

# NEWSLETTER

European Union Reference Laboratory for *Salmonella*

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# Contents

<b>EDITORIAL NOTE</b> .....	<b>4</b>
<b>CONTRIBUTION OF THE EURL-SALMONELLA</b> .....	<b>6</b>
TIMETABLE EURL- <i>SALMONELLA</i> PROFICIENCY TEST PRIMARY PRODUCTION STAGE 2023 .....	6
TIMETABLE EURL- <i>SALMONELLA</i> PROFICIENCY TEST TYPING 2023 .....	7
<b>FROM THE LITERATURE</b> .....	<b>8</b>

## Editorial Note

Bilthoven, 3 October 2023

Dear colleagues,

I do hope you had a good summer, despite the quite extreme situations in some countries due to climate change. This year we have seen many forest fires due to long dry and hot periods in some countries and floodings due to heavy rain falls in other countries. Hopefully you are all doing well and still had a good rest during the summer break.

At the EURL-*Salmonella* we are again full up and running with the organisation of the Proficiency Tests (PTs) of this autumn.

At first some information on the **PT on typing of *Salmonella* 2022** for which the interim summary report on the optional part on cluster analysis was published in July 2023 (also see <https://www.eurilsalmonella.eu/media/3611>). The individual cluster analysis results as well as the results on the obligatory part on serotyping were already shared earlier this year.

Currently the **PT on detection of *Salmonella* in samples from the primary production stage (PPS) 2023** is organised. This PT focuses on the detection of *Salmonella* in chicken faeces and the samples were sent to the participants on 25 September. For information, the time table for this PT is (again) included in this Newsletter.

In November, the **2023 PT on typing of *Salmonella*** will be organised. Like former years, this study will contain an obligatory part on serotyping of *Salmonella*, and a voluntary part on cluster analysis (NGS only). The timetable for this PT is also (again) included in this Newsletter.

During the summer we also discussed internally the set-up of the **EURL-*Salmonella* PTs in 2024**. As it has been quite a while since we organised the last (and first) PT on detection of *Salmonella* in live bivalve molluscs (2020), we have decided that we will focus the PT in spring 2024 on this matrix. As the NRLs-*Salmonella* for live bivalve molluscs generally differ from the other NRLs for detection of *Salmonella* in food samples, we also consider to organise in September/October 2024 again a combined PT for NRLs-*Salmonella* for Food and PPS.

As you may know, the EURL-*Salmonella* is member of the **inter EURLs working group on NGS**. This working group exists of 8 biological EURLs and aims to promote the use of NGS across the EURLs' networks, build NGS capacity within the EU and ensure liaison with EFSA and ECDC on the NGS mandate of the EC. This year, the inter EURLs WG has been active in several NGS areas.

Several **documents which provide guidance** to the laboratories in the area of application of NGS have been updated and published at the websites of the EURLs. Please have a look at: <https://www.eurilsalmonella.eu/publications/analytical-methods> - Next Generation Sequencing (NGS).

In June 2023, a **joint EURLs training on NGS** was organised for the NRL networks at the premises of the EURL-*Salmonella* in Bilthoven the Netherlands. The presentations will soon become available at the website(s) of the EURLs. Quite likely a similar training will be organised at the premises of EURL-*Campylobacter* in Sweden in 2024. Information about this training will be shared with the NRL networks as soon as more details are available.

In the past year the working group drafted a manuscript on the role of the joint EURLs working group on the use of NGS. In July 2023, this **article** was published in *Microbial Genomics*, entitled: European Union Reference Laboratories support the National food, feed and veterinary Reference Laboratories with rolling out Whole Genome Sequencing in Europe. The publication can be found at the following link: <https://doi.org/10.1099/mgen.0.001074>

On 5 and 6 September 2023 the inter-EURLs WG on NGS and EFSA jointly organised a second event in the series of Science Meets Policy Conferences, entitled: '**Science Meets Policy conference: Using Next Generation Sequencing to tackle foodborne threats**'. The conference was well attended with approximately 150 in person and 250 virtual participants. The presentations given at this conference can be found at the website of EFSA, through this link: <https://www.efsa.europa.eu/en/events/science-meets-policy-conference-using-next-generation-sequencing-tackle-foodborne-threats#documents>

On 29 September 2023, the inter EURLs WG NGS organised a free **webinar entitled 'Proficiency Tests on Next Generation Sequencing: approaches in use at the European Union Reference Laboratories.'** The aim of this webinar was to present the approaches used by EURLs for Proficiency Tests on NGS and to share experiences and serve as guidelines for the organisation of PTs on NGS at the Member State level. We were happy to notice that there was a large interest from the NRL networks as approximately 170 participants joined this webinar. It is planned to also make the presentations of this webinar available at the websites of the EURLs.

In the previous Newsletter you were informed about the request to publish **ISO/TS 6579-4** ('Identification of monophasic *Salmonella* Typhimurium by PCR') as a full ISO instead of a Technical Specification (TS). During summer 2023, ISO central secretariat agreed to change the status of the document to a full ISO and recently also CEN agreed to publish the document as a full standard (EN ISO 6579-4). Hence, we now can proceed to launch the public enquiry or DIS voting (Draft International Standard) for this document.

In July 2023, the following EURL-*Salmonella* report was published: Pol-Hofstad, I.E. and Mooijman, K.A., 2022. Combined EURL-*Salmonella* Proficiency Test Primary Production Stage and Food, 2022. Detection of *Salmonella* in hygiene swab samples. RIVM report 2022-0108. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. <https://www.rivm.nl/bibliotheek/rapporten/2022-0108.pdf>

Best wishes,  
Kirsten Mooijman  
Coordinator EURL-*Salmonella*

## Contribution of the EURL-*Salmonella*

### Timetable EURL- *Salmonella* Proficiency Test Primary Production Stage 2023 Detection of *Salmonella* in chicken faeces

Week	Date	Subject
27-35		E-mailing the link to the registration form for the Proficiency Test. Please <b>register by 31 August 2023</b> at the latest.
39		E-mailing the link for the result form to the participants. E-mailing the protocol and instructions for the result form to the NRLs. Preparation of media by the NRLs.
39	Monday 25 September 2023	Shipment of the parcels to the participants as Biological Substance Category B (UN 3373).
40	<b>Monday 2 October 2023</b>	<b>Start performance of the Proficiency Test.</b>
43	27 October at the latest	Deadline for completing the result form: <b>27 October 2023</b> (23:59h CET) After this deadline the result form will be closed.
	December 2023	Interim summary report

If you have questions or remarks about this Proficiency Test, please contact:

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## Timetable EURL- *Salmonella* Proficiency Test Typing 2023 Serotyping and optional part NGS Cluster analysis

Week	Date	Subject
39	Week of 25 September	Emailing of the link to the registration form for the typing study. Please <b>register by 20 October 2023</b> at the latest.
43	Week of 23 October	Emailing of the protocol 2023.
45	Monday 6 November	Shipment of the parcels to the participants as Biological Substance Category B (UN 3373).
45	Week of 6 November	<i>Upon receipt:</i> Starting the identification of the strains, according to the usual practice of the laboratory. Sending the link for the result form on Serotyping to the participants. Sending the link for the result form on NGS Cluster Analysis to the participants in a separate email.
50	15 December 2023 at the latest	Deadline for completing the electronic submission of <b>Serotyping</b> results: <b>15 December 2023</b> . After this deadline, the result form for serotyping will be closed.
5	31 January 2024 at the latest	Deadline for completing the electronic submission of <b>NGS Cluster Analysis</b> results: <b>31 January 2024</b> .
	February 2024	Serotyping: Evaluation of individual laboratory results and Interim summary report.
	April/May 2024	NGS Cluster Analysis: Evaluation of individual laboratory results and Interim summary report.

