboratory comparison' is not wrong, but as we also evaluate the performance of the participants it may be better to speak about Proficiency Testing. For that reason we decided to change the expression 'EURL-*Salmonella* interlaboratory comparison studies', into 'EURL-*Salmonella* Proficiency Tests'. We did not change the activities; we just changed the wording.

In relation to these EURL-*Salmonella* Proficiency Tests, we have been busy with the following activities in the first quarter of this year

In January/February 2019, the analysis of the serotyping results of the **EURL-*Salmonella* Proficiency Test Serotyping 2018** 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/sites/default/files/2019-02/Interim%20Summary%20Report%20EURL-Salmonella%20PT%20Serotyping%202018.pdf Two participants did not meet the level of good performance at the first stage of the study and a follow-up study for these laboratories is currently ongoing. The results of the PFGE typing part of this PT are still under analysis and will soon be reported to the participants.

By the end of March 2019, the combined **EURL-*Salmonella* Proficiency Test for Food and Feed 2019** was organised and the results of this study can still be submitted until 16 April 2019.

Currently we are also busy with the preparation of the **EURL-*Salmonella* workshop of 2019**. The draft program is almost finalised and more information will be sent to the participants as soon as it is available.

By the end of 2018 we were informed about a **multi-country outbreak of *Salmonella* Coeln,** investigated by ECDC and EFSA. After a call for information among the NRLs-*Salmonella*, sequence data as well as isolates (to be sequenced by the EURL) have been received. With agreement of the relevant NRLs, the sequence data have been shared with EFSA and ECDC for further analysis (still ongoing). I would like to thank all NRLs-*Salmonella* for their fast and comprehensive replies to this call.

For your information, in February 2019 **Regulation (EU) 2019/229** was published. This Regulation is amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. In this amendment the references to EN ISO methods have been updated. For example, for the detection of *Salmonella* reference is made to EN ISO 6579-1 (2017) replacing the former version EN ISO 6579 (2002). Regulation (EU) 2019/229 can be found at the following

link: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32019R0229&qid=1554122191521&from=EN

In February 2019, the following report was published:
Kuijpers A.F.A. and Mooijman, K.A, 2019. 4th EURL-*Salmonella* interlaboratory comparison study animal feed 2018. Detection of *Salmonella* in chicken feed. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM Report no.: 2018-0023.
http://www.rivm.nl/bibliotheek/rapporten/2018-0023.pdf

Best wishes,
Kirsten Mooijman
Coordinator EURL-*Salmonella*

From the Literature

Salmonella-related Literature from Scopus: January - March 2019

Zhai, L., Liu, H., Chen, Q., Lu, Z., Zhang, C., Lv, F., Bie, X.
Development of a real-time nucleic acid sequence–based amplification assay for the rapid detection of Salmonella spp. from food
(2019) Brazilian Journal of Microbiology, 50 (1), pp. 255-261.
ABSTRACT: Salmonella spp. is one of the most common foodborne infectious pathogen. This study aimed to develop a real-time nucleic acid sequence–based amplification (NASBA) assay for detecting Salmonella in foods. Primers and a molecular beacon targeting the Salmonella-specific xcd gene were designed for mRNA transcription, and 48 Salmonella and 18 non-Salmonella strains were examined. The assay showed a high specificity and low detection limit for Salmonella (7 × 10 −1 CFU/mL) after 12 h of pre-enrichment. Importantly, it could detect viable cells. Additionally, the efficacy of the NASBA assay was examined in the presence of pork background microbiota; it could detect Salmonella cells at 9.5 × 10 3 CFU/mL. Lastly, it was successfully used to detect Salmonella in pork, beef, and milk, and its detection limit was as low as 10 CFU/25 g (mL). The real-time NASBA assay developed in this study may be useful for rapid, specific, and sensitive detection of Salmonella in food of animal origin. ISSN: 15178382

Guttula, D., Yao, M., Baker, K., Yang, L., Goult, B.T., Doyle, P.S., Yan, J.
Calcium-mediated Protein Folding and Stabilization of Salmonella Biofilm-associated Protein A
(2019) Journal of Molecular Biology, 431 (2), pp. 433-443.
ABSTRACT: Biofilm-associated proteins (BAPs) are important for early biofilm formation (adhesion) by bacteria and are also found in mature biofilms. BapA from Salmonella is a ~ 386-kDa surface protein, comprising 27 tandem repeats predicted to be bacterial Ig-like (BIg) domains. Such tandem repeats are conserved for BAPs across different bacterial species, but the function of these domains is not completely understood. In this work, we report the first study of the mechanical stability of the BapA protein. Using magnetic tweezers, we show that the folding of BapA BIg domains requires calcium binding and the folded domains have differential mechanical stabilities. Importantly, we identify that > 100 nM concentration of calcium is needed for folding of the BIg domains, and the stability of the folded BIg domains is regulated by calcium over a wide concentration range from sub-micromolar (μM) to millimolar (mM). Only at mM calcium concentrations, as found in the extracellular environment, do the BIg domains have the saturated mechanical stability. BapA has been suggested to be involved in Salmonella invasion, and it is likely a crucial mechanical component of biofilms. Therefore, our results provide new insights into the potential roles of BapA as a structural maintenance component of Salmonella biofilm and also Salmonella invasion. ISSN: 00222836

Ung, A., Baidjoe, A.Y., Van Cauteren, D., Fawal, N., Fabre, L., Guerrisi, C., Danis, K., Morand, A., Donguy, M.-P., Lucas, E., Rossignol, L., Lefèvre, S., Vignaud, M.-L., Cadel-Six, S., Lailler, R., Jourdan-Da Silva, N., Le Hello, S.
Disentangling a complex nationwide Salmonella dublin outbreak associated with raw-milk cheese consumption, France, 2015 to 2016
(2019) Eurosurveillance, 24 (3), art. no. 1700703, .
ABSTRACT: On 18 January 2016, the French National Reference Centre for Salmonella reported to Santé publique France an excess of Salmonella enterica serotype Dublin (S. Dublin) infections. We investigated to identify the source of infection and implement control measures. Whole genome sequencing (WGS) and multilocus variable-number tandem repeat analysis (MLVA) were performed to identify microbiological clusters and links among cases, animal and food sources. Clusters were defined as isolates with less than 15 single nucleotide polymorphisms determined by WGS and/or with identical MLVA pattern. We compared different clusters of cases with other cases (case-case study) and controls recruited from a web-based cohort (case-control study) in terms of food consumption. We interviewed 63/83 (76%) cases; 2,914 controls completed a questionnaire. Both studies' findings indicated that successive S. Dublin outbreaks from different sources had occurred between November 2015 and March 2016. In the case-control study, cases of distinct WGS clusters were more likely to have consumed Morbier (adjusted odds ratio (aOR): 14; 95% confidence interval (CI): 4.8-42) or Vacherin Mont d'Or (aOR: 27; 95% CI: 6.8-105), two bovine raw-milk cheeses. Based on these results,

the Ministry of Agriculture launched a reinforced control plan for processing plants of raw-milk cheeses in the production region, to prevent future outbreaks. ISSN: 1025496X

Mohammad, Z., Kalbasi-Ashtari, A., Riskowski, G., Castillo, A.
Reduction of Salmonella and Shiga toxin-producing Escherichia coli on alfalfa seeds and sprouts using an ozone generating system
(2019) International Journal of Food Microbiology, 289, pp. 57-63.
ABSTRACT: Several outbreaks of illness have been associated with consumption of alfalfa sprouts contaminated with Shiga toxin-producing Escherichia coli (STEC) and Salmonella. The ozone application was investigated as an intervention. Alfalfa seeds were inoculated with cocktails of 3 Salmonella strains, including serotypes Typhimurium, Agona and Saintpaul, and 3 strains of Shiga toxin-producing Escherichia coli (STEC) including serotypes O104:H4, O157:H7 and O121:H19 with a final load of 7.0 log CFU/ml. Then, the inoculated seeds, and the sprouts obtained from these seeds were separately subjected to aqueous ozone treatment containing (5 mg/L) ozone for varied times of exposure. The mean log reductions for Salmonella achieved on seeds after 10, 15, and 20 min of ozone exposure were 1.6 ± 0.5, 1.7 ± 0.3, 2.1 ± 0.5, respectively and 1.5 ± 0.4, 1.6 ± 0.4, 2.1 ± 0.5 for STEC, respectively. For sprouts obtained from the inoculated seed, the mean log reductions for Salmonella after 10, 15, and 20 min exposure times were 0.7 ± 0.2, 1.1 ± 0.4, 3.6 ± 0.2, respectively, whereas the mean log reductions for STEC were 0.7 ± 0.1, 1.2 ± 0.3 and 1.8 ± 0.2, respectively. At each contact time, there were no differences in log reductions between pathogens on seeds ($P > 0.05$), whereas on sprouts, the reductions obtained at 20 min were significantly greater ($P < 0.05$) for Salmonella than for STEC. On both seeds and sprouts, the exposure time had significant ($P < 0.05$) effects on log reductions of Salmonella and STEC. The weight, color properties and shelf life of ozonated sprouts were also tested. The ozonation did not have negative effects on germination (%), color and mass of sprouts in comparison with the controls. This study confirmed that it is possible to substantially reduce Salmonella and STEC by using a low ozone concentration (5 mg/L) and reduce food safety risk with less concern about the safety for processing workers of this treatment, this without affecting seed germination. This procedure may be a promising intervention to reduce Salmonella and STEC from alfalfa seeds and sprouts. ISSN: 01681605

