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Contents

Editorial Note

Bilthoven, 7 January 2022

Dear colleague,

First of all I would like to **wish you a good, healthy and, if ever possible, a virus-free 2022**! I hope you have had time to relax during the Christmas break and had the change to have a good time with family and friends, despite any limitations due to, as it seems, the never ending COVID-19 pandemic.

In the last quarter of 2021 we organised two Proficiency Tests (PTs):

In September 2021 the **PT on detection of *Salmonella* in samples from the primary production stage (PPS)** was organised. The samples concerned boot socks with chicken faeces. The results of this PT, the interim summary as well as the NRLs' own results, were reported to the participants early December 2021. The interim summary is also available at our website: https://www.eurlsalmonella.eu/documenten/interim-summary-report-eurl-salmonella-pt-pps-2021.

In November the **PT on typing of *Salmonella*** was organised, containing an obligatory part on serotyping of *Salmonella*, and a voluntary part on cluster analysis (using MLVA and/or WGS). The deadline for submission of the serotyping results was shortly before the Christmas break and the deadline for submission of the cluster analysis is by the end of January 2022. Soon we will start the analysis of the results of the serotyping part, after which you will be informed about the results.

Mid-December 2021 we have discussed with the members of ISO/TC34/SC9-WG10, the comments on **draft ISO/CD TS 6579-4 on identification of monophasic *Salmonella* Typhimurium**, as well as the next (draft) version of this ISO document (draft ISO/DTS 6579-4). At the meeting of ISO-WG10 it was agreed to still amend some (small) parts of the ISO document, including better clarification of the scope. The final draft ISO/DTS 6579-4 is planned to become available by the end of January/early February 2022. At the meeting of ISO-WG10, also the set-up and planning of the **Interlaboratory Study (ILS) for determination of the performance characteristics** of the 3 PCR protocols described in this draft ISO/DTS 6579-4 was discussed. For the set-up of this ILS, the information described in EN ISO 16140-6 is followed. This includes that each PCR protocol has to be tested with 16 target strains and 8 non-target strains by at least 10 collaborators. Each participant can choose to analyse one, two or all three PCR protocols with the set of strains, as long as in the end at least 10 valid data sets are available for each PCR protocol. The set-up of the ILS will also be sent for comments to the ISO working group on statistics and validation of microbiological methods, to make sure that the set-up is in line with what has been agreed in ISO/TC34/SC9. In February/March 2022 a call for participants in the ILS will be sent to the members of ISO/TC34/SC9, to the members of ISO-WG10 and to the NRLs-*Salmonella*. The ILS is planned to be organised in the period May-July 2022. As this ILS will take much capacity of the EURL staff, as well as of several NRLs-*Salmonella*, we have decided to move the EURL-*Salmonella* **PT on detection of *Salmonella* in Food** to **September** 2022 (normally organised in March). This September-PT will then become a **combined Primary Production Stage and Food study**.

Currently we are also discussing the organisation of the **EURL-*Salmonella* workshop 2022**. We have chosen the following (tentative) dates for the workshop: 24 and 25 May 2022. Depending on the situation with SARS-CoV-2 we will decide in the coming months whether the workshop will become a physical meeting, an online meeting or a so-called hybrid meeting (a combination of physical and online).

In December 2021, the following EURL-*Salmonella* reports were published:

Jacobs-Reitsma, W.F., Verbruggen, A., Diddens, R.E., van Hoek, A.H.A.M., Mooijman, K.A., 2021. EURL-*Salmonella* Proficiency Test Typing 2019. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM report no.: 2020-0084. https://www.rivm.nl/bibliotheek/rapporten/2020-0084.pdf

Jacobs-Reitsma, W.F., Verbruggen, A., Diddens, R.E., van Hoek, A.H.A.M., Mooijman, K.A., 2021. EURL-*Salmonella* Proficiency Test Typing 2020. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM report no.: 2021-0126. https://www.rivm.nl/bibliotheek/rapporten/2021-0126.pdf

Diddens, R.E. and Mooijman, K.A, 2021. EURL-*Salmonella* Proficiency Test Food 2021. Detection of *Salmonella* in liquid whole egg. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM Report no.: 2021-0128. https://www.rivm.nl/bibliotheek/rapporten/2021-0128.pdf

Best wishes,
Kirsten Mooijman
Coordinator EURL-*Salmonella*

From the Literature

Salmonella-related Literature from Scopus: October – December 2021

Carroll, L.M., Buehler, A.J., Gaballa, A., Siler, J.D., Cummings, K.J., Cheng, R.A., Wiedmann, M.
Monitoring the Microevolution of Salmonella enterica in Healthy Dairy Cattle Populations at the Individual Farm Level Using Whole-Genome Sequencing
(2021) Frontiers in Microbiology, 12, art. no. 763669, .
ABSTRACT: Livestock represent a possible reservoir for facilitating the transmission of the zoonotic foodborne pathogen Salmonella enterica to humans; there is also concern that strains can acquire resistance to antimicrobials in the farm environment. Here, whole-genome sequencing (WGS) was used to characterize Salmonella strains (n = 128) isolated from healthy dairy cattle and their associated environments on 13 New York State farms to assess the diversity and microevolution of this important pathogen at the level of the individual herd. Additionally, the accuracy and concordance of multiple in silico tools are assessed, including: (i) two in silico serotyping tools, (ii) combinations of five antimicrobial resistance (AMR) determinant detection tools and one to five AMR determinant databases, and (iii) one antimicrobial minimum inhibitory concentration (MIC) prediction tool. For the isolates sequenced here, in silico serotyping methods outperformed traditional serotyping and resolved all un-typable and/or ambiguous serotype assignments. Serotypes assigned in silico showed greater congruency with the Salmonella whole-genome phylogeny than traditional serotype assignments, and in silico methods showed high concordance (99% agreement). In silico AMR determinant detection methods additionally showed a high degree of concordance, regardless of the pipeline or database used (≥98% agreement among susceptible/resistant assignments for all pipeline/database combinations). For AMR detection methods that relied exclusively on nucleotide BLAST, accuracy could be maximized by using a range of minimum nucleotide identity and coverage thresholds, with thresholds of 75% nucleotide identity and 50–60% coverage adequate for most pipeline/database combinations. In silico characterization of the microevolution and AMR dynamics of each of six serotype groups (S. Anatum, Cerro, Kentucky, Meleagridis, Newport, Typhimurium/Typhimurium variant Copenhagen) revealed that some lineages were strongly associated with individual farms, while others were distributed across multiple farms. Numerous AMR determinant acquisition and loss events were identified, including the recent acquisition of cephalosporin resistance-conferring blaCMY- and blaCTX–M-type beta-lactamases. The results presented here provide high-resolution insight into the temporal dynamics of AMR Salmonella at the scale of the individual farm and highlight both the strengths and limitations of WGS in tracking zoonotic pathogens and their associated AMR determinants at the livestock-human interface. ISSN: 1664302X

An, B., Zhang, H., Su, X., Guo, Y., Wu, T., Ge, Y., Zhu, F., Cui, L.
Rapid and Sensitive Detection of Salmonella spp. Using CRISPR-Cas13a Combined With Recombinase Polymerase Amplification
(2021) Frontiers in Microbiology, 12, art. no. 732426, .
ABSTRACT: Salmonella spp. is one of the most common foodborne disease-causing pathogens that can cause severe diseases in very low infectious doses. Rapid and sensitive detecting Salmonella spp. is advantageous to the control of its spread. In this study, a conserved short fragment of the Salmonella invA gene was selected and used to design primers and specific crRNA (CRISPR RNA) for establishing a one-tube and two-step reaction system for Salmonella spp. detection, by combining recombinase polymerase amplification (RPA) with CRISPR-Cas13a (Clustered Regularly Interspaced Short Palindromic Repeats associated protein 13a) cleavage. The established one-tube RPA-Cas13a method can complete the detection within 20 min and the two-step RPA-Cas13a method detection time within 45 min. The designed primers were highly specific to Salmonella spp. and had no cross-reaction with the other nine diarrheal bacteria. The one-tube RPA-Cas13a could detect the Salmonella genome with the limit of 102 copies, which was the same as real-time polymerase chain reaction (PCR), but less sensitive than two-step RPA-Cas13a (100 copies). The detection results of one-tube or two-step RPA-Cas13a and real-time PCR were highly consistent in clinical samples. One-tube RPA-Cas13a developed in this study provides a simple, rapid, and specific detection method for Salmonella spp. While two-step assay was more sensitive and suitable for samples at low abundance. ISSN: 1664302X

Castrica, M., Andoni, E., Intraina, I., Curone, G., Copelotti, E., Massacci, F.R., Terio, V., Colombo, S., Balzaretti, C.M.
Prevalence of listeria monocytogenes and salmonella spp. In different ready to eat foods from large retailers and canteens over a 2-year period in northern italy
(2021) International Journal of Environmental Research and Public Health, 18 (20), art. no. 10568, .
ABSTRACT: This study aims to give an overview of the prevalence of Listeria monocytogenes and Salmonella spp. in 9727 samples (2996 for L. monocytogenes and 6731 for Salmonella spp.) from different categories of ready-to-eat (RTE) foods, collected over 2 years from 28 large retailers and 148 canteens in the regions of northern Italy. The RTE samples were classified into two groups according to the preparation methods: (i) multi-ingredient preparations consisting of fully cooked food ready for immediate consumption, or with minimal further handling before consumption (Group A), and (ii) multi-ingredient preparations consisting of cooked and uncooked food, or preparations consisting of only raw ingredients (Group B). L. monocytogenes and Salmonella spp. were investigated in both of these categories. The overall prevalence of L. monocytogenes and Salmonella spp. was 0.13% and 0.07%, respectively. More specifically, L. monocytogenes was found in 0.04% of 2442 analysed RTE food samples belonging to group A and in 0.54% of 554 samples belonging to group B. Furthermore, 0.03% of 5367 RTE food samples from group A and 0.21% of 1364 samples from group B tested positive for Salmonella spp. In conclusion, the results obtained in this study can provide a significant contribution to L. monocytogenes and Salmonella spp. risk analysis in RTE foods.
ISSN: 16617827

Gu, Y., Lü, Z., Cao, C., Sheng, H., Li, W., Cui, S., Li, R., Lü, X., Yang, B.
Cunning plasmid fusion mediates antibiotic resistance genes represented by ESBLs encoding genes transfer in foodborne Salmonella
(2021) International Journal of Food Microbiology, 355, art. no. 109336, .
ABSTRACT: Foodborne disease caused by antibiotic resistant Salmonella is quite difficult to deal with. In order to further explore the antibiotic resistance associated with gene transfer among foodborne Salmonella, several wild-type Salmonella strains were used as donors and recipients, respectively, to investigate how extended spectrum β-lactamases (ESBLs) encoding genes co-transfer with transposable elements to transmit antibiotic resistance. Antibiotic susceptibility was determined by agar dilution method, the transposase encoding gene was detected via PCR combined with DNA sequencing, S1 nuclease and pulsed field gel electrophoresis (S1-PFGE), and southern-blot. Illumina HiSeq 4000 platform and Nanopore MinION long-read sequencing technology were used to determine the antibiotic resistance encoding genes (ARGs) and their surrounding gene environment. The results indicated that the conjugation frequency was from ×10−4 to ×10−5 per recipient cell. A 185,608-bp-long DNA fragment and two short backbone protein encoding regions in pG19 in the donor fused with part genes in pS3 in the recipient during conjugation, the size of this fusion plasmid is as same as that of pG19. Cefoxitin resistance of the transconjugant was mediated by a tnpA21-related blaDHA-1 transfer. Resistance of Salmonella to ceftriaxone, cefoperazone and ceftiofur was mediated by a tnpU1548 related blaTEM-1B and blaCTX-M-3 transfer. The study indicated that transposase synergy and plasmid selective fusion act as important roles for foodborne Salmonella gathering ARGs. The consistent size of the plasmid before and after fusion suggested the invisibility and complexity of bacterial conjugation without DNA sequencing, the fact reminded us that the rampant transmission of antibiotic-resistance encoding genes would pose tremendous threat to food safety. ISSN: 01681605

