

NEWSLETTER

European Union Reference Laboratory for *Salmonella*

Vol. 30 No. 2
June 2024

ISSN 2211-6877



Continuation of Newsletter Community Reference Laboratory for *Salmonella*
ISSN 1572-3836

Produced by

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This activity is co-funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the granting authority European Health and Digital Executive Agency (HaDEA). Neither the European Union nor the granting authority can be held responsible for them.



**Co-funded by
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Editorial Note

Bilthoven, 2 July 2024

Dear colleagues,

It was so nice to meet many of you in person again at the **29th EURL-*Salmonella* workshop**, which we organised as hybrid meeting in Leiden, the Netherlands on 28 and 29 May 2024! After 4 years of only online meetings, it was really nice to shake hands again and to have real life discussions in a meeting room. It was a challenge to organise the workshop as a hybrid meeting, with approx. 43 participants onsite and 32 online, but luckily we were well supported by an IT organization. When looking at the evaluation of the workshop, I am happy to notice that the majority of participants was also satisfied with the workshop. The presentations and group picture of the workshop were published at our website shortly after the meeting and can be found at the following link: <https://www.eurlsalmonella.eu/workshop-2024>

At the workshop you were also informed by Kris de Smet of DG SANTE about the proposal of the European Commission to make **WGS analysis**, and reporting to EFSA, mandatory of at least one (pre-screened) isolate from sampling of food, animals, feed or related environment within the frame of (a suspicion of) a foodborne outbreak. In relation to this, DG SANTE asked for additional information on costs, experiences and capacity of WGS analyses at the NRLs-*Salmonella*. For this, we sent out a short questionnaire from mid-April to the end of May 2024. In total 16 different NRLs-*Salmonella* of 14 different countries completed this questionnaire and we have sent a summary of the outcome to DG SANTE early June 2024. Thank you very much for your cooperation!

Also in relation to WGS, we organised a **joint-EURLs training course on NGS** for 28 participants of 8 EURL/NRL networks at the premises of the EURL-*Campylobacter* and EURL-foodborne viruses in Uppsala, Sweden on 25 and 26 June 2024. The training was well received and the presentations were shared with the participants and will also soon become available through the websites of the relevant EURLs.

In the previous Newsletter, I informed you that (finally) the Grant Agreement for the EURL-*Salmonella* activities 2023-2024 was signed by all parties early 2024. Only a few months later, we now have also received the **call for submission** of the proposal for the **activities of EURL-*Salmonella* for 2025-2027**. Currently, we are busy with describing the workplan and estimating the budget for this 3 years' period to make sure to submit it all before the end of August in the portal of HaDEA.

To keep an overview on the EURL-*Salmonella* activities of this year, we include again the table with main activities performed and planned in 2024 in this Newsletter.

As usual, an important activity is the organisation of the EURL-*Salmonella* Proficiency Tests (PTs):

Very recently, the interim summary report of the **WGS cluster analysis** part of the **2023 PT on typing of *Salmonella*** was shared with the participants. This report is also available at the EURL-*Salmonella* website:

<https://www.eurlsalmonella.eu/documenten/interim-summary-report-eurl-salmonella-pt-cluster-analysis-2023>

In February-March 2024, the **2024 PT on the detection of *Salmonella* in live bivalve molluscs (LBM)** was organised. We were happy to notice that all participants scored a good performance in this PT. The individual results as well as the interim summary of this PT was sent to the participants early May 2024. The interim summary is also available at the EURL-*Salmonella* website through the following link: <https://www.euralsalmonella.eu/media/3881>

Currently we are busy with the preparation of the **combined PT for NRLs-*Salmonella* for Food and for primary production stage (PPS)**. This PT will focus on the detection of *Salmonella* in environmental samples from the food and animal production and will be organised in September/October 2024. As indicated before in the Newsletter and at the workshop, this PT will not only be a combined PT for the matrix, but it will also be combined for testing the performance of the method ('interlaboratory study'/ILS). It is planned to also use the results of this PT-ILS for the generation of (missing) performance characteristics of ISO 6579-1. You will soon be informed about the registration for this combined PT-ILS. The timetable for this combined PT-ILS is included in this Newsletter.

In November, the **2024 PT on typing of *Salmonella*** will be organised. Like in former years, this study will contain an obligatory part on serotyping of *Salmonella*, and a voluntary part on NGS cluster analysis. The timetable for this PT is also included in this Newsletter.

From 10 until 14 June 2024, **the annual meetings of CEN/TC463 and ISO/TC34/SC9** (Microbiology of the Food chain) were organised. This year's meeting was organised as an hybrid meeting in St. Louis, USA. As convener of an ISO Ad hoc group (AHG 'Guidance document for drafting ISO/CEN standards') and of two *Salmonella* ISO working groups for detection (WG9) and typing of *Salmonella* (WG10), the EURL-*Salmonella* participated (online) in these annual meetings and presented the progress of the AHG and WGs. The progress in the two ISO working groups for *Salmonella* is summarized below.

ISO/TC34/SC9-WG9 ('Detection of *Salmonella*'). The next steps for WG9 are:

- Organisation of PT-ILS for detection of *Salmonella* in environmental samples in September/October 2024. Results are expected at the beginning of 2025.
- Analysis of literature data for performance characteristics of remaining categories. Results are expected at the beginning of 2025.
- Discussion with a WG9-subgroup on the set-up of interlaboratory studies for validation of larger test portion sizes (approx. September 2024).
- The 4th meeting WG9 is planned approx. February/March 2025.

ISO/TC34/SC9-WG10 ('Typing of *Salmonella*'). This working group is in charge of the development of ISO 6579-4 ('Identification of monophasic *Salmonella* Typhimurium by PCR'). The Draft International Standard, **ISO/DIS 6579-4**, was published in December 2023, for voting and comments until 15 March 2024. The results of this ballot was 100% approval in CEN as well as in ISO. In total, 19 pages of comments (mainly editorial) were received. In March-April 2024, draft observations were given to the comments of ISO/DIS 6579-4 and draft ISO/FDIS 6579-4 was prepared. These documents were discussed at the 5th meeting (online) of ISO-WG10 on 23 April 2024. After this meeting, the observations to the comments as well as ISO/FDIS 6579-4 were finalized and submitted to the committee manager of SC9 for launching the FDIS-ballot. It is expected that the FDIS ballot of ISO 6579-4 will soon be launched.

Finally, I would like to wish you all a nice, sunny and relaxing summer period and hopefully you will have the opportunity to take a good rest!

Best wishes,
Kirsten Mooijman
Coordinator EURL-*Salmonella*

Contribution of the EURL-*Salmonella*

EURL- *Salmonella* activities in 2024

Date(s)	Activity
26 February – 22 March 2024	EURL- <i>Salmonella</i> Proficiency Test Live Bivalve Molluscs; Detection of <i>Salmonella</i> in mussels.
28 – 29 May 2024	EURL- <i>Salmonella</i> workshop in Leiden, the Netherlands.
June 2024	Joint Training Course of the inter EURLs Working Group on NGS in Sweden.
September – October 2024	Combined EURL- <i>Salmonella</i> Proficiency Test PPS-Food.
November 2024 – January 2025	EURL- <i>Salmonella</i> Proficiency Test Typing; Serotyping and (optional) NGS Cluster analysis.