Silva, P.L.A.P.A., Goulart, L.R., Reis, T.F.M., Mendonça, E.P., Melo, R.T., Penha, V.A.S., Peres, P.A.B.M., Hoepers, P.G., Beletti, M.E., Fonseca, B.B.
Biofilm Formation in Different Salmonella Serotypes Isolated from Poultry
(2019) Current Microbiology, 76 (1), pp. 124-129.
ABSTRACT: Little is known about Salmonella biofilm assembly, making the prevention of the disease a challenge in the poultry production chain. The objective of the present study was then to evaluate biofilm formation from different serotypes of Salmonella spp. in both polystyrene plates and eggshells. Salmonella Gallinarum and S. Minnesota were both classified as producers of biofilms of moderate intensity. Interestingly, S. Gallinarum produces biofilm even though being a serotype without flagellum and not having the lux gene in its genome, suggesting that there might be other important structures and genes associated with biofilm formation. Regarding Enteritidis, Typhimurium, Typhimurium variant, and Heidelberg serotypes, despite having high counts, BFI (Biofilm Formation Index) showed low biofilm production, probably due to the scarcity of extracellular matrix produced by such strains. A turkey eggshell model was then used for S. Enteritidis and S. Heidelberg biofilm formation. The results from the microbial count and scanning electron microscopy showed that Salmonella serotypes were also able to generate biofilm in eggshells, suggesting the presence of biofilms in poultry producing farms, a main concern for the poultry production industry. ISSN: 03438651

Zhou, X., Zhang, Z., Suo, Y., Cui, Y., Zhang, F., Shi, C., Shi, X.
Effect of sublethal concentrations of ceftriaxone on antibiotic susceptibility of multiple antibiotic-resistant Salmonella strains
(2019) FEMS Microbiology Letters, 366 (2), art. no. fny283, .
ABSTRACT: The aim of this study was to determine whether sublethal concentrations of ceftriaxone could alter antibiotic resistance patterns in Salmonella strains. Three multiple antibiotic-resistant Salmonella isolates and the control strain ATCC 13076 were subjected to induction experiments by stepwise increases in sublethal concentrations of ceftriaxone. Sublethal levels of ceftriaxone induced antibiotic resistance but not control Salmonella isolates to ceftriaxone and to other antibiotics. After 100 generations in 2 months when the antibiotic stress was removed, only one isolate (Salmonella Typhimurium 11202) maintained the induction changes in antibiotic resistance phenotype (tetracycline from resistance to sensitive and ampicillin from sensitive to resistance). Consistent with its

stable phenotypic resistance changes, expression of the tetracycline and β-lactam resistance-related genes tetA and blaTEM were >10-fold down- and upregulated, respectively. Moreover, this strain had increased mRNA levels of efflux pump associated genes acrB and tolC and the SOS response regulator lexA and downregulation of the porin gene ompC. We found no overt changes in plasmid profiles before and after resistance induction. In all, sublethal concentrations of ceftriaxone induced alterations in Salmonella isolates to multiple antibiotics and some of them kept stable maintenance. The increased blaTEM expression may pose a potential danger for new generation β-lactam antibiotics. © FEMS 2019. ISSN: 03781097

Cadel-Six, S., Vignaud, M.-L., Mohammed, M.
Draft Genome Sequences of Salmonella enterica subsp. Enterica Serovar Dublin Strains from St. Nectaire and Morbier cheeses characterized by multilocus variable-number tandem-repeat analysis profiles associated with two fatal outbreaks in France (2019) Microbiology Resource Announcements, 8 (1), art. no. e01361-18, .
ABSTRACT: We report here the draft genome sequences of 2 Salmonella enterica subsp. enterica serovar Dublin strains from St. Nectaire and Morbier cheeses having multilocus variable-number tandem-repeat analysis (MLVA) profiles identified during the fatal outbreaks that occurred in France in 2012 and 2015 to 2016, respectively. These draft genome sequences will help uncover the virulence determinants in invasive S. Dublin strains. Copyright ISSN: 2576098X

Mooijman, K.A., Pielaat, A., Kuijpers, A.F.A.
Validation of EN ISO 6579-1 - Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 1 detection of Salmonella spp. (2019) International Journal of Food Microbiology, 288, pp. 3-12.
ABSTRACT: The European and International Standard method for the detection of Salmonella spp. in samples from the primary production stage, EN ISO 6579:2002/Amd.1:2007, was validated by an interlaboratory study in the frame of Mandate M/381, ordered by the European Commission and accepted by the European Standardisation Organisation (CEN). In addition to this study, results from two interlaboratory studies organised earlier by the European Union Reference Laboratory (EURL) for Salmonella were used for determination of the performance characteristics. Parallel to the performance evaluation for the Mandate, the revision of EN ISO 6579:2002 started. Part of this revision was the incorporation of the standardised method for detection of Salmonella in samples from the primary production stage (EN ISO 6579:2002/Amd.1:2007) and its performance characteristics in the new part 1 of EN ISO 6579. The 2002 version of EN ISO 6579 already contained performance characteristics for the detection of Salmonella in food samples, but LOD 50 values (contamination level at which 50% of the samples are found positive) were not yet included. To be in line with the performance characteristics determined for detection of Salmonella spp. in samples from the primary production stage, LOD 50 values for detection of Salmonella in food samples were calculated from the raw data of the validation studies performed in 2000. In this paper, the performance characteristics of EN ISO 6579-1:2017 are determined based not only on the results of the interlaboratory study carried out in 2013 under the Mandate, but also on several other interlaboratory studies. These performance characteristics consist of specificity, sensitivity and LOD 50. ISSN: 01681605

Kirkland, C., Black, E., Forghani, F., Pomraning, A., Sadowsky, M.J., Diez-Gonzalez, F.
Room temperature growth of salmonella enterica serovar saintpaul in fresh Mexican salsa (2019) Journal of Food Protection, 82 (1), pp. 102-108.
ABSTRACT: Salsa-associated outbreaks, including the large multistate outbreak in the United States in 2008 caused by jalapeño and serrano peppers contaminated with Salmonella Saintpaul, have raised concerns about salsa as a potential vehicle for transmission. Despite these events, there has been relatively limited research on the potential growth of pathogenic bacteria in salsa. The aim of this study was to characterize the survival and growth of Salmonella, including the outbreak strain of Salmonella Saintpaul (E2003001236), in freshly made salsa and its main ingredients. Chopped tomatoes, jalapeño peppers, cilantro, and onions were tested individually or mixed according to different salsa recipes. Samples were inoculated with five Salmonella serotypes at 3 log CFU/g: Saintpaul (various strains), Typhimurium, Montevideo, Newport, or Enteritidis. Samples were then stored at room temperature (23°C) for up to 12 h or 3 days. The Salmonella Saintpaul levels reached approximately 9 log CFU/g after 2 days in tomato, jalapeño pepper, and cilantro. Growth was slower in onions, reaching 6 log CFU/g by day 3. Salsa recipes, with or without lime juice, supported the growth of Salmonella

Saintpaul, and final levels were approximately 7 log CFU/g after 3 days at 23°C. In contrast, the counts of Salmonella Typhimurium, Salmonella Montevideo, Salmonella Newport, and Salmonella Enteritidis increased only 2 log CFU/g after 3 days in any of the salsas. Other Salmonella Saintpaul strains were able to grow in salsas containing 10% lime juice, but their final levels were less than 5 log CFU/g. These findings indicate the enhanced ability of the Salmonella Saintpaul outbreak strain to grow in salsa compared with other Salmonella strains. Recipe modifications including but not limited to adding lime juice (at least 10%) and keeping fresh salsa at room temperature for less than 12 h before consumption are strategies that can help mitigate the growth of Salmonella in salsa. ISSN: 0362028X