García-Soto, S., Tomaso, H., Linde, J., Methner, U.
Epidemiological analysis of salmonella enterica subsp. Enterica serovar Dublin in German cattle herds using whole-genome sequencing
(2021) Microbiology Spectrum, 9 (2), art. no. e00332-21, .
ABSTRACT: Salmonella enterica subsp. enterica serovar Dublin is a cattle-adapted serovar that causes enteritis and systemic diseases in animals. In Germany, S. Dublin is not detected or is very rarely detected in some federal states but is endemic in certain regions. Information on detailed genetic characteristics of S. Dublin is not available. An understanding of the paths and spreading of S. Dublin within and between regions and over time is essential to establish effective control strategies. Whole-genome sequencing (WGS) and bioinformatic analysis were used to explore the genetic traits of S. Dublin and to determine their epidemiological context. Seventy-four S. Dublin strains collected in 2005 to 2018 from 10 federal states were studied. The phylogeny was analyzed using core-genome single-nucleotide polymorphisms (cgSNPs) and core-genome multilocus

sequence typing. Genomic clusters at 100 cgSNPs, 40 cgSNPs, and 15 cgSNPs were selected for molecular epidemiology. WGS-based genoserotyping confirmed serotyping. Important specific virulence determinants were detected in all strains, but multidrug resistance in German S. Dublin organisms is uncommon. Use of different thresholds for cgSNP analysis enabled a broad view and also a detailed view of the occurrence of S. Dublin in Germany. Genomic clusters could be allocated nationwide, to a limited number of federal states, or to special regions only. Results indicate both persistence and spread of S. Dublin within and between federal states in short and longer time periods. However, to detect possible routes of infection or persistence of S. Dublin indicated by genomic analysis, information on the management of the cattle farms and contacts with corresponding farms are essential. IMPORTANCE Salmonella enterica subsp. enterica serovar Dublin is a bovine host-adapted serovar that causes up to 50% of all registered outbreaks of salmonellosis in cattle in Germany. S. Dublin is not detected or is only rarely detected in some federal states but has been endemic in certain regions of the country for a long time. Information on genetic traits of the causative strains is essential to determine routes of infection. WGS and bioinformatic analysis should be used to explore the genetic characteristics of S. Dublin. Combining the genomic features of S. Dublin strains with information on the management of the cattle farms concerned should enable the detection of possible routes of infection or persistence of S. Dublin. This approach is regarded as a prerequisite to developing effective intervention strategies. ISSN: 21650497

Foster, N., Tang, Y., Berchieri, A., Geng, S., Jiao, X., Barrow, P.
Revisiting persistent salmonella infection and the carrier state: What do we know?
(2021) Pathogens, 10 (10), art. no. 1299, .
ABSTRACT: One characteristic of the few Salmonella enterica serovars that produce typhoid-like infections is that disease-free persistent infection can occur for months or years in a small number of individuals post-convalescence. The bacteria continue to be shed intermittently which is a key component of the epidemiology of these infections. Persistent chronic infection occurs despite high levels of circulating specific IgG. We have reviewed the information on the basis for persistence in S. Typhi, S. Dublin, S. Gallinarum, S. Pullorum, S. Abortusovis and also S. Typhimurium in mice as a model of persistence. Persistence appears to occur in macrophages in the spleen and liver with shedding either from the gall bladder and gut or the reproductive tract. The involvement of host genetic background in defining persistence is clear from studies with the mouse but less so with human and poultry infections. There is increasing evidence that the organisms (i) modulate the host response away from the typical Th1-type response normally associated with immune clearance of an acute infection to Th2-type or an anti-inflammatory response, and that (ii) the bacteria modulate transformation of macrophage from M1 to M2 type. The bacterial factors involved in this are not yet fully understood. There are early indications that it might be possible to remodulate the response back towards a Th1 response by using cytokine therapy. ISSN: 20760817

Wang, M., Zhang, Y., Tian, F., Liu, X., Du, S., Ren, G.
Overview of rapid detection methods for salmonella in foods: Progress and challenges
(2021) Foods, 10 (10), art. no. 2402, .
ABSTRACT: Salmonella contamination in food production and processing is a serious threat to con-sumer health. More and more rapid detection methods have been proposed to compensate for the inefficiency of traditional bacterial cultures to suppress the high prevalence of Salmonella more effi-ciently. The contamination of Salmonella in foods can be identified by recognition elements and screened using rapid detection methods with different measurable signals (optical, electrical, etc.). Therefore, the different signal transduction mechanisms and Salmonella recognition elements are the key of the sensitivity, accuracy and specificity for the rapid detection methods. In this review, the bioreceptors for Salmonella were firstly summarized and described, then the current promising Salmonella rapid detection methods in foodstuffs with different signal transduction were objectively summarized and evaluated. Moreover, the challenges faced by these methods in practical monitoring and the development prospect were also emphasized to shed light on a new perspective for the Salmonella rapid detection methods applications. ISSN: 23048158

Delannoy, S., Cadel-Six, S., Bonifait, L., Tran, M.-L., Cherchame, E., Baugé, L., Romero, K., Rouxel, S., Thépault, A., Cordevant, C., Chemaly, M., Brisabois, A., Fach, P.
Closed genome sequence of a Salmonella enterica serovar bovismorbificans strain isolated from dried pork sausage associated with an outbreak in France
(2021) Microbiology Resource Announcements, 10 (40), art. no. e00662-21, .

ABSTRACT: We report here the closed genome sequence of one Salmonella enterica subsp. enterica serovar Bovismorbificans strain isolated from dried pork sausage consumed by a patient suffering from salmonellosis. ISSN: 2576098X

Li, I.-C., Wu, R., Hu, C.-W., Wu, K.-M., Chen, Z.-W., Chou, C.-H.
Comparison of conventional molecular and whole-genome sequencing methods for differentiating salmonella enterica serovar schwarzengrund isolates obtained from food and animal sources
(2021) Microorganisms, 9 (10), art. no. 2046, .
ABSTRACT: Over the last decade, Salmonella enterica serovar Schwarzengrund has become more prev-alent in Asia, Europe, and the US with the simultaneous emergence of multidrug-resistant isolates. As these pathogens are responsible for many sporadic illnesses and chronic complications, as well as outbreaks over many countries, improved surveillance is urgently needed. For 20 years, pulsed-field gel electrophoresis (PFGE) has been the gold standard for determining bacterial relatedness by targeting genome-wide restriction enzyme polymorphisms. Despite its utility, recent studies have reported that PFGE results correlate poorly with that of closely related outbreak strains and clonally dominant endemic strains. Due to these concerns, alternative amplification-based molecular methods for bacterial strain typing have been developed, including clustered regular interspaced short palindromic repeats (CRISPR) and multilocus sequence typing (MLST). Furthermore, as the cost of sequencing continues to decrease, whole genome sequencing (WGS) is poised to replace other molecular strain typing methods. In this study, we assessed the discriminatory power of PFGE, CRISPR, MLST, and WGS methods to differentiate between 23 epidemiologically unrelated S. en-terica serovar Schwarzengrund isolates collected over an 18-year period from distinct locations in Taiwan. The discriminatory index (DI) of each method for different isolates was calculated, resulting in values between 0 (not discriminatory) and 1 (highly discriminatory). Our results showed that WGS has the greatest resolution (DI = 0.982) compared to PFGE (DI = 0.938), CRISPR (DI = 0.906), and MLST (DI = 0.463) methods. In conclusion, the WGS typing approach was shown to be the most sensitive for S. enterica serovar Schwarzengrund fingerprinting. ISSN: 20762607

Gargano, V., Gambino, D., Migliore, S., Vitale, M., Sciortino, S., Costa, A., Vicari, D.
Can human handling increase the presence of multidrug resistance (Mdr) in salmonella spp. isolated from food sources?
(2021) Microorganisms, 9 (10), art. no. 2018, .
ABSTRACT: The spread of antibiotic resistance (AR) among zoonotic pathogens is a serious health problem, especially because in the last decade the massive use of antibiotics has favored the emergence of Multidrug Resistance (MDR) strains. Some species of the Salmonella genus are among the major causes of foodborne infections worldwide and could represent reservoirs of AR. For these reasons, the susceptibility to six antibiotic classes of 63 strains isolated from animals and food was determined to assess the presence of MDR strains. In addition, the detection of resistance genes was done for strains that resulted in MDR. A statistically significant difference was found when comparing the presence of Salmonella spp. MDR strains between strains isolated from animals and strains isolated from food. Our data seem to indicate that MDR occurs mostly in Salmonella strains isolated from food. ISSN: 20762607

Stevens, M.P., Kingsley, R.A.
Salmonella pathogenesis and host-adaptation in farmed animals
(2021) Current Opinion in Microbiology, 63, pp. 52-58.
ABSTRACT: Salmonella is an animal and zoonotic pathogen of global importance. Depending on pathogen and host factors, infections can be asymptomatic or involve acute gastroenteritis or invasive disease. Genomic signatures associated with host-range, tissue tropism or differential virulence of Salmonella enterica serovars, and their variants, have emerged. In turn, it is becoming feasible to predict invasive potential, host-adaptation and zoonotic risk of Salmonella from sequence data to improve outbreak investigation, risk assessment and control strategies. Functional annotation of Salmonella genomes has accelerated with the screening of high-density mutant libraries, revealing host-specific, niche-specific and serovar-specific virulence factors. As natural hosts and reservoirs, farmed animals provide powerful insights into host-adaptation and pathogenesis of Salmonella not always evident from surrogate rodent or cell-based models.
ISSN: 13695274

Correia-Gomes, C., Leonard, F., Graham, D.
Description of control programmes for Salmonella in pigs in Europe. Progress to date?

(2021) Journal of Food Safety, 41 (5), art. no. e12916, .
ABSTRACT: Salmonella spp. are one of the main causes of foodborne disease in Europe and have been associated with consumption of pig meat. However, Salmonella monitoring programmes in pigs and pig meat are not harmonized between those European countries where they exist. In general, current control programmes in Europe can be split into those aiming for: (a) elimination of infection and (b) control and reduction. Elimination programmes for Salmonella were introduced by Sweden, Norway, and Finland several decades ago. They have several elements in common including: prevalence at farm level at the start of the programmes was low, the programmes focus on the entire food chain and use bacteriology as the main detection method. If Salmonella contamination is detected stringent measures are applied. These programmes have achieved their target of a very low level of positive carcase swabs. Other European countries (e.g., Denmark, Germany, Netherlands, Belgium, UK, Ireland) have aimed for control and reduction of Salmonella in pigs rather than elimination. The main component that these programmes have in common is that their monitoring system is based on serology and farms are assigned to risk categories based on their serological profile, with control measures being targeted to high seroprevalence farms. The degree of success of each programme has varied but overall, the programmes described here have not achieved a consistent reduction of farm-level prevalence. This review describes these programmes in detail and discusses reasons for the failure to achieve the desired outcome of reducing Salmonella prevalence at farm level. ISSN: 01496085

Possas, A., Posada-Izquierdo, G.D., Zurera, G., Pérez-Rodríguez, F.
Evaluating the fate of Escherichia coli O157:H7 and Salmonella spp. on cucumbers
(2021) Food Microbiology, 99, art. no. 103830, .
ABSTRACT: The occurrence of various foodborne disease outbreaks linked to the consumption of cucumbers worldwide in the last years raised concerns regarding the survival ability of foodborne pathogens on this food matrix. This work aimed at evaluating and quantifying the survival of Escherichia coli O157:H7 and Salmonella spp. on cucumber surfaces. Cucumbers were inoculated with a 5-strain cocktail of each microorganism and kept at 25 °C. The survival ability of two green fluorescent protein (GFP) labelled Salmonella strains inoculated individually on cucumbers was also evaluated. The inoculated areas were swabbed at different time intervals (maximum of 72 h) and cells were enumerated by plate count method (log CFU/cm2). The population of both pathogens decreased significantly on cucumber surfaces over time. E. coli O157:H7 could only be recovered up to 8 h while Salmonella spp. could be detected up to 24 h. The GFP-labelled Salmonella strains showed similar behaviour on cucumbers compared to the evaluated Salmonella cocktail. Survival kinetic parameters were estimated by fitting the Weibull model to the survival data. The data obtained in this study indicate that despite of the rapid decrease on concentrations of both pathogens evaluated on cucumbers surfaces, strategies to avoid their contamination during the supply chain as well as proper cleaning and disinfection protocols must be put forward to mitigate both E. coli O57:H7 and Salmonella on cucumbers and therefore, to decrease the exposure of consumers to microbial hazards and to avoid cross-contamination events during distribution, retail and in domestic environments. ISSN: 07400020