**Timetable EURL-*Salmonella* combined PT-ILS
Primary Production Stage (PPS) and Food 2024.
Detection of *Salmonella* in fabric swabs.**

Week	Date	Subject
28-35		E-mailing the link to the registration form for the Proficiency Test. Please register by 31 August 2024 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.
40	Monday 30 September 2024	Shipment of the parcels to the participants as Biological Substance Category B (UN 3373).
40	Wednesday 2 October 2024	Start performance of the combined PT-ILS PPS-Food. In case of late arrival of the parcel, start performance of the combined PT-ILS immediately after arrival of the parcel.
44	Friday 1 November at the latest	Deadline for completing the result form: 1 November 2023 (23:59h CET) After this deadline the result form will be closed.
	December 2024	Interim summary report

If you have questions or remarks about this combined PT-ILS, please contact:

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RIVM / Z&O (internal Pb 63) EURL- *Salmonella*

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Timetable EURL- *Salmonella* Proficiency Test Typing 2024 Serotyping and optional part NGS Cluster analysis

Week	Date	Subject
38	Week of 16 September	Emailing of the link to the registration form for the PT Typing. Please register by 18 October 2024 at the latest.
43	Week of 21 October	Emailing of the protocol 2024.
45	Monday 4 November 2024	Shipment of the parcels to the participants as Biological Substance Category B (UN 3373).
45	Week of 4 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	Friday 13 December 2024 at the latest	Deadline for completing the electronic submission of Serotyping results: 13 December 2024. After this deadline, the result form for serotyping will be closed.
5	Friday 31 January 2025 at the latest	Deadline for completing the electronic submission of NGS Cluster Analysis results: 31 January 2025.
	February 2025	Serotyping: Evaluation of individual laboratory results and Interim summary report.
	April/May 2025	NGS Cluster Analysis: Evaluation of results and Interim summary report.

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

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<http://www.eurlsalmonella.eu/>

From the Literature

Salmonella-related Literature selection from Scopus: April – June 2024

Altissimi C., Primavilla S., Roila R., Gavaudan S., Morandi B., Di Lullo S., Coppini M., Baldinelli C., Cai D., Branciaro R., Valiani A., Paulsen P., Ranucci D.

Salmonella in Wild Boar Meat: Prevalence and Risk Assessment in Central Italy (Umbria and Marche Region)

(2024) *Foods*, 13 (8), art. no. 1156

ABSTRACT: A survey was conducted from 2018 to 2023 to assess the presence of *Salmonella* in 280 hunted wild boar (carcasses after evisceration and skinning, N = 226; liver, N = 258; and fecal samples, N = 174). The overall prevalence was 2.86% (confidence interval 95%, 1.45–5.45%) with five positive samples detected in carcasses, three in the liver, and one in a fecal sample. This prevalence was in line with those found in nearby areas denoting a low number of positive samples. Positive animals were over 24 months of age and weighed, before skinning, 59.00 ± 9.11 Kg and no difference was detected in microbial loads between samples positive and negative for *Salmonella* (aerobic colony count of 4.59 and 4.66 log CFU/400 cm², and Enterobacteriaceae count of 2.89 and 2.73 log CFU/400 cm² (mean values) in positive and negative subjects, respectively). *Salmonella* Stanleyville was the most frequently isolated serotype. A semiquantitative risk assessment was conducted for the first time in game meat considering two products, meat cuts intended for cooking and fermented dry sausages. Only proper cooking can reduce the risk of ingestion of *Salmonella* to the minimum for consumers, whereas ready-to-eat dry sausages constitute risk products in terms of foodborne Salmonellosis (risk score of 64 out of 100). ISSN: 23048158

Duggan K., Shepherd M., Maillard J.-Y.

Susceptibility of Salmonella enterica Typhimurium dry surface biofilms to disinfection

(2024) *Journal of Food Safety*, 44 (2), art. no. e13117

ABSTRACT: In food preparation and manufacturing environments, surfaces contaminated with *Salmonella* can lead to outbreaks of Salmonellosis. We hypothesise that *Salmonella* resides on dry surfaces in a biofilm form leading to potential environmental persistence and transfer following contact. This is the first study reporting that *Salmonella* Typhimurium can form dry surface biofilm (DSB). Six disinfectants commonly used in the food industry were evaluated for their efficacy against the DSB. The two most efficacious formulations reduced bacterial viability in DSB by >99.99% when combined with mechanical removal (5 sec wiping; 300 g weight). Five out of six formulations significantly reduced bacterial transfer when combined with wiping. Complete eradication of *Salmonella* Typhimurium DSB was challenging, and mechanical removal was essential to produce a >99.99% reduction in bacterial viability within DSB. This study highlights a potential mode of survival of *Salmonella* Typhimurium on food-contact surfaces and DSB challenges for disinfection. ISSN: 01496085

Petrin S., Tiengo A., Longo A., Furlan M., Marafin E., Zavagnin P., Orsini M., Losasso C., Barco L.

Uncommon Salmonella Infantis Variants with Incomplete Antigenic Formula in the Poultry Food Chain, Italy

(2024) *Emerging Infectious Diseases*, 30 (4), pp. 795 - 799

ABSTRACT: Uncommon *Salmonella* Infantis variants displaying only flagellar antigens phenotypically showed identical incomplete antigenic formula but differed by molecular serotyping. Although most formed rough colonies, all shared antimicrobial resistances and the presence of *usg* gene with wild-type *Salmonella* Infantis. Moreover, they were undistinguishable wild-type *Salmonella* Infantis by whole-genome sequencing. ISSN: 10806040

An J.-H., Lee H.-S.

Effect of the storage temperature on the quality of eggs inoculated with Salmonella Enteritidis onto shell

(2024) *Food Science and Biotechnology*, 33 (5), pp. 1255 - 1260

ABSTRACT: This study explored the temperature-dependent effect on the growth characteristics of *Salmonella* Enteritidis (SE) on eggshell toward identifying an appropriate storage temperature for unwashed eggs in an actual distribution environment. Among the

test storage temperatures (10 °C, 25 °C, and 35 °C), 25 °C was determined to be an appropriate storage temperature, with no effect of changing temperature on the control of SE on eggshell. Regarding the effect of the temperature on egg quality, the quality indicators of egg such as Haugh unit, yolk index, albumin index, and albumin pH were significantly maintained. These results indicated that unwashed eggs should be distributed at 25 °C for SE control, and the storage temperature should be below 10 °C from at least day 4 onward after the start of distribution to maintain egg quality. This study will assist for safety management of unwashed egg in an actual distribution environment.
ISSN: 12267708

Gędas A., Schmidt H., Weiss A.

Suitability of Escherichia coli ATCC 11229 as Salmonella enterica surrogate for strawberry nectar pasteurization
(2024) *LWT*, 198, art. no. 116056

ABSTRACT: Heat resistance, D- and z-values of five *Salmonella enterica* strains, namely S. Senftenberg LTH 5703, S. Typhimurium ATCC 13311 and ATCC 14028, S. Saintpaul LTH 6494, and S. Enteritidis ATCC 13076, as well as of a *Salmonella* cocktail and three potential surrogate strains were investigated in phosphate buffered saline and strawberry nectar (12° Brix). Thermal inactivation was performed at 60 °C, 65 °C, and 72 °C. Moreover, the influence of pre-incubation under stressful environmental conditions on the heat resistance was tested. The results identified *Escherichia coli* ATCC 11229 as a suitable surrogate candidate for *Salmonella* strains, as its calculated D-values in both matrices were higher or statistically the same than those of the examined pathogens. The safety analysis showed no virulence factors that could classify *E. coli* ATCC 11229 as a pathogen. Furthermore, the results show significant differences in D-values of all strains in different matrices, which indicates a clear influence of the environment on heat resistance of bacteria. The pre-incubation under stress conditions had no significant effect on the heat resistance of *E. coli* ATCC 11229. However, further research is needed to explore the influence of stress conditions and duration on bacterial heat resistance. ISSN: 00236438

Borovikov S., Kuibagarov M., Akibekov O., Muranets A.