Dhakal, J., Sharma, C.S., Nannapaneni, R., McDaniel, C.D., Kim, T., Kiess, A.
Effect of chlorine-induced sublethal oxidative stress on the biofilm-forming ability of salmonella at different temperatures, nutrient conditions, and substrates
(2019) Journal of Food Protection, 82 (1), pp. 78-92.
ABSTRACT: The present study was conducted to evaluate the effect of chlorine-induced oxidative stress on biofilm formation by various Salmonella strains on polystyrene and stainless steel (SS) surfaces at three temperatures (30, 25 [room temperature], and 4°C) in tryptic soy broth (TSB) and 1/10 TSB. Fifteen Salmonella strains (six serotypes) were exposed to a sublethal chlorine concentration (150 ppm of total chlorine) in TSB for 2 h at the predetermined temperatures. The biofilm-forming ability of the Salmonella strains was determined in 96-well polystyrene microtiter plates by using a crystal violet staining method and on SS coupons in 24-well tissue culture plates. All tested strains of Salmonella produced biofilms on both surfaces tested at room temperature and at 30°C. Of the 15 strains tested, none (chlorine stressed and nonstressed) formed biofilm at 4°C. At 30°C, Salmonella Heidelberg (ID 72), Salmonella Newport (ID 107), and Salmonella Typhimurium (ATCC 14028) formed more biofilm than did their respective nonstressed controls on polystyrene ($P \leq 0.05$). At room temperature, only stressed Salmonella Reading (ID 115) in 1/10 TSB had significantly more biofilm formation than did the nonstressed control cells ($P \leq 0.05$). Salmonella strains formed more biofilm in nutrient-deficient medium (1/10 TSB) than in full-strength TSB. At 25°C, chlorine-stressed Salmonella Heidelberg (ATCC 8326) and Salmonella Enteritidis (ATCC 4931) formed stronger biofilms on SS coupons ($P \leq 0.05$) than did the nonstressed cells. These findings suggest that certain strains of Salmonella can produce significantly stronger biofilms on plastic and SS upon exposure to sublethal chlorine. ISSN: 0362028X

Santillana Farakos, S.M., Pouillot, R., Davidson, G.R., Johnson, R., Son, I., Anderson, N., VAN DOREN, J.M.
A quantitative risk assessment of human salmonellosis from consumption of walnuts in the United States
(2019) Journal of Food Protection, 82 (1), pp. 45-57.
ABSTRACT: We assessed the risk of human salmonellosis from consumption of shelled walnuts in the United States and the impact of 0-to 5-log reduction treatments for Salmonella during processing. We established a baseline model with Salmonella contamination data from 2010 to 2013 surveys of walnuts from California operations to estimate baseline prevalence and levels of Salmonella during preshelling storage and typical walnut processing stages, considered U.S. consumption data, and applied an adapted dose-response model from the Food and Agriculture Organization and the World Health Organization to evaluate risk of illness per serving and per year. Our baseline model predicted 1 case of salmonellosis per 100 million servings (95% confidence interval [CI], 1 case per 3 million to 1 case per 2 billion servings) of walnuts untreated during processing and uncooked by consumers, resulting in an estimated 6 cases of salmonellosis per year (95% CI,,1 to 278 cases) in the United States. A minimum 3-log reduction treatment for Salmonella during processing of walnuts eaten alone or as an uncooked ingredient resulted in a mean risk of,1 case per year. We modeled the impact on risk per serving of three atypical situations in which the Salmonella levels were increased by 0.5 to 1.5 log CFU per unit pretreatment during processing at the float tank or during preshelling storage or posttreatment during partitioning into consumer packages. No change in risk was associated with the small increase in levels of Salmonella at the float tank, whereas an increase in risk was estimated for each of the other two atypical events. In a fourth scenario, we estimated the risk per serving associated with consumption of walnuts with Salmonella prevalence and levels from a 2014 to 2015 U.S. retail survey. Risk per serving estimates were two orders of magnitude larger than those of the baseline model without treatment. Further research is needed to determine whether this finding reflects variability in Salmonella contamination across the supply or a rare event affecting a portion of the supply. ISSN: 0362028X

Calhoun, S.
Prevalence and concentration of salmonella on raw, shelled peanuts in the United States (Journal of Food Protection) 81(11), 1755–1760, (2018), 10.4315/0362-028X.JFP-18-114) (2019) Journal of Food Protection, 82 (1), p. 6.
ABSTRACT: ''Prevalence and Concentration of Salmonella on Raw, Shelled Peanuts in the United States'' In the article ''Prevalence States'' by Stephen Calhoun, Laurie Post, Benjamin Warren, Sterling Thompson, and Ann Rogers Bontempo, Journal of Food Protection 81 (11):1755–1760, 2018, doi:10.4315/0362-028X.JFP-18-114, Table 2 (p. 1758) was incomplete as published. It should read as follows: (Table presented). ISSN: 0362028X

Collins, J., Simpson, K.M.J., Bell, G., Durrheim, D.N., Hill-Cawthorne, G.A., Hope, K., Howard, P., Kohlenberg, T., Lawrence, K., Lilly, K., Porigneaux, P., Sintchenko, V., Wang, Q., Ward, M.P., Wiethoelter, A., Mor, S.M., Flint, J.
A One Health investigation of Salmonella enterica serovar Wangata in north-eastern New South Wales, Australia, 2016-2017
(2019) Epidemiology and infection, 147, p. e150.
ABSTRACT: Salmonella enterica serovar Wangata (S. Wangata) is an important cause of endemic salmonellosis in Australia, with human infections occurring from undefined sources. This investigation sought to examine possible environmental and zoonotic sources for human infections with S. Wangata in north-eastern New South Wales (NSW), Australia. The investigation adopted a One Health approach and was comprised of three complimentary components: a case-control study examining human risk factors; environmental and animal sampling; and genomic analysis of human, animal and environmental isolates. Forty-eight human S. Wangata cases were interviewed during a 6-month period from November 2016 to April 2017, together with 55 Salmonella Typhimurium (S. Typhimurium) controls and 130 neighbourhood controls. Indirect contact with bats/flying foxes (S. Typhimurium controls (adjusted odds ratio (aOR) 2.63, 95% confidence interval (CI) 1.06-6.48)) (neighbourhood controls (aOR 8.33, 95% CI 2.58-26.83)), wild frogs (aOR 3.65, 95% CI 1.32-10.07) and wild birds (aOR 6.93, 95% CI 2.29-21.00) were statistically associated with illness in multivariable analyses. S. Wangata was detected in dog faeces, wildlife scats and a compost specimen collected from the outdoor environments of cases' residences. In addition, S. Wangata was detected in the faeces of wild birds and sea turtles in the investigation area. Genomic analysis revealed that S. Wangata isolates were relatively clonal. Our findings suggest that S. Wangata is present in the environment and may have a reservoir in wildlife populations in north-eastern NSW. Further investigation is required to better understand the occurrence of Salmonella in wildlife groups and to identify possible transmission pathways for human infections. ISSN: 14694409

Chattaway, M.A., Chandra, N., Painset, A., Shah, V., Lamb, P., Acheampong, E., Lo, J., Patel, B., Larkin, L., Sergeant, M., Cormican, M., Litrup, E., Crook, P.
Genomic approaches used to investigate an atypical outbreak of Salmonella Adjame (2019) Microbial genomics, 5 (1), .
ABSTRACT: In 2017, an outbreak of gastroenteritis in England attributed to Salmonella Adjame was detected and investigated. With the introduction of whole genome sequencing (WGS) for microbial typing, methods for comparing international outbreak data require evaluation. A case was defined as a person resident in England with a clinical sample from 1 June 2017 to 27 July 2017 from whom S. Adjame was isolated. Cases were interviewed and exposures analysed. Backward tracing of food provenance was undertaken. WGS was performed on isolates from cases and historical isolates and compared using Public Health England's SnapperDB high-quality SNP pipeline and Enterobase's Salmonella core genome multi-locus sequence typing (cgMLST) scheme. In total, 14 cases were identified. The majority were vegetarian, probably of South Asian descent, with a median age of 66.5 years with no recent international travel reported. Cases consumed a range of fresh food products including herbs and spices bought from South Asian grocers. Backward tracing did not identify a common source. WGS typing showed sub-clustering and considerable genetic variation across human samples. cgMLST allele-based analysis was comparable to SNP-derived phylogenetic analysis and clusters were defined using each method. Imported herbs or spices were suspected vehicles. The cases were linked in time and place but WGS showed marked heterogeneity, atypical of a point source Salmonella outbreak. The application of incorporating SNP or allelic differences into the case definition may not always be appropriate. With further validation, cgMLST could be used for international outbreak alerts when WGS analysis is being undertaken to facilitate comparison. ISSN: 20575858