If you have questions or remarks about this Proficiency Test, please contact:

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## From the Literature

### Salmonella-related Literature selection from Scopus: July – September 2023

**Akomea-Frempong S., Skonberg D.I., Arya R., Perry J.J.**

*Survival of Inoculated Vibrio spp., Shigatoxigenic Escherichia coli, Listeria monocytogenes, and Salmonella spp. on Seaweed (Sugar Kelp) During Storage*  
(2023) *Journal of Food Protection*, 86 (7), art. no. 100096

ABSTRACT: Bacteria including *Vibrio* spp. persist in coastal waters and can contaminate edible seaweeds. Pathogens such as *Listeria monocytogenes*, shigatoxigenic *Escherichia coli* (STEC), and *Salmonella* have been associated with and present serious health risks in minimally processed vegetables including seaweeds. This study evaluated the survival of four pathogens inoculated onto two product forms of sugar kelp subjected to different storage temperatures. The inoculation comprised of a cocktail of two *Listeria monocytogenes* and STEC strains, two *Salmonella* serovars, and two *Vibrio* species. STEC and *Vibrio* were grown and applied in salt-containing media to simulate preharvest contamination, whereas *L. monocytogenes* and *Salmonella* inocula were prepared to simulate postharvest contamination. Samples were stored at 4°C and 10°C for 7 days, and 22°C for 8 h. Microbiological analyses were performed periodically (1, 4, 8, 24 h, etc.) to evaluate the effects of storage temperature on pathogen survival. Pathogen populations decreased under all storage conditions, but survival was greatest for all species at 22°C, with STEC exhibiting significantly less reduction (1.8 log CFU/g) than *Salmonella*, *L. monocytogenes*, and *Vibrio* (3.1, 2.7, and 2.7 log CFU/g, respectively) after storage. The largest population reduction (5.3 log CFU/g) was observed in *Vibrio* stored at 4°C for 7 days. Regardless of storage temperature, all pathogens remained detectable at the end of the study duration. Results emphasize the need for strict adherence to temperature control for kelp as temperature abuse may support pathogen survival, especially STEC, during storage, and the need for prevention of postharvest contamination, particularly with *Salmonella*. ISSN: 0362028X

**Smith R.P., May H.E., Burow E., Meester M., Tobias T.J., Sassu E.-L., Pavoni E., Di Bartolo I., Prigge C., Wasyl D., Zmudzki J., Viltrop A., Nurmoja I., Zoche-Golob V., Alborali G.L., Romantini R., Dors A., Krumova-Valcheva G., Koláčková I., Aprea G., Daskalov H.**

*Assessing pig farm biosecurity measures for the control of Salmonella on European farms*  
(2023) *Epidemiology and Infection*, 151, art. no. e130

ABSTRACT: *Salmonella* spp. is a common zoonotic pathogen, causing gastrointestinal infections in people. Pigs and pig meat are a major source of infection. Although farm biosecurity is believed to be important for controlling *Salmonella* transmission, robust evidence is lacking on which measures are most effective. This study enrolled 250 pig farms across nine European countries. From each farm, 20 pooled faecal samples (or similar information) were collected and analysed for *Salmonella* presence. Based on the proportion of positive results, farms were categorised as at higher or lower *Salmonella* risk, and associations with variables from a comprehensive questionnaire investigated. Multivariable analysis indicated that farms were less likely to be in the higher-risk category if they had '<400 sows'; used rodent baits close to pig enclosures; isolated stay-behind (sick) pigs; did not answer that the hygiene lock/ anteroom was easy to clean; did not have a full perimeter fence; did apply downtime of at least 3 days between farrowing batches; and had fully slatted flooring in all fattener buildings. A principal components analysis assessed the sources of variation between farms, and correlation between variables. The study results suggest simple control measures that could be prioritised on European pig farms to control *Salmonella*. ISSN: 09502688

**Bloomfield S.J., Janecko N., Palau R., Alikhan N.-F., Mather A.E.**

*Genomic diversity and epidemiological significance of non-typhoidal Salmonella found in retail food collected in Norfolk, UK*  
(2023) *Microbial Genomics*, 9 (7), art. no. 001075

ABSTRACT: Non-typhoidal *Salmonella* (NTS) is a major cause of bacterial gastroenteritis. Although many countries have implemented whole genome sequencing (WGS) of NTS, there is limited knowledge on NTS diversity on food and its contribution to human disease. In this study, the aim was to characterise the NTS genomes from retail foods in a particular region of the UK and assess the contribution to human NTS infections. Raw food samples were collected at retail in a repeated cross-sectional design in Norfolk, UK,

including chicken (n=311), leafy green (n=311), pork (n=311), prawn (n=279) and salmon (n=157) samples. Up to eight presumptive NTS isolates per positive sample underwent WGS and were compared to publicly available NTS genomes from UK human cases. NTS was isolated from chicken (9.6 %), prawn (2.9 %) and pork (1.3 %) samples and included 14 serovars, of which Salmonella Infantis and Salmonella Enteritidis were the most common. The S. Enteritidis isolates were only isolated from imported chicken. No antimicrobial resistance determinants were found in prawn isolates, whilst 5.1 % of chicken and 0.64 % of pork samples contained multi-drug resistant NTS. The maximum number of pairwise core non-recombinant single nucleotide polymorphisms (SNPs) amongst isolates from the same sample was used to measure diversity and most samples had a median of two SNPs (range: 0–251). NTS isolates that were within five SNPs to clinical UK isolates belonged to specific serovars: S. Enteritidis and S. Infantis (chicken), and S. I 4,[5],12:i-(pork and chicken). Most NTS isolates that were closely related to human-derived isolates were obtained from imported chicken, but further epidemiological data are required to assess definitively the probable source of the human cases. Continued WGS surveillance of Salmonella on retail food involving multiple isolates from each sample is necessary to capture the diversity of Salmonella and determine the relative importance of different sources of human disease. ISSN: 20575858

**Marmion M., Soro A.B., Whyte P., Scannell A.G.M.**

*Green label marinades: A solution to salmonella and campylobacter in chicken products? (2023) Heliyon, 9 (7), art. no. e17655*

ABSTRACT: Introduction: The presence of meat-borne pathogens entering the home remains a concern for consumers, despite advances made in improving antimicrobial interventions and systems within the processing line. Naturally antibacterial food ingredients including citrus juice and essential oils have been proven to inhibit the proliferation of microbial growth with varying success. Aims: This study aims to investigate the antimicrobial and sensory effects of mixtures of essential oils, fruit juices and herbs at established Minimum Inhibitory Concentrations (MICs) for their biopreservative effect on general microbiota of chicken and against chicken challenged with selected pathogenic/surrogate microorganisms. Materials and methods: Three marinade compositions were designed for use on chicken meat; lemon juice, thyme oil and black pepper (M1), lime juice, lemongrass oil and chilli paste (M2), and olive oil, oregano oil, basil oil and garlic paste (M3). These marinades were assessed for antibacterial effects against Salmonella enterica, Campylobacter jejuni and Listeria innocua on marinated chicken drumsticks stored in aerobic conditions at 4 °C. Consumer tasting sessions were also conducted with a small focus group using selected final marinades. Results: M1 and M2 were effective at significantly reducing initial pathogen carriage from 6 Log CFU/g to 2 Log CFU/g on refrigerated chicken meat as well as increasing the shelf-life of the product during cold-storage from 2 days to 7 days. However, consumer studies indicate that the flavours these marinades impart to treated products can be strong. Conclusion: These findings indicate that these designed marinades have shown excellent potential to improve food safety as well as shelf-life for the consumer, particularly in settings where food safety is often compromised such as barbecuing or in care settings. However, further recipe optimisation is required to make these marinades acceptable to consumers. ISSN: 24058440