Clinical Case of Salmonella Detected in an Aborted Mare Fetus and its Characteristics
(2024) *International Journal of Veterinary Science*, 13 (3), pp. 357 - 361

ABSTRACT: In articles and reports by researchers from different countries, *Salmonella enterica* subsp. *enterica* serovar *Abortus equi* is often cited as the cause of abortion in mares. Here we report a clinical case of *Salmonella* isolated from an aborted mare fetus, and the results of its typing and examination of antibiotic resistance. Abortions were reported in a herd of local Jabe horses. Vaginal smears and internal organ (heart, spleen) samples of an aborted horse fetus from a farm in the Karaganda region were obtained. The mare aborted in the stage of deep pregnancy, and the fetus that formed had fur and hooves. Three different isolates were obtained and classified as *Salmonella*. The identical antimicrobial resistance pattern and sequencing type were seen in all three samples. The isolates were subsequently genotyped and found to be 100% identical to *S. enterica* subsp. *enterica*. Based on the results of bacteriological and molecular genetic analyses of cultures isolated from the samples, it was established that the cause of abortion in mares was an infection related to salmonella etiology and was caused by the pathogen *S. enterica* subsp. *enterica* serovar *Enteritidis*. ISSN: 23043075

Jacqueline C., Samper-Cativiela C., Fernandez S.M., Ugarte-Ruiz M., Plaza I.C.D.L., Alvarez J., Herrera-Leon S.

Phenotypic and genetic characterization of antimicrobial resistance in Salmonella enterica serovar Choleraesuis isolates from humans and animals in Spain from 2006 to 2021
(2024) *Journal of Antimicrobial Chemotherapy*, 79 (4), pp. 790 - 800

ABSTRACT: Objectives: While an increase in the levels of MDR in *Salmonella enterica* serovar *Choleraesuis* has been reported in Europe, little is known about the situation in Spain. Therefore, we first aimed to assess the phenotypic resistance profile and to determine the presence of genetic determinants of resistance of *S. Choleraesuis* isolates collected in animal and human. Our second objective was to identify and characterize clusters of highly related isolates. Methods: We analysed 50 human and 45 animal isolates retrieved from 2006 to 2021 using the disc diffusion method and performed WGS followed by analyses of genetic determinants and phylogenetic analysis. Results: All isolates were of ST145 and corresponded to the variant Kunzendorf. Swine isolates harboured a significantly higher number of antimicrobial resistance genes than human isolates, and often carried plasmid replicons of the IncHI2/IncHI2A type (42% of all animal isolates). In addition, we identified several MDR *S. Choleraesuis* strains circulating in humans and swine between 2006 and 2021. The phylogenetic analyses identified four clades associated with

specific patterns of resistance genes and plasmid replicons. The clades also included isolates that differed in terms of year and region of isolation as well as host of origin. Conclusions: This One Health approach highlights that reducing human MDR *S. Choleraesuis* infections may require the adoption of strategies that not only seek to prevent cases in humans but also to characterize and reduce the infection burden in swine. ISSN: 03057453

Maćkiw E., Kowalska J., Korsak D., Stasiak M., Antoszevska A., Ławrynowicz-Paciorek M., Postupolski J.

Thermal resistance of selected strains of Salmonella spp. isolated from eggs and sesame seeds

(2024) *LWT*, 198, art. no. 115907

ABSTRACT: In this study, we investigated the effect of food matrices on the heat tolerance of *Salmonella* strains and evaluated the impact of sugar and fat on the heat resistance of these strains. We tested ten strains of *Salmonella* spp. isolated from eggs and ten from sesame seeds. The conducted research revealed that *S. Poona* 4193 and *S. Oranienburg* 4182, isolated from sesame seeds, exhibited the lowest average decimal reduction times (D-values) at 60 °C and 65 °C. Conversely, strains of *S. Enteritidis* 3927 (eggs), and *S. Mbandaka* 4297 (sesame seeds), demonstrated the highest D-values. In the next stage we examined the effect of different concentrations of sucrose (5%, 10%, 15%) and sesame oil (10%, 25%, 50%) on the tolerance of *Salmonella* to high temperatures. We then selected specific concentrations of sucrose and oil in the medium for each strain based on the highest D-value obtained. The 4297 strain exhibited the highest D-value with 5% sucrose and 10% oil, 4193 strains with 10% sucrose and 25% oil, 4182 with 10% sucrose and 10% oil, and 3927 strain with 10% sucrose, and 50% oil. Our studies indicated that the heat resistance of *Salmonella* is highly dependent on the specific strain. ISSN: 00236438

Mattock J., Chattaway M.A., Hartman H., Dallman T.J., Smith A.M., Keddy K., Petrovska L., Manners E.J., Duze S.T., Smouse S., Tau N., Timme R., Baker D.J., Mather A.E., Wain J., Langridge G.C.

A One Health Perspective on Salmonella enterica Serovar Infantis, an Emerging Human Multidrug-Resistant Pathogen

(2024) *Emerging Infectious Diseases*, 30 (4), pp. 701 - 710

ABSTRACT: *Salmonella enterica* serovar *Infantis* presents an ever-increasing threat to public health because of its spread throughout many countries and association with high levels of antimicrobial resistance (AMR). We analyzed whole-genome sequences of 5,284 *Salmonella Infantis* strains from 74 countries, isolated during 1989-2020 from a wide variety of human, animal, and food sources, to compare genetic phylogeny, AMR determinants, and plasmid presence. The global *Salmonella Infantis* population structure diverged into 3 clusters: a North American cluster, a European cluster, and a global cluster. The levels of AMR varied by *Salmonella Infantis* cluster and by isolation source; 73% of poultry isolates were multidrug resistant, compared with 35% of human isolates. This finding correlated with the presence of the pESI megaplasmid; 71% of poultry isolates contained pESI, compared with 32% of human isolates. This study provides key information for public health teams engaged in reducing the spread of this pathogen. © 2024 Centers for Disease Control and Prevention (CDC). ISSN: 10806040

Jones S.L., Chandran S., Gibson K.E.

Impact of food residue on recovery of Listeria monocytogenes and Salmonella Typhimurium from stainless steel surface

(2024) *Journal of Food Safety*, 44 (2), art. no. e13125

ABSTRACT: This study evaluated the impact of different food residues on the recovery of *Listeria monocytogenes* and *Salmonella Typhimurium* from stainless steel surfaces. Food residues tested include lettuce rinsate, blended lettuce, low-fat milk, and whole milk for *Listeria monocytogenes*, and powdered infant formula, all-purpose flour, and whole milk dairy powder for *Salmonella Typhimurium*. Bacterial suspensions were inoculated on stainless steel surfaces with or without food residues and held for different time periods. Significant differences ($P < 0.0001$) in *Salmonella Typhimurium* recovery were observed between the no food residue control and all food residues over 24 hours. For *Listeria monocytogenes*, minimal variability in recovery was observed among food residue types, with significant differences from the no food residue control ($P < 0.05$) observed after 24 hours. The study also found that surface sampling can spread *Listeria monocytogenes* and *Salmonella Typhimurium* on stainless steel surfaces, suggesting that food residue type may affect microbial recovery during environmental monitoring. ISSN: 01496085

Hobbelen P., Hagens T., Markovich M.P., Bellaiche M., Giovannini A., De Massis F., de Koeijer A.