Bourassa, D.V., Lapidus, J.L., Kennedy-Smith, A.E., Morey, A.
Efficacy of neutralizing buffered peptone water for recovery of Salmonella, Campylobacter, and Enterobacteriaceae from broiler carcasses at various points along a commercial immersion chilling process with peroxyacetic acid
(2019) Poultry Science, 98 (1), pp. 393-397.
ABSTRACT: In 2016, USDA-Food Safety and Inspection Service began using a neutralizing buffered peptone water (nBPW) to rinse broiler carcasses for Salmonella and Campylobacter performance standard testing. The nBPW contains standard buffered peptone water (BPW) with compounds to neutralize residual antimicrobials that may be transferred from the carcass to the sample rinsate. However, a direct comparison of nBPW and BPW on carcasses commercially treated with antimicrobials has not been conducted. On 3 replicate days in a commercial broiler processing plant, an immersion chilling biomap using whole carcass rinse samples taken prior to any chilling treatment (30), after pre-chill treatment (30), after primary chill (30), after secondary chill (30), after post-chill treatment (50), and after post-chill treatment without the pre-chill treatment (49) were tested. Carcasses were rinsed with either BPW (without neutralizer) or nBPW. Rinsates were sampled for Salmonella and Campylobacter prevalence and both Enterobacteriaceae (EB) prevalence and counts. No significant differences were observed between sampling sites or rinse media for Salmonella due to an overall low prevalence (4 positive/219 samples). Campylobacter prevalence significantly decreased from prior to chilling (93%) to after all chilling steps (47%) as anticipated ($P < 0.0001$); however, overall significantly fewer Campylobacter positive carcasses were detected when nBPW was used (55%) in comparison to BPW (70%, $P = 0.0258$). Both EB prevalence and counts significantly decreased (both $P < 0.0001$) from prior to chilling (100%, 2.35 log10 CFU/mL) through after all chilling steps (52%, 0.47 log10 CFU/mL). The use of nBPW versus BPW did not impact EB prevalence; however, samples rinsed with nBPW had significantly higher overall counts (1.26 vs. 1.00 log10 CFU/mL, $P = 0.0134$). The results from this study indicate that the use of a PAA pre-chill treatment did not significantly impact bacteria recovery following all chilling steps. The use of nBPW was effective in neutralizing residual PAA in carcass rinsates when sampling for EB counts; however, nBPW may lessen the ability to detect Campylobacter in these same samples. ISSN: 00325791

Karacan Sever, N., Akan, M.
Molecular analysis of virulence genes of Salmonella Infantis isolated from chickens and Turkeys
(2019) Microbial Pathogenesis, 126, pp. 199-204.
ABSTRACT: In this study, virulence genes of S. Infantis strains, which are commonly isolated from chickens and turkeys in Turkey, were analyzed, and the virulence genes of S. Infantis and other common serovars aside from S. Infantis were compared. In this study, 200 S. Infantis strains isolated from litter, powder, environmental sources, rodent samples and broiler chicken carcasses from a chicken slaughterhouse obtained from chickens (broiler chickens, breeders, laying hens) and 24 S. Infantis strains isolated and identified from litter, powder, environmental and rodent samples obtained from turkeys were analyzed. A total of 40 strains, comprising 10 strains from each Salmonella serovar (S. Typhimurium, S. Enteritidis, S. Kentucky and S. Hadar) (chicken-origin) were also selected from the collection of strains for comparison with S. Infantis strains. The virulence genes of 264, comprising 224 S. Infantis strains and 40 strains from common serovars other than S. Infantis were analyzed. A conventional polymerase chain reaction (PCR) was used to analyze the 11 genes associated with the virulence (sipA, sipD, sopD, sopB, sopE, sopE2, sitC, ssaR, sifA, spvC and pefA) in these strains. SipA, ssaR and sopE genes were found in the 209 S. Infantis strains (93.3%), sipD in 208 (92.85%), sopB in 207 (92.41%), sitC in 206 (91.96%), sifA in 203 (90.62%), sopD in 198 (88.39%), sopE2 in 166 (74.1%), spvC in 20 (8.92%) and the pefA virulence gene in one strain (0.44%). It was found that 74.55% of S. Infantis strains were distributed in gene patterns 1 and 2. In this study, the sopE2 virulence gene in S. Infantis strains was analyzed for the first time. The involvement of the dominant gene patterns of the S. Infantis strains isolated from broiler chicken and broiler chicken carcasses, and the fact that none of S. Infantis strains belonging to the breeders and laying hens were included in these patterns, indicated that S. Infantis entered the broilers not through the breeders, but through environmental factors. The presence of sipA, sipD, sopD, ssaR, sopB, sopE, sifA and sitC virulence genes in S. Infantis strains were found to be similar, but remarkable differences were found compared to the S. Typhimurium and S. Enteritidis strains in the presence of sopE2, spvC and pefA virulence genes. This study examining the virulence genes of S. Infantis strains provides detailed information aimed at providing an understanding of the pathogenesis and epidemiology of Salmonella in poultry. ISSN: 08824010

Yokoyama, E., Torii, Y., Shigemura, H., Ishige, T., Yanagimoto, K., Uematsu, K., Ando, N., Murakami, S.
Isolation of Salmonella enterica serovar Agona strains and their similarities to strains derived from a clone caused a serovar shift in broilers
(2019) Journal of Infection and Chemotherapy, 25 (1), pp. 71-74.
ABSTRACT: Salmonella enterica serovar Agona strains isolated from human cases were compared to strains that were derived from a clone caused a serovar shift in broilers. Pulsed field gel electrophoresis (PFGE) analysis with XbaI or BlnI digestion showed that three of seven strains from human case strains and most of the 81 strains from broilers were clustered in single complex in a minimum spanning tree (MST) reconstructed from the PFGE data. All the strains from human cases and 22 randomly selected strains from broilers were also analyzed by whole genome sequencing (WGS). Analysis of single nucleotide polymorphism (SNP) in the S. Agona core genes showed that four strains from human cases and all the strains from broilers were clustered in a maximum likelihood phylogenetic tree (ML tree) and an MST. These results indicated that the strains derived from the clone caused the serovar shift had already spread to humans. PFGE analysis with XbaI showed that four strains from broilers did not cluster with the other strains in an MST, though all those strains clustered in an ML tree and an MST reconstructed from SNP data. Moreover, three strains from broilers did not cluster in an MST reconstructed from PFGE with BlnI digestion, though those strains clustered in an ML tree and an MST reconstructed from SNP data. Therefore, it was suggested that S. Agona strains derived from a particular clone could not be traced by PFGE analysis but can be investigated by WGS analysis. ISSN: 1341321X

Springer, H.R., Denagamage, T.N., Fenton, G.D., Haley, B.J., Van Kessel, J.A.S., Hovingh, E.P.
Antimicrobial resistance in fecal escherichia coli and salmonella enterica from dairy calves: A systematic review
(2019) Foodborne Pathogens and Disease, 16 (1), pp. 23-34.
ABSTRACT: The discovery of antibiotics brought with it many advances in the health and well-being of humans and animals; however, in recent years development of antimicrobial resistance (AMR) has increasingly become a concern. Much of the antibiotic use on dairy farms is for disease management in mature cattle, and AMR in fecal organisms is relatively rare in this group. However, young dairy calves often carry high levels of AMR in their fecal Escherichia coli and Salmonella enterica, which could provide a potential reservoir of AMR genes on dairy farms. To develop practical and effective antibiotic stewardship policies for dairy calf rearing, it is vital to have a solid understanding of the current state of knowledge regarding AMR in these animals. A systematic review process was used to summarize the current scientific literature regarding AMR in fecal S. enterica and E. coli and associations between management practices and AMR prevalence in dairy calves in the United States and Canada. Seven online databases were searched for literature published from 1997 to 2018. Multiple studies indicated an association between preweaned calves and increased risk of fecal shedding of resistant bacteria, compared to other animal groups on dairy farms. There also was evidence, although less consistent, of an impact of antibiotic treatment, antibiotic-containing milk replacer feeding, and feeding nonsalable or waste milk (WM) on the presence of AMR bacteria. Overall, the research summarized in this systematic review highlights the need for continued research on the impact of management practices, including antibiotic use, WM feeding, and disease prevention practices in reducing AMR in E. coli and S. enterica in dairy calves. In addition, few data were available on physiological and microbiological factors that may contribute to the high relative populations of resistant bacteria in young calves, suggesting another valuable area of future research. ISSN: 15353141

Gil Molino, M., Risco Pérez, D., Gonçalves Blanco, P., Fernandez Llario, P., Quesada Molina, A., García Sánchez, A., Cuesta Gerveno, J.M., Gómez Gordo, L., Martín Cano, F.E., Pérez Martínez, R., Varela Fernández, E., Rey Pérez, J.
Outbreaks of antimicrobial resistant Salmonella Choleraesuis in wild boars piglets from central-western Spain
(2019) Transboundary and Emerging Diseases, 66 (1), pp. 225-233.
ABSTRACT: Salmonella enterica serovar Choleraesuis is the aetiological agent of swine paratyphoid being a highly invasive zoonotic pathogen. Wild boar natural populations are experiencing a demographical expansion as well as some farms are breeding this species to release for hunting with management sometimes identical to that of domestic pigs, including supplementation, grouping, and antibiotic treatments. This situation increases the chance of contact between wild boars and livestock, and potentially induces stress,

with different sanitary consequences. The present work aims to describe the clinical features of recent outbreaks caused by S. Choleraesuis in wild boar from central-western Spain, as well as the antimicrobial resistance and phylogenetic relationships of isolates involved. 28 strains of S. Choleraesuis were isolated from 28 different wild boars belonging to 10 different game states located in central western Spain and submitted to the Clinical Veterinary Hospital (CVH) of the University of Extremadura. Samples were taken from different organs and cultured according to the ISO 6579:2002 procedure. Suspicious colonies were identified by PCR and antimicrobial resistance was evaluated by disc diffusion susceptibility test and the presence of the main resistance genes as well as 18 plasmid replicons frequently found among the Enterobacteriaceae was verified by PCR. Pulsed field gel electrophoresis was applied to determine the genetic relationship between isolates. The outbreaks under study were characterized by high mortality (35%–84%) and a septicaemic presentation. S. Choleraesuis was isolated from all the wild boars analysed, and 26 of the 28 isolates presented resistance to at least one antibiotic. The predominant resistances found were against sulphonamide, streptomycin, tetracycline, and doxicicline and sul1, strA-strB, and tetA were the most prevalent resistance genes among isolates. 10 strains carried FIIA, FIB+H/1 or FIIA+H/1 plasmids. PFGE classified the isolates into four different profiles, grouped into two clusters. This results show that prevention against S. Choleraesuis must be considered in the sanitary programs of the wild boar breeders.
ISSN: 18651674