**Montoro-Dasi L., Lorenzo-Rebenaque L., Marco-Fuertes A., Vega S., Marin C.**

*Holistic Strategies to Control Salmonella Infantis: An Emerging Challenge in the European Broiler Sector (2023) Microorganisms, 11 (7), art. no. 1765*

ABSTRACT: Salmonella spp. has been globally recognized as one of the leading causes of acute human bacterial gastroenteritis resulting from the consumption of animal-derived products. Salmonella Enteritidis, S. Typhimurium, and its monophasic variant are the main serovars responsible for human disease. However, a serovar known as S. Infantis has emerged as the fourth most prevalent serovar associated with human disease. A total of 95% of isolated S. Infantis serovars originate from broilers and their derived products. This serovar is strongly associated with an elevated antimicrobial (AMR) and multidrug resistance, a resistance to disinfectants, an increased tolerance to environmental mercury, a heightened virulence, and an enhanced ability to form biofilms and attach to host cells. Furthermore, this serovar harbors genes that confer resistance to colistin, a last-resort antibiotic in human medicine, and it has the potential to acquire additional transferable AMR against other critically important antimicrobials, posing a new and significant challenge to global public health. This review provides an overview of the current status of the S. Infantis serovar in the poultry sector, focusing on its key virulence factors, including its virulence genes, antimicrobial resistance, and biofilm formation. Additionally, novel

holistic strategies for controlling *S. Infantis* along the entire food chain are presented in this review. ISSN: 20762607

**Sarrami Z., Sedghi M., Mohammadi I., Bedford M., Miranzadeh H., Ghasemi R.**

*Effects of bacteriophage on Salmonella Enteritidis infection in broilers*

(2023) *Scientific reports*, 13 (1), pp. 12198

ABSTRACT: Bacteriophages (BP) are viruses that can infect bacteria. The present study evaluated the effect of BP on *Salmonella* infected broilers. A number of 150 day-old broilers were used in a completely randomized design with five treatments that included: (1) basal diet from day 0 to 28; (2) basal diet + 0.3 g/kg of colistin from day 0 to 28; (3) basal diet from day 1 to 13, and basal diet + 0.4 g/kg of colistin from day 14 to 28; (4) basal diet + 1 g/kg of BP from day 0 to 28; (5) basal diet + 1.5 g/kg of BP from day 0 to 28. On day 13, 15 chickens from each treatment were challenged by *Salmonella* Enteritidis (SE), while fifteen from each treatment were not; instead, they were kept in the same cage with the challenged chickens (exposed chickens). At 7 and 14 days post-challenge, the number of SE and coliform bacteria in the cecum and liver of colistin and BP-fed birds was lower than the control treatment. In exposed and challenged chickens, the height and surface area of villus were greater in the BP and colistin-supplemented groups. Serum concentrations of aspartate aminotransferase and alanine transaminase were greater, while serum albumin and triglycerides concentrations were lower in the control treatment. The liver of the challenged chickens had more pathological lesions than exposed birds. BP significantly decreased PPAR $\gamma$  gene expression in exposed chickens. In the challenged and exposed chickens, TLR4 gene expression was lower in BP and colistin-treated birds as compared to the control. In conclusion, adding BP to the diet from the day of age prevents the spread of *Salmonella*. ISSN: 20452322

**Cobo-Simón M., Hart R., Ochman H.**

*Gene flow and species boundaries of the genus Salmonella*

(2023) *mSystems*, 8 (4)

ABSTRACT: The genus *Salmonella* comprises two species, *Salmonella bongori* and *Salmonella enterica*, which are infectious to a wide variety of animal hosts. The diversity within *S. enterica* has been further partitioned into 6–10 subspecies based on such features as host range, geography, and most recently, genetic relatedness and phylogenetic affiliation. Although *Salmonella* pathogenicity is attributable to large numbers of acquired virulence factors, the extent of homologous exchange in the species at large is apparently constrained such that the species and subspecies form distinct clusters of strains. To explore the extent of gene flow within and among subspecies, and to ultimately define true biological species, we evaluated patterns of recombination in over 1,000 genomes currently assigned to the genus. Those *Salmonella* subspecies containing sufficient numbers of sequenced genomes to allow meaningful analysis—i.e., subsp. *enterica* and *diarizonae*—were found to be reproductively isolated from one another and from all other subspecies. Based on the configuration of genomic sequence divergence among subspecies, it is expected that each of the other *Salmonella* subspecies will also represent a biological species. Our findings argue against the application of prescribed nucleotide-identity thresholds to delineate bacterial species and contend that the Biological Species Concept should not be disregarded for bacteria, even those, like *Salmonella*, that demonstrate complex patterns of species and subspecies divergence. **IMPORTANCE** The Biological Species Concept (BSC), which defines species boundaries based on the capacity for gene exchange, is widely used to classify sexually reproducing eukaryotes but is generally thought to be inapplicable to bacteria due to their completely asexual mode of reproduction. We show that the genus *Salmonella*, whose thousands of described serovars were formerly considered to be strictly clonal, undergoes sufficient levels of homologous recombination to be assigned to species according to the BSC. Aside from the two recognized species, *Salmonella enterica* and *Salmonella bongori*, several (and likely all) of the subspecies within *S. enterica* are reproductively isolated from one another and should each be considered a separate biological species. These findings demonstrate that species barriers in bacteria can form despite high levels of nucleotide identity and that commonly applied thresholds of genomic sequence identity are not reliable indicators of bacterial species status. ISSN: 23795077

**Homann C., Eckey I., Chuppava B., Teich K., Buch J., Zimmermann A., Kaltschmitt M., Grone R., Wilke V., Visscher C.**

*Rye and Rye Bran as Components of Diets in Piglet Production—Effects on Salmonella Prevalence*

(2023) *Animals*, 13 (14), art. no. 2262

**ABSTRACT:** The nutritional benefits of rye (and therefore rye bran) are mainly due to its high content of fermentable dietary fiber, the non-starch polysaccharides (NSP). Microorganisms in the large intestine are able to convert these into short-chain fatty acids (SCFA), including butyrate. Butyrate strengthens the epithelial barrier function in the colon by nourishing the enterocytes and inhibiting the spread of Salmonella in the intestinal tract. Therefore, the aim of this study was to test under field conditions whether a diet with rye or rye bran as the main ingredient for gilts, sows, and weaned piglets is associated with a lower Salmonella prevalence. Depending on the age groups, between 20–30% rye or between 15–20% rye bran was used in the experimental group. A total of  $n = 1983$  boot swabs,  $n = 356$  fecal samples, and  $n = 1909$  serum samples were examined. The results of this study show that rye or rye bran at the levels used had no apparent effect on the number of positive Salmonella samples. However, the Salmonella OD values in the experimental groups were significantly lower than in the control group. This suggests that the use of rye leads to a lower incidence of infection, but this effect could not be proven from swabs. ISSN: 20762615

**Benson H.E., Reeve L., Findlater L., Vusirikala A., Pietzsch M., Olufon O., Matthews E., Hoban A., Painset A., Team I.M., Balasegaram S., Larkin L., Weir S., Heinsbroek E.**

*Local Salmonella Enteritidis restaurant outbreak investigation in England provides further evidence for eggs as source in widespread international cluster, March to April 2023 (2023) Eurosurveillance, 28 (27)*

**ABSTRACT:** We report a 5-single nucleotide polymorphism cluster of Salmonella Enteritidis in England, part of a global cluster of S. Enteritidis ST11. Forty-seven confirmed cases have been investigated of whom 25 were linked to a restaurant. In addition, there were 18 probable cases with restaurant exposure. Epidemiological investigations suggested eggs or chicken as the most likely cause of the outbreak but were unable to distinguish between those two food vehicles. Ongoing food chain investigations indicated links to imported eggs from Poland. ISSN: 1025496X