Zaiko, E.V., Bataeva, D.S., Yushina, Y.K., Grudistova, M.A., Velebit, B.
Prevalence, serovar, and antimicrobial resistance of Salmonella isolated from meat and minced meat used for production smoked sausage
(2021) IOP Conference Series: Earth and Environmental Science, 854 (1), art. no. 012108.
ABSTRACT: The objective of this study was to research the prevalence, serovars, and antimicrobial resistance profiles of Salmonella isolated from meat and minced meat used for the production of fermented sausage. A total of 116 samples were tested, and among them, 20 (17.2%) were positive. Salmonella was detected in 3 (10.3%) beef samples, 5 (19.2%) pork samples, and 6 (20.7%) poultry samples. In minced meat, the Salmonella prevalence was 18.8%. Salmonella enterica serovar Agama (5.2%) was the most commonly identified serovar, followed by S. Enteritidis (4.3%), S. Typhimurium (3.4%), S. Infantis (2.6%), and S. Lindenburg (1.7%). Most of the serovars identified in the present study are recognized as frequent causes of human salmonellosis. Thus, the presence of these serovars means foods with these meats are a likely source of human infections. We found the Salmonella isolates exhibited high rates of resistance to antimicrobials tetracycline, ampicillin, streptomycin, and ciprofloxacin. The highest level of resistance was to tetracycline (75%), followed by resistance to ampicillin (50%), streptomycin (30%), ciprofloxacin (20%), gentamicin (20%), and neomycin (10%). The high-level resistance observed for some of the serovars calls for concern. Salmonella with multidrug resistance

in meat used to produce fermented sausages is considered a high additional risk for human health. ISSN: 17551307

Sargeant, J.M., Totton, S.C., Plishka, M., Vriezen, E.R.
Salmonella in Animal Feeds: A Scoping Review
(2021) Frontiers in Veterinary Science, 8, art. no. 727495, .
ABSTRACT: The objective of this study was to describe the volume and nature of published literature on Salmonella in animal feeds using a formal scoping review methodology. A structured search followed by eligibility screening resulted in the identification of 547 relevant studies, encompassing studies conducted in the fields in which animal feeds are grown (15 studies), the manufacturing sector (106), during transportation (11), in the retail sector (15), and on-farm (226), with the sector not described for 204 studies. The most common study purposes were to estimate the prevalence of Salmonella in animal feeds (372 studies) and to identify serovars (195). The serovars that were found in animal feeds included serovars associated with human illness, with animal illness, and with serovars identified in food (livestock and poultry) intended for human consumption. There were 120 intervention studies and 83 studies conducted to evaluate potential risk factors. Within intervention and risk factor studies, there may be sufficient depth to warrant synthesis research in the areas of heat interventions, fermentation and ensiling, organic acids, season, and geographic region. Some deficiencies were identified in the completeness of reporting of key features in the relevant studies. ISSN: 22971769

Roche, S.M., Holbert, S., Le Vern, Y., Rossignol, C., Rossignol, A., Velge, P., Virlogeux-Payant, I.
A large panel of chicken cells are invaded in vivo by Salmonella Typhimurium even when depleted of all known invasion factors
(2021) Open biology, 11 (11), p. 210117.
ABSTRACT: Poultry are the main source of human infection by Salmonella. As infected poultry are asymptomatic, identifying infected poultry farms is difficult, thus controlling animal infections is of primary importance. As cell tropism is known to govern disease, our aim was therefore to identify infected host-cell types in the organs of chicks known to be involved in Salmonella infection and investigate the role of the three known invasion factors in this process (T3SS-1, Rck and PagN). Chicks were inoculated with wild-type or isogenic fluorescent Salmonella Typhimurium mutants via the intracoelomic route. Our results show that liver, spleen, gall bladder and aortic vessels could be foci of infection, and that phagocytic and non-phagocytic cells, including immune, epithelial and endothelial cells, are invaded in vivo in each organ. Moreover, a mutant defective for the T3SS-1, Rck and PagN remained able to colonize organs like the wild-type strain and invaded non-phagocytic cells in each organ studied. As the infection of the gall bladder had not previously been described in chicks, invasion of gall bladder cells was confirmed by immunohistochemistry and infection was shown to last several weeks after inoculation. Altogether, for the first time these findings provide insights into cell tropism of Salmonella in relevant organs involved in Salmonella infection in chicks and also demonstrate that the known invasion factors are not required for entry into these cell types. ISSN: 20462441

Mei, X., Ma, B., Zhai, X., Zhang, A., Lei, C., Zuo, L., Yang, X., Zhou, C., Wang, H.
Florfenicol enhances colonization of a salmonella enterica serovar enteritidis flor mutant with major alterations to the intestinal microbiota and metabolome in neonatal chickens
(2021) Applied and Environmental Microbiology, 87 (24), art. no. e01681-21, .
ABSTRACT: Florfenicol is an important antibiotic commonly used in poultry production to prevent and treat Salmonella infection. However, oral administration of florfenicol may alter the animals' natural microbiota and metabolome, thereby reducing intestinal colonization resistance and increasing susceptibility to Salmonella infection. In this study, we determined the effect of florfenicol (30 mg/kg of body weight) on gut colonization of neonatal chickens challenged with Salmonella enterica subsp. enterica serovar Enteritidis. We then analyzed the microbial community structure and metabolic profiles of cecal contents using microbial 16S amplicon sequencing and liquid chromatography-mass spectrometry (LC-MS) untargeted metabolomics, respectively. We also screened the marker metabolites using a multi-omics technique and assessed the effect of these markers on intestinal colonization by S. Enteritidis. Florfenicol administration significantly increased the loads of S. Enteritidis in cecal contents, spleen, and liver and prolonged the residence of S. Enteritidis. Moreover, florfenicol significantly affected cecal colony structures, with reduced abundances of Lactobacillus and Bacteroidetes and increased levels of Clostridia, Clostridium, and Dorea. The metabolome was greatly influenced by florfenicol administration, and perturbation in metabolic pathways related to linoleic acid metabolism (linoleic acid, conjugated linoleic acid [CLA], 12,13-EpOME, and 12,13-

diHOME) was most prominently detected. We screened CLA and 12,13-diHOME as marker metabolites, which were highly associated with Lactobacillus, Clostridium, and Dorea. Supplementation with CLA maintained intestinal integrity, reduced intestinal inflammation, and accelerated Salmonella clearance from the gut and remission of enteropathy, whereas treatment with 12,13-diHOME promoted intestinal inflammation and disrupted intestinal barrier function to sustain Salmonella infection. Thus, these results highlight that florfenicol alters the intestinal microbiota and metabolism of neonatal chickens and promotes Salmonella infection mainly by affecting linoleic acid metabolism.
ISSN: 00992240

Wang, Z., Zhu, T., Chen, Z., Meng, J., Simpson, D.J., Gänzle, M.G.
Genetic determinants of stress resistance in desiccated Salmonella enterica
(2021) Applied and Environmental Microbiology, 87 (23), art. no. e01683-21, .
ABSTRACT: Enteric pathogens, including Salmonella, are capable of long-term survival after desiccation and resist heat treatments that are lethal to hydrated cells. The mechanisms of dry-heat resistance differ from those of wet-heat resistance. To elucidate the mechanisms of dry-heat resistance in Salmonella, screening of the dry-heat resistance of 108 Salmonella strains, representing 39 serotypes, identified the 22 most resistant and the 8 most sensitive strains for comparative genome analysis. A total of 289 genes of the accessory genome were differently distributed between resistant and sensitive strains. Among these genes, 28 proteins with a putative relationship to stress resistance were selected for to quantify relative gene expression before and after desiccation and expression by solid-state cultures on agar plates relative to cultures growing in liquid culture media. Of these 28 genes, 15 genes were upregulated (P, 0.05) after desiccation or by solid-state cultures on agar plates. These 15 genes were cloned into the low-copy-number vector pRK767 under the control of the lacZ promoter. The expression of 6 of these 15 genes increased (P, 0.05) resistance to dry heat and to treatment with pressure of 500 MPa. Our finding extends the knowledge of mechanisms of stress resistance in desiccated Salmonella to improve control of this bacterium in dry food. IMPORTANCE This study directly targeted an increasing threat to food safety and developed knowledge and targeted strategies that can be used by the food industry to help reduce the risk of foodborne illness in their dry products and thereby reduce the overall burden of foodborne illness. Genomic and physiological analyses have elucidated mechanisms of bacterial resistance to many food preservation technologies, including heat, pressure, disinfection chemicals, and UV light; however, information on bacterial mechanisms of resistance to dry heat is scarce. Mechanisms of tolerance to desiccation likely also contribute to resistance to dry heat, but this assumption has not been verified experimentally. It remains unclear how mechanisms of resistance to wet heat relate to dry-heat resistance. Thus, this study will fill a knowledge gap to improve the safety of dry foods.
ISSN: 00992240

Dégi, J., Imre, K., Herman, V., Bucur, I., Radulov, I., Petrec, O.-C., Cristina, R.T.
Antimicrobial drug-resistant salmonella in urban cats: Is there an actual risk to public health?
(2021) Antibiotics, 10 (11), art. no. 1404, .
ABSTRACT: The present study was undertaken to investigate the presence of Salmonella spp. in the faeces of client-owned cats in urban areas and to evaluate the risk that is posed to public health. Fresh faecal samples were collected directly from the rectums from 53 diarrhoeic and 32 non-diarrhoeic cats. The samples were individually screened for the presence of Salmonella spp. using standard methods and, in the case of positive findings, the resulting typical colonies were then biochemically confirmed using the VITEK® 2 automated system. Subsequently, all of the Salmonella spp. isolates were molecularly tested for the presence of the invA gene. All of the isolates were serotyped using the slide agglutination technique according to the White–Kauffmann–Le Minor scheme. The phenotypic antimicrobial susceptibility profile of the isolated strains was obtained from the VITEK® 2 system using specific cards from the Gram-negative bacteria. A total of 16 of the samples (18.82%) tested positive for Salmonella spp. according to conventional and molecular testing methods. Serotyping of the Salmonella isolates showed the presence of three serotypes, namely S. enteritidis (n = 9; 56.3%), S. typhimurium (n = 4; 25%), and S. kentucky (n = 3; 18.8%). All of the tested strains showed strong resistance towards cefazolin, cefepime, ceftazidime, and ceftriaxone. Additionally, resistance (listed in descending order of strength) was observed to trimethoprim/sulfamethoxazole (11/16; 68.8%), ampicillin (10/16; 62.5%), ampicillin/sulbactam (9/16; 56.3%), gentamicin (9/16; 56.3%), nitrofuran-toin (8/16; 50.0%), and amikacin (5/16; 31.3%). No resistance was expressed against ciprofloxacin, ertapenem, imipenem, levofloxacin, piperacillin/tazobactam, and tobramycin. The results of this study highlight a substantial

public health issue and medical concern, especially in vulnerable people, such as children, the elderly, and immunocompromised individuals. ISSN: 20796382