Siira, L., Naseer, U., Alfsnes, K., Hermansen, N.O., Lange, H., Brandal, L.T.
Whole genome sequencing of Salmonella Chester reveals geographically distinct clusters, Norway, 2000 to 2016
(2019) Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin, 24 (4), .
ABSTRACT: IntroductionDuring summer 2016, Norway observed an increase in Salmonella enterica subsp. enterica serovar Chester cases among travellers to Greece.AimOur aim was to investigate genetic relatedness of S. Chester for surveillance and outbreak detection by core genome multilocus sequence typing (cgMLST) and compare the results to genome mapping.MethodsWe included S. Chester isolates from 51 cases of salmonellosis between 2000 and 2016. Paired-end sequencing (2 × 250 bp) was performed on Illumina MiSeq. Genetic relatedness by cgMLST for Salmonellaenterica subsp. enterica, including 3,002 genes and seven housekeeping genes, was compared by reference genome mapping with CSI Phylogeny version 1.4 and conventional MLST.ResultsConfirmed travel history was available for 80% of included cases, to Europe (n = 13), Asia (n = 12) and Africa (n = 16). Isolates were distributed into four phylogenetic clusters corresponding to geographical regions. Sequence type (ST) ST411 and a single-locus variant ST5260 (n = 17) were primarily acquired in southern Europe, ST1954 (n = 15) in Africa, ST343 (n = 11) and ST2063 (n = 8) primarily in Asia. Part of the European cluster was further divided into a Greek (n = 10) and a Cypriot (n = 4) cluster. All isolates in the African cluster displayed resistance to ≥ 1 class of antimicrobials, while resistance was rare in the other clusters.ConclusionWhole genome sequencing of S. Chester in Norway showed four geographically distinct clusters, with a possible outbreak occurring during summer 2016 related to Greece. We recommend public health institutes to implement cgMLST-based real-time Salmonellaenterica surveillance for early and accurate detection of future outbreaks and further development of cluster cut-offs. ISSN: 15607917

Santos, S.A.O., Martins, C., Pereira, C., Silvestre, A.J.D., Rocha, S.M.
Current Challenges and Perspectives for the Use of Aqueous Plant Extracts in the Management of Bacterial Infections: The Case-Study of Salmonellaenterica Serovars
(2019) International journal of molecular sciences, 20 (4), .
ABSTRACT: Worldwide, foodborne diseases are a growing public health problem. Among the infectious bacteria, non-typhoidal Salmonella enterica serovars (NTS) are the major cause of hospitalization and death, and the emergence and spread of their antibiotic-resistance is becoming a worldwide health issue. This, coupled with the restrictions of antibiotics use in agriculture and animal production, calls for alternative approaches to solve this problem. Plant-derived aqueous extracts compounds could provide novel straightforward approaches to control pathogenic bacteria. This review discusses the antimicrobial activity of aqueous plant extracts against Salmonella serovars, the possible mechanisms of action involved, which components/structures might be responsible for such activity, and the current challenges for the use of these extracts/components in Salmonella infection management and their application perspectives.
ISSN: 14220067

Di Febo, T., Schirone, M., Visciano, P., Portanti, O., Armillotta, G., Persiani, T., Di Giannatale, E., Tittarelli, M., Luciani, M.
Development of a Capture ELISA for Rapid Detection of Salmonella enterica in Food Samples
(2019) Food Analytical Methods, 12 (2), pp. 322-330.
ABSTRACT: In this study, an immunology-based assay that employed specific monoclonal antibodies binding with somatic or flagella antigens of Salmonella enterica subsp. enterica was performed. As this pathogen is one of the most important bacterial species responsible for foodborne outbreaks, its detection in food by rapid and easy methods is properly suitable. After a first screening by indirect ELISA, three monoclonal antibodies (1B6D9, 1B6C11, 1D12F11) versusS. enterica subsp. enterica serovar Typhimurium ATCC 14028 (whole antigen) and another one (4E6F11) versusS. enterica flagellin were further characterized by immunoblotting and mass spectrometry analysis. Then, a total of 84 food samples (dairy products, meat, pasta and flour, eggs, and animal feed) were analyzed by both the official method ISO 6579:2002 and S. enterica capture ELISA. For the standardization of the last method, the specific monoclonal antibody 4E6F11 was selected. The developed Salmonella capture ELISA showed a significant agreement with the official method (ISO 6579:2002). Relative sensitivity, specificity, and accuracy were 100%, 81.0%, and 90.5%, respectively. Therefore, this assay could represent a valid alternative to conventional methods able to detect this pathogen in food. ISSN: 19369751

Huang, X., Zhou, X., Jia, B., Li, N., Jia, J., He, M., He, Y., Qin, X., Cui, Y., Shi, C., Liu, Y., Shi, X.
Transcriptional Sequencing Uncovers Survival Mechanisms of Salmonella enterica Serovar Enteritidis in Antibacterial Egg White
(2019) mSphere, 4 (1), .
ABSTRACT: The survival mechanism of Salmonella enterica serovar Enteritidis in antibacterial egg white is not fully understood. In our lab, an egg white-resistant strain, S. Enteritidis SJTUF 10978, was identified. Cell envelope damage and osmotic stress response (separation of cell wall and inner membrane as well as cytoplasmic shrinkage) of this strain surviving in egg white were identified through microscopic observation. RNA-Seq analysis of the transcriptome of Salmonella survival in egg white showed that a considerable number of genes involved in DNA damage repair, alkaline pH adaptation, osmotic stress adaptation, envelope damage repair, Salmonella pathogenicity island 2 (SPI-2), iron absorption, and biotin synthesis were significantly upregulated (fold change ≥ 2) in egg white, indicating that these pathways or genes might be critical for bacterial survival. RNA-Seq results were confirmed by qRT-PCR, and the survival analysis of six gene deletion mutants confirmed their importance in the survival of bacteria in egg white. The importance of alkaline pH adaptation and envelope damage repair for Salmonella to survive in egg white were further confirmed by analysis of nhaA, cpxR, waaH, and eco deletion mutants. According to the RNA-Seq results, we propose that alkaline pH adaptation might be the cause of bacterial osmotic stress phenotype and that the synergistic effect between alkaline pH and other inhibitory factors can enhance the bacteriostatic effect of egg white. Moreover, cpxR and sigE were recognized as the central regulators that coordinate bacterial metabolism to adapt to envelope damage and alkaline pH.IMPORTANCESalmonella enterica serovar Enteritidis is a major foodborne pathogen that causes salmonellosis mainly through contaminated chicken eggs or egg products and has been a worldwide public health threat since 1980. Frequent outbreaks of this serotype through eggs correlate significantly with its exceptional survival ability in the antibacterial egg white. Research on the survival mechanism of S. Enteritidis in egg white will help to further understand the complex and highly effective antibacterial mechanisms of egg white and lay the foundation for the development of safe and effective vaccines to prevent egg contamination by this Salmonella serotype. Key pathways and genes that were previously overlooked under bactericidal conditions were characterized as being induced in egg white, and synergistic effects between different antimicrobial factors appear to exist according to the gene expression changes. Our work provides new insights into the survival mechanism of S. Enteritidis in egg white. ISSN: 23795042