The optimisation of Salmonella surveillance programmes for pullet and layer farms using local farm density as a risk factor

(2024) *PLoS ONE*, 19 (4), art. no. e0291896

ABSTRACT: Human salmonellosis cases are often caused by *Salmonella* serovars Enteritidis and Typhimurium and associated with the consumption of eggs and egg products. Many countries therefore implemented general surveillance programmes on pullet and layer farms. The identification of risk factors for *Salmonella* infection may be used to improve the performance of these surveillance programmes. The aims of this study were therefore to determine 1) whether local farm density is a risk factor for the infection of pullet and layer farms by *Salmonella* Enteritidis and Typhimurium and 2) whether the sampling effort of surveillance programmes can be reduced by accounting for this risk factor, while still providing sufficient control of these serovars. We assessed the importance of local farm density as a risk factor by fitting transmission kernels to Israeli surveillance data during the period from June 2017 to April 2019. The analysis shows that the risk of infection by serovars Enteritidis and Typhimurium significantly increased if infected farms were present within a radius of approximately 4 km and 0.3 km, respectively. We subsequently optimized a surveillance programme that subdivided layer farms into low and high risk groups based on the local farm density with and allowed the sampling frequency to vary between these groups. In this design, the pullet farms were always sampled one week prior to pullet distribution. Our analysis shows that the risk-based surveillance programme is able to keep the between-farm R0 of serovars Enteritidis and Typhimurium below 1 for all pullet and layer farms, while reducing the sampling effort by 32% compared to the currently implemented surveillance programme in Israel. The results of our study therefore indicate that local farm density is an important risk factor for infection of pullet and layer farms by *Salmonella* Enteritidis and Typhimurium and can be used to improve the performance of surveillance programmes.

FDM, MB and AK were funded in the context of the EU Animal Health and Welfare (ANIHWA) ERA-Net initiative via the Epi-Risk Project (<https://www.anihwa.eu/>). The ANIHWA funds for AK were provided by the Dutch Ministry of Agriculture, Nature and Food Quality via project number BO-43-013.04-001 (<https://www.government.nl/ministries/ministry-of-agriculture-nature-and-food-quality>). In addition, FDM was co-funded by Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Teramo, Italy (<https://www.izs.it/IZS/>). MB was co-funded by the Kimron Veterinary Institute, Beit Dagan, Israel (<https://www.gov.il/en/departments/Units/2vet>). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. ISSN: 19326203

Lin A., Singh A., Allred A., Allard M., Waltman D., Imanian B., Ng J.H.J., Sanahmadi Y., Khaksar R.

Targeted Next-Generation Sequencing Assay for Direct Detection and Serotyping of Salmonella from Enrichment

(2024) *Journal of Food Protection*, 87 (4), art. no. 100256

ABSTRACT: In this study, an automated, targeted next-generation sequencing (tNGS) assay to detect and serotype *Salmonella* from sample enrichments was evaluated. The assay generates millions of reads to detect multiple *Salmonella*-specific genes and serotype-specific alleles, detecting all *Salmonella* spp. tested to date, and serotyping 62 common *Salmonella* serotypes. Accuracy was tested on 291 pure reference cultures (251 *Salmonella*, 40 non-*Salmonella*), 21 artificially contaminated poultry carcass rinse samples, and 363 naturally contaminated poultry environmental samples. Among the 291 pure reference cultures, the automated tNGS assay resulted in 100% detection accuracy, 100% serotyping accuracy for the claimed serotypes, and 0% false positives. The limit of detection was estimated at 5×10^4 CFU/mL by testing enumerated cultures of strains representative of six serotypes. In cocontamination studies with mixtures of two serotypes (Enteritidis, Typhimurium, Kentucky, Infantis, and Newport) at a 1:1 ratio, tNGS detected both serotypes with 100% accuracy. The assay demonstrated 100% accuracy in artificially contaminated poultry carcass rinse sample enrichments. Targeted NGS was highly effective in detecting *Salmonella* in samples collected from poultry production facilities. Results demonstrated that tNGS could detect *Salmonella* and provide accurate serotyping information consistent with conventional serology. These findings highlight the reliable and efficient performance of a fully automated tNGS *Salmonella* assay in detecting and identifying *Salmonella* strains in complex matrices, reducing the time to results from 4 to 5 days required by the traditional isolation and serotyping to 10–12 h for tNGS after primary enrichment. ISSN: 0362028X

Ribeiro-Almeida M., Mourão J., Magalhães M., Freitas A.R., Novais C., Peixe L., Antunes P.

Raw meat-based diet for pets: a neglected source of human exposure to Salmonella and pathogenic Escherichia coli clones carrying mcr, Portugal, September 2019 to January 2020

(2024) *Eurosurveillance*, 29 (18), art. no. .2300561

ABSTRACT: Background: The pet industry is expanding worldwide, particularly raw meat-based diets (RMBDs). There are concerns regarding the safety of RMBDs, especially their potential to spread clinically relevant antibiotic-resistant bacteria or zoonotic pathogens. Aim: We aimed to investigate whether dog food, including RMBD, commercially available in Portugal can be a source of *Salmonella* and/or other Enterobacteriaceae strains resistant to last-line antibiotics such as colistin. Methods: Fifty-five samples from 25 brands (21 international ones) of various dog food types from 12 suppliers were screened by standard cultural methods between September 2019 and January 2020. Isolates were characterised by phenotypic and genotypic methods, including whole genome sequencing and comparative genomics. Results: Only RMBD batches were contaminated, with 10 of 14 containing poly-clonal multidrug-resistant (MDR) *Escherichia coli* and one MDR *Salmonella*. One turkey-based sample contained MDR *Salmonella* serotype 1,4,[5],12:i:- ST34/cgST142761 with similarity to human clinical isolates occurring worldwide. This *Salmonella* exhibited typical antibiotic resistance (blaTEM + strA-strB+ sul2+ tet(B)) and metal tolerance profiles (pco+ sil+ ars) associated with the European epidemic clone. Two samples (turkey/veal) carried globally dispersed MDR *E. coli* (ST3997-complexST10/cgST95899 and ST297/ cgST138377) with colistin resistance (minimum inhibitory concentration: 4 mg/L) and mcr-1 gene on IncX4 plasmids, which were identical to other IncX4 circulating worldwide. Conclusion: Some RMBDs from European brands available in Portugal can be a vehicle for clinically relevant MDR *Salmonella* and pathogenic *E. coli* clones carrying genes encoding resistance to the last-line antibiotic colistin. Proactive actions within the One Health context, spanning regulatory, pet-food industry and consumer levels, are needed to mitigate these public health risks. ISSN: 1025496X

Dishan A., Hizlisoy H., Onmaz N.E., Yildirim Y., Gonulalan Z., Al S.

Comprehensive analysis of Salmonella in poultry meat and products in Türkiye: Prevalence, antibiotic susceptibility and genomic characterisation

(2024) *International Journal of Food Science and Technology*, 59 (5), pp. 3412 - 3422

ABSTRACT: The study aimed to determine the prevalence and characteristics of *Salmonella* isolated from raw chicken meat and products. For this purpose, a total of 293 samples were collected, including chicken breast (n = 90), skinned drumstick (n = 80), skinned chicken chop (n = 42), wing (n = 32), chicken offal (n = 27) and chicken patty (n = 22). The samples were subjected to *Salmonella enterica*. detection and the obtained suspicious isolates were confirmed by conventional PCR. Their phenotypical antibiotic resistance profiles were subsequently determined. The prevalence of *Salmonella* Enteritidis and Typhimurium serovars among *S. enterica* isolates were investigated using TaqMan probe Real-Time PCR (qPCR) analysis, and the detected serovars were evaluated with whole genome sequencing. In the study, 112 (38.22%) of the 293 chicken samples contained *S. enterica*, with five (4.46%) and one (0.89%) of the isolates identified as *Salmonella* Enteritidis and Typhimurium, respectively. Antibiotic resistance analysis revealed that all isolates were sensitive to Meropenem and Aztreonam, while the most resistant antibiotics were Doxycycline (96.42%) and Trimethoprim-sulfamethoxazole (71.42%). Whole genome sequencing, specifically SNP-based phylogenetic analyses, indicated that *Salmonella* Enteritidis and Typhimurium isolates were distinct clones. All *Salmonella* Enteritidis isolates shared the same antigenic profiles (9: g, m: -) and cgMLST types of 11, while the *Salmonella* Typhimurium isolate had cgMLST type 19 and a 4:i:1,2 antigenic profile. It was observed that the phenotypic resistance profiles of the isolates were consistent with the whole genome characterisation. The data obtained in the study reveal the continued importance of *Salmonella* monitoring for the poultry industry across different regions of Türkiye to maintain food safety. Chicken meat and products are indispensable to public health in providing healthy nutrition and access to animal protein. The microbiological and epidemiological risks observed in mass production can be minimised, particularly by integrating epidemiological and molecular findings with an effective strategy. ISSN: 09505423