**Bianchi D.M., Barzanti P., Adriano D., Martucci F., Pitti M., Ferraris C., Floris I., La Brasca R., Ligotti C., Morello S., Scardino G., Musolino N., Tramuta C., Maurella C., Decastelli L.**

*Food Safety Monitoring of Salmonella spp. in Northern Italy 2019–2021 (2023) Pathogens, 12 (7), art. no. 963*

**ABSTRACT:** Salmonella is the second most frequent bacterial pathogen involved in human gastrointestinal outbreaks in the European Union; it can enter the food-production chain from animal or environmental sources or from asymptomatic food operators. European food legislation has established microbiological criteria to ensure consumer protection. Salmonella is listed under both process hygiene criteria and food safety criteria. Each EU member state designates an agency to organize or perform controls and other official activities. This paper describes the official control plans performed by competent authorities in Northern Italy in the three-year period 2019–2021. A total of 4413 food samples were delivered to the IZS Food Safety laboratories for Salmonella detection, of which 36 (0.8%) tested positive. Salmonella was most frequently detected in poultry meat samples (25/36 positive samples) followed by other meat products and pork products. The official controls for the protection of consumer health apply the EU's farm-to-fork approach: the samples were collected during production (food production plants), from products on the market, and from collective catering (restaurants, cafeterias, canteens). This manuscript will provide information about the presence of Salmonella in foodstuffs that can help competent authorities to set control plans based on risk assessments. ISSN: 20760817

**Possas A., Posada-Izquierdo G.D., Tarlak F., Jiménez-Jiménez F., Pérez-Rodríguez F.**

*Inactivation of Salmonella Typhimurium in fresh-cut lettuce during chlorine washing: Assessing the impacts of free chlorine concentrations and exposure times (2023) LWT, 184, art. no. 115069*

**ABSTRACT:** The aim of this study was to evaluate, quantify and model the inactivation of Salmonella in fresh-cut lettuce during washings with chlorinated water at different free chlorine concentrations (FCC, 0–150 mg/L). Individual fresh-cut lettuce samples (4 cm<sup>2</sup>) were inoculated with a Salmonella culture (ca. 4 log CFU/cm<sup>2</sup>) and washed with 100-mL solutions with different FCC for different times (0–150 s). The surviving Salmonella cells recovered from samples were enumerated by plate count methodology. A fast decay on Salmonella counts was marked in the first 20 s of washing, followed by a slowing down on reductions. A maximum of 2.6 log-decrease was observed after 2.5-min washing

regardless of FCC. The log-linear with tail primary model coupled with a linear secondary model was fitted to inactivation data obtained at FCC from 50 to 150 mg/L through global regression analysis, yielding a suitable model to describe Salmonella concentrations as a function of FCC and washing times (RMSE = 0.34, R<sub>2</sub>adj = 0.84). Simulations using the developed model showed inactivation rates varying from 0.17 log CFU/s at 50 mg/L to 0.86 log CFU/s at 150 mg/L. Disinfection models on lettuce are valuable tools for the validation of control measures in the fresh-cut produce industry and for quantitative risk assessments. ISSN: 00236438

**Arispón F., Cano N., Jordano R., Medina L.M.**

*Monitoring Hygienic Measures for Decreasing Salmonella Occurrence in Scalding Tank Water of a Turkey Slaughterhouse*

(2023) *Applied Sciences (Switzerland)*, 13 (14), art. no. 8103

ABSTRACT: The objective of this work was to test different treatments based on the temperature and acidification of scalding tank water throughout the day in a turkey slaughterhouse under industrial conditions in order to decrease the occurrence of Salmonella. After controlling the scalding tank water under usual conditions, the following measures were taken: (a) the temperature was increased to 60 °C and 70 °C for 15 min at the halfway point of the day; (b) the scalding water was acidified and six different initial pH levels were tested. Both measures which were tested (heating and acidification of scalding water) showed efficiency in reducing the occurrence of Salmonella during the scalding step. In order to prevent the disadvantages associated with the hardest measures in each case, we propose that scalding water be heated to 70 °C for 15 min without carcasses, which can be repeated if the disadvantages of the exposed costs and resources of processing are acceptable. Regarding acidification, a suitable measure would be an initial pH of 4.0 or any treatment that keeps the pH of the scalding water below 4.5, using acid that does not affect the final quality of the products and/or the elements involved. ISSN: 20763417

**Elbashir S.M., Adnan A.M., Bowers J., DePaola A., Jahncke M., Punchihewage-Don A.J., Da Silva L.V., Hashem F., Parveen S.**

*Antimicrobial Resistance, Virulence Properties and Genetic Diversity of Salmonella Typhimurium Recovered from Domestic and Imported Seafood*

(2023) *Pathogens*, 12 (7), art. no. 897

ABSTRACT: The quantity of seafood imported and produced by domestic aquaculture farming has increased. Recently, it has been reported that multidrug-resistant (MDR) Salmonella Typhimurium may be associated with seafood. However, information is limited to the antimicrobial resistance, virulence properties, and genetic diversity of S. Typhimurium recovered from imported and domestic seafood. This study investigated the antimicrobial resistance, virulence properties, and genetic diversity of S. Typhimurium isolated from domestic and imported catfish, shrimp, and tilapia. A total of 127 isolates were tested for the presence of multidrug-resistance (MDR), virulence genes (*invA*, *pagC*, *spvC*, *spvR*), and genetic diversity using the Sensititre micro-broth dilution method, PCR, and pulsed-field gel electrophoresis (PFGE), respectively. All isolates were uniformly susceptible to six (amoxicillin/clavulanic acid, ceftiofur, ceftriaxone, imipenem, nitrofurantoin, and trimethoprim/sulfamethoxazole) of the 17 tested antimicrobials and genetically diverse. Fifty-three percent of the Salmonella isolates were resistant to at least one antimicrobial and 49% were multidrug resistant. Ninety-five percent of the isolates possessed the *invA* gene, 67% *pagC*, and 43% for both *spvC*, and *spvR*. The results suggest that S. Typhimurium recovered from seafood is frequently MDR, virulent, and have the ability to cause salmonellosis. ISSN: 20760817

**Olaimat A.N., Al-Holy M.A., Abughoush M.H., Daseh L., Al-Nabulsi A.A., Osaili T.M., Al-Rousan W., Maghaydah S., Ayyash M., Holley R.A.**

*Survival of Salmonella enterica and Listeria monocytogenes in date palm paste and syrup at different storage temperatures*

(2023) *Journal of Food Science*, 88 (7), pp. 2950 - 2959

ABSTRACT: Abstract: This study aimed to investigate the behavior of Salmonella enterica and Listeria monocytogenes in processed date paste and syrup at different temperatures. Commercial products were inoculated with approximately 6 log CFU/mL of S. enterica or L. monocytogenes and stored at 4, 10, and 24°C for 90 days. S. enterica was able to survive in date products until the end of storage at 4°C. At this temperature, numbers decreased by 2.1 log CFU/g in date paste and by 3.4 log CFU/g in date syrup; however, at 10°C, cells were reduced >4.2 log CFU/g and were undetectable by direct plating in date paste or by enrichment (complete elimination) in syrup. Further, at 24°C, complete elimination of S. enterica was achieved in date paste and syrup by 30 and 7 days, respectively. L.