Boer, M.D.-D., de Boer, R.F., Lameijer, A., Sterne, E., Skidmore, B., Suijkerbuijk, A.W.M., Heck, M., van der Zanden, A.G.M.
Selenite enrichment broth to improve the sensitivity in molecular diagnostics of Salmonella (2019) Journal of Microbiological Methods, 157, pp. 59-64.
ABSTRACT: Selenite enrichment broth (SEB) is used to optimize the recovery of Salmonella enterica subspecies enterica from stool samples. Compared to a direct culture approach, it enhances culture yield by reducing growth of faecal coliforms and faecal streptococci. Over the course of seven years from 2000 to 2017, 47,235 faecal samples

were tested with a Salmonella PCR. We investigated the added value of using SEB in combination with faeces for DNA extraction, in order to improve the sensitivity of molecular diagnostics for detection of Salmonella. A Salmonella enterica subspecies enterica strain was tested for growth characteristics, with and without incubation in SEB, to determine the impact of Selenite enrichment in the Salmonella PCR. Retrospectively, a total of 102 Salmonella enterica subspecies enterica PCR positive faecal samples were re-analysed. DNA extraction was performed with the EasyMag® and MagNaPure96® system using three different input volumes of faeces and SEB. Prospectively, 114 Salmonella PCR positive faecal samples were retested within 2 days using five different input volumes for DNA extraction. Retrospectively, PCR that used SEB as part of input in the DNA extraction, 7/102 (7%) Salmonella PCR positive samples were additionally detected compared to no use of SEB. Of these, Salmonella enterica subspecies enterica serovariation Thompson, Enteritidis, 9,12:l.v and Senftenberg have been outbreak related in the past. Prospectively results were combined in collaboration with another microbiology laboratory, 15/114 (13.2%) additional specimens were detected with the Salmonella PCR, including processing Selenite enrichment broth. In conclusion, of the total 47,235 feacal samples, with SEB the prevalence of a positive PCR for Salmonella is 2.2%. Of these 2.2% positive Salmonella PCRs, 0.4% was not detected in culture. By using SEB an improved detection of Salmonella diagnostics could be realized and a substantial part of 13,2% additional Salmonella cases could be detected. ISSN: 01677012

González-López, C., Martínez-Peniche, R.A., Iturriaga, M.H., Arvizu-Medrano, S.M.
Attachment and colonization of Salmonella on 'Rayada', 'Golden Delicious', and 'Red Delicious' apples
(2019) Journal of the Science of Food and Agriculture, 99 (3), pp. 1166-1171.
ABSTRACT: BACKGROUND: Fruits and vegetables have been associated with outbreaks of disease in different countries. The apple (Malus domestica Borkh) and its products have been reported as vehicles for illness outbreaks. To create strategies to prevent pathogen survival it is necessary to understand how pathogens persist on fruit. This paper assessed the ability of Salmonella to attach to, and to colonize, the surface of three apple cultivars: 'Rayada', 'Golden Delicious' and 'Red Delicious'. RESULTS: Salmonella was able to colonize and generate biofilms on the surface of apples with a soil suspension as the only source of nutrients. Significant differences in Salmonella attachment were seen among the three cultivars of apple studied. Using SEM, attached cells and the formation of exopolysaccharides and biofilms on the three apple cultivars were demonstrated. In all cultivars, the development of Salmonella was only seen in apples stored at 15 and 22 °C, with average increases in the population of 1.4 and 2.3 Log CFU/apple, respectively. At 5 °C, Salmonella growth was inhibited. CONCLUSION: Salmonella can colonize apple surfaces under environmental conditions (relative humidity, temperature and nutrients) occurring in primary apple production. ISSN: 00225142

Crabb, H.K., Gilkerson, J.R., Browning, G.F.
Does only the age of the hen matter in Salmonella enterica contamination of eggs?
(2019) Food Microbiology, 77, pp. 1-9.
ABSTRACT: Contamination of eggs with Salmonella enterica is a significant risk factor contributing to foodborne disease. Periods of peak egg contamination were identified by conducting longitudinal environmental and egg sampling in 7 layer flocks until they were 50 weeks of age. A total of 714 environmental samples and 8958 eggs were cultured using standard methods for the detection of salmonellae. Pooled egg contamination with Salmonella Typhimurium or Salmonella Infantis was detected at a true prevalence (TP) of 0.002 (95% CI = 0.001, 0.004) or 0.005 (95% CI = 0.004, 0.007), respectively. S. Typhimurium and S. Infantis were detected in individual egg components; in shell rinse at a TP of 0.014 (95% CI = 0.005, 0.038), in shell and membrane at a TP of 0.01 (95% CI = 0.003, 0.032), and in albumen and yolk content at a TP of 0.007 (95% CI = 0.001, 0.027). The concentration of salmonellae in all fractions was <1 CFU/mL. The TP of Salmonella enterica in eggs was highest at the onset of lay. Higher egg prevalence was associated with a lower body weight, higher egg production, higher egg weight and mass than the breed standard for age, and poorer feed conversion efficiency. Flock physiology appears to have an important influence on the detection of eggs contaminated with Salmonella enterica. ISSN: 07400020

Langlais, M., Thibodeau, A., Fravalo, P.
A metagenomic analysis of the pre-enrichment step for the isolation of Salmonella spp. from pig feces
(2019) Journal of Microbiological Methods, 157, pp. 43-46.

ABSTRACT: The bacterial short pre-enrichment culture step is important for the proper detection and isolation of Salmonella spp. from pig feces. Using metagenomics, we showed that pre-enrichment of Salmonella was favored not only by inhibiting the growth of competing bacteria but also by increasing its fitness. ISSN: 01677012

Neri, D., Antoci, S., Iannetti, L., Ciorba, A.B., D'Aurelio, R., Del Matto, I., Di Leonardo, M., Giovannini, A., Prencipe, V.A., Pomilio, F., Santarelli, G.A., Migliorati, G.
EU and US control measures on Listeria monocytogenes and Salmonella spp. in certain ready-to-eat meat products: An equivalence study
(2019) Food Control, 96, pp. 98-103.
ABSTRACT: European Union (EU) and United States (US) regulations implement different food safety standards, particularly relating to the presence of Listeria monocytogenes in certain ready-to-eat (RTE) meat products. Compared to EU, the US adopt different procedures and methods, and establish zero tolerance limits to ensure consumers protection from Listeria monocytogenes in RTE products. A complete equivalence evaluation of the EU and US food inspection and certification systems has not been carried out yet. This study was funded by the Italian Central Competent Authority in the field of food safety (Ministry of Health) and included a total of 164 Italian establishments, 81 authorized for export of pork meat products to US ("US establishments") and 83 only authorized for EU internal trade ("EU establishments"). Establishments were stratified according to production volume, type of RTE products manufactured, presentation and processing, in order to include two homogeneous groups (US and EU). Sampling was carried out by NAS (Carabinieri Corps for the prevention of the adulteration of beverages and foodstuffs) without any given notice. Sampling, detection and identification of Listeria monocytogenes and Salmonella spp. took place according to US Food Safety and Inspection Service (FSIS) directives and procedures. All 1124 samples tested negative for Salmonella spp., while 5 samples tested positive for Listeria monocytogenes. Among the latter, 4 samples were collected in 3 US establishments (US products prevalence 4/556 = 0.72%, 95% CL: 0.29–1.83%; US establishments prevalence 3/81 = 3.70%, 95% CL: 1.34–10.32%) and 1 was collected in a EU establishment (EU products prevalence 1/568 = 0.18%, 95% CL: 0.04–0.98%; EU establishment prevalence 1/83 = 1.20%, 95% CL: 0.29–0.46%). Bilateral Fisher's exact test showed no differences between EU and US products and establishments prevalence (P = 0.213 and P = 0.364, respectively), corroborating the existence of equivalent Listeria monocytogenes and Salmonella spp. contamination in the products under these two control systems. ISSN: 09567135

Pigłowski, M.
Pathogenic and non-pathogenic microorganisms in the rapid alert system for food and feed (2019) International Journal of Environmental Research and Public Health, 16 (3), art. no. 477, .
ABSTRACT: The most frequently notified pathogenic microorganisms in the RASFF in 1980–2017 were Salmonella sp., Listeria, Escherichia and Vibrio, whereas, among the notified non-pathogenic microorganisms were unspecified microorganisms, Enterobacteriaceae, Salmonella sp. and Coliforms. Microorganisms were reported mainly in poultry meat, meat, fish, molluscs, crustaceans, fruits, vegetables, herbs, spices, nuts, milk, cereals (in food) and in feed materials and pet food (in feed). The number of notifications decreased at the turn of 2005 and 2006, but has steadily increased since then. The notification basis were official controls, border controls and company's checks. Products were notified mainly by Italy, France, United Kingdom, Germany and Netherlands. The reported products originated from Brazil, European Union countries and India, Thailand and Vietnam. The notification types were alerts, information and border rejections. The distribution status was often not specified or distribution on the market was possible. The risk decision was usually not made. Products were re-dispatched, import was not authorised or products were withdrawn from the market, destroyed and recalled from the market. Proper cooperation within the framework of the RASFF can contribute to shaping public health law and reducing outbreaks associated with microorganisms. ISSN: 16617827