Brooks, L.A., Bailey, M.A., Krehling, J.T., Chasteen, K.S., Macklin, K.S.
A comparison of colonizing ability between salmonella enteritidis and salmonella heidelberg in broiler chickens challenged through feed administration
(2021) Foodborne Pathogens and Disease, 18 (11), pp. 784-789.
ABSTRACT: With over 1 million estimated cases per year in the United States, foodborne salmonellosis is an important public health issue. Chicken products are frequent sources of foodborne Salmonella infection. These bacteria readily colonize the gastrointestinal tract of broiler chickens, and feed is a known vector. Past research has demonstrated that the survivability of Salmonella in feed is dependent on the serovar and strain. Therefore, the objective of this research was to compare colonization incidence of these two serovars in broiler chicken tissues by administration of feed contaminated with Salmonella enterica serovar Enteritidis (SE) or Salmonella enterica serovar Heidelberg (SH). A comparison was made with equal conditions so that there was no influence of other factors. Birds were inoculated by addition of Salmonella to the feed (1 × 104 colony-forming unit [CFU]/g of feed) at 14 days of age, and the following tissue samples were collected from each bird after grow-out (days 34-41 depending on the trial): abdominal cavity swab, bone marrow swab, cloaca swab, lung swab, breast, bursa and thymus, ceca, crop, kidney, liver and spleen, skin, spinal cord, thigh, and trachea. A higher percentage of birds inoculated with SE were positive in at least one tissue compared with SH (68% and 9%, respectively), and the SE inoculated birds also showed a higher number of positive tissue samples than SH (13.1% and 0.7%, respectively). Recovery of SH was low for all tissue samples. However, recovery of SE was variable between samples, with ceca showing the highest percentage (50%). These results indicate that challenge at day 14 through feed administration results in greater colonization by SE compared with SH, suggesting that monitoring and control methods for Salmonella in feed should focus on SE to have the greatest positive effect. ISSN: 15353141

Kulshreshtha, G., Benavides-Reyes, C., Rodriguez-Navarro, A.B., Diep, T., Hincke, M.T.
Impact of different layer housing systems on eggshell cuticle quality and salmonella adherence in table eggs
(2021) Foods, 10 (11), art. no. 2559, .
ABSTRACT: The bacterial load on the eggshell surface is a key factor in predicting the bacterial penetration and contamination of the egg interior. The eggshell cuticle is the first line of defense against vertical penetration by microbial food-borne pathogens such as Salmonella Enteritidis. Egg producers are increasingly introducing alternative caging systems into their production chain as animal welfare concerns become of greater relevance to today's consumer. Stress that is introduced by hen aggression and modified nesting behavior in furnished cages can alter the physiology of egg formation and affect the cuticle deposition/quality. The goal of this study was to determine the impact of caging systems (conventional, enriched, free-run, and free-range), on eggshell cuticle parameters and the eggshell bacterial load. The cuticle plug thickness and pore length were higher in the free-range eggs as compared to conventional eggs. The eggshells from alternative caging (enriched and free-range) had a higher total cuticle as compared to conventional cages. A reduction in bacterial cell counts was observed on eggshells that were obtained from free-range eggs as compared to the enriched systems. An inverse correlation between the contact angle and Salmonella adherence was observed. These results indicate that the housing systems of layer hens can modify the cuticle quality and thereby impact bacterial adherence and food safety. ISSN: 23048158

Withenshaw, S.M., Cawthraw, S., Gosling, B., Newton, K., Oastler, C.E., Smith, R.P., Davies, R.H.
Risk factor analysis for Salmonella contamination of broiler chicken (Gallus gallus) hatcheries in Great Britain
(2021) Preventive Veterinary Medicine, 196, art. no. 105492, .
ABSTRACT: Salmonellosis is the second most commonly reported zoonosis in the European Union and contaminated meat from broiler chickens (Gallus gallus) is an important source of human infection. In Great Britain (GB), prevalence of Salmonella enterica in broiler flocks is low, having declined considerably since the introduction of the Salmonella National Control Programme in 2010. However, this decreasing trend has stabilised in recent years and serovars with known ability to persistently colonise hatcheries have been isolated from broiler flocks with increasing frequency, indicating that further controls on hatchery contamination are required. The broiler industry in GB has changed dramatically over the

last 15 years, with greater intensification and dominance by a small number of very large companies which rely on relatively few hatcheries. An investigation of risk factors for Salmonella contamination in GB broiler hatcheries was therefore carried out so that relevant up-to-date advice on Salmonella control can be provided. Twenty-two hatcheries, representing most commercial scale GB broiler hatcheries, were visited between 2015 and 2018. Salmonella contamination was comprehensively investigated at each hatchery by collecting between 108 and 421 environmental swab samples per hatchery (6990 samples in total from all hatcheries). An in-depth questionnaire on hatchery operations was completed for each hatchery, and results were incorporated into a risk factor analysis (univariable followed by multivariable mixed effects logistic regression) to identify factors associated with Salmonella occurrence. Overall, 6.0 % (416/6990) of environmental samples were Salmonella-positive and Salmonella was isolated from 17/22 hatcheries. Ten different serovars were isolated, the most common being S. Senftenberg and S. Mbandaka which are known hatchery colonisers. Sixty-four risk factor variables were investigated. Twenty-two of these were initially retained based on univariable analyses ($p \leq 0.25$) and six were ultimately left in the final multivariable model ($p \leq 0.05$). Salmonella detection was positively associated with having ≥30 hatchers in regular use compared to fewer (Odds ratio [OR] 23.7, 95 % confidence interval [CI] 6.7–84.2), storing trays in process rooms (OR 28.8, CI 7.8–106.3), drying set-up trolleys in corridors (OR 15.6, CI 5.9–41.4) and having skips located in enclosed areas (OR 8.99, CI 5.89–41.35). Using a closed waste disposal system was negatively associated with Salmonella detection (OR 0.08, CI 0.04−0.18) and the odds of detecting Salmonella in hatcheries with 31−60 total workers was lower compared to hatcheries with ≤30 staff (OR 0.16, CI 0.06−0.40). Despite the complexities of hatchery enterprises, changes to a relatively small number of features may significantly reduce the occurrence of hatchery contamination. ISSN: 01675877

De Smet, J., Vandeweyer, D., Van Moll, L., Lachi, D., Van Campenhout, L.
Dynamics of Salmonella inoculated during rearing of black soldier fly larvae (Hermetia illucens)
(2021) Food Research International, 149, art. no. 110692, .
ABSTRACT: The black soldier fly is currently the most produced edible insect on industrial scale, with its larval stage being processed into animal feed as the main application. As this insect species enters the feed and food chain, good hygiene and monitoring practices are needed to avoid the entrance of foodborne pathogens via the larvae. However, insufficient data on the risk of such introductions via industrial larvae production are available. To address this gap, a range of rearing trials were conducted in which the substrate, chicken feed, was inoculated with different levels of Salmonella and in which total viable counts and Salmonella counts were determined during the following days. The outgrowth of Salmonella was slower in those experiments with a lower initial contamination level than in experiments with a higher level. No significant reducing effect originating from the larvae on the substrate Salmonella counts was observed, in contrast to previous studies using other substrates. Our study also revealed that airborne transmission of Salmonella is possible under rearing conditions corresponding to those applied at industrial production sites. Based on our results, we recommend insect producers to use substrate ingredients free of Salmonella, and not to count on the antimicrobial activities that BSFL may exert in some situations towards food pathogens. More inoculation studies using other Salmonella serotypes, other zoonotic bacteria, other substrates, larvae of other ages and including variations on rearing protocols are needed in order to obtain a general view on the dynamics of food pathogens in this insect species and to support comprehensive risk assessments. ISSN: 09639969

Gast, R.K., Jones, D.R., Guraya, R., Anderson, K.E., Karcher, D.M.
Research Note: Contamination of eggs by Salmonella Enteritidis and Salmonella Typhimurium in experimentally infected laying hens in indoor cage-free housing
(2021) Poultry Science, 100 (11), art. no. 101438, .
ABSTRACT: Contaminated eggs are a leading source of human Salmonella infections and this problem continues to challenge public health authorities and egg industries around the world. Salmonella invasion of the ovaries and oviducts of infected laying hens can result in bacterial deposition inside the edible portions of developing eggs. The introduction, persistence, and transmission of salmonellae in commercial egg-laying flocks are influenced by flock management practices, but the food safety ramifications of different types of laying hen housing remain unresolved. The present study assessed the frequency of internal contamination of eggs after experimental Salmonella Enteritidis and S. Typhimurium infection of laying hens in indoor cage-free housing. Groups of 72 hens were housed on wood shavings in isolation rooms simulating commercial cage-free barns with community kick-out nest boxes and perches and 1/3 of the hens in each room were orally

inoculated with 8.0 × 107 cfu of 2-strain mixtures of either S. Enteritidis (2 rooms) or S. Typhimurium (2 rooms), and the entire internal contents of all eggs laid 5 to 30 d postinoculation in nest boxes or on the flooring substrate were cultured to detect Salmonella. Contaminated eggs were laid between 8 and 28 d postinoculation. The overall incidence of S. Enteritidis isolation from eggs (3.41%) was significantly (P = 0.0005) greater than S. Typhimurium (1.19%). The contamination frequencies associated with the 2 egg collection locations were not significantly different (P > 0.05). These results demonstrate that oral infection of a relatively small proportion of laying hens in indoor cage-free housing with invasive Salmonella serovars can result in the production of internally contaminated eggs at low frequencies over a period of nearly a month postinoculation. ISSN: 00325791

Chakroun, I., Fedhila, K., Mahdhi, A., Mzoughi, R., Saidane, D., Esteban, M.Á., Bakhrouf, A.
Atypical Salmonella Typhimurium persistence in the pacific oyster, Crassostrea gigas, and its effect on the variation of gene expression involved in the oyster's immune system
(2021) Microbial Pathogenesis, 160, art. no. 105185, .
ABSTRACT: Salmonella is one of the most important pathogens involved in food intoxication outbreaks, and in many cases, the intoxication has been linked to shellfish which is typically consumed raw. While much is understood about the interactions between Salmonella and vertebrates, much less is known about its relationships with invertebrates, which could be an overlooked and important aspect to better understand the Salmonella interaction with its diversified hosts. The aim of this study was to investigate the effect of preadaptation in seawater microcosms during 12 months on Salmonella Typhimurium by determining its survival capacity within this mollusk over a period of 30 days. The results showed that the stressed bacteria are able to survive in this mollusk at a higher concentration even after thirty days of infection compared to bacteria in the normal state. In order to minimize the effect of an experimental device for one month on the survival of Salmonella, we carried out an in vitro study to determine the number of viable Salmonella in the hemocytes of oysters. Interestingly, we evaluated the effect of the antibacterial activity of different extracts of C. gigas using the solvents (Methanol, Ethanol and acetic acid) specifically against stressed and unstressed Salmonella. Furthermore, we compared the expression of three genes in the oyster Cg-big-def1, timp and sod in response to experimental infections of this mollusk with Vibrio splendidus kb133 and S. Typhimurium LT2DT104 in normal and stressed states. These findings are very important to contribute to explaining several questions about the persistence of S. Typhimurium for a long time in C. gigas and the host's immune response to this microorganism which is considered to be non-virulent for molluscs. ISSN: 08824010

Singh, A., Rahman, M.A., Sharma, R., Yemmireddy, V.
Papaya ripeness and post-harvest storage conditions affect growth, survival and death kinetics of Salmonella and spoilage organisms
(2021) Postharvest Biology and Technology, 181, art. no. 111659, .
ABSTRACT: The purpose of this study was to determine the effect of papaya ripeness level (0, 25, 50, 75 and/or 100 %) and post-harvest storage conditions (i.e., Temperature: 4, 12 and 21 °C, and RH: 55 and 90 %) on the survival kinetics of Salmonella spp., and spoilage organisms. In addition, the effect of test conditions on the physico-chemical properties of fresh-cut papayas was also determined. Maradol papayas of different commercial ripeness levels were cut into 3 cm2 cubes either with or without a peel. The samples were spot inoculated with 25 μL of nalidixic acid adopted Salmonella spp (4-strain) to achieve 4–5 log CFU g−1. The inoculated samples and uninoculated controls were stored in an environmental chamber for up to 14 d. Papaya ripeness level in combination with storage temperature and RH have shown to affect Salmonella survival on both fresh-cut papaya and papaya peel. Increasing the fruit ripeness level from 0 to 100 %, storage temperature from 4 to 21 °C and RH from 55 to 90 % increased the log survival. Samples at low ripeness levels showed slower growth of yeast and molds compared to samples at higher ripeness levels. Ripeness levels also showed an effect on the total soluble solids content of fresh-cut papaya during the storage. Based on these findings, papaya ripeness level in combination with post-harvest storage conditions need to be considered to maximize the microbiological safety while maintaining the quality. ISSN: 09255214

Lopes, S.M., Batista, A.C.F., da Silva, D.C., Rodrigues, R.D.Q., Tondo, E.C.
Salmonella survival during soft-cooked eggs processing by steam oven
(2021) LWT, 151, art. no. 112167, .