monocytogenes numbers decreased by 1.4, 4.4, and >4.6 log CFU/g in date paste stored at 4, 10, and 24°C for 90 days, respectively. In date syrup, numbers of *L. monocytogenes* decreased to undetectable levels by 50, 14, and 4 days at 4, 10, and 24°C, respectively, by direct plating and complete elimination was observed at 10 and 24°C by 50 and 30 days of storage, respectively. The initial pH values of date paste and syrup were 4.7 and 4.8, respectively, and remained stable until the end of storage except for *L. monocytogenes*-inoculated syrup. Practical Application: *Salmonella enterica* and *Listeria monocytogenes* can easily survive in date paste and syrup particularly at refrigerator temperature, which explains the necessity of preventing the contamination of date products with foodborne pathogens. ISSN: 00221147

**Balala L.M., Mendoza B.C., Baldrias L.R., Masangkay J.S.**

*Salmonella* in free-range chickens: pathology of subclinical persistent infection (2023) *International Journal of Agricultural Technology*, 19 (4), pp. 1447 - 1458

ABSTRACT: Testing of apparently healthy free-range chickens revealed the detection of *Salmonella* in 2.11% (5/237) and 8.04% (16/199) of the samples by culture method and PCR, respectively, with an overall detection rate of 8.86% (21/237). Primary histopathological lesions consistent with *Salmonella* infection were observed in the liver and spleen at Days 10 through 150 and in the intestines at Days 120 through 150. Sinusoidal congestion (83.3%) and lymphoid hyperplasia (66.7%) were the most predominant lesions in the spleen persisting from Day 10 until Day 150. Cloudy swelling (40%) with cytoplasmic granulation and typhoid nodules (26.7%) were observed in the liver beginning Day 10. Cecal tonsil activation was observed at Day 10, while structural changes and infiltration of inflammatory cells in the submucosa were the significant histopathological changes in the intestines throughout Day 150. *Salmonella* is a silent threat to public health in subclinical infections. Active surveillance and monitoring of this pathogen should be carried out continuously to improve detection and diagnosis. Sustainable mitigating strategies should be designed for free-range poultry to control *Salmonella* and achieve food security and safety. ISSN: 26300192

**Ashish Singh K., Nair S.S., Rai R.**

Survival of *Salmonella* spp. and Pathogenic *Escherichia coli* in Food Matrixes and Its Relevance in the Development of Proficiency Testing Samples (2023) *Journal of AOAC International*, 106 (4), pp. 956 - 969

ABSTRACT: Monitoring of food products by government agencies for their compliance to regulatory limits is an essential step in controlling foodborne outbreaks. For monitoring purposes, an extensive setup of the surveillance system is used, which involves ISO 17025:2017 accredited laboratories for food testing. Participation in proficiency testing (PT) programs is a requirement of ISO 17025:2017, which ensures data accuracy and analyst competency. Participation in PT schemes is costly for laboratories in developing countries as most of the commercial suppliers are situated in the United States and Europe. The literature or data available on creation of microbiological proficiency testing is scanty as much of the data available with commercial suppliers are trade secrets, and there is only 0.06% of research articles available in the Scopus database on the topic. In this review article, an attempt is made to understand the factors impacting the survival of two important foodborne pathogens, i.e., *Escherichia coli* and *Salmonella* spp., by extracting information available from growth studies and root-cause analysis of various food safety incidents and recalls. Utilization of this information in the development of PT samples is discussed in this review article along with a focus on the availability of PT samples and associated ISO standards to formulate homogeneous and stable PT samples. This review article elaborates on the focus areas that can be considered by PT providers (PTP)-for example, initial inoculum level and preparation, strain type, microbial growth phase, the impact of different types of food matrixes including low-moisture food, antimicrobial components, pH, presence of competitor microbes, and environmental conditions involving storage temperature, time, and relative humidity. These focus areas can be used to successfully create PT samples by PTP in developing countries. ISSN: 19447922

**Michelacci V., Asséré A., Cacciò S., Cavaiuolo M., Mooijman K., Morabito S., Pedersen S.K., Sayeb M., Segerman B., Simonsson M., Skarin H., Tozzoli R., van Hoek A., Hendriksen R.S.**

*European Union Reference Laboratories support the National food, feed and veterinary Reference Laboratories with rolling out whole genome sequencing in Europe* (2023) *Microbial genomics*, 9 (7)

ABSTRACT: The Inter European Union Reference Laboratories (EURLs) Working Group on Next Generation Sequencing (NGS) involves eight EURLs for microbiological food and feed

hazards and has been working since 2017 to promote the adoption of NGS by the National Reference Laboratories (NRLs) in the European Union. This work illustrates the results of the first 5 years of activity. By working together, the EURLs involved have released guidance documents for assisting NRLs in all the steps of NGS, helping the transition from classical molecular methods towards whole genome sequencing while ensuring harmonization, with the final aim of improving preparedness in the use of NGS to characterize microbial hazards and trace the sources of infection. ISSN: 20575858

**Krishnan A., Xu X., Tamayo M.S., Mishra A., Critzer F.**

*Impact of chlorine or peracetic acid on inactivation of Salmonella, Escherichia coli, and Listeria monocytogenes in agricultural water*  
(2023) *Science of the Total Environment*, 885, art. no. 163884

ABSTRACT: Preharvest agricultural water has been recognized as one of the routes of contamination for foodborne pathogens during fruit and vegetable production. Several strategies have been proposed to reduce the risk of pathogens, including preharvest water chemigation, but literature is lacking with regards to microbiological inactivation of common bacterial foodborne pathogens associated with fresh produce contamination, *Salmonella enterica*, Shiga-toxigenic *Escherichia coli* (STEC), and *Listeria monocytogenes*, in surface irrigation water after exposure to chlorine and peracetic acid (PAA). Surface water supplied by a local irrigation district was collected over the summer of 2019. Water was autoclaved, divided into 100 mL samples, and inoculated with a cocktail of five *Salmonella*, STEC, or *Listeria monocytogenes* strains or a single strain non-pathogenic *E. coli*. Samples were then treated with 3, 5, or 7 ppm of free chlorine or PAA, and surviving populations were evaluated using a time-kill assay. A first-order kinetic model was used to fit the inactivation data and obtain the D-values. A secondary model was used to explain the changes due to the type of water, treatment, and microorganism. At a concentration of 3 ppm, the observed and predicted D-values of free chlorine treatments were higher than that of PAA treatments for ground and surface water. Results indicated that PAA was more effective inactivating bacteria than sodium hypochlorite at concentrations of 3 and 5 ppm for both water sources (surface and ground). However, at 7 ppm, the effectiveness of PAA and sodium hypochlorite showed no statistically significant difference for both surface and groundwater. Findings will provide information regarding efficacy of chemical sanitizers like chlorine and PAA for inactivation of *Salmonella*, *Listeria*, and STEC in surface water from which treatments can be derived. Ultimately benefitting growers in the selection of an appropriate method for in-field treatment of irrigation water if deemed necessary. ISSN: 00489697

**Pacholewicz E., Wisselink H.J., Koene M.G.J., van der Most M., Gonzales J.L.**

*Environmental Sampling Methods for Detection of Salmonella Infections in Laying Hens: A Systematic Review and Meta-Analysis*  
(2023) *Microorganisms*, 11 (8), art. no. 2100

ABSTRACT: Salmonellosis is the second most commonly reported foodborne gastrointestinal infection in humans in the European Union (EU). Most outbreaks are caused by *Salmonella* Enteritidis, present in contaminated food products, particularly in egg and egg products. In recent years, an increase in the prevalence of *Salmonella* in laying hen flocks in the EU has been observed. For the effective control of infection, adequate detection is key. In laying hen flocks, the occurrence of *Salmonella* in the EU is monitored by the culture of environmental samples (dust, faeces, and boot swabs). The performance of sampling procedures described in the literature for the detection of *Salmonella* in laying hens was reviewed. In total, 924 abstracts were screened, resulting in the selection of 87 abstracts and 18 publications for qualitative and quantitative analyses, respectively. Sample sizes and sampling locations of faecal material and dust were variable and poorly described. Microbiological culture methods used to detect *Salmonella* were variably described in the literature and were often incomplete. Overall, the available literature indicates higher sensitivity of environmental versus individual hen matrices and points to differences in sensitivity between environmental matrices. For non-cage housing systems, boot swabs are the preferred samples, while for cage housing systems dust might be a more reliable sample. ISSN: 20762607