McGeoch L.J., Hoban A., Sawyer C., Rabie H., Painset A., Browning L., Brown D., Mccarthy C., Nelson A., Firme A., Pista Â., Moreno J., Martins J.V., Silveira L., Machado J., Vasconcelos P., Olufon O., Inzungou-Massanga C., Douglas A., McCormick J., Larkin L., Balasegaram S.

Salmonella Saintpaul outbreak associated with cantaloupe consumption, the United Kingdom and Portugal, September to November 2023
(2024) *Epidemiology and Infection*, 152, art. no. e78

ABSTRACT: In September 2023, the UK Health Security Agency identified cases of *Salmonella* Saintpaul distributed across England, Scotland, and Wales, all with very low genetic diversity. Additional cases were identified in Portugal following an alert raised by the United Kingdom. Ninety-eight cases with a similar genetic sequence were identified, 93 in the United Kingdom and 5 in Portugal, of which 46% were aged under 10 years. Cases formed a phylogenetic cluster with a maximum distance of six single nucleotide polymorphisms (SNPs) and average of less than one SNP between isolates. An outbreak investigation was undertaken, including a case-control study. Among the 25 UK cases included in this study, 13 reported blood in stool and 5 were hospitalized. One hundred controls were recruited via a market research panel using frequency matching for age. Multivariable logistic regression analysis of food exposures in cases and controls identified a strong association with cantaloupe consumption (adjusted odds ratio: 14.22; 95% confidence interval: 2.83-71.43; p-value: 0.001). This outbreak, together with other recent national and international incidents, points to an increase in identifications of large outbreaks of *Salmonella* linked to melon consumption. We recommend detailed questioning and triangulation of information sources to delineate consumption of specific fruit varieties during *Salmonella* outbreaks. ISSN: 09502688

Aarø N.S., Torpdahl M., Rasmussen T., Jensen M., Nielsen H.L.

Salmonella infections in Denmark from 2013–2022 with focus on serotype distribution, invasiveness, age, sex, and travel exposition
(2024) *European Journal of Clinical Microbiology and Infectious Diseases*, 43 (5), pp. 947 - 957

ABSTRACT: Purpose: To analyze the nationwide incidence of *Salmonella* infections in Denmark from 2013 to 2022. Methods: Confirmed cases of *Salmonella* enterica subsp. enterica were examined using the National Register of Enteric Pathogens during 2013–2022. Proportions, incidence rates (IR), relative risk (RR), and 95% confidence intervals (CI) were calculated to assess differences in serotypes, invasiveness, age, sex, and travel exposure. Results: We identified 9,944 Danish *Salmonella* enterica subsp. enterica cases, with an average annual incidence rate of 16.9 per 100,000 inhabitants, declining during the COVID-19 pandemic. Typhoidal cases totaled 206, with an average annual IR of 0.35 per 100,000 inhabitants. Enteric fever patients had a median age of 24 years (IQR: 17–36). Leading non-typhoid *Salmonella* (NTS) serotypes were *S. Enteritidis* (26.4%), monophasic *S. Typhimurium* (16.5%), and *S. Typhimurium* (13.5%). Median age for NTS cases was 42 (IQR: 18–62), with even sex distribution, and a third reported travel prior to onset of disease. The overall percentage of invasive NTS (INTS) infection was 8.1% (CI: 7.6–8.7). Eleven serotypes were associated with higher invasiveness, with *S. Dublin* and *S. Panama* having the highest invasiveness with age and sex-adjusted RR of 7.31 (CI: 6.35–8.43) and 5.42 (CI: 3.42–8.60), respectively, compared to all other NTS serotypes. Increased age was associated with higher RR for INTS infection. Conclusion: During the decade, there was a limited number of typhoidal cases. The dominant NTS serotypes were *S. Enteritidis* and monophasic *S. Typhimurium*, whereas *S. Dublin* and *S. Panama* exhibited the highest invasive potential. ISSN: 09349723

Patà Z., Faré P.B., Lava S.A.G., Milani G.P., Bianchetti M.G., Janett S., Hunjan I., Kottanattu L.

Nontyphoidal Salmonella Outbreaks Associated with Chocolate Consumption: A Systematic Review

(2024) *Pediatric Infectious Disease Journal*, 43 (5), pp. 420 - 424

ABSTRACT: Background: A large, cross-border outbreak of nontyphoidal salmonellosis connected to chocolate product consumption was recently reported. This occurrence motivated us to conduct a comprehensive review of existing literature concerning outbreaks of nontyphoidal salmonellosis associated with chocolate consumption. Methods: We performed a systematic review following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (PROSPERO CRD42022369023) in 3 databases: U.S. National Library of Medicine, Web of Science and Excerpta Medica. Google Scholar and the bibliography of each identified report were also screened. Eligible were articles published after 1970, describing outbreaks of more than 10 patients with a nontyphoidal salmonellosis associated with chocolate consumption. Results: Twenty-three articles were included, which described 12 outbreaks involving a total of 3266 patients. All outbreaks occurred in high-income countries: 1 was limited to 1 city, 6 involved 1 country and the remaining 5 involved 2 or more countries. Six outbreaks peaked in winter, 3 in autumn, 2 in spring and 1 in summer. Children were mainly affected. No predominant

serotype was identified. Conclusions: Our data documents that chocolate is an optimal medium for the transmission of nontyphoidal salmonellosis. A connected worldwide reporting system including high-income, middle-income and low-income countries is crucial to detect infectious diseases outbreaks in an early phase and avoid their spread.
ISSN: 08913668

Ianiro G., Pavoni E., De Sabato L., Monini M., Delibato E., Perrone V., Ostanello F., Niine T., Di Bartolo I.

Investigation of Salmonella, hepatitis E virus (HEV) and viral indicators of fecal contamination in four Italian pig slaughterhouses, 2021–2022
(2024) *Research in Veterinary Science*, 171, art. no. 105209