ABSTRACT: Worldwide, despite concerns about the possibility of Salmonella presence in eggs and the recommendations of regulatory agencies, soft-cooked eggs are served. Studies indicate that eggs cooked at low temperature for long times are able to inactivate Salmonella at safe levels. There are two main low cooking methods used in food services, thermocirculator (tested in a previous study) and steam oven, not yet validated. This study was undertaken to analyze the survival of Salmonella during soft-cooked eggs processing by steam oven. Five strains of Salmonella were inoculated in egg yolks and incubated at 37 °C, for 18 h, reaching 8.1 ± 0.1 log10 CFU/g. Contaminated eggs were processed at 62 °C for 52 min in asteam oven and samples were collected for the purpose of investigating Salmonella survival. Results indicated that the egg's center temperature reached 58.7 ± 0.4 °C after 17 min and no Salmonella was detected. After 52 min of cooking, yolk remained liquid. The results of this study demonstrated that soft-cooked eggs processing by steam oven showed similar results to the processing by thermocirculator and can also be used by chefs and food processors in order to validate another way of serving soft-cooked eggs safely. ISSN: 00236438

Kuus, K., Kramarenko, T., Sõgel, J., Mäesaar, M., Fredriksson-Ahomaa, M., Roasto, M.
Prevalence and serotype diversity of Salmonella enterica in the Estonian meat production chain in 2016–2020
(2021) Pathogens, 10 (12), art. no. 1622, .
ABSTRACT: Background: Salmonella enterica represents a considerable public concern worldwide, with farm animals often recognised as an important reservoir. This study gives an overview of the prevalence and serotype diversity of Salmonella over a 5-year period in the meat production chain in Estonia. Data on human salmonellosis over the same period are provided. Methods: Salmonella surveillance data from 2016 to 2020 were analysed. Results: The prevalence of Salmonella at the farm level was 27.7%, 3.3% and 0.1% for fattening pigs, cattle and poultry, respectively. S. Derby was the most prevalent serotype at the farm level for fattening pigs and S. Dublin for cattle. The top three serotypes isolated at the slaughterhouse and meat cutting levels were S. Derby, monophasic S. Typhimurium and S. Typhimurium with proportions of 64.7%, 9.4% and 7.0%, respectively. These serotypes were the top five most common Salmonella serotypes responsible for human infections in Estonia. S. Enteritidis is the main cause (46.9%) of human salmonellosis cases in Estonia, but in recent years, Enteritidis has not been detected at the slaughterhouse or meat cutting level. Conclusion: In recent years, monophasic S. Typhimurium has become epidemiologically more important in Estonia, with the second-highest cause in human cases and third-highest among the most prevalent serotypes of Salmonella enterica in the meat chain. ISSN: 20760817

Lauer, J.R., Simsek, S., Bergholz, T.M.
Fate of Salmonella and Enterohemorrhagic Escherichia coli on Wheat Grain
(2021) Journal of food protection, 84 (12), pp. 2109-2115.
ABSTRACT: ABSTRACT: Wheat flour has been connected to outbreaks of foodborne illnesses with increased frequency in recent years, specifically, outbreaks involving Salmonella enterica and enterohemorrhagic Escherichia coli (EHEC). However, there is little information regarding the survival of these pathogens on wheat grain during long-term storage in a low-moisture environment. This study aims to evaluate the long-term survival of these enteric pathogens on wheat grain over the course of a year. Hard red spring wheat was inoculated with strains of four serovars of Salmonella (Enteritidis, Agona, Tennessee, and Montevideo) and six serotypes of EHEC (O157:H7, O26:H11, O121:H19, O45:NM, O111:H8, and O103:H2) in triplicate, sealed in Mylar bags to maintain the water activity, and stored at room temperature (22 ± 1°C). The survival of each pathogen was evaluated by plating onto differential media. Viable counts of strains from all four serovars of Salmonella (Enteritidis, Agona, Tennessee, and Montevideo) were detected on wheat grain stored at room temperature (22 ± 1°C) for the duration of the study (52 weeks). Viable counts of strains from EHEC serotypes O45:NM, O111:H8, and O26:H11 were only detected for 44 weeks, and strains from serotypes O157:H7, O121:H19, and O103:H2 were only detected for 40 weeks until they passed below the limit of detection (2.0 log CFU/g). The D-values were found to be significantly different between Salmonella and EHEC (adjusted $P \leq 0.05$) with Salmonella D-values ranging from 22.9 ± 2.2 weeks to 25.2 ± 1.0 weeks and EHEC D-values ranging from 11.4 ± 0.6 weeks to 13.1 ± 1.8 weeks. There were no significant differences among the four Salmonella strains or among the six EHEC strains (adjusted $P > 0.05$). These observations highlight the wide range of survival capabilities of enteric pathogens in a low-moisture environment and confirm these pathogens are a food safety concern when considering the long shelf life of wheat grain and its products. ISSN: 19449097

Hu, J., Che, C., Zuo, J., Niu, X., Wang, Z., Lian, L., Jia, Y., Zhang, H., Zhang, T., Yu, F., Nawaz, S., Han, X.
Effect of Antibiotics on the Colonization of Live Attenuated Salmonella Enteritidis Vaccine in Chickens
(2021) Frontiers in Veterinary Science, 8, art. no. 784160, .
ABSTRACT: Salmonellosis, caused by Salmonella Enteritidis, is a prevalent zoonosis that has serious consequences for human health and the development of the poultry sector. The Salmonella Enteritis live vaccine (Sm24/Rif12/Ssq strain) is used to prevent Salmonella Enteritidis around the world. However, in some parts of the world, poultry flocks are frequently raised under intensive conditions, with significant amounts of antimicrobials to prevent and treat disease and to promote growth. To investigate whether antibiotic use influences the colonization of orally administered Salmonella live vaccines, 240 1-day-old specific pathogen-free chicks were randomly divided into 24 groups of 10 animals for this study. The different groups were treated with different antibiotics, which included ceftiofur, amoxicillin, enrofloxacin, and lincomycin–spectinomycin. Each group was immunized 2, 3, 4, and 5 days after withdrawal, respectively. At 5 days after immunization, the blood, liver, and ceca with contents were collected for the isolation of the Salmonella live vaccine strain. The result showed that no Salmonella vaccine strain was isolated in the blood and liver of the chicks in those groups. The highest number of Salmonella vaccine strains was isolated in the cecum from chicks vaccinated 2 days after ceftiofur withdrawal, and no Salmonella vaccine strain was isolated from the cecum in chicks immunized 3 days after ceftiofur withdrawal. Among the chickens immunized 4 days after the withdrawal of amoxicillin, enrofloxacin, and lincomycin–spectinomycin, the number of Salmonella vaccine colonization in the cecum was the highest, which was higher than that of the chickens immunized at other withdrawal interval (2, 3, and 5 days) groups and was higher than that of the chickens without treatment ($P < 0.05$). This study provides a reference for the effective use of the Salmonella Enteritidis live vaccine and key antibiotics commonly utilized in the poultry industry. ISSN: 22971769

Osaili, T.M., Al-Nabulsi, A.A., Al Sheikh, Y.M., Alaboudi, A.R., Olaimat, A.N., Al-Holy, M., Al-Rousan, W.M., Holley, R.
Inactivation of salmonella spp., escherichia coli o157:H7 and listeria monocytogenes in tahini by microwave heating
(2021) Foods, 10 (12), art. no. 2972, .
ABSTRACT: Tahini (sesame paste) is a traditional food. Numerous foodborne outbreaks have been associated with it. This study aimed to (i) explore the efficiency of 2450 MHz microwave heating at 220, 330, 440, 550, and 660 W on the inactivation of Salmonella spp, Escherichia coli O157:H7, and Listeria monocytogenes in tahini; (ii) determine the impact of desiccation and starvation stresses on pathogen survival; (iii) assess the impact of microwave heating on the physicochemical characteristics of tahini. The inoculated microorganisms in tahini were reduced with higher microwave power levels (p < 0.05) and longer exposure times. The D-values of unstressed Salmonella spp., Escherichia coli O157:H7, and L. monocytogenes ranged from 6.18 to 0.50 min, 6.08 to 0.50 min, and 4.69 to 0.48 min, respectively, at power levels of 220 to 660 W, with z-values of 410, 440, and 460 W, respectively. Generally, desiccation and starvation stress levels prior to heating increased microbial resistance to heat treatment. Microwave heating did not affect acid, peroxide, p-anisidine, or color values of tahini up to 90◦C. These findings reveal microwave heating as a potential method for lowering the risk of Salmonella spp., E. coli O157:H7 and L. monocytogenes in tahini with no compromise on quality.
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Napoleoni, M., Villa, L., Barco, L., Busani, L., Cibin, V., Lucarelli, C., Tiengo, A., Dionisi, A.M., Conti, F., Nunes, F.R.D.S., Tantucci, L., Staffolani, M., Silenzi, V., Fraticelli, R., Morandi, B., Blasi, G., Rocchegiani, E., Fisichella, S., Enter-Net and Enter-Vet Peripheral Laboratories Referents for Marche Region
A strong evidence outbreak of salmonella enteritidis in central italy linked to the consumption of contaminated raw sheep milk cheese
(2021) Microorganisms, 9 (12), art. no. 2464, .
ABSTRACT: Salmonellosis is the second most commonly reported gastrointestinal infection in humans after campylobacteriosis, and an important cause of foodborne outbreaks in the EU/EEA. The vast majority (72.4%) of the salmonellosis foodborne outbreaks reported in EU in 2019 were caused by Salmonella Enteritidis, even if their total number due to this serovar decreased. In spring 2020, a foodborne outbreak of S. Enteritidis occurred in the Marche region (Central Italy), involving 85 people. The common exposure source was a cheese, pecorino “primo sale”, produced with raw sheep milk. The cheese batches were

produced by two local dairies, with a livestock production facility, also including a sheep farm, being part of one dairy. Bacteriological analysis of samples collected allowed the detection of S. Enteritidis in animal faeces, environmental samples, raw-milk bulk tanks and milk taken from single animals. These data confirm that, despite the scarce scientific evidence, S. Enteritidis can infect sheep and be shed into the animals' milk. Hence, this is a real risk for public health when unpasteurized milk is used in production of such cheese. The present paper describes the results of the investigations conducted to clarify this outbreak. ISSN: 20762607

Foddai, A., Nielsen, J., Nielsen, L.R., Rattenborg, E., Murillo, H.E., Ellis-Iversen, J.
Evaluation of risk-based surveillance strategies for Salmonella Dublin in Danish dairy herds by modelling temporal test performance and herd status classification errors
(2021) Microbial Risk Analysis, 19, art. no. 100184, .
ABSTRACT: The potential risk-based improvement of the Salmonella Dublin surveillance programme in Danish dairy herds was investigated, considering herd status misclassifications due to testing errors. The programme started in October 2002. Currently (early 2021) all dairy herds are classified based on quarterly bulk tank milk (BTM) testing with an indirect antibody ELISA (iELISA). Over the last two decades, the prevalence of herds classified as "likely infected" (levels 2,3) reduced remarkably. However, since 2015, the apparent prevalence has increased again, calling for improved surveillance and control to protect animal and human health. A deterministic simulation model based on data (2018–2019) from 2283 dairy herds in level 1 ("most likely free from infection"), was developed to estimate status misclassifications as false negative (FN) and false positive (FP) herds, under two testing strategies. These were: (A) the current system based on quarterly BTM testing only, and (B) an alternative strategy based on additional blood testing of up to eight calves, within herds at high risk of infection (HR). Both strategies were evaluated using three risk classification methods (I to III) and four sensitivity analysis scenarios (SA1-4), where different temporal performances were simulated for the iELISA in BTM. To apply strategy B, the best high-risk classification method (II), which combined managerial applicability and minimized errors, would require testing approximately 1000 calves across 127 HR herds. In that case, strategy A would cause 3 FNs and 67 FPs, by assuming annual BTM sensitivity (BTMSe) 95% conditional on a 1-year disease history and specificity (BTMSp) 97%. Whereas strategy B could cause a similar number of FNs, but 7 FPs more, assuming a sensitivity (Se) of 77% and specificity (Sp) of 99% in individual blood-samples (SA1). Assuming also quarterly BTMSe 53% and BTMSp 99.9% (SA4), strategy A derived 28 FNs and 2 FPs, while strategy B resulted in 6 FNs less and 8 FPs more. Therefore, strategy B could improve early detection of infected HR herds, while strategy A would avoid more unnecessary restrictions in false-positive herds. This improves knowledge on the potential use of additional blood testing in HR herds and illustrates how deterministic modelling can be used to improve disease surveillance and control. ISSN: 23523522