**Ahmed M.F.E., Chuppava B., Lingens J.B., Hankel J., El-Wahab A.A., Münster P., Antakli A., Radko D., Visscher C.**

*Survival persistence of the 3 common Salmonella enterica serotypes isolated from broilers' in different matrices*  
(2023) *Poultry Science*, 102 (8), art. no. 102788

ABSTRACT: Broiler meat is the predominant source of *Salmonella* as a foodborne pathogen. Several control strategies have focused on the reduction of *Salmonella* spp.

levels at different production stages. However, the persistence of *Salmonella* between consecutive flocks is still of great concern. This study was designed to understand the cause of reinfection in broiler flocks due to survival of *Salmonella* in feeding lines of related matrices. *Salmonella* (S.) Enteritidis, S. Infantis, and S. Typhimurium isolated from broiler farms in North-West Germany were used. Four types of matrices (phosphate buffer saline (PBS), dietary plant fat, fat with feed mixture, and feed) were applied to evaluate *Salmonella* survival (with the initial dose about  $8.0 \log_{10}$  CFU/mL) during a simulation of 4 production cycles. To evaluate the growth and survival status of *Salmonella* ISO 6579-1:2017 were performed (quantitatively by plate count method (PCM) and most probable number method (MPN)) and qualitatively) at 5 defined time points (-7, 0, 4, 7, and 35 d). In all matrices and for the 3 serovars, the *Salmonella* count decreased at the end of the fourth cycle in comparison to the beginning of the experimental infection, and was still cultivated except for fat matrix. The PBS matrices showed the highest survival level of *Salmonella* and did not decline drastically by the end of the fourth cycle ( $5.93 \pm 0.00$ ,  $5.87 \pm 0.02$ ,  $5.73 \pm 0.05 \log_{10}$  CFU/mL, respectively). However, the fat matrices showed the lowest survival level for the 3 isolates at d 35 since the first cycle ( $0 \log_{10}$  CFU/mL using PCM). Regarding the fat-feed mixture, and feed matrices, there was a fluctuation in the survival rate of *Salmonella* (all serovars) within each cycle. For the qualitative method, the 3 serovars persisted in all matrices until the end of the fourth cycle except for fat matrices. The present study highlights the ability of *Salmonella* to survive for a long time in different temperatures and matrices despite efficient cleaning and disinfection processes in the feeding lines, which may influence reinfection with *Salmonella* in poultry houses.  
ISSN: 00325791

**McMillan E.A., Hiott L.M., Carrico J.A., Machado M.P., Pouseele H., Jackson C.R., Frye J.G.**

*Polymerase chain reaction for the in vitro detection of the pESI plasmid associated with the globally circulating Salmonella Infantis outbreak strain*  
(2023) *Letters in applied microbiology*, 76 (8)

ABSTRACT: A globally circulating strain of *Salmonella enterica* serotype *Infantis* containing the pESI plasmid has increased in prevalence in poultry meat samples and cases of human infections. In this study, a polymerase chain reaction (PCR) protocol was designed to detect the pESI plasmid and confirm the *Infantis* serotype of *Salmonella* isolates. Primers were tested bioinformatically to predict specificity, sensitivity, and precision. A total of 54 isolates of *Salmonella* serotypes *Infantis*, *Senftenberg*, and *Alachua* were tested, with and without the pESI plasmid carriage. Isolates of 31 additional serotypes were also screened to confirm specificity to *Infantis*. Specificity, sensitivity, and precision of each primer were  $>0.95$ . All isolates tested produced the expected band sizes. This PCR protocol provides a rapid and clear result for the detection of the pESI plasmid and serotype *Infantis* and will allow for the in vitro detection for epidemiological studies where whole-genome sequencing is not available. ISSN: 1472765X

**Münster P., Pöppel L., Antakli A., Müller-Doblies D., Radko D., Kemper N.**

*The Detection of Salmonella Enteritidis on German Layer Farms after Cleaning and Disinfection*  
(2023) *Animals*, 13 (16), art. no. 2588

ABSTRACT: The presence of *Salmonella* Enteritidis in poultry houses after cleaning and disinfection can pose a potential risk to public health, as *Salmonella* remains one of the most important causes of foodborne diseases. This study focused on ten German layer farms (including floor-reared and free-range systems) with a recent history of *Salmonella* Enteritidis, and samples were collected from July 2018 to March 2021 after the cleaning and disinfection process. A total of 244 swab samples were tested for the presence of *Salmonella* using real-time PCR, followed by a culture of positive samples. Results revealed that 61 out of the 244 swab samples tested positive for *Salmonella*, indicating a prevalence of 25% in the samples examined. Among the *Salmonella*-positive swab samples identified with the PCR assay, 65.6% (40 out of 61) were confirmed by the culture. Of the 40 isolates obtained from the culture, 36 were identified as *Salmonella* Enteritidis, while 4 were categorized as rough *Salmonella* strains. This study emphasizes the importance of both the surrounding area of the poultry houses in terms of infection carry-over and the meticulous implementation of cleaning and disinfection procedures to eliminate any remaining infection within the houses. To mitigate the risk of further *Salmonella* spread on layer farms, additional investigations are recommended to focus on the existing transmission pathways of *Salmonella* and their genetic diversity.  
ISSN: 20762615

**Gül E., Bakkeren E., Salazar G., Steiger Y., Younes A.A., Clerc M., Christen P., Fattinger S.A., Nguyen B.D., Kiefer P., Slack E., Ackermann M., Vorholt J.A., Sunagawa S., Diard M., Hardt W.-D.**

*The microbiota conditions a gut milieu that selects for wild-type Salmonella Typhimurium virulence*

(2023) *PLoS Biology*, 21 (8), art. no. e3002253

ABSTRACT: Salmonella Typhimurium elicits gut inflammation by the costly expression of HilD-controlled virulence factors. This inflammation alleviates colonization resistance (CR) mediated by the microbiota and thereby promotes pathogen blooms. However, the inflamed gut-milieu can also select for hilD mutants, which cannot elicit or maintain inflammation, therefore causing a loss of the pathogen's virulence. This raises the question of which conditions support the maintenance of virulence in S. Typhimurium. Indeed, it remains unclear why the wild-type hilD allele is dominant among natural isolates. Here, we show that microbiota transfer from uninfected or recovered hosts leads to rapid clearance of hilD mutants that feature attenuated virulence, and thereby contributes to the preservation of the virulent S. Typhimurium genotype. Using mouse models featuring a range of microbiota compositions and antibiotic inflammation-inflicted microbiota disruptions, we found that irreversible disruption of the microbiota leads to the accumulation of hilD mutants. In contrast, in models with a transient microbiota disruption, selection for hilD mutants was prevented by the regrowing microbiota community dominated by Lachnospirales and Oscillospirales. Strikingly, even after an irreversible microbiota disruption, microbiota transfer from uninfected donors prevented the rise of hilD mutants. Our results establish that robust S. Typhimurium gut colonization hinges on optimizing its manipulation of the host: A transient and tempered microbiota perturbation is favorable for the pathogen to both flourish in the inflamed gut and also minimize loss of virulence. Moreover, besides conferring CR, the microbiota may have the additional consequence of maintaining costly enteropathogen virulence mechanisms. Copyright: ISSN: 15449173