ABSTRACT: In the pork production chain, the control at slaughterhouse aims to ensure safe food thanks to proper hygienic conditions during all steps of the slaughtering. *Salmonella* is one of the main foodborne pathogens in the EU causing a great number of human cases, and pigs also contribute to its spreading. Pig is the main reservoir of the zoonotic hepatitis E virus (HEV) that can be present in liver, bile, feces and even rarely in blood and muscle. The aim of this study was to assess the presence of both *Salmonella* and HEV in several points of the slaughtering chain, including pig trucks. Other viruses hosted in the gut flora of pigs and shed in feces were also assayed (porcine adenovirus PAdV, rotavirus, norovirus, and mammalian orthoreovirus MRV). Torque teno sus virus (TTSuV) present in both feces, liver and blood was also considered. Four Italian pig abattoirs were sampled in 12 critical points, 5 of which were the outer surface of carcasses before processing. HEV and rotavirus (RVA) were not detected. Norovirus was detected once. *Salmonella* was detected in two of the 4 abattoirs: in the two lairage pens, in the site of evisceration and on one carcass, indicating the presence of *Salmonella* if carcass is improperly handled. The sampling sites positive for *Salmonella* were also positive for PAdV. MRV was detected in 10 swabs, from only two abattoirs, mainly in outer surface of carcasses. TTSuV was also detected in all abattoirs. Our study has revealed a diverse group of viruses, each serving as indicator of either fecal (NoV, RVA, PAdV, MRV) or blood contamination (TTSuV). TTSuV could be relevant as blood contamination indicators, crucial for viruses with a viremic stage, such as HEV. The simultaneous presence of PAdV with *Salmonella* is relevant, suggesting PAdV as a promising indicator for fecal contamination for both bacterial and viruses. In conclusion, even in the absence of HEV, the widespread presence of *Salmonella* at various points in the chain, underscores the need for vigilant monitoring and mitigation strategies which could be achieved by testing not only bacteria indicators as expected by current regulation, but also some viruses (PAdV, TTSuV, MRV) which could represent other sources of fecal contamination. ISSN: 00345288

Gorski L., Shariat N.W., Richards A.K., Siceloff A.T., Aviles Noriega A., Harhay D.M.

Growth assessment of Salmonella enterica multi-serovar populations in poultry rinsates with commonly used enrichment and plating media
(2024) *Food Microbiology*, 119, art. no. 104431

ABSTRACT: Isolation of *Salmonella* from enrichment cultures of food or environmental samples is a complicated process. Numerous factors including fitness in various selective enrichment media, relative starting concentrations in pre-enrichment, and competition among multi-serovar populations and associated natural microflora, come together to determine which serovars are identified from a given sample. A recently developed approach for assessing the relative abundance (RA) of multi-serovar *Salmonella* populations (CRISPR-SeroSeq or Deep Serotyping, DST) is providing new insight into how these factors impact the serovars observed, especially when different selective enrichment methods are used to identify *Salmonella* from a primary enrichment sample. To illustrate this, we examined *Salmonella*-positive poultry pre-enrichment samples through the selective enrichment process in Tetrathionate (TT) and Rappaport Vassiliadis (RVS) broths and assessed recovery of serovars with each medium. We observed the RA of serovars detected post selective enrichment varied depending on the medium used, initial concentration, and competitive fitness factors, all which could result in minority serovars in pre-enrichment becoming dominant serovars post selective enrichment. The data presented provide a greater understanding of culture biases and lays the groundwork for investigations into robust enrichment and plating media combinations for detecting *Salmonella* serovars of greater concern for human health. ISSN: 07400020

Hu L., Zhang G.

Effect of Selective Enrichment Storage Temperature and Duration Time on the Detection of Salmonella in Food
(2024) *Journal of AOAC International*, 107 (3), pp. 471 - 478

ABSTRACT: Background: For pathogen detection in food, there are occasions where samples cannot be processed immediately after selective enrichment or need to be reexamined days or weeks later for confirmation or retest. Objective: This study aimed to investigate the effect of different prolonged period of storage of selective enrichments of food at 4 ± 2 C and room temperature (20–22 C) on the detection and isolation of *Salmonella*. Method: This study included two experiments involving 34 types of foods to compare the effect of 4 ± 2 C and room temperature storage on the detection of *Salmonella* in 204 selective enrichments (Rappaport-Vassiliadis [RV] and Tetrathionate [TT] broths) during a 42-day storage (Experiment I); and to monitor the survival of *Salmonella* in 300 selective enrichments (RV and TT) with different preenrichment broths (Lactose broth [LB] or Buffered peptone water broth [BPW]), stored at 4 C for 60 days (Experiment II). All the samples were subjected to *Salmonella* analysis following the FDA BAM method. Results: During multiple samplings, the positive detection rate for *Salmonella* remained consistent through Day 42 after selective enrichment, irrespective of *Salmonella* serotype, storage temperature, pre-enrichment broth, or selective enrichment broth in both Experiment I and II. However, on Day 60 sampling in Experiment II, seven previously positive results turned to negatives. These data indicated that storage of RV and TT enrichments at 4 ± 2 C or room temperature for up to 42 days after selective enrichment did not compromise the detection of *Salmonella* in the tested food categories, regardless of *Salmonella* serotypes and the broths used for pre-enrichment and selective enrichment. Conclusions: At least for the food types studied in this experiment, the recovery of *Salmonella* from selective enrichments could be postponed for a limited period of time (e.g., <42 days) if needed without adversely affecting the test results. However, the delayed analysis of TT and RV enrichments does pose a risk of reduced detection sensitivity, as evidenced by the seven negative results on Day 60 compared to previous positives. We do not recommend or endorse delaying the analysis of TT and RV enrichments. Highlights: In the food matrixes investigated in this experiment, the plating and isolation of *Salmonella* from selective TT and RV enrichments stored at 4 ± 2 C or room temperature could be deferred for a period (up to 42 days) without any negative effect on the test results, if necessary. ISSN: 10603271

Demircioglu A., Coskun A.G., Kanar T.S., Eyigor A., Temelli S.

High Salmonella load with serovar virchow dominance pose major public safety risk in postchill broiler carcasses
(2024) *Poultry Science*, 103 (5), art. no. 103584

ABSTRACT: The objective of this study was to determine *Salmonella* contamination levels, presence and serovar distribution in broiler carcasses before and after chilling, as well as to evaluate the effectiveness of chilling process. A total of 96 pooled neck skin samples (PNSS) of 48 prechill (PreC) and 48 postchill (PosC) carcasses, representing 480 broilers collected in 6 mo' period were analyzed using ISO 6579-2:2012 Miniaturized Most Probable Number (ISO-mMPN) technique. Species confirmation and serovar identification was performed by *Salmonella*-specific real-time PCR (Salm-PCR) and conventional serotyping, respectively. Mean *Salmonella* count was 1.84 log₁₀ MPN/g in PreC, and 1.48 log₁₀ MPN/g in PosC samples, indicating a statistically significant reduction of 0.36 log₁₀ MPN/g ($p < 0.05$) in the counts by plant's air chill system. *Salmonella* positivity reduced from 97.9% (47/48) in PreC to 85.42% (41/48) in PosC samples, confirmed by Salm-PCR with identified serovars as *S. Virchow* (89.77 %) followed by *S. Schwarzengrund* (9.09%) and *S. Bredeney* (1.14%). Persistence of high load and prevalence of *Salmonella* with serovar *Virchow* dominance (other than the ones mandated in current guidelines) in the final product contributes significant and up to date data to relevant literature, and provides unbiased epidemiological reference to legal authorities for future relevant revisions. ISSN: 00325791

da Costa F.K.C., Carciofi B.A.M., de Aragão G.M.F., Ienczak J.L.

Modeling the influence of propionic acid concentration and pH on the kinetics of Salmonella Typhimurium
(2024) *International Journal of Food Microbiology*, 416, art. no. 110662

ABSTRACT: *Salmonella Typhimurium* is a foodborne pathogen often found in the poultry production chain. Antibiotics have been used to reduce *S. Typhimurium* contamination in poultry aviaries and improve chicken growth. However, antibiotics were banned in several countries. Alternatively, organic acids, such as propionic acid (PA), can control pathogens. This study determined the PA minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and mathematically modeled *S. Typhimurium* growth/inactivation kinetics under the influence of PA at different pH values (4.5, 5.5, and 6.5) which are within the pH range of the chicken gastrointestinal tract. The PA MIC against *S. Typhimurium* was pH-dependent, resulting in 5.0, 3.5 and 9.0 mM undissociated

PA at pH 4.5, 5.5, and 6.5, respectively. The Baranyi and Roberts and the Weibull model fit growth and inactivation data well, respectively. Secondary models were proposed. The validated model predicted 3-log reduction of *S. Typhimurium* in 3 h at 68.2 mM of undissociated PA and pH 4.5. The models presented a good capacity to describe the kinetics of *S. Typhimurium* subjected to PA, representing a useful tool to predict PA antibacterial action depending on the pH. ISSN: 01681605

Patil K., Adhikari M., Rubinelli P., Desiree K., Vierck K.R., Acuff J.C.