Choong, F.X., Huzell, S., Rosenberg, M., Eckert, J.A., Nagaraj, M., Zhang, T., Melican, K., Otzen, D.E., Richter-Dahlfors, A.
A semi high-throughput method for real-time monitoring of curli producing Salmonella biofilms on air-solid interfaces
(2021) Biofilm, 3, art. no. 100060, .
ABSTRACT: Biofilms enable bacteria to colonize numerous ecological niches. Bacteria within a biofilm are protected by the extracellular matrix (ECM), of which the fibril-forming amyloid protein curli and polysaccharide cellulose are major components in members of Salmonella, Eschericha and Mycobacterium genus. A shortage of real-time detection methods has limited our understanding of how ECM production contributes to biofilm formation and pathogenicity. Here we present optotracing as a new semi-high throughput method for dynamic monitoring of Salmonella biofilm growth on air-solid interfaces. We show how an optotracer with binding-induced fluorescence acts as a dynamic fluorescent reporter of curli expression during biofilm formation on agar. Using spectrophotometry and microscopic imaging of fluorescence, we analyse in real-time the development of the curli architecture in relation to bacterial cells. With exceptional spatial and temporal precision, this revealed a well-structured, non-uniform distribution of curli organised in distally projecting radial channel patterns. Dynamic monitoring of the biofilm also showed defined regions undergoing different growth phases. ECM structures were found to assemble in regions of late exponential growth phase, suggesting that ECM forms on site after bacteria colonize the surface. As the optotracer biofilm method expedites screening of curli production, providing exceptional spatial-temporal understanding of the surface-associated biofilm lifestyle, this method adds a new technique to further our understanding of bacterial biofilms. ISSN: 25902075

Papić, B., Kušar, D., Mićunović, J., Vidrih, Š., Pirš, M., Ocepek, M., Avberšek, J.
Genomic insights into Salmonella Choleraesuis var. Kunzendorf outbreak reveal possible interspecies transmission
(2021) Veterinary Microbiology, 263, art. no. 109282, .
ABSTRACT: Salmonella enterica serovar Choleraesuis is a host-adapted serovar that causes serious infections in domestic pigs and wild boars. Here, we investigated an outbreak of salmonellosis in domestic pigs in Slovenia, 2018–2019. To assess the outbreak, 18 isolates from domestic pigs, wild boars, wild boar meat and a human patient underwent whole-genome sequencing (WGS). All isolates were of sequence type (ST) 145 and harbored no antimicrobial resistance genes or AMR-associated mutations. A single transmission cluster (≤ 6 alleles) of spatially (< 100 km) and temporally linked isolates was observed, comprising isolates of pig (n = 9), wild boar (n = 2) and human (n = 1) origin, and suggesting possible interspecies transmission. In all outbreak-related animal cases, septicemic salmonellosis was observed, accompanied in some cases by enteric symptoms. All pig isolates were linked to a single intensive breeding farm that distributed growers to small family farms. The same transport vehicles were used to distribute growers to family farms and also to transport livestock between neighboring countries. Both isolates that originated from the imported wild boar meat were genetically distant (≥ 122 alleles) from the outbreak cluster. The present results indicate the importance of screening domestic pigs and proper disinfection of transport vehicles to control the spread of S. Choleraesuis. ISSN: 03781135

Pal, A., Riggs, M.R., Urrutia, A., Osborne, R.C., Jackson, A.P., Bailey, M.A., Macklin, K.S., Price, S.B., Buhr, R.J., Bourassa, D.V.
Investigation of the potential of aerosolized Salmonella Enteritidis on colonization and persistence in broilers from day 3 to 21
(2021) Poultry Science, 100 (12), art. no. 101504, .
ABSTRACT: The presence of Salmonella in air of poultry houses has been previously confirmed. Therefore, it is important to investigate the entry of Salmonella into broilers through air. The present study aimed to evaluate different levels of Salmonella Enteritidis aerosol inoculations in broiler chicks for colonization of ceca, trachea, and liver/spleen and persistence over time. In 3 independent trials, 112 one-day-old birds were randomly divided into 4 groups (n = 28/group). On d 1 of age, one group was exposed to an aerosol of sterile saline and the remaining three groups were exposed to an aerosol generated from one of 3 doses (103, 106, or 109 CFU/mL) of S. Enteritidis inoculum. Aerosol exposure time was 30 min/group and was performed using a nebulizer. On d 3, 7, 14, and 21 of age, ceca, trachea, and liver/spleen were aseptically removed. Ceca were cultured for Salmonella counts (log10 CFU/g) and all tissues were cultured for Salmonella prevalence. All tissues from the control group were Salmonella negative for all sampling days. On sampling d 3 and 7, ceca Salmonella counts were highest (5.14 and 5.11, respectively) when challenged with 109 Salmonella (P ≤ 0.0281). Ceca Salmonella counts increased from d 3 (2.43) to d 7 (4.43), then remained constant when challenged at 103 Salmonella, and counts decreased over time for all other groups. Tissue Salmonella prevalence increased with increasing challenge levels at all sampling timepoints (P ≤ 0.0213). Salmonella prevalence was low (0/18 to 4/18) and did not change over time following 103 Salmonella challenge (P ≥ 0.2394). Prevalence decreased over time in ceca and trachea following 106 and 109 Salmonella challenge (P ≤ 0.0483). Liver/spleen Salmonella prevalence increased from d 3 (13/18) to d 14 (18/18) and then decreased at d 21 (10/18) in birds exposed to an aerosol of 109 Salmonella but remained constant over time for rest of the Salmonella inoculated groups. Overall, this study demonstrated the Salmonella colonization and persistence in different tissues in broilers following exposure to aerosolized Salmonella. ISSN: 00325791

Larsen, B.R., Richardson, K.E., Obe, T., Schaeffer, C., Shariat, N.W.
Mixed Salmonella cultures reveal competitive advantages between strains during pre-enrichment and selective enrichment
(2021) Journal of Food Safety, 41 (6), art. no. e12934, .
ABSTRACT: Culture-based Salmonella isolation consists of nonselective pre-enrichment, followed by selective enrichment in Rappaport–Vassiliadis (RV) or tetrathionate (TT) broths, and subsequent plating on selective indicator agar. This study aimed to assess the recovery of two strains belonging to serovars Montevideo (strain ATCC-8387) and Typhimurium (strain ATCC-14028) when grown together in different media. The two strains were co-inoculated 1:1, 10:1, 100:1, and the reciprocal, and CRISPR-SeroSeq was used to assess the relative frequency of both strains after each culture step. Individually, there was no growth difference between both strains in universal pre-enrichment (UP) or

RV broths, though both strains had a higher growth in the former. In TT, both strains had reduced growth, especially ser. Montevideo-8387. When the strains were combined, the growth of ser. Typhimurium-14028 strain was higher in UP and TT broths, while the ser. Montevideo strain was higher in RV broth. The ser. Typhimurium strain also grew better on xylose lysine tergitol-4 (XLT-4) agar. Using media that are commonly used for Salmonella isolation, this work reveals strain-to-strain differences in growth and that in three conditions (UP, RV, XLT-4), these differences were only manifested when the strains were in competition with each other. These findings illustrate the importance of dual selective enrichments to be able to capture all Salmonella present in a sample. ISSN: 01496085

Newton, K., Withenshaw, S.M., Cawthraw, S.A., Davies, R.
In-depth farm investigations and an exploratory risk factor analysis for the presence of Salmonella on broiler farms in Great Britain
(2021) Preventive Veterinary Medicine, 197, art. no. 105498, .
ABSTRACT: Salmonella is a major cause of foodborne illness across Europe but there has been little recent research on its control in broiler production in Great Britain. Investigations of Salmonella presence on 20 broiler farms and a separate exploratory risk factor analysis involving 36 Salmonella-positive farms and 22 Salmonella-negative farms were carried out to investigate Salmonella contamination and control on broiler farms in Great Britain. Sources of Salmonella persistence on farm and potential risk factors for on-farm contamination were identified, enabling provision of up-to-date advice on Salmonella control to farmers. Twenty broiler farms across England and Wales were intensively sampled over time. Most farms were included in the study after routine testing as part of the Salmonella National Control Programmes (NCPs) identified regulated Salmonella serovars or potential associations with outbreak cases of significance for human health. Across all farms and visits, the highest proportion of Salmonella-positive samples were from areas exterior to broiler houses compared to anterooms or house interiors. Exterior Salmonella-positive samples were primarily collected from the immediate areas around the houses, with the highest proportions being from drainage, farm tracks/driveways, and pooled water. Elimination of Salmonella was variable but was most successful inside affected houses (compared to exterior areas) and for regulated Salmonella serovars under the Salmonella NCPs and high priority Salmonella strains with multi-drug resistances. It is likely that the financial and reputational concerns associated with regulated Salmonella serovars and those of greater public health significance underlie the reason that these serovars were more effectively controlled at farm level, as effective elimination of Salmonella can involve a considerable investment in infrastructure, time and resources. Without perceived direct benefits in eliminating non-regulated Salmonella serovars at farm level it can be challenging to maintain the required motivation and investment. A separate farm-level risk factor analysis was carried out using data collected from 58 broiler farms representing six GB broiler companies. Risk of testing positive for Salmonella via NCP sampling in the previous year was greater in the absence of house-specific anterooms and if at least some poultry houses were surrounded by soil/grass compared to if all were surrounded by concrete or a mixture of concrete and stones/gravel. Odds of testing positive for Salmonella in the previous year was also greater for farms whose maximum holding capacity was >100,000 birds, and farms where the usual number of visitors per day was 0−1 compared to 2−3. The analysis was exploratory and caution is required with interpretation, but results provide preliminary insight into aspects of farm management that may be important, practicable targets for Salmonella control on broiler farms in GB. ISSN: 01675877

Khan, S., McWhorter, A.R., Moyle, T.S., Chousalkar, K.K.
Refrigeration of eggs influences the virulence of Salmonella Typhimurium
(2021) Scientific Reports, 11 (1), art. no. 18026, .
ABSTRACT: Salmonella Typhimurium is a human pathogen associated with eggs and egg-derived products. In Australia, it is recommended that eggs should be refrigerated to prevent condensation that can enhance bacterial penetration across the eggshell. Except for the United States, the guidelines on egg refrigeration are not prescriptive. In the current study, in-vitro and in-vivo experiments were conducted to understand the role of egg storage temperatures (refrigerated vs ambient) on bacterial load and the virulence genes expression of Salmonella Typhimurium. The in-vitro egg study showed that the load of Salmonella Typhimurium significantly increased in yolk and albumen stored at 25 °C. The gene expression study showed that ompR, misL, pefA, spvA, shdA, bapA, and csgB were significantly up-regulated in the egg yolk stored at 5 °C and 25 °C for 96 h; however, an in-vivo study revealed that mice infected with egg yolk stored at 25 °C, developed salmonellosis from day 3 post-infection (p.i.). Mice fed with inoculated egg yolk, albumen, or eggshell wash stored at refrigerated temperature did not show signs of salmonellosis

during the period of the experiment. Data obtained in this study highlighted the importance of egg refrigeration in terms of improving product safety. ISSN: 20452322