**Terio V., Manfredi A., Dimuccio M.M., Casalino G., Luposella F., Losacco C., Bonerba E., Ceci E., Circella E., Corrente M., Bozzo G.**

*Management of Laying Hen Farms in France Infected by Salmonella spp.: Comparison of Three Strategies*

(2023) *Agriculture (Switzerland)*, 13 (8), art. no. 1490

ABSTRACT: The presence of Salmonella spp. on laying hen farms turns out to be a major problem for public health reasons, mainly related to foodborne illnesses that may arise due to ingestion of contaminated eggs. When Salmonella spp. infection on farms is confirmed by laboratory analysis, animal health measures are applied on the establishment, including total elimination of animals. The aim of this study is to describe three different methods of managing Salmonella enteritidis-infected laying hen farms, all of which were used within the Moselle department (France). The methods chosen, culling, use of a mobile CO<sub>2</sub> container, or lethal injection with T-61, depend on specific starting conditions, including the number of birds and proximity to an authorized slaughterhouse. This study, therefore, helps to identify the main problems with each method and provides recommendations for improving the management of Salmonella spp.-infected laying hen farms, where antibiotics cannot be used. ISSN: 20770472

**Lentsch V., Aslani S., Echtermann T., Preet S., Cappio Barazzone E., Hoces D., Moresi C., Kümmerlen D., Slack E.**

*"EvoVax" – A rationally designed inactivated Salmonella Typhimurium vaccine induces strong and long-lasting immune responses in pigs*

(2023) *Vaccine*, 41 (38), pp. 5545 - 5552

ABSTRACT: Salmonella enterica subspecies enterica serovar Typhimurium (S.Tm) poses a considerable threat to public health due to its zoonotic potential. Human infections are mostly foodborne, and pork and pork products are ranked among the top culprits for transmission. In addition, the high percentage of antibiotic resistance, especially in monophasic S.Tm, limits treatment options when needed. Better S.Tm control would therefore be of benefit both for farm animals and for safety of the human food chain. A promising pre-harvest intervention is vaccination. In this study we tested safety and immunogenicity of an oral inactivated S.Tm vaccine, which has been recently shown to generate an "evolutionary trap" and to massively reduce S.Tm colonization and transmission in mice. We show that this vaccine is highly immunogenic and safe in post-weaning pigs and that administration of a single oral dose results in a strong and long-lasting serum IgG response. This has several advantages over existing – mainly live – vaccines against S.Tm, both in improved seroconversion and reduced risk of vaccine-strain persistence and reversion to virulence. ISSN: 0264410X

**Alegbeleye O., Sant'Ana A.S.**

*Survival of Salmonella spp. under varying temperature and soil conditions (2023) Science of the Total Environment, 884, art. no. 163744*

ABSTRACT: Soils can serve as suitable reservoirs for or barriers against microbial contamination of water resources and plant produce. The magnitude of water or food contamination risks through soil depends on several factors, including the survival potential of microorganisms in the soil. This study assessed and compared the survival/persistence of 14 *Salmonella* spp. strains in loam and sandy soils at 5, 10, 20, 25, 30, 35, 37 °C and under uncontrolled ambient temperature conditions in Campinas Sao Paulo. The ambient temperature ranged from 6 °C (minimum) to 36 °C (maximum). Bacterial population densities were determined by the conventional culture method (plate counts) and monitored for 216 days. Statistical differences among the test parameters were determined by Analysis of Variance, while relationships between temperature and soil type were evaluated using Pearson correlation analysis. Similarly, relationships between time and temperature for survival of the various strains were evaluated using Pearson correlation analysis. Results obtained indicate that temperature and soil type influence the survival of *Salmonella* spp. in soils. All 14 strains survived for up to 216 days in the organic-rich loam soil under at least three of the temperature conditions evaluated. However, comparatively lower survival rates were recorded in sandy soil, especially at lower temperature. The optimum temperature for survival varied among the strains, where some survived best at 5 °C and others between 30 and 37 °C. Under uncontrolled temperature conditions, the *Salmonella* strains survived better in loam than in sandy soils. Bacterial growth over post inoculation storage period was overall more impressive in loam soil. In general, the results indicate that temperature and soil type can interact to influence the survival of *Salmonella* spp. strains in soil. For the survival of some strains, there were significant correlations between soil type and temperature, while for some others, no significant relationship between soil and temperature was determined. A similar trend was observed for the correlation between time and temperature. ISSN: 00489697

**Leekitcharoenphon P., Vigre H., Kaas R.S., Aarestrup F.M.**

*Trends in Salmonella Dublin over time in Denmark from food and animal related isolates (2023) Infection, Genetics and Evolution, 113, art. no. 105475*

ABSTRACT: *Salmonella enterica* serovar Dublin is highly adapted to cattle and a relatively rare cause of human infections. In Denmark *S. Dublin* has been endemic in the cattle population for many years. A national surveillance program in the cattle population was established at herd-level to reduce the occurrence of *S. Dublin*. In this study, we analyzed 421 *S. Dublin* genomes from cattle and food in order to determine the trend of *S. Dublin*'s population size over time in Denmark and the impact of intervention in the cattle industry on the bacterial population size. A phylogenetic tree based on SNPs exhibited two major clades and one small cluster. All isolates were ST10. The temporal phylogenetic tree for the *S. Dublin* isolates showed that the most recent common ancestor was estimated to be in ~1980 for the two major clades. An effective population size over time based on a Bayesian skyline plot showed that the population size of *S. Dublin* decreased significantly between 2014 and 2019 in both major clades. This result was concordant with the decrease of infected human cases by *S. Dublin* in Denmark. The strengthening of a surveillance program in Denmark could be the cause for the reduction of *S. Dublin*'s effective population size. This study showed that whole genome sequencing combined with computer intensive phylogenetic analysis estimating the effective size of the *S. Dublin*'s population over time is a strongly relevant measure with respect to assessing the impact of control measures aiming to reduce the bacterial population in the reservoir and the risk for human infection. ISSN: 15671348

**Lee A., Maks-Warren N., Aguilar V., Piszczor K., Swicegood B., Ye M., Warren J., O'Neill E., Fleck M., Tejayadi S.**

*Inactivation of Salmonella, Shiga Toxin-producing E. coli, and Listeria monocytogenes in Raw Diet Pet Foods Using High-Pressure Processing (2023) Journal of Food Protection, 86 (9), art. no. 100124*

ABSTRACT: Pet food formulated with raw meat can pose health risks to pets and humans. High-pressure processing (HPP) was evaluated to achieve a 5-log reduction of *Salmonella*, *E. coli* STEC, and *L. monocytogenes* in commercial raw pet foods and maintain a 5-log reduction throughout post-HPP storage. Three formulation types that varied in the amounts of striated meat, organ meat, bone, seeds, and other ingredients (fruits, vegetables, and minor ingredients) designated as A-, S-, and R-formulations were used. Eight raw diet pet foods, consisting of three beef formulations (A-, S- and R-Beef), three chicken formulations (A-, S-, and R-Chicken), and two lamb formulations (A- and S-Lamb),

were inoculated with 7 log CFU/g cocktails of Salmonella, E. coli STEC or L. monocytogenes, HPP at 586 MPa for 1–4 min, and stored refrigerated (4°C) or frozen (–10 to –18°C) for 21 days with microbiological analyses at various time intervals. A-formulations (20–46% meat, 42–68% organs, 0.9–1.3% seeds, and 10.7–11.1% fruits, vegetables, and minor ingredients) inoculated with Salmonella and treated at 586 MPa for at least 2 min achieved a 5-log reduction 1 day post-HPP and maintained that inactivation level during frozen storage. A- and S-formulations inoculated with E. coli STEC and treated at 586 MPa for at least 2 min achieved a 5-log reduction from day 6 of frozen storage. L. monocytogenes was more HPP resistant than Salmonella and E. coli STEC. S-formulations containing chicken or beef and stored frozen post-HPP had lower inactivation of L. monocytogenes compared to A-formulations containing chicken or beef. S-Lamb had higher frozen storage inactivation ( $5.95 \pm 0.20$  log CFU/g) compared to chicken ( $2.52 \pm 0.38$  log CFU/g) or beef ( $2.36 \pm 0.48$  log CFU/g). HPP coupled with frozen storage time was effective in achieving and maintaining a 5-log reduction of Salmonella and E. coli STEC while L. monocytogenes was more resistant and requires further optimization to achieve a 5-log reduction. ISSN: 0362028X