Evaluating the Safety of Sous-Vide Cooking for Beef Products Inoculated with Single Strains of Salmonella enterica and Escherichia coli O157
(2024) *Journal of Food Protection*, 87 (5), art. no. 100252

ABSTRACT: Sous-vide cooking is a growing trend among retailers and consumers. Foodborne pathogens may survive the cooking if nonvalidated parameters are used or if pathogens have enhanced thermal resistance. Pathogen inactivation from sous-vide cooking was determined when introduced directly to beef products or via contaminated spices, and with or without a finishing step. Beef products (ground beef, tenderized, and nontenderized steaks) were inoculated with pathogens (*Salmonella* Montevideo and *Escherichia coli* O157:NM) in three ways: 1) directly onto the meat 2) ground black pepper incorporated into the recipe 3) ground pepper equilibrated at 30% RH (4 d) prior to incorporation. Beef samples were vacuum-packaged and submerged in a 62.5°C water bath for 120 min. Samples were sampled at 5, 10, 20, and 120 min (recommended from a partner quality study), and a duplicate was grilled to a specific internal temperature (74°C for ground beef, 57°C for steaks) and sampled. Sous-vide cooking reduced pathogen populations by >5 log CFU/g after most treatment times, but less than grilled counterparts (ca. 1–2 log CFU/g difference; $p < 0.05$). There were no statistically significant differences between inoculation methods, but the tenderization of steaks resulted in significantly lower reductions of pathogens from sous-vide cooking ($p < 0.05$). This research challenged sous-vide cooking parameters (120 min, 62.5°C). It showed sous-vide alone lowered pathogens by >4 log CFU/g after most 20-min treatments, but 120-min sous-vide treatments or grilling would be needed for >5-log reductions. Contaminated pepper led to less consistent reductions during the cooking process, yet 2-h sous-vide still achieved a 5-log reduction. Sous-vide cooking instructions must be validated as more products and recipes are marketed. ISSN: 0362028X

Garcia-Vozmediano A., Maurella C., Ceballos L.A., Crescio E., Meo R., Martelli W., Pitti M., Lombardi D., Meloni D., Pasqualini C., Ru G.

Machine learning approach as an early warning system to prevent foodborne Salmonella outbreaks in northwestern Italy
(2024) *Veterinary research*, 55 (1), pp. 72

ABSTRACT: Salmonellosis, one of the most common foodborne infections in Europe, is monitored by food safety surveillance programmes, resulting in the generation of extensive databases. By leveraging tree-based machine learning (ML) algorithms, we exploited data from food safety audits to predict spatiotemporal patterns of salmonellosis in northwestern Italy. Data on human cases confirmed in 2015-2018 ($n = 1969$) and food surveillance data collected in 2014-2018 were used to develop ML algorithms. We integrated the monthly municipal human incidence with 27 potential predictors, including the observed prevalence of *Salmonella* in food. We applied the tree regression, random forest and gradient boosting algorithms considering different scenarios and evaluated their predictivity in terms of the mean absolute percentage error (MAPE) and R^2 . Using a similar dataset from the year 2019, spatiotemporal predictions and their relative sensitivities and specificities were obtained. Random forest and gradient boosting ($R^2 = 0.55$, MAPE = 7.5%) outperformed the tree regression algorithm ($R^2 = 0.42$, MAPE = 8.8%). *Salmonella* prevalence in food; spatial features; and monitoring efforts in ready-to-eat milk, fruits and vegetables, and pig meat products contributed the most to the models' predictivity, reducing the variance by 90.5%. Conversely, the number of positive samples obtained for specific food matrices minimally influenced the predictions (2.9%). Spatiotemporal predictions for 2019 showed sensitivity and specificity levels of 46.5% (due to the lack of some infection hotspots) and 78.5%, respectively. This study demonstrates the added value of integrating data from human and veterinary health services to develop predictive models of human salmonellosis occurrence, providing early warnings useful for mitigating foodborne disease impacts on public health. ISSN: 12979716

Nichols M., Stapleton G.S., Rotstein D.S., Gollarza L., Adams J., Caidi H., Chen J., Hodges A., Glover M., Peloquin S., Payne L., Norris A., DeLancey S., Donovan D., Dietrich S., Glaspie S., McWilliams K., Burgess E., Holben B., Pietrzen K., Benko S., Feldpausch E., Orel S., Neises D., Kline K.E., Tobin B., Caron G., Viveiros B., Miller

A., Turner C., Holmes-Talbot K., Mank L., Nishimura C., Nguyen T.N., Hale S., Francois Watkins L.K.

Outbreak of multidrug-resistant Salmonella infections in people linked to pig ear pet treats, United States, 2015–2019: results of a multistate investigation (2024) The Lancet Regional Health - Americas, 34, art. no. 100769

ABSTRACT: Background: International distribution of contaminated foods can be a source of *Salmonella* infections in people and can contribute to the spread of antimicrobial-resistant bacteria across countries. We report an investigation led by the United States Centers for Disease Control and Prevention, the Food and Drug Administration (FDA), and state governmental officials into a multistate outbreak of salmonellosis linked to pig ear pet treats. Methods: Pig ear treats and companion dogs were tested for *Salmonella* by state officials and the FDA. Products were traced back to the country of origin when possible. Cases were defined as outbreak illnesses in people associated with one of seven *Salmonella* serotypes genetically related to samples from pig ear pet treats, with isolation dates from June 2015 to September 2019. Whole genome sequencing (WGS) of isolates was used to predict antimicrobial resistance. Findings: The outbreak included 154 human cases in 34 states. Of these, 107 of 122 (88%) patients reported dog contact, and 65 of 97 (67%) reported contact with pig ear pet treats. *Salmonella* was isolated from 137 pig ear treats, including some imported from Argentina, Brazil, and Colombia, and from four dogs. WGS predicted 77% (105/137) of human and 43% (58/135) of pig ear treat isolates were resistant to ≥ 3 antimicrobial classes. Interpretation: This was the first documented United States multistate outbreak of *Salmonella* infections linked to pig ear pet treats. This multidrug-resistant outbreak highlights the interconnectedness of human health and companion animal ownership and the need for zoonotic pathogen surveillance to prevent human illness resulting from internationally transported pet food products. Funding: Animal Feed Regulatory Program Standards award. Animal and product testing conducted by FDA Vet-LIRN was funded by Vet-LIRN infrastructure grants (PAR-22-063).
ISSN: 2667193X

Pérez-Lavalle L., Valero A., Cejudo-Gómez M., Carrasco E.