Obe, T., Berrang, M.E., Cox, N.A., House, S.L., Shariat, N.W.
Comparison of selective enrichment and plating media for Salmonella isolation from broiler carcasses
(2021) Journal of Food Safety, 41 (6), art. no. e12928, .
ABSTRACT: Salmonella detection and isolation rely on different selective enrichment media, which can influence which serovars are detected. The objective of this study was to compare Salmonella recovery from broiler carcass rinses using three different selective enrichment protocols and three differential plating agars. Eight prechill broiler carcasses were collected at a commercial slaughter facility. Each carcass was subjected to whole carcass rinse procedure in buffered peptone water (BPW). An aliquot of the rinse and whole carcasses in the remaining rinse were incubated as a pre-enrichment before subculturing in selective enrichment broths (Rappaport Vassiliadis [RV], Tetrathionate Hajna [TT], and TT to RV in series [TT-to-RV]). Enriched samples were streaked on the three differential agars (Hektoen Enteric [HE], Brilliant Green Sulfa [BGS], and Xylose-Lysine-Tergitol-4 [XLT-4]). Salmonella was isolated from all eight carcasses. Considering all sample preparations as independent subsamples, Salmonella was detected in 88% (128/144) of subsamples with a 100% recovery from the TT-to-RV enrichment, and 92 and 71% from RV and TT broths, individually. A high concordance in recovery on BGS versus XLT-4 agar plates was observed compared to HE versus BGS and HE versus XLT-4 plates. These data suggest that choice of pre-enrichment method, selective enrichment medium, and differential agar can influence the recovery of Salmonella from poultry samples. ISSN: 01496085

Regmi, P., Jones, D.R., Gast, R.K., Guard, J.Y., Karcher, D.M.
Egg carton and eggshell: is there a possibility of Salmonella cross-contamination?
(2021) Journal of Applied Poultry Research, 30 (4), art. no. 100185, .
ABSTRACT: Producers and consumers associated with small scale backyard egg production tend to reuse egg cartons. Egg cartons are also reused for arts and craft projects, gardening, and organization units for small items. The reuse of egg cartons is primarily driven by economic or ecological reasons. The ability of zoonotic bacteria, such as Salmonella Enteritidis (SE), to survive on the eggshell surface and a variety of food packaging materials makes the reuse of egg carton risky. This study was aimed at determining the scope of cross-contamination of SE between eggshell and different egg carton types using 2 experiments. Unwashed eggs from end-of-lay white Leghorn hens were used in the experiments. Two different SE strains were used with 3 independent tubes of inocula from each strain as replicates. In Experiment 1, 216 eggs from each SE strain (72/replicate) were inoculated with 10 µL of SE inoculum (~ 9.95 log cfu/mL), allowed to dry in room temperature, and placed in nonadjacent wells of noninoculated plastic, polystyrene foam, and pulp egg cartons. Egg cartons of each type were then stored either at refrigeration (4°C) or room temperature (25°C). After 24 h eggs were discarded and the carton-wells were swabbed for SE recovery. In Experiment 2, wells of egg cartons were inoculated with SE and uninoculated eggs were placed in them and stored similar to Experiment 1. A total of 216 wells within the egg cartons were inoculated for each SE strain (72/replicate). Eggshell samples were collected for SE recovery. Only 3 samples were detected positive for SE in Experiment 1 and no effect of carton type, SE strain, or incubation temperature was observed. In Experiment 2, 8 eggshell samples were SE positive – 6 from polystyrene foam and 2 from plastic carton. Statistical difference was observed for pulp versus polystyrene foam only (P < 0.05). These results indicate that transfer of SE between egg carton and eggshell surface is possible and that the risk of cross-contamination is associated with type of carton material. ISSN: 10566171

Ingle, D.J., Ambrose, R.L., Baines, S.L., Duchene, S., Gonçalves da Silva, A., Lee, D.Y.J., Jones, M., Valcanis, M., Taiaroa, G., Ballard, S.A., Kirk, M.D., Howden, B.P., Pearson, J.S., Williamson, D.A.
Evolutionary dynamics of multidrug resistant Salmonella enterica serovar 4,[5],12:i:- in Australia
(2021) Nature Communications, 12 (1), art. no. 4786, .
ABSTRACT: Salmonella enterica serovar 4,[5],12:i:- (Salmonella 4,[5],12:i:-) is a monophasic variant of Salmonella Typhimurium that has emerged as a global cause of multidrug resistant salmonellosis. We used Bayesian phylodynamics, genomic epidemiology, and phenotypic characterization to describe the emergence and evolution of Salmonella 4,[5],12:i:- in Australia. We show that the interruption of the genetic region surrounding the phase II flagellin, FljB, causing a monophasic phenotype, represents a

stepwise evolutionary event through the accumulation of mobile resistance elements with minimal impairment to bacterial fitness. We identify three lineages with different population dynamics and discrete antimicrobial resistance profiles emerged, likely reflecting differential antimicrobial selection pressures. Two lineages are associated with travel to South-East Asia and the third lineage is endemic to Australia. Moreover antimicrobial-resistant Salmonella 4,[5],12:i- lineages efficiently infected and survived in host phagocytes and epithelial cells without eliciting significant cellular cytotoxicity, suggesting a suppression of host immune response that may facilitate the persistence of Salmonella 4,[5],12:i:-. ISSN: 20411723

Kim, J.-H., Oh, S.-W.
A colorimetric lateral flow assay based on multiplex PCR for the rapid detection of viable Escherichia coli O157:H7 and Salmonella Typhimurium without enrichment
(2021) LWT, 152, art. no. 112242, .
ABSTRACT: Foodborne pathogens are a food safety problem, and there is demand for rapid and sensitive diagnostic methods. Therefore, multiplex PCR-lateral flow assay (mPCR-LFA) with concentration methods was studied for detecting Escherichia coli O157:H7 and Salmonella Typhimurium. To sensitively detect only viable bacteria in cabbage and reduce time required for results, propidium monoazide was used to selectively inhibit DNA amplification and was subjected to filtration and DNA concentration. This increased the sensitivity by 10−100-fold. E. coli O157:H7 and S. Typhimurium were then simultaneously amplified using mPCR and detected using dual LFA. Our results showed that mPCR-LFA detected 102 CFU/25 g and 101 CFU/25 g of E. coli O157:H7 and S. Typhimurium in 100 min, respectively. This demonstrated that mPCR-LFA and concentration can potentially be performed without cultural enrichment to detect foodborne pathogens. ISSN: 00236438

Rincón-Gamboa, S.M., Poutou-Piñales, R.A., Carrascal-Camacho, A.K.
Analysis of the assessment of antimicrobial susceptibility. Non-typhoid Salmonella in meat and meat products as model (systematic review)
(2021) BMC Microbiology, 21 (1), art. no. 223, .
ABSTRACT: Background: The scientific publications of antimicrobial susceptibilities and resistance must be precise, with interpretations adjusted to the standard. In this frame, knowledge of antimicrobial resistance is fundamental in pathogenic microorganisms such as Salmonella spp., known for many annual deaths worldwide. The objective of this work was to compare the interpretation of standards, the concentrations, and the breakpoints, to study antimicrobial resistance in Non-Typhoidal Salmonella (NTS) isolated from beef, pork, and chicken meat, meat products, and propose additional considerations that improve the use and usefulness of published results. Results: After refining the search based on meeting the inclusion and exclusion criteria, 48 papers were selected. In 33 (68.8%) of them, the disc diffusion method was used, in 11 (22.9%) the MIC determination method, and in 4 (8.33%) were used both. In 24 (50%) of the articles, the selection of a different (correct) standard could have had an impact on the interpretation of antimicrobial susceptibility, which observed when considering three scenarios, i) comparison between the year of the isolation versus the implemented standard, ii) comparison between the year of submission versus implemented standard and iii) comparison between the year of publication versus implemented standard. Conclusions: The most frequent scenario was the inadequate selection of standards, indicating that some studies had not ensured that applied standards kept in line with the date of isolation, date of publication and interpretation of susceptibilities. We proposed 2 years for standards use for resistance and multi-resistance interpretations. On the other hand, we invite researchers to publish their results in the shortest possible time, and editors and reviewers of scientific journals to prioritise these types of studies and verify the correspondence between the standard cited and the one used and the one to be taken into account. ISSN: 14712180

van der Wolf, P., Meijerink, M., Libbrecht, E., Tacken, G., Gijsen, E., Lillie-Jaschniski, K., Schüller, V.
Salmonella Typhimurium environmental reduction in a farrow-to-finish pig herd using a live attenuated Salmonella Typhimurium vaccine
(2021) Porcine Health Management, 7 (1), art. no. 43, .
ABSTRACT: Background: Salmonella Typhimurium is an important zoonotic pathogen in pigs, that can cause clinical disease. Many sow herds and finishing herds are infected with Salmonella, and therefore pose a threat for the contamination of pork and pork products and ultimately consumers. Case presentation: This case study describes a farrow-to-finish pig herd, producing its own replacement gilts, which had experienced clinical outbreaks of salmonellosis since 2002. Outbreaks were characterised by profuse diarrhoea, dead pigs

and high antimicrobial use (colistin sulphate). The aim of this study was to see whether using vaccination of sows and piglets with Salmoporc®, a live attenuated Salmonella Typhimurium vaccine, in combination with standard hygienic precautions, it was possible to reduce Salmonella Typhimurium to below the bacteriological detection limit. Monitoring of the presence of Salmonella was done using a total of 20 pooled faecal, sock and dust samples per herd visit in the period from September 2016 to October 2020. Within the first 10 months after the start of vaccination in August 2016, there was a rapid reduction in clinical symptoms, antimicrobial usage and the number of Salmonella-positive samples. During the winters of 2017/2018 and 2018/2019 the number of positive samples increased again, however with minimal need to use antimicrobials to treat the affected animals. In July 2019, only two samples from a corridor were positive. In September and November 2019 and in October 2020 all three samplings were completely negative for S. Typhimurium. Conclusions: This case, together with other longitudinal studies, can be seen as a proof of the principle that long term vaccination with a live attenuated S. Typhimurium vaccine can reduce the level of S. Typhimurium in the herd environment to very low levels within a farrow-to-finish herd initially suffering from clinical salmonellosis. Also, clinical symptoms indicating salmonellosis were no longer observed and antimicrobials to treat clinically diseased pigs were no longer needed. ISSN: 20555660

Channaiah, L.H., Michael, M., Acuff, J.C., Phebus, R.K., Thippareddi, H., Milliken, G.
Thermal inactivation of Salmonella during hard and soft cookies baking process
(2021) Food Microbiology, 100, art. no. 103874, .
ABSTRACT: This study validated a simulated commercial baking processes for hard and soft cookies to control Salmonella, and determined D- and z-values of 7-serotype Salmonella (Newport, Senftenberg, Tennessee, Typhimurium, and three isolates from dry pet food) cocktail in cookie doughs. Cookie doughs were prepared using flour mist-inoculated with the Salmonella cocktail. Hard and soft cookies were baked at 185 °C for 16 min and 165.6 °C for 22 min, respectively, followed by 30 min of ambient air cooling. D-values of the cocktail in cookie doughs were determined using thermal-death-time disks. Studies were designed as randomized complete blocks with three replications as blocks (α = 0.05). Salmonella populations decreased by > 5 log CFU/g in hard and soft cookies at 11.5 and 20.5 min of baking, respectively. Salmonella was not detected in hard cookies at the end of baking (as determined by enrichment), whereas in soft cookies, 0.6 log CFU/g Salmonella was present at the end of baking and cooling. Salmonella D-values in hard cookie dough at 60, 65 and 70 °C were 59.6, 28.1 and 11.9 min, respectively; while in soft cookie dough they were 62.3, 28.6 and 14.4 min, respectively. The Salmonella z-values in hard and soft cookie doughs were 14.5 and 15.8 °C, respectively. ISSN: 07400020