**da Silva D.C., Lopes S.M., Tondo E.C.**

*Influence of heat treatments on Salmonella survival in eggnog drink*

(2023) *International Journal of Gastronomy and Food Science*, 33, art. no. 100792

ABSTRACT: Eggnog is a well-known creamy drink prepared with sugar, milk, spices, egg yolks, and in some cases added alcoholic beverages. However, concerns were raised about its safety because egg yolk can be contaminated by Salmonella. In this study inoculated egg yolks (8.5 log CFU/ml) were used to prepare eggnog forming a drink with 0.4%ABV., pH 6.31, 7.40 log CFU/ml of Salmonella and tested in three different scenarios: A = without any heat treatment; B = mild heat treatment (until 70 °C); C = high heat treatments (until 80 and 70 °C). Samples were collected at seven times after rum was added at 5 °C, and 24 h at 25 °C. Microbiological results demonstrated that in scenario A, the counts did not show a significant reduction. In scenario B, Salmonella counts were reduced by approximately 2.6 log CFU/ml after the heat treatment. Eggnog prepared according to scenario C demonstrated Salmonella reductions of more than 5 log CFU/ml. After storage at 25 °C for 24 h, Salmonella counts reached 8.39, 8.40 and 4.34 log CFU/ml in scenarios A, B and C, respectively. Based on the results, we suggest the safe way to consume the eggnog drink is to purchase eggs that came from industries inspected and supervised by regulatory agencies, to prepare it with high heat treatment and to consume it immediately. ISSN: 1878450X

**Nuanmuang N., Leekitcharoenphon P., Njage P.M.K., Gmeiner A., Aarestrup F.M.**

*An Overview of Antimicrobial Resistance Profiles of Publicly Available Salmonella Genomes with Sufficient Quality and Metadata*

(2023) *Foodborne Pathogens and Disease*, 20 (9), pp. 405 - 413

ABSTRACT: Salmonella enterica (S. enterica) is a commensal organism or pathogen causing diseases in animals and humans, as well as widespread in the environment. Antimicrobial resistance (AMR) has increasingly affected both animal and human health and continues to raise public health concerns. A decade ago, it was estimated that the increased use of whole genome sequencing (WGS) combined with sharing of public data would drastically change and improve the surveillance and understanding of Salmonella epidemiology and AMR. This study aimed to evaluate the current usefulness of public WGS data for Salmonella surveillance and to investigate the associations between serovars, antibiotic resistance genes (ARGs), and metadata. Out of 191,306 Salmonella genomes deposited in European Nucleotide Archive and NCBI databases, 47,452 WGS with sufficient minimum metadata (country, year, and source) of S. enterica were retrieved from 116 countries and isolated between 1905 and 2020. For in silico analysis of the WGS data, KmerFinder, SISTR, and ResFinder were used for species, serovars, and AMR identification, respectively. The results showed that the five common isolation sources of S. enterica are human (29.10%), avian (22.50%), environment (11.89%), water (9.33%), and swine (6.62%). The most common ARG profiles for each class of antimicrobials are  $\beta$ -lactam (blaTEM-1B; 6.78%), fluoroquinolone [(parC[T57S], qnrB19); 0.87%], folate pathway antagonist (sul2; 8.35%), macrolide [mph(A); 0.39%], phenicol (floR; 5.94%), polymyxin B (mcr-1.1; 0.09%), and tetracycline [tet(A); 12.95%]. Our study reports the first overview of ARG profiles in publicly available Salmonella genomes from online databases. All data sets from this study can be searched at Microreact. ISSN: 15353141

**Bernad-Roche M., Marín-Alcalá C.M., Vico J.P., Mainar-Jaime R.C.**

*Salmonella Control in Fattening Pigs through the Use of Esterified Formic Acid in Drinking Water Shortly before Slaughter*

(2023) *Animals*, 13 (18), art. no. 2814

**ABSTRACT:** The presence of *Salmonella* in pig feces is a major source of abattoir and carcass contamination, and one of the main sources of human salmonellosis. This study assessed whether using a form of esterified formic acid (30% formic acid) in drinking water (10 kg/1000 L) 5 days before slaughter could be a helpful strategy to mitigate this public health issue. Thus, 240 pigs from three *Salmonella*-positive commercial fattening farms were selected. From each farm, 40 pigs were allocated to a control group (CG) and 40 to a treatment group (TG). At the abattoir, fecal samples from both groups were collected for *Salmonella* detection (ISO 6579-1:2017) and quantification (ISO/TS 6579-2:2012). *Salmonella* was present in 35% (95% IC = 29.24–41.23) of the samples collected. The prevalence was significantly higher in the CG than in the TG (50% vs. 20%;  $p < 0.001$ ). In all farms, the TG showed a lower percentage of shedders than the CG. A random-effects logistic model showed that the odds of shedding *Salmonella* were 5.63 times higher (95% CI = 2.92–10.8) for the CG than for the TG. Thus, the proportion of pigs shedding *Salmonella* that was prevented in the TG due to the use of this form of organic acid was 82.2%. In addition, a Chi-squared analysis for trends showed that the higher the *Salmonella* count, the higher the odds of the sample belonging to the CG. These results suggest that adding this type of acid to drinking water 5 days before slaughter could reduce the proportion of *Salmonella*-shedding pigs and the *Salmonella* loads in the guts of shedder pigs. ISSN: 20762615

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*Synergy between polypyrrol and benzoic acid against antibiotic-resistant Salmonella spp* (2023) *Journal of applied microbiology*, 134 (9)

**ABSTRACT: AIMS:** The purpose was to characterize *Salmonella* Heidelberg (SH) and Minnesota (SM) isolates in terms of their resistance and persistence profile and to assess the antimicrobial effect of benzoic acid (BA) and polypyrrole (PPy). **METHODS AND RESULTS:** The 20 isolates from broiler litter drag swabs were submitted to antibiogram and efflux pump expression. The minimum inhibitory/bactericidal concentration (MIC/MBC) of the compounds, synergistic activity, time kill, biofilm production, presence of related genes, and molecular docking between compounds and bacterial target sites were evaluated. All isolates showed multidrug resistance (MDR) and BA and PPy showed mean MIC (1750 and 342  $\mu\text{g ml}^{-1}$ ) and MBC (3167 and 1000  $\mu\text{g ml}^{-1}$ ), respectively. None of the isolates expressed an efflux pump. The compounds showed synergism against an SH isolate and reduced the count by 3 logs in the presence of the compounds after 4 h. Most isolates (16/20) produced weak to moderate biofilm and 17 showed genes related to biofilm. The compounds interacted with two essential proteins, 3,4-dihydroxy-2-butanone 4-phosphate synthase proteins and ferritin-like domain-containing protein, in bacterial metabolism at different target sites. **CONCLUSIONS:** It can be concluded that BA and PPy showed activity on SH and SM, MDR, and biofilm producers, with a potential synergistic effect. ISSN: 13652672