Assessment of the efficacy of decontamination treatments against Salmonella enterica subsp. enterica serovar Thompson on strawberries at different storage conditions (2024) Postharvest Biology and Technology, 212, art. no. 112907

ABSTRACT: The risk of outbreaks associated with strawberries consumption has led to the evaluation of different antimicrobial strategies. The effect of disinfectants, i.e. chlorine (100 and 200 mg L⁻¹), peracetic acid (40 and 80 mg L⁻¹), lactic acid and hydrogen peroxide (2.5 and 5%), on strawberries contaminated with *Salmonella enterica* subsp. *enterica* serovar Thompson during 2 min and stored at different times at two temperatures (20 and 4 °C) was studied. Likewise, the influence of disinfectants on the physicochemical quality of fruits was addressed. The efficacy of 100 mg L⁻¹ chlorine or 40 mg L⁻¹ peracetic acid decreased as storage time at 20 °C increased. Most of the physicochemical parameters were not affected by the treatments, except for 5% hydrogen peroxide, which caused discoloration of the strawberries. 2.5% lactic acid treatment was selected for the evaluation of its antimicrobial effect on strawberries stored at 4 °C for 6 d after treatment. This treatment achieved an efficient reduction of *S. Thompson* which was maintained over time, while for the case of mesophilic bacteria and yeasts and molds, differences with control or water-washed samples were levelled out over time. No changes in physicochemical parameters were observed during the storage time. 2.5% lactic acid is proposed for decontamination of strawberry-derived products. ISSN: 09255214

Khan S., McWhorter A.R., Andrews D.M., Underwood G.J., Moore R.J., Van T.T.H., Gast R.K., Chousalkar K.K.

Dust sprinkling as an effective method for infecting layer chickens with wild-type Salmonella Typhimurium and changes in host gut microbiota (2024) Environmental Microbiology Reports, 16 (3), art. no. e13265

ABSTRACT: Role of dust in *Salmonella* transmission on chicken farms is not well characterised. *Salmonella Typhimurium* (ST) infection of commercial layer chickens was investigated using a novel sprinkling method of chicken dust spiked with ST and the uptake compared to a conventional oral infection. While both inoculation methods resulted in colonisation of the intestines, the *Salmonella* load in liver samples was significantly higher at 7 dpi after exposing chicks to sprinkled dust compared to the oral infection group. Infection of chickens using the sprinkling method at a range of doses showed a threshold for colonisation of the gut and organs as low as 1000 CFU/g of dust. Caecal content microbiota analysis post-challenge showed that the profiles of chickens infected by the sprinkling and oral routes were not significantly different; however, both challenges induced differences when compared to the uninfected negative controls. Overall, the study

showed that dust sprinkling was an effective way to experimentally colonise chickens with *Salmonella* and alter the gut microbiota than oral gavage at levels as low as 1000 CFU/g dust. This infection model mimics the field scenario of *Salmonella* infection in poultry sheds. The model can be used for future challenge studies for effective *Salmonella* control. ISSN: 17582229

Perry J., Arnold K., Satuchne C., Koren O., Kenigswald G., Elnekave E.

Accumulation of resistance genes in Salmonella Typhimurium transmitted between poultry and dairy farms increases the risk to public health

(2024) *Applied and environmental microbiology*, 90 (6), pp. e0229723

ABSTRACT: *Salmonella Typhimurium* is a zoonotic pathogen that poses a major threat to public health. This generalist serotype can be found in many hosts and the environment where varying selection pressures may result in the accumulation of antimicrobial resistance determinants. However, the transmission of this serotype between food-producing hosts, specifically between poultry layer flocks and nearby dairy herds, was never demonstrated. We investigated an outbreak at a dairy in Israel to determine the role of nearby poultry houses to be sources of infection. The 2-month outbreak resulted in a 47% mortality rate among 15 calves born in that period. Routine treatment of fluid therapy, a nonsteroidal anti-inflammatory, and cefquinome was ineffective, and control was achieved by the introduction of vaccination of dry cows against *Salmonella* (Bovivac S, MSD Animal Health) and a strict colostrum regime. Whole genome sequencing and antimicrobial sensitivity tests were performed on *S. Typhimurium* strains isolated from the dairy (n = 4) and strains recovered from poultry layer farms (n = 10). We identified acquired antimicrobial-resistant genes, including the blaCTX-M-55 gene, conferring resistance to extended-spectrum cephalosporins, which was exclusive to dairy isolates. Genetic similarity with less than five single nucleotide polymorphism differences between dairy and poultry strains suggested a transmission link. This investigation highlights the severe impact of *S. Typhimurium* on dairy farms and the transmission risk from nearby poultry farms. The accumulation of potentially transferable genes conferring resistance to critically important antimicrobials underscores the increased public health risk associated with *S. Typhimurium* circulation between animal hosts. IMPORTANCESalmonella Typhimurium is one of the major causes of food-borne illness globally. Infections may result in severe invasive disease, in which antimicrobial treatment is warranted. Therefore, the emergence of multi-drug-resistant strains poses a significant challenge to successful treatment and is considered one of the major threats to global health. *S. Typhimurium* can be found in a variety of animal hosts and environments; however, its transmission between food-producing animals, specifically poultry layers flocks and dairy herds, was never studied. Here, we demonstrate the transmission of the pathogen from poultry to a nearby dairy farm. Alarmingly, the multi-drug-resistant strains collected during the outbreak in the dairy had acquired resistance to extended-spectrum cephalosporins, antibiotics critically important in treating Salmonellosis in humans. The findings of the study emphasize the increased risk to public health posed by zoonotic pathogens' circulation between animal hosts. ISSN: 10985336

Korkeala H., Koskela T., Jalava K.

Salmonella Typhimurium outbreak caused by contaminated chocolate highlights the importance of food hygiene and a socially responsible food chain

(2024) *Hygiene and Environmental Health Advances*, 10, art. no. 100096

ABSTRACT: Recent global salmonella outbreak was linked to chocolate products but potential role of cocoa beans has been neglected. Chocolate related salmonella outbreaks have occurred mostly during low cocoa price levels. Food business operators should ensure hygiene at all production stages. These outbreaks highlight corporate social responsibility while demonstrating the need of multi-professional approaches. ISSN: 27730492

Santos M.R.B.D., Moreira Filho A.L.D.B., Freitas Neto O.C., Andrade M.D.F.D.S., Silva N.M.V.D., Sales G.F.C., Oliveira C.J.B.D., Givisiez P.E.N.

Shifts in microbiota and gene expression of nutrient transporters, mucin and interleukins in the gut of fast-growing and slow-growing chickens infected by Salmonella Enteritidis (2024) *Brazilian Journal of Microbiology*, 55 (2), pp. 1987 - 1996

ABSTRACT: Slow-growing breeds are more resistant to *Salmonella* infection compared to fast-growing broilers. However, it is unclear whether that is associated with innate resistance or rather rely on differences in *Salmonella*-induced gut responses. We investigated the microbial composition and gene expression of nutrient transporters, mucin, and interleukin in the gut of a fast-growing (Cobb500) and a slow-growing naked neck (NN) chicken breeds challenged with *Salmonella Enteritidis*. Hatchlings were inoculated at two days of age using sterile broth (sham) or *Salmonella Enteritidis* (SE) and

distributed according to a completely randomized design into four treatments: Cobb-sham; Cobb-SE; NN-sham; and NN-SE. Cecal SE counting and microbial composition by 16 S rRNA sequencing were determined at 24-, 96-, and 168-hours post-inoculation (hpi). Gene expression of amino acid (Asct1) and peptide transporters (PepT1), glucose transporters (Sglt1, Glut2 and Glut5) and mucin (Muc2) in the jejunum and expression of interleukins (IL1 beta, IL8, IL17 and IL22) in the cecum was assessed by qPCR at 24 and 168 hpi. NN birds were colonized by SE just as Cobb birds but showed innate upregulation of Muc2, IL8 and IL17 in comparison to Cobb. While nutrient transporter mRNA expression was impaired in SE-challenged Cobb birds, the opposite was observed in NN. There were no differences in microbial diversity at different sampling times for Cobb-SE, whereas the other groups had higher diversity and lower dominance at 24 hpi compared with 96 hpi and 168 hpi. NN birds apparently develop earlier gut microbial stability, have higher basal level of mucin gene expression as well as differential nutrient transporter and interleukin gene expression in the presence of SE which might mitigate the effects of SE infection compared to Cobb birds. ISSN: 15178382