Sanaa, M., Pouillot, R., Vega, F.G., Strain, E., Van Doren, J.M.
GenomeGraphR: A user-friendly open-source web application for foodborne pathogen whole genome sequencing data integration, analysis, and visualization
(2019) PLoS ONE, 14 (2), art. no. e0213039, .
ABSTRACT: Food safety risk assessments and large-scale epidemiological investigations have the potential to provide better and new types of information when whole genome sequence (WGS) data are effectively integrated. Today, the NCBI Pathogen Detection

database WGS collections have grown significantly through improvements in technology, coordination, and collaboration, such as the GenomeTrakr and PulseNet networks. However, high-quality genomic data is not often coupled with high-quality epidemiological or food chain metadata. We have created a set of tools for cleaning, curation, integration, analysis and visualization of microbial genome sequencing data. It has been tested using Salmonella enterica and Listeria monocytogenes data sets provided by NCBI Pathogen Detection (160,000 sequenced isolates in 2018). GenomeGraphR presents foodborne pathogen WGS data and associated curated metadata in a user-friendly interface that allows a user to query a variety of research questions such as, transmission sources and dynamics, global reach, and persistence of genotypes associated with contamination in the food supply and foodborne illness across time or space. The application is freely available (https://fda-riskmodels.foodrisk.org/ genomegraphr/). This is an open access article, free of all copyright, and may be freely reproduced, distributed, transmitted, modified, built upon, or otherwise used by anyone for any lawful purpose. The work is made available under the Creative Commons CC0 public domain dedication. ISSN: 19326203

Jansen, W., Müller, A., Grabowski, N.T., Kehrenberg, C., Muylkens, B., Al Dahouk, S.
Foodborne diseases do not respect borders: Zoonotic pathogens and antimicrobial resistant bacteria in food products of animal origin illegally imported into the European Union (2019) Veterinary Journal, 244, pp. 75-82.
ABSTRACT: Globalisation, international trade and the ever-growing flow of goods and people enable animal diseases and zoonotic pathogens to travel worldwide. The risk of reintroducing previously eradicated animal diseases into the European Union is omnipresent as considerable amounts of food products of animal origin (POAO) from endemic countries are continuously imported legally and illegally into the EU. Additionally, these products may be potential vectors for emerging foodborne zoonoses, which are of public health concern due to their significant morbidity and mortality rates. This review summarises the legal background of veterinary public health measures and provides a critical overview on recent epidemiological studies, which analysed 1577 illegally imported POAO for major foodborne zoonotic pathogens and antimicrobial resistance in indicator bacteria. The samples rarely exceeded microbiological contamination levels of domestic products for Salmonella, Verotoxin-producing Escherichia coli and thermophilic Campylobacter spp. However, Listeria monocytogenes and Staphylococcus aureus were the most frequently detected pathogens in illegally imported meat and meat products (5% and 4.3%, respectively) and S. aureus in milk and milk products (7.4%). The most likely source of those zoonotic pathogens in illegally imported POAO are cross contamination and improper hygiene measures while handling, processing and storage. Moreover, uncommon and genetically distant variants including antimicrobial resistant foodborne pathogens such as methicillin-resistant S. aureus or extended-spectrum β-lactamase-producing Enterobacteriaceae were discovered. The introduction of POAO poses a largely underestimated threat, both to animal and public health. ISSN: 10900233

Brawand, S.G., Keller, C., Overesch, G.
Outbreak of salmonellosis in cattle caused by the unusual Salmonella serotype Stockholm (2019) Veterinary Record Case Reports, 7 (1), art. no. e000544, .
ABSTRACT: Salmonellosis in livestock is not only a problem for farmers due to economic losses but is also a human health concern because of its zoonotic nature. In Europe, bovine enteric salmonellosis is known to be caused by a limited number of serotypes, that is, Salmonella enterica subspecies enterica (S.) serotypes Typhimurium and Dublin. Here, we describe an outbreak of salmonellosis in a Swiss cattle herd caused by S. Stockholm. To the authors' knowledge, in cattle, this serotype has hitherto only been described once: isolated from beef cattle in a slaughterhouse in India. On the other hand, S. Stockholm has been isolated at least once from the stool of a patient suffering from gastroenteritis (Kantele 2011). This outbreak demonstrates that all known non-typhoidal S enterica subspecies enterica serotypes, despite their rare detection, have to be considered pathogenic and potentially zoonotic agents. ISSN: 20526121

Vinayaka, A.C., Ngo, T.A., Kant, K., Engelsmann, P., Dave, V.P., Shahbazi, M.-A., Wolff, A., Bang, D.D.
Rapid detection of Salmonella enterica in food samples by a novel approach with combination of sample concentration and direct PCR
(2019) Biosensors and Bioelectronics, 129, pp. 224-230.
ABSTRACT: Foodborne salmonellosis remains a major economic burden worldwide and particularly for food industries. The diverse and complexity of food matrices pose great challenges for rapid and ultra-sensitive detection of Salmonella in food samples. In this

study, combination of pathogen pre-concentration with rapid molecular identification is presented to overcome these challenges. This combination enabled effective real-time PCR detection of low levels of Salmonella enterica serovar Typhimurium without culture enrichment. Anti-salmonella antibody, immobilized on protein AG-magnetic beads, could efficiently concentrate Salmonella Typhimurium with a capturing efficiency of 95%. In the direct PCR, a strong linear relationship between bacteria concentration and the number of cycles was observed with a relative PCR efficiency of ~92% resulting in a limit of detection (LoD) of ~2 CFU/mL. Analysis of spiked food samples that include vegetable salad, egg yolk, egg white, whole egg and minced pork meat has validated the precision of the method. A relative accuracy of 98.3% with a sensitivity of 91.6% and specificity of 100% was achieved in the Salmonella spiked food samples. The use of a Phusion hot start DNA polymerase with a high tolerance to possible PCR inhibitors allowed the integration of direct PCR, and thereby reducing the duration of analysis to less than 3 h. The Cohen's kappa index showed excellent agreement (0.88) signifying the capability of this method to overcome the food matrix effects in rapid and ultra-sensitive detection of Salmonella in food. This approach may lay a future platform for the integration into a Lab-on-a-chip system for online monitoring of foodborne pathogens. ISSN: 09565663

Schut, C.H., Farzan, A., Ainslie-Garcia, M.H., Friendship, R.M., Lillie, B.N.
Antibody Responses to Salmonella in Pigs from Weaning Up to Marketing and Presence of Salmonella at Slaughter
(2019) Foodborne Pathogens and Disease, 16 (3), pp. 187-194.
ABSTRACT: Salmonella is estimated to be one of the leading causes of enteric illness worldwide. Human salmonellosis is most frequently related to contaminated food products, particularly those of animal origin, such as pork. Pigs are often asymptomatic carriers of Salmonella, highlighting the importance of identifying high-prevalence farms and effective detection methods. The objectives of this study were to investigate Salmonella antibody responses and their association with on-farm shedding and Salmonella isolation at slaughter. Fourteen groups of pigs from eight farrowing sources were followed from birth to slaughter (totaling 796 pigs). Information about farm management was collected through a questionnaire. Blood and fecal samples were collected four times at different stages of production, and palatine tonsils/submandibular lymph nodes were obtained at slaughter. Sera were tested for Salmonella antibodies by enzyme-linked immunosorbent assay, and fecal/tissue samples were cultured for Salmonella. Data were analyzed using a mixed-effect multivariable modeling method with farm, litter, and pig as random effects. Salmonella seropositivity rates were 20.3%, 5.8%, 15.9%, and 37.3% at weaning, at the end of nursery, at end of grower, and at end of finisher, respectively. Salmonella seropositivity and shedding increased with age ($p < 0.05$), and pigs shedding Salmonella were more likely to test seropositive ($p = 0.02$). Antibody response and shedding on-farm had no significant association with isolation of Salmonella from tissues harvested at slaughter. The variation in Salmonella seropositivity due to farm was 28.9% of total variation. These findings indicate that on-farm intervention may be a more effective approach to control Salmonella and to reduce the presence of Salmonella at slaughter. Additionally, the observation that some pigs in this study were Salmonella-negative throughout production and at slaughter is promising with regard to food safety, and studies are needed to explore the genotypes of those pigs. ISSN: 15353141

Shah, M.K., Bradshaw, R., Nyarko, E., Millner, P.D., Neher, D., Weicht, T., Bergholz, T.M., Sharma, M.
Survival and growth of wild-type and rpoS-deficient salmonella newport strains in soil extracts prepared with heat-treated poultry pellets
(2019) Journal of Food Protection, 82 (3), pp. 501-506.
ABSTRACT: Manure runoff can transfer pathogens to farmlands or to water sources, leading to subsequent contamination of produce. Untreated biological soil amendments, like manure, can be contaminated with foodborne pathogens, such as Salmonella Newport, which may lead to transfer of the pathogen to fruits or vegetables. Studies have reported the occurrence and survival of Salmonella in manure or manure slurries. However, data on the survival and growth of Salmonella Newport is lacking in matrices simulating runoff. We quantified the survival and growth of wild-type (WT) Salmonella Newport and rpoS-deficient (ΔrpoS) strains in sterile and nonsterile soil extracts prepared with (amended) or without (unamended) heat-treated poultry pellets at 258C. Salmonella Newport WT and ΔrpoS populations reached a maximum cell density of 6 to 8 log CFU/mL in 24 to 30 h in amended and unamended soil extracts and remained in stationary phase for up to 4 days. Salmonella Newport in amended soil extracts exhibited a decreased lag phase (λ, 2.87 ± 1.01 h) and greater maximum cell densities (N_{max}, 6.84 ± 1.25 CFU/mL) compared with λ (20.10 ± 9.53 h) and N_{max} (5.22 ± 0.82 CFU/mL) in unamended soil extracts. In

amended soil extract, the ΔrpoS strain had no measurable λ, similar growth rates (μ max) compared with WT, and a lower N max compared with the WT strain. Unamended, nonsterile soil extracts did not support the growth of Salmonella Newport WT and led to a decline in populations for the ΔrpoS strain. Salmonella Newport had lower cell densities in nonsterile soil extracts (5.94 ± 0.95 CFU/mL) than it did in sterile soil extracts (6.66 ± 1.50 CFU/mL), potentially indicating competition for nutrients between indigenous microbes and Salmonella Newport. The most favorable growth conditions were provided by amended sterile and nonsterile soil extracts, followed by sterile, unamended soil extracts for both Salmonella Newport strains. Salmonella Newport may grow to greater densities in amended extracts, providing a route for increased Salmonella levels in the growing environments of produce. ISSN: 0362028X