Xu, H., Zhang, W., Zhang, K., Zhang, Y., Wang, Z., Zhang, W., Li, Y., Li, Q.
Characterization of Salmonella serotypes prevalent in asymptomatic people and patients
(2021) BMC Infectious Diseases, 21 (1), art. no. 632, .
ABSTRACT: Background: Infection with Salmonella enterica usually results in diarrhea, fever, and abdominal cramps, but some people become asymptomatic or chronic carrier as a source of infection for others. This study aimed to analyze the difference in serotype, antimicrobial resistance, and genetic profiles between Salmonella strains isolated from patients and those from asymptomatic people in Nantong city, China. Methods: A total of 88 Salmonella strains were collected from patients and asymptomatic people from 2017 to 2018. Serotyping, antimicrobial susceptibility testing, and PFGE analysis were performed to analyze the characteristics of these strains. Results: Twenty serotypes belonging to 8 serogroups were identified in the 88 Salmonella strains. S. Typhimurium remained to be the predominant serotype in strains from both patients and asymptomatic people. Among the 27 strains from patients, S. Enteritidis and S. Rissen were shown as the other two major serotypes, while S. London, S. Derby, and S. Meleagridis were demonstrated as the other significant serotypes among the 61 strains from asymptomatic people. Antimicrobial resistance testing revealed that 84.1% of strains from both resources were multi-drug resistant. PFGE displayed a highly discriminative ability to differentiate strains belonging to S. Derby, S. Typhimurium, etc., but could not efficiently differentiate serotypes like S. Enteritidis. Conclusions: This study's results demonstrated that S. Typhimurium could cause human infection in both symptomatic and asymptomatic state; S. London, S. Derby, and S. Meleagridis usually cause asymptomatic infection, while S. Enteritidis infection mainly results in human diseases. The high multi-drug resistance rate detected in the antimicrobial resistance and diverse PFGE profiles of these strains implied that the strains were isolated from different sources, and the increased surveillance of Salmonella from both patients and asymptomatic people should be taken to control the disease.
ISSN: 14712334

Babu, U.S., Harrison, L.M., Patel, I.R., Mammel, M.K., Bigley, E., III, Balan, K.V.
Development and validation of an improved method for the detection of Salmonella in cinnamon bark and oregano leaves using the adsorbent beta zeolite in the pre-enrichment media
(2021) Food Microbiology, 100, art. no. 103852, .
ABSTRACT: The detection of Salmonella in spices is challenging due to the presence of antibacterial components. In this study, we evaluated the use of an adsorbent beta zeolite in pre-enrichment media to improve the recovery of Salmonella from cinnamon bark and oregano leaves. Samples (25 g) were spiked with varying levels of S. Montevideo or S. Senftenberg. After 2 weeks of stabilization at RT, betazeolite was added to cinnamon and oregano samples prior to the addition of 225 mL or 475 mL of pre-enrichment media, respectively. Detection sensitivity and rate of the test method were compared to the FDA Bacteriological Analytical Manual (BAM) method which requires the use of 2.5 L pre-enrichment broth. While Salmonella could not be detected in the test method using the reduced volume of pre-enrichment media alone, the addition of beta zeolite resulted in a positivity rate of 62% and 72.6% for cinnamon bark and oregano leaves respectively (all spike levels and both serovars combined). Furthermore, while there were differences in the LOD50 compared to the BAM method, there was no significant difference in the minimum level of detection between the betazeolite and the BAM methods. Our results demonstrate that the use of betazeolite in the pre-enrichment media offers a method with reduced media volumes without compromising on the sensitivity or efficiency of Salmonella detection in cinnamon bark and oregano leaves. ISSN: 07400020

Vázquez, X., García, P., García, V., de Toro, M., Ladero, V., Heinisch, J.J., Fernández, J., Rodicio, R., Rodicio, M.R.
Genomic analysis and phylogenetic position of the complex IncC plasmid found in the Spanish monophasic clone of Salmonella enterica serovar Typhimurium
(2021) Scientific Reports, 11 (1), art. no. 11482, .
ABSTRACT: pUO-STmRV1 is an IncC plasmid discovered in the Spanish clone of the emergent monophasic variant of Salmonella enterica serovar Typhimurium, which has probably contributed to its epidemiological success. The sequence of the entire plasmid determined herein revealed a largely degenerated backbone with accessory DNA incorporated at four different locations. The acquired DNA constitutes more than two-thirds of the pUO-STmRV1 genome and originates from plasmids of different incompatibility groups, including IncF (such as R100 and pSLT, the virulence plasmid specific of S. Typhimurium), IncN and IncI, from the integrative element GIsul2, or from yet unknown sources. In addition to pSLT virulence genes, the plasmid carries genes conferring resistance to widely-used antibiotics and heavy metals, together with a wealth of genetic elements involved in DNA mobility. The latter comprise class 1 integrons, transposons, pseudo-transposons, and insertion sequences, strikingly with 14 copies of IS26, which could have played a crucial role in the assembly of the complex plasmid. Typing of pUO-STmRV1 revealed backbone features characteristically associated with type 1 and type 2 IncC plasmids and could therefore be regarded as a hybrid plasmid. However, a rooted phylogenetic tree based on core genes indicates that it rather belongs to an ancient lineage which diverged at an early stage from the branch leading to most extant IncC plasmids detected so far. pUO-STmRV1 may have evolved at a time when uncontrolled use of antibiotics and biocides favored the accumulation of multiple resistance genes within an IncC backbone. The resulting plasmid thus allowed the Spanish clone to withstand a wide variety of adverse conditions, while simultaneously promoting its own propagation through vertical transmission. ISSN: 20452322

D'Incau, M., Salogni, C., Giovannini, S., Ruggeri, J., Scali, F., Tonni, M., Formenti, N., Guarneri, F., Pasquali, P., Alborali, G.L.
Occurrence of Salmonella Typhimurium and its monophasic variant (4, [5],12:i:-) in healthy and clinically ill pigs in northern Italy
(2021) Porcine Health Management, 7 (1), art. no. 34, .
ABSTRACT: Background: The serovar Typhimurium (4, [5],12:i:1,2), is the most frequently isolated serovar in case of salmonellosis in pigs in Europe and its monophasic variant (4, [5],12:i:-) has been increasingly responsible for Salmonella outbreaks in humans. A total of 25,215 samples were collected, during the years 2002–2017, from 1359 pig farms located in Northern Italy. Samples were collected from different material sources including fecal samples, rectal swabs, gut content and different organs. Results: Salmonella was isolated in 15.80% of samples and, among the isolates, 733 were typed as Salmonella Typhimurium (ST) or its monophasic variant (MST). Over time, there was an increase of isolation of MST which outnumbered ST. Most of the strains were isolated in animals during the weaning stage and the growing – fattening period whereas the clinical

cases were mainly present in young pigs after weaning. Conclusions: This study confirms the presence of ST and MST in pig farms although, considering the total of isolated serotypes, with lower percentages than previously reported. In the last few years, ST has increasingly been replaced by MST suggesting that MST has a competitive advantage over ST, probably due to its different antigenicity and pathogenicity which renders the infection stealthier to recognize and control. ISSN: 20555660

Baert, L., Gimonet, J., Barretto, C., Fournier, C., Jagadeesan, B.
Genetic changes are introduced by repeated exposure of Salmonella spiked in low water activity and high fat matrix to heat
(2021) Scientific Reports, 11 (1), art. no. 8144, .
ABSTRACT: WGS is used to define if isolates are “in” or “out” of an outbreak and/or microbial root cause investigation. No threshold of genetic differences is fixed and the conclusions on similarity between isolates are mainly based on the knowledge generated from previous outbreak investigations and reported mutation rates. Mutation rates in Salmonella when exposed to food processing conditions are lacking. Thus, in this study, the ability of heat and dry stress to cause genetic changes in two Salmonella serotypes frequently isolated from low moisture foods was investigated. S. enterica serovars S. Agona ATCC 51,957 and S. Mbandaka NCTC 7892 (ATCC 51,958) were repeatedly exposed to heat (90 °C for 5 min) in a low water activity and high fat matrix. No increased fitness of the strains was observed after 10 repeated heat treatments. However, genetic changes were introduced and the number of genetic differences increased with every heat treatment cycle. The genetic changes appeared randomly in the genome and were responsible for a population of diverse isolates with 0 to 28 allelic differences (0 to 38 SNPs) between them. This knowledge is key to interpret WGS results for source tracking investigations as part of a root cause analysis in a contamination event as isolates are exposed to stress conditions. ISSN: 20452322

Chattaway, M.A., Langridge, G.C., Wain, J.
Salmonella nomenclature in the genomic era: a time for change
(2021) Scientific Reports, 11 (1), art. no. 7494, .
ABSTRACT: Salmonella enterica nomenclature has evolved over the past one hundred years into a highly sophisticated naming convention based on the recognition of antigens by specific antibodies. This serotyping scheme has led to the definition of over 2500 serovars which are well understood, have standing in nomenclature and, for the majority, biological relevance. Therefore, it is highly desirable for any change in naming convention to maintain backwards compatibility with the information linked to these serovars. The routine use of whole genome sequencing and the well-established link between sequence types and serovars presents an opportunity to update the scheme by incorporating the phylogenetically relevant sequence data whilst preserving the best of serotyping nomenclature. Advantages include: overcoming the variability in antibody preparations; removing the need to use laboratory animals and implementing a truly universal system. However, the issue of trying to reproduce the phenotyping gold standard needs to be relaxed if we are to fully embrace the genomic era. We have used whole genome sequence data from over 46,000 isolates of Salmonella enterica subspecies enterica to define clusters in two stages: Multi Locus Sequence Typing followed by antigen prediction. Sequence type—serotype discrepancies were resolved using core SNP clustering to determine the phylogenetic groups and this was confirmed by overlaying the antigenic prediction onto the core SNP clusters and testing the separation of clusters using cgMLST Hierarchical Clustering. This allowed us to define any major antigenic clusters within an ST—here called the MAC type and written as ST-serovar. Using this method, 99.96% of Salmonella isolates reported in the UK were assigned a MAC type and linked to a serovar name taken from the Kauffmann and White scheme. We propose a change for reporting of Salmonella enterica sub-types using the ST followed by serovar. ISSN: 20452322

Scharling, F.S., Bertelsen, M.F., Sós, E., Bojesen, A.M.
Prevalence of salmonella species, clostridium perfringens, and clostridium difficile in the feces of healthy elephants (loxodonta species and elephas maximus) in Europe
(2021) Journal of Zoo and Wildlife Medicine, 51 (4), pp. 752-888.
ABSTRACT: Pathogenic Salmonella spp., Clostridium perfringens, and Clostridium difficile have been reported to infect and cause severe enteritis and enterotoxemia in African (Loxodonta spp.) and Asian elephants (Elephas maximus). However, little information exists on whether healthy elephants carry and possibly shed these gastrointestinal organisms. This study was conducted to investigate the prevalence of all three bacteria in feces from healthy elephants in European zoos. Bacterial identification was performed by selective culture on fecal samples and a polymerase chain reaction (PCR) amplification

protocol, on the basis of primers targeting the hilA gene (Salmonella spp.), the cpa gene (C. perfringens), and the tpi gene (C. difficile) from deoxyribonucleic acid extracted from elephant feces. The PCR protocol was validated prior to initiation of the investigation. Fecal samples collected from 50 African and 86 Asian elephants originating from 30 European zoologic institutions were investigated. The PCR validation revealed detection limits ranging from 104 to 106 colony-forming units per gram of feces of each gene. Only C. perfringens (one type A and two type E) was detected in the initial sampling (2.2%, three Asian elephants), whereas no Salmonella spp. or C. difficile was detected. At a follow-up sampling from C. perfringens-positive animals and relatives, 2 mo after the initial sampling, three animals were culture positive for Salmonella enterica spp. enterica. All positive samples were obtained with bacterial culture, whereas no PCR reactions were positive. Despite carrying these pathogens, all culture-positive animals were clinically healthy and did not develop signs of gastrointestinal disease during the study period. The findings indicate that prevalence of Salmonella spp., C. perfringens, and C. difficile in feces from healthy Asian and African elephants in Europe is very low. ISSN: 10427260