Jalal Uddin, Md., Jeon, G., Ahn, J.
Variability in the adaptive response of antibiotic-resistant Salmonella typhimurium to environmental stresses
(2019) Microbial Drug Resistance, 25 (2), pp. 182-192.
ABSTRACT: This study was designed to evaluate the resistance phenotype and genotype of wild type (WT)-, cefotaxime (CET)-, and ciprofloxacin (CIP)-induced Salmonella Typhimurium ATCC 19585, CIP-resistant Salmonella Typhimurium ATCC 19585, Salmonella Typhimurium CCARM 8009, and Salmonella Typhimurium KCCM 40253 before and after exposure to pH 4.5, 4% NaCl, and heat at 42C. The susceptibilities of WT Salmonella Typhimurium ATCC 19585 and WT Salmonella Typhimurium KCCM 40253 to all antibiotics tested in this study were decreased after CET and CIP induction with the exception with kanamycin, meropenem, and polymyxin B. The highest β-lactamase activities were 2.8 and 3.3 nmol/(min$mL), respectively, at the WT- and CET-induced Salmonella Typhimurium CCARM 8009. FT-IR spectra were found to be dominant at the region from 1,700 to 1,500 cm -1 corresponding to proteins such as amides I, II, and III. The relative expression levels of efflux pump-related genes (acrA, acrB, and TolC), porin-related gene (ompC), virulence-related gene (stn), adhesion-related gene (fimA), and stress-induced alternative sigma factor (rpoS) varied in the antibiotic resistance and stress exposure. This study provides useful information for understanding the antibiotic resistance profile, physicochemical property, and gene expression pattern in Salmonella Typhimurium in association with the induction of antibiotic resistance and exposure to environmental stresses. ISSN: 10766294

Keefer, A.B., Xiaoli, L., M'ikanatha, N.M., Yao, K., Hoffmann, M., Dudley, E.G.
Retrospective whole-genome sequencing analysis distinguished PFGE and drug-resistance-matched retail meat and clinical Salmonella isolates
(2019) Microbiology (United Kingdom), 165 (3), art. no. 000768, pp. 270-286.
ABSTRACT: Non-typhoidal Salmonella is a leading cause of outbreak and sporadic-associated foodborne illnesses in the United States. These infections have been associated with a range of foods, including retail meats. Traditionally, pulsed-field gel electrophoresis (PFGE) and antibiotic susceptibility testing (AST) have been used to facilitate public health investigations of Salmonella infections. However, whole-genome sequencing (WGS) has emerged as an alternative tool that can be routinely implemented. To assess its potential in enhancing integrated surveillance in Pennsylvania, USA, WGS was used to directly compare the genetic characteristics of 7 retail meat and 43 clinical historic Salmonella isolates, subdivided into 3 subsets based on PFGE and AST results, to retrospectively resolve their genetic relatedness and identify antimicrobial resistance (AMR) determinants. Single nucleotide polymorphism (SNP) analyses revealed that the retail meat isolates within S. Heidelberg, S. Typhimurium var. O5- subset 1 and S. Typhimurium var. O5- subset 2 were separated from each primary PFGE pattern-matched clinical isolate by 6-12, 41-96 and 21-81 SNPs, respectively. Fifteen resistance genes were identified across all isolates, including fosA7, a gene only recently found in a limited number of Salmonella and a ≥95%phenotype to genotype correlation was observed for all tested antimicrobials. Moreover, AMR was primarily plasmid-mediated in S. Heidelberg and S. Typhimurium var. O5- subset 2, whereas AMR was chromosomally carried in S. Typhimurium var. O5- subset 1. Similar plasmids were identified in both the retail meat and clinical isolates. Collectively, these data highlight the utility of WGS in retrospective analyses and enhancing integrated surveillance for Salmonella from multiple sources. ISSN: 13500872

Peeters, L., Mostin, L., Wattiau, P., Boyen, F., Dewulf, J., Maes, D.
Efficacy of Clostridium butyricum as probiotic feed additive against experimental Salmonella Typhimurium infection in pigs
(2019) Livestock Science, 221, pp. 82-85.

ABSTRACT: Salmonella Typhimurium (S. Typhimurium) infections in pigs constitute a risk for human salmonellosis. The use of probiotics may be a promising tool to reduce Salmonella infections in pigs. The present study investigated the efficacy of Clostridium butyricum (C. butyricum), at two different dosages, as probiotic feed additive against S. typhimurium infection in experimentally challenged pigs. After weaning, 35 Salmonella negative pigs were randomly divided into 4 groups; Negative control: no feed additive (n = 5), Positive control: no feed additive (n = 10), CB-H: ±2 × 10 6 CFU C. butyricum/g feed (n = 10), CB-L: ± 5 × 10 5 CFU C. butyricum/g feed (n = 10). Pigs were fed ad libitum with the experimental feed, including the probiotic feed additive according to the group, from arrival (day -7) until euthanasia (day 42). One week after arrival (day 0), pigs in the positive control group, CB-H and CB-L were orally inoculated with 2 × 10 8 CFU/mL nalidixic acid resistant S. typhimurium strain 112910a (1 mL/pig). Fecal excretion, serological response, intestinal carriage and prevalence of S. typhimurium positive ileocecal lymph nodes were evaluated. Under the present conditions, the probiotic feed additive C. butyricum did not significantly reduce fecal excretion, serological response, intestinal carriage and prevalence of S. typhimurium in the ileocecal lymph nodes in experimentally challenged pigs. Further research is needed to investigate the results under field conditions and to detect possible additional effects of the application of the probiotic for a longer time period. ISSN: 18711413

Octavia, S., Zulaina, S., Seet, S.K., Tien, W.S., Thu, M., Ooi, P.L., Cui, L., Lin, R.T.P.
Whole-genome sequencing of the rare Salmonella enterica serovar Anfo isolated from food handlers
(2019) Journal of medical microbiology, 68 (3), pp. 429-431.
ABSTRACT: Field investigations were conducted after a small cluster of food poisoning involving six cases was reported. While no stool samples were available from the cases for microbiological testing, Salmonella species was found to be present in the stools of food handlers with gastroenteritis symptoms. Four Salmonella isolates recovered from the food handlers were retrospectively investigated at the genome level using whole-genome sequencing (WGS). WGS showed that S. Anfo (antigenic formulae 39:y:1,2), a rarely isolated serovar, caused infections in the food handlers. S. Anfo analysed in this study contained virulence factors required for causing disease. They did not contain any antibiotic resistance genes or plasmid. The epidemiologically related isolates differed to each other by a maximum of one single nucleotide polymorphism. WGS was useful in identifying rare Salmonella serovars and it is potentially more cost-effective than traditional serotyping methods. It can also confidently group epidemiologically related isolates belonging to S. Anfo. ISSN: 14735644

Jones, J.L., Wang, L., Ceric, O., Nemser, S.M., Rotstein, D.S., Jurkovic, D.A., Rosa, Y., Byrum, B., Cui, J., Zhang, Y., Brown, C.A., Burnum, A.L., Sanchez, S., Reimschuessel, R.
Whole genome sequencing confirms source of pathogens associated with bacterial foodborne illness in pets fed raw pet food
(2019) Journal of Veterinary Diagnostic Investigation, 31 (2), pp. 235-240.
ABSTRACT: Reports of raw meat pet food containing zoonotic foodborne bacteria, including Salmonella, Escherichia coli, and Listeria monocytogenes, are increasing. Contaminated raw pet food and biological waste from pets consuming those diets may pose a public health risk. The U.S. Food and Drug Administration Veterinary Laboratory Investigation and Response Network conducted 2 case investigations, involving 3 households with animal illnesses, which included medical record review, dietary and environmental exposure interviews, animal sample testing, and whole genome sequencing (WGS) of bacteria isolated from the pets and the raw pet food. For each case investigation, WGS with core genome multi-locus sequence typing analysis showed that the animal clinical isolates were closely related to one or more raw pet food bacterial isolates. WGS and genomic analysis of paired animal clinical and animal food isolates can confirm suspected outbreaks of animal foodborne illness. ISSN: 